Asiatic Citrus Canker Detected in a Pummelo Orchard in Northern Australia

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ABSTRACT

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Raised, roughly circular, corky scabs 4–5 mm in diameter and typical of citrus canker were observed on spring flush leaves, twigs, and fruits of pummelos (*Citrus grandis*) in a young orchard near Darwin, Australia, during a survey conducted in 1991 as part of the Northern Australia Quarantine Strategy. The causal agent was identified as *Xanthomonas campestris* pv. *citri* (Asiatic group, or group A), using pathogenicity in a series of hosts, fatty acid profiles, and DNA fingerprints. The 10 strains from Darwin were compared with two previously identified strains of *X. c. citri* (group A) from a canker outbreak (which has since been eradicated) on Thursday Island in the Torres Strait. Symptoms on inoculated leaves of sweet orange, West Indian lime, sour orange, and Duncan grapefruit included lesions of eruptive, calluslike white tissue and were produced by all strains. Lesions were larger and more erumpent on seedlings or detached leaves of West Indian lime, sweet orange, and Duncan grapefruit. On citrange C-35 (*Poncirus trifoliata* × *C. sinensis*) leaves, calluslike lesions were produced by Thursday Island strains, and small, light tan, necrotic areas were produced by the Darwin strains. The fatty acid profiles of the Thursday Island strains were similar to a library generated from the fatty acid profiles of the Darwin strains (with similarity indices of 0.610 and 0.810). All Darwin strains had identical DNA restriction patterns, which were similar (with a similarity coefficient of 94%) but not identical to those produced from the reference Thursday Island strains. These results confirm that the canker outbreak near Darwin was caused by *X. c. citri* (group A). All citrus trees within the diseased orchard have been destroyed. No further outbreaks have been detected.

During a survey of citrus in northern Australia in April 1991, as part of the Northern Australia Quarantine Strategy, the senior author observed symptoms consistent with citrus canker in a young pummelo (*Citrus grandis* (L.) Osbeck) orchard of 150 trees. Symptoms were raised, roughly circular, corky scabs 4–5 mm in diameter, with a yellow halo on the leaves and twigs of the spring flush.

Australia has had three previous outbreaks of citrus canker: in the Northern Territory in 1912 (10), on Home Island in the Cocos (Keeling) Islands in 1981 (21), and on Thursday Island in the Torres Strait in 1984 (12). The 1912 outbreak apparently originated from Japan or China, since citrus trees were imported into Darwin from these sources, and nearly every ship trading between these countries and Australia landed small consignments of citrus fruits there. In 1915–1916 proclamations were issued that prohibited both the importation of citrus trees and fruit into Australia and the removal of citrus trees and fruit from the Northern Territory to any other part of the country. Material from California and Arizona was later exempted. Although symptoms of citrus canker were detected in the Northern Territory in 1912 by Hill (10), the causal agent was not confirmed until 1916, when it was identified by C. C. Brittlebank of the Victorian Department of Agriculture and by H. E. Stephens of Florida. As far as possible, every citrus tree in the Territory was carefully examined for canker in 1918. All citrus trees in proximity to canker-affected trees were destroyed. Land in the vicinity of areas in which canker had been detected was placed under quarantine for a period of 5 yr.

By 1922 it was obvious that the initial eradication campaign was not successful. Hill then recommended that all citrus trees in the Northern Territory be destroyed. The destruction campaign was modified, in that only trees north of the 19th parallel were dug out and burned. It was not until 1925 that permission was granted to grow citrus trees again in the Northern Territory. Plantings were stringently inspected, and reinfection of trees was not found. No symptoms of the disease were found during a survey of 4,400 trees in 1950 in regions that were featured in the original surveys (18).

No further outbreaks of citrus canker occurred in Australia until 1981, when the disease was found on West Indian limes (*C. aurantifolia* (Christm.) Swing.) on Home Island in the Cocos Islands (21). In May–June 1984 the A form of citrus canker was detected on three West Indian lime and two sweet orange (*C. sinensis* (L.) Osbeck) trees in gardens on Thursday Island in the Torres Strait (12). Four additional trees with the disease were found in the near vicinity in 1985. All diseased trees were destroyed. By September 1988, the Commonwealth Department of Primary Industries and Energy considered this outbreak to have been eradicated (3). No further evidence of canker was found during a survey of citrus on Torres Strait Islands and Cape York Peninsula carried out by the senior author in 1990 as part of the Northern Australia Quarantine Strategy.

In this paper we report the fourth outbreak of citrus canker in Australia and its identification as Asiatic citrus canker. The pathogen was identified as *Xanthomonas campestris* pv. *citri* (Hasse) Dye group A by pathogenicity in a series of hosts (2,13), fatty acid profiles (20), and a genomic fingerprinting technique (9).

