Citrus Bacteriosis in Mexico

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ABSTRACT

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A leaf- and twig-spotting disease was first recognized in Colima, Mexico, late in 1981 on Mexican lime trees (*Citrus aurantifolia*). The disease has since been found in Mexican lime groves in several Pacific Coast states of Mexico as well as on other citrus varieties located near affected Mexican lime groves. "Citrus bacteriosis" (CB) is characterized by tan or light brown raised lesions (1–4 mm in diameter) with chlorotic halos and limited development. Lesions occur primarily on young succulent leaves and green shoots. No symptoms have been observed on citrus fruits. Bacteria identified as *Xanthomonas campestris* pv. *citri* (*X. c. citri*) on the basis of physiological, biochemical, serological, and pathogenicity tests, as well as other bacteria, have been associated with CB. Isolation of *X. c. citri* strains has been inconsistent, although bacteria in disease lesions often have been serologically linked to *X. c. citri*. Internal and external quarantines on movement of citrus from CB-affected areas have been in effect since 1982. In addition, a program to reduce levels of CB pathogen(s) in Colima by spraying groves periodically with copper oxychloride (2.5 g a