MATERIALS AND METHODS

Sample collection. Leaves and twigs with canker symptoms were collected in April 1991 by the senior author from 4-yr-old pummelo (cv. Termat) trees at Lambell's Lagoon, near Darwin. Specimens were sealed in plastic bags and immediately transported to the Berrimah Research Station of the Northern Territory Department of Primary Industry and Fisheries, where the samples were divided and some material forwarded on to the Biological and Chemical Research Institute (BCRI), Rydalmere, New South Wales.

Bacterial isolations. Isolations were carried out under quarantine conditions at BCRI Rydalmere and at Berrimah Research Station. Individual lesions were dissected from 10 different leaves, finely chopped in separate drops of sterile water in sterile petri dishes, and left for 15 min. before streaking the liquid from the macerate onto sucrose peptone agar (SPA) (sucrose at 20 g/L, peptone at 5 g/L, K_2HPO_4 at 0.5 g/L, $MgSO_4 \cdot 7H_2O$ at 0.25 g/L, and agar at 15 g/L, pH 7.2–7.4). Single yellow, domed, mucoid colonies indicative of *Xanthomonas* were selected after 3 days at 28 C and were restreaked twice to obtain pure strains. Nine strains (DAR 65859–65867) were identified as typical xanthomonads by fatty-acid profiling and were retained for further testing. An additional *Xanthomonas* strain (DAR 65868) was obtained from a lesion on a leaf surface-sterilized with 75% alcohol prior to maceration.

Two strains of *X. c. citri* group A from the canker outbreak on Thursday Island (12) were obtained as lyophilized cultures

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from A. Diatloff, Queensland Department of Primary Industries, Indooroopilly. The strains had been identified by M. Goto at Shizuoka University, Japan, as belonging to group A on the basis of pathogenicity and phage sensitivity tests. Strain DPI 1484 (DAR 65869) was from a lesion on sweet orange and DPI 1847 (DAR 65870) from West Indian lime.

A citrus canker specimen (DAR 65859) from the Darwin outbreak and bacterial strains (DAR 65859–70) were lodged with the Australian National Collection of Plant Pathogenic Bacteria in the NSW Agriculture Plant Pathology Branch Herbarium (DAR).

Pathogenicity tests. Aggressiveness of each *Xanthomonas* strain was evaluated with detached-leaf assays and attached-leaf inoculations. A strain of *Pseudomonas saccharophila* Doudoroff (BCRI Bacteriology Number B983.13), which had been isolated from canker-affected pummelo leaves, was used as the negative control. All inoculations were carried out in the quarantine laboratory at the Quarantine Station, BCRI Rydalmere.

Detached-leaf assays. Young (two thirds to fully expanded) terminal leaves from glasshouse-grown plants of sweet orange, Duncan grapefruit (*C.* × *paradisi* Macfady), C-35 citrange (*Poncirus trifoliata* (L.) Raf. × *C. sinensis*), West Indian lime, and sour orange (*C. aurantium* L.) cultivars Bitter Sweet Seville and Gou Tou, were washed for 10 min in running tap water, surface-disinfected by dipping in 1% sodium hypochlorite for 2 min, and rinsed thoroughly with sterile distilled water. C-35 citrange was used as a substitute for *P. trifoliata*, since the latter species was dormant during the test period. Each leaf was placed on 1% water agar in a petri dish with the lower surface up and was wounded by puncturing with an insulin syringe (0.36-mm-diameter needle). Droplets (10–20 µl) of an aqueous bacterial suspension (~1×10^8 cfu/ml, determined by plate count) were placed on five needle puncture wounds on the lower surface of the detached leaf tissue. Bacteria had grown on SPA at 28 C for 48 hr. Plants were maintained in an illuminated controlled environment cabinet at 26 C. The detached-leaf assay was performed twice, with an average of three leaves used per bacterial strain. After 5–7 days the needle puncture wounds were examined and photographed under 10× magnification of a Zeiss Stemi SV8 microscope.

Attached-leaf inoculations. An attached-leaf assay was performed to determine if the detached-leaf assay correlated with more natural inoculation methods. Aqueous cell suspensions of the bacteria (~10^8 cfu/ml) were prepared from a 48-hr culture on SPA at 28 C and either injected through the lower surface of half to fully expanded leaves with an insulin syringe or applied to needle puncture wounds on the lower surface of the leaf. The test plants were 9-mo-old glasshouse seedlings of Duncan grapefruit, West Indian lime, sweet orange, sour orange, rough lemon (*C. jambhiri* Lush.), and Swingle citrumelo (*P. trifoliata* × *C. paradisi*). In the first test, inoculated plants were placed in sealed plastic bags for 2 days, whereas in the second experiment the plants were bagged for 7 days. Plants were incubated in an illuminated controlled-environment cabinet (at 26 C) within the Quarantine Station at BCRI Rydalmere. The attached-leaf assay was conducted twice with two plants (approximately eight leaves) per test plant for each *Xanthomonas* strain. Reactions to wounding were assessed and photographed 7–10 days after inoculation.

Fatty acid analyses. Each strain was grown on SPA for 2–3 days at 28 C to obtain actively growing colonies, then subcultured on BBL trypticase soy broth with 1.5% Difco Bacto Agar (TSBA). The TSBA plate cultures were grown at 28 ± 1 C for 24 ± 1 hr under aerobic conditions. Approximately 40 mg of bacterial culture was harvested from each plate with a platinum loop. Fatty acids were extracted from each culture, converted to fatty acid methyl esters, and assayed by the method of Sasser (20).

The fatty acid methyl ester extracts were injected into a Hewlett-Packard 5898A gas–liquid chromatograph, where component fatty acids were separated by a 25 m × 0.2 mm high resolution capillary column with 0.33-µm cross-linked 5% phenyl methyl silicone coating prior to flame ionization detection. Separated peaks were identified by the MIDI Microbial Identification System (MIS) standards and software (Microbial ID,

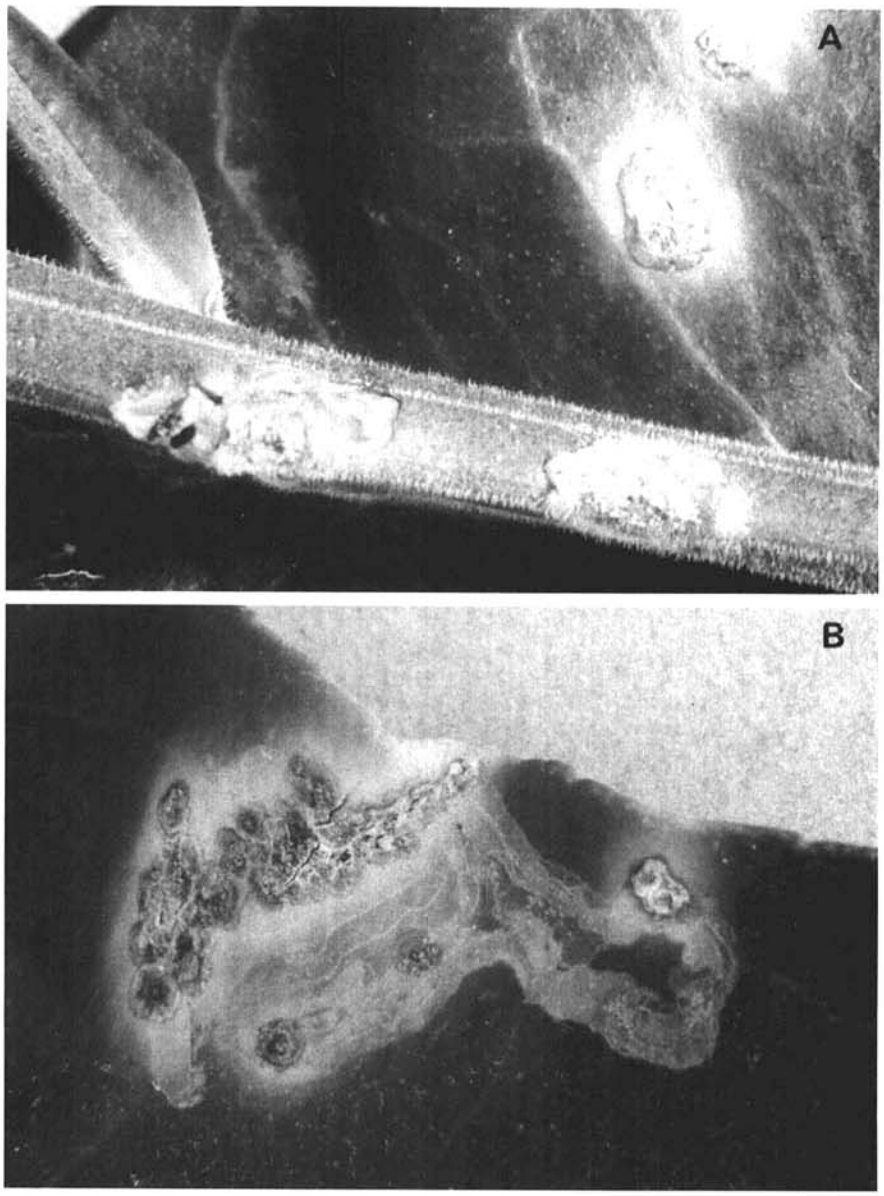

Fig. 1. Symptoms of canker **(A)** on a leaf and twig of the spring flush of a 4-yr-old pummelo tree at Darwin, Australia, and **(B)** following the channels made by leaf miners (*Phyllocnistis citrella*).

Inc., Newark, Delaware) (1). Each extract was named by comparing the calculated percentage area of each of the fatty acid peaks with profiles of known bacteria held in the computer systems library database, linked to the gas–liquid chromograph, using the MIDI software. A similarity index was produced for each strain by matching to the closest species in the MIDI MIS TSBA aerobe library version 3.0. The similarity index is based on statistical similarity, which expresses how closely the composition of an unknown isolate compares with the fatty acid composition of the library matches. The library of fatty acid profiles is computer-searched using a pattern recognition program. A similarity index of 0.6–1.0 is reported to be an excellent match (1).

Following fatty acid analyses of the Darwin strains DAR 65859–65867, a new library entry profile was generated with the MIDI Library Generation Software (1). The profiles of the two reference strains DPI 1484 and DPI 1847 of *X. c. citri* group A from the canker outbreak on Thursday Island were compared to the new library entry profile.

DNA fingerprinting. *DNA isolation.* A loopful (40 mg) of bacterial culture (grown for 2 days on SPA) was suspended in 500 µl of TNE buffer (25 mM Tris, 100 mM NaCl, 100 mM EDTA, pH 8.0). To the suspension 100 µl of 20% (w/v) aqueous sodium dodecyl sulfate (SDS) and 500 µl of phenol was added (17). The tube was vortexed and cells left to lyse at room temperature for 30 min, then 250 µl of chloroform–isoamyl alcohol (24:1, v/v) was mixed in. The lysate was centrifuged (microcentrifuge, 15 min), and 2 vol of cold (−20 C) ethanol was added to the resulting supernatant to precipitate the DNA. The DNA precipitate was allowed to develop on ice for 30 min and was collected by centrifugation. The DNA pellets were washed with a solution of 100 mM Na acetate in 70% ethanol, air-dried, and resuspended in 100 µl of TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.4) overnight at 4 C.

Restriction digests. Forty microliters

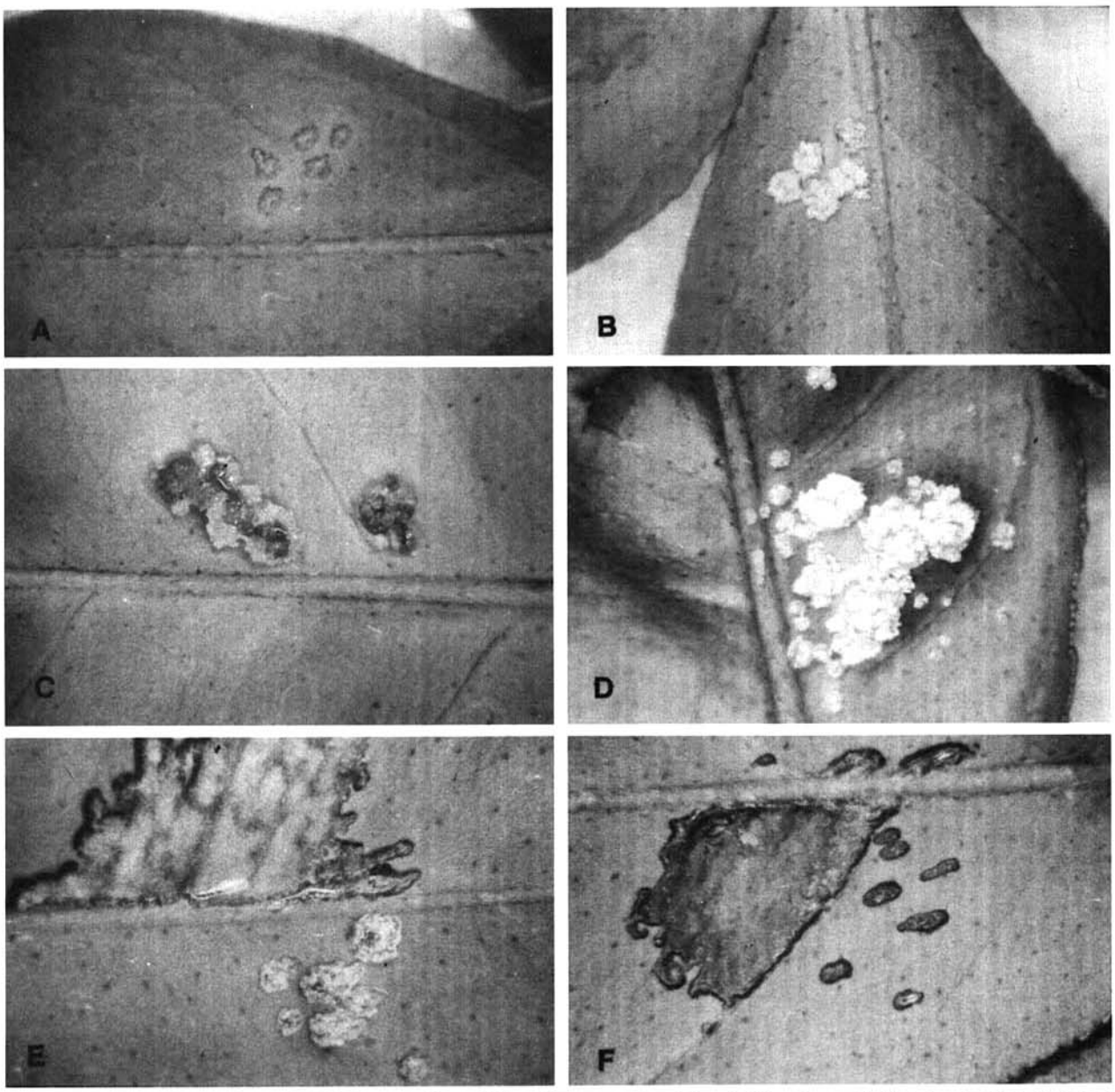

Fig. 2. Lesions on detached leaves of C-35 citrange **(A and B)** and sweet orange **(C and D)** and on attached leaves of sweet orange **(E)** and Swingle citrumelo **(F)** following wound inoculation with Darwin and Thursday Island strains of *Xanthomonas campestris* pv *citri* group A. Lesions on detached leaves 7 days after inoculation: lesions produced by **(A)** Darwin strain DAR 65861 and **(B)** Thursday Island strain DPI 1847 at wound sites on C-35 citrange and Thursday Island strains **(C)** DPI 1484 and **(D)** DPI 1847 on sweet orange. Lesions on attached leaves 7 days after inoculation: Darwin strains **(E)** DAR 68560 on sweet orange and **(F)** DAR 65859 on Swingle citrumelo. Note "big vein" effect produced by injection of inoculum, with calluslike lesions around pinpricks.

of the DNA prepared in the section above were digested in a total volume of 100 µl at 37 C for 4 hr using 25 units of the restriction enzyme *Hae* III (Promega). Digests of DNA were performed according to the manufacturer's instructions, except that bovine serum albumin (BSA) and RNase A were added to 50 and 10 µg/ml, respectively. Digests were terminated by extraction with 40 µl of phenol chloroform. In all cases, duplicate digests of independent DNA extractions were performed.

Polyacrylamide gel electrophoresis. Digested samples were made up to 10% sucrose, 20 mM EDTA, and 40 mM Tris-acetate (pH 7.2). Aliquots (30 µl) of the digests prepared in the section above were electrophoresed in 3-mm thick 5% polyacrylamide gels (a 30:1 mixture of acrylamide and bis-acrylamide) cast and run in Tris-acetate buffer (40 mM Tris-acetate and 1 mM EDTA, pH 7.2), using a vertical slab gel apparatus (Pharmacia GE 2/4). Samples were electrophoresed for 17 hr at 25 mA per 8 × 14 cm gel cassette. Under these conditions restriction fragments smaller than about 500 base pairs migrated off the end of the gel. Gels were silver-stained using a method modified from that of Igloi (11). Digests were assessed as complete if no differences could be discerned between duplicates prepared from independent DNA extractions. The restriction patterns generated were matched by visual inspection. Similarity coefficients were calculated using the formula $F = (2Nxy/Nx + Ny) \times 100$, where Nx and Ny were the number of restriction fragments produced by digestion of isolates x and y, respectively, and Nxy was the number of fragments common to both x and y (19).

RESULTS

Field symptomatology. The affected pummelo trees were growing in an orchard of mixed tropical fruits at Lambell's Lagoon, 40 km southeast of Darwin. There were 25 vigorous 4-yr-old pummelo trees and an additional 125 2-yr-old trees. Leaves, twigs (Fig. 1), and occasional fruits had symptoms when inspected in late April 1991 at the end of the wet season. Only the growth flush produced in early summer, at the beginning of the wet season, was affected.

Lesions that were still active had a dark green, oily ring at the margin and a prominent yellow halo. Raised, roughly circular, corky scabs 4–5 mm in diameter, sometimes coalescing, were common on leaves and some twigs (Fig. 1A). Frequently the infection of leaves followed the course taken by leaf-mining caterpillars (*Phyllocnistis citrella* Stainton) (Fig. 1B) and other injuries. Exudation from lesions was occasionally seen. Leaf fall was commencing prematurely from badly affected twigs.

Pathogenicity tests. *Detached-leaf assays.* With most test hosts, reactions were similar for the Darwin and Thursday Island strains. The exception was C-35 citrange, in which the Darwin strains produced small, light tan, necrotic lesions with slight corking and a chlorotic margin around each pinprick; a water-soaked margin occurred around some lesions (Fig. 2A). By contrast, Thursday Island strains produced small, eruptive lesions of white callus, some with water-soaked margins (Fig. 2B), which were more severe on younger leaves.

On detached sweet orange leaves the symptoms varied from sunken, water-soaked areas to erumpent lesions with water soaking (Fig. 2C) to white calluslike eruptions (Fig. 2D). After 7 days the erumpent, calluslike lesions had become light tan and had split. The most extensive and erumpent lesions were produced by Thursday Island strains (DPI 1484 and DPI 1847) and the Darwin strain DAR 65868.

Similar severe symptoms were produced on detached leaves of Duncan grapefruit. Strain DPI 1484 ex Thursday Island produced more severe symptoms of calluslike tissue, with and without water soaking, than other strains. On West Indian lime, lesions were similar to those produced on sweet orange, but smaller in size. On rough lemon leaves, lesions were small and calluslike. No differences between strains occurred for the reaction on sour orange, and symptoms were less severe than on sweet orange, West Indian, grapefruit, or rough lemon.

Attached-leaf inoculations. Symptoms were more severe in the second experiment than in the first, where plants were kept bagged (and consequently the humidity was high) for 7 days instead of 2 days. Some secondary infection occurred around wounded sites under high humidity. Generally symptoms were similar, but less severe, on attached than detached leaves. The exception was for C-35 citrange, where the symptoms were at least as severe as on detached leaves. Injections resulted in a chlorosis of infiltrated areas within 24 hr, which later became dark and greasy in appearance. The severity of the reaction was reflected in a "big vein" effect and an enlargement of tissue at the margin of the infiltrated area (Fig. 2E).

Symptoms on Swingle citrumelo were similar in severity to those produced on sour orange. The lesions produced following wounding were slightly erumpent, with a water-soaked margin (Fig. 2F), and became light tan and cracked after 7 days.

Fatty acid analyses. Darwin and Thursday Island strains were compared with entries in the MIDI MIS TSBA aerobe library version 3.0 to obtain similarity indices to *X. c. citri*. Similarity indices varied from 0.420 to 0.615 (mean = 0.524 ± SD 0.072) for Darwin strains and 0.387 and 0.424 (mean = 0.406 ± SD 0.026) for the two Thursday Island strains (Table 1).

Thursday Island strains DPI 1484 and DPI 1847 were matched (similarity indices 0.610 and 0.810, respectively) to a library generated from the fatty acid profiles of the 10 Darwin strains (Table 1). The mean fatty acid profile for the Darwin strains was similar to a previously published (20) fatty acid profile for *X. c. citri*, developed using the same MIDI system (Table 2).

DNA fingerprinting. Digestion of DNA from *Xanthomonas* strains with *Hae* III produced a complex restriction

Table 1. Comparison of similarity indices for fatty acid methyl ester profiles generated from *Xanthomonas campestris* pv. *citri* strains from Darwin and Thursday Island, Australia, against MIDI MIS TSBA aerobe library version 3.0 (1) and a library generated from the Darwin strains (Darwin LIB)

	Similarity indices	
	Fatty acid library	
Culture[a]	TSBA 3.0	Darwin LIB
DAR 65859	0.494	0.824
DAR 65860	0.468	0.910
DAR 65861	0.584	0.870
DAR 65862	0.492	0.911
DAR 65863	0.569	0.956
DAR 65864	0.402	0.882
DAR 65865	0.572	0.946
DAR 65866	0.447	0.855
DAR 65867	0.597	0.834
DAR 65868	0.615	0.863
DPI 1484	0.387	0.610
DPI 1847	0.424	0.810

[a] DAR 65859–65868 are from Darwin, Australia; DPI 1484 and 1847, from Thursday Island.

Table 2. Comparison of mean fatty acid composition of Darwin strains of *Xanthomonas campestris* pv. *citri* with *X. c. citri* profile from Sasser[a]

	Total fatty acids (%)	
Fatty acids	Darwin	Sasser
14:0	0.8	0.9
15:0	1.1	1.1
16:0	2.9	4.6
16:1 CIS 9	14.1	15.2
12:0 30H	1.8	2.1
11:0 ISO	4.2	4.8
15:0 ISO	31.3	34.4
16:0 ISO	4.2	3.0
17:0 ISO	8.9	7.5
17:1 ISO F	9.1	7.1
15:0 ANTEISO	10.2	8.5
17:0 ANTEISO	0.5	0.4
11:0 ISO 30H	1.8	1.6
13:0 ISO 30H	4.0	4.1
16:1 B	1.2	...[b]
17:1 B	1.1	...
Unknown ECL[c] 11.798	1.4	...

[a] Fatty acid profile for *X. c. citri* previously published by Sasser (20).
[b] Not given.
[c] Equivalent chain length.

pattern of about 50 resolvable bands between 2,000 and 500 base pairs. Nine bacterial strains from the suspected citrus canker outbreak at Darwin had identical restriction patterns, and these were similar but not identical to those produced by digestion of two known strains of *X. c. citri* (group A) that had been isolated from Thursday Island (Fig. 3). The similarity coefficient comparing Thursday Island and Darwin strains was 94%. This very close relationship confirms that the canker outbreak at Darwin was caused by a strain of *X. c. citri* (group A).

DISCUSSION

Asiatic citrus canker is the most devastating form of the citrus bacterial canker diseases and affects most *Citrus* species and hybrids as well as other rutaceous and nonrutaceous hosts (4). Grapefruit, *P. trifoliata*, sweet oranges, Mexican limes, and lemons and some mandarins, tangerines, and tangelos may be severely affected (14). Pummelos also may become severely diseased, but Koizumi and Kuhara (15) recognized both susceptible and resistant cultivars, according to disease severity on the foliage and twigs of field trees. The Darwin outbreak of citrus canker involved only one small orchard of 150 pummelo trees. The disease was widely distributed among the 2- and 4-yr-old trees, but symptoms were seen only in the one growth flush, produced at the beginning of the 1990–1991 summer monsoon season. *X. c. citri* may have been present for a longer period, surviving epiphytically in low numbers without symptom development (8) or as subclinical infections. The severity of leaf symptoms was related to the abundance of channels produced by leaf miners, and these insects may have been responsible for short-distance dissemination of the pathogen.

The origin of the outbreak is unknown. DNA fingerprints, fatty acid profiles, and pathogenicity, while confirming the identity of the causal agent as a member of group A of *X. c. citri*, also provided evidence for differences between the Darwin and Thursday Island strains. It seems unlikely that the two outbreaks were in any way related, given the geographical isolation of the two sites and the nature of citrus production in the two areas. In the Darwin area there are small mixed orchards of tropical and subtropical fruits, whereas on Thursday Island there are no commercial orchards, and most citrus are yard trees of seedling West Indian lime or sweet orange. Because few pummelo cultivars have been legally imported into Australia, and given that the pummelo is indigenous to the Malayan and East Indian archipelagos, where canker is endemic, it is possible that an illegal introduction may have resulted in the current outbreak.

Reactions on wound-inoculated detached leaves proved a rapid and reliable method of characterizing the strains of *X. c. citri*. Disease development was more rapid on detached leaves and was correlated with that on wound-inoculated attached leaves, provided the latter were maintained at high relative humidity for 7 days.

Analysis of bacterial DNA by Southern transfer or by examination of total genomic DNA digests has been used successfully to identify infrasubspecific categories within plant pathogenic bacterial species of various genera, including *Pseudomonas* (5) and *Xanthomonas* (7,9,16). DNA-based approaches provide a rapid and sensitive means of identifying clonal bacterial lines for epidemiological, quarantine, and evolutionary investigations. Here we found that DNA extracts from the Darwin strains had identical *Hae* III restriction patterns that were very similar but not identical to those from the Thursday Island strains of *X. c. citri*.

The fatty acid profile library developed from the Darwin strains is similar but not identical to the profile published by Sasser (20). The differences are minor and probably reflect the much narrower base of the Darwin library, which was generated by analysis of strains from a single outbreak. The homogeneity of the Darwin strains is shown by the identical DNA fingerprint results and the negligible variation in the strain matches to the library. The lower match of the fatty acid profiles of Thursday Island strains to both the TSBA and Darwin libraries may reflect a difference in origin of these strains to those included in the two

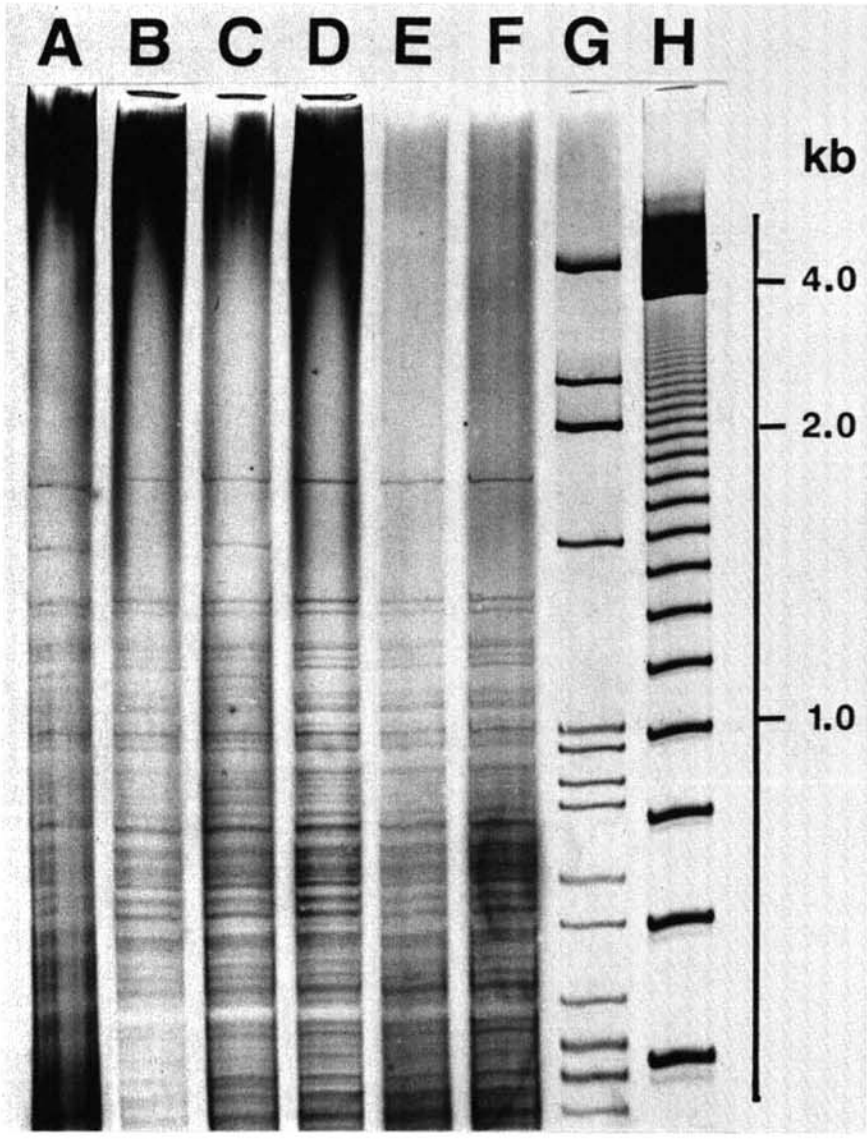

Fig. 3. DNA fingerprinting analysis of the Darwin and Thursday Island strains of *Xanthomonas campestris* pv. *citri*. *Hae* III digests of total DNA were separated by electrophoresis on 5% polyacrylamide and stained with silver. Individual tracks are as follows: **(A)** DPI 1847 (Thursday Island), **(B)** DAR 65862 (Darwin), **(C)** DPI 1484, **(D)** DAR 65863, **(E)** DAR 65865, **(F)** DAR 65866, **(G)** *Hae* III digested lambda DNA, **(H)** BRL 123 bp ladder. Sizes in kilobases (kb) are given at right.

libraries. Variation in library matches between the two Thursday Island strains, even though they had identical DNA fingerprints, may reflect slight differences in fatty acid components of the two strains. A similar phenomenon occurs with *Pseudomonas solanacearum* (Smith) Smith biovar III, in which strains with identical DNA fingerprints but different colony morphology show minor differences in their fatty acid profiles (6). The low matches of the Darwin and Thursday Island strains to *X. c. citri* in the TSBA library most probably reflect the different geographical origins of the TSBA entries and the narrow base of the Darwin library.

The slight but consistent differences between Thursday Island and Darwin strains in pathogenicity tests, DNA fingerprints, and fatty acid profiles suggest that host or geographical clonal lines of citrus canker group A may exist. Analysis of a greater range of group A strains could reveal yet more clonal lines within the group and the possible origins of the Australian canker A outbreaks. Strict quarantine regulations on the importation of major exotic pathogens precluded comparisons of Australian strains of *X. c. citri* with strains from other countries.

Citrus canker has been successfully eradicated in Australia on two previous occasions (3,18). Following identification of the 1991 outbreak at Darwin, all citrus trees in the affected orchard were eradicated by burning in situ. No commercial citrus may be replanted in the orchard for 2 yr. Movement of citrus fruits and citrus propagating material from the Northern Territory to other states has been prohibited by the Northern Territory Department of Primary Industries. Surveys of all orchards in the areas surrounding Darwin and Katherine have been carried out and will be repeated at the end of the next wet season.

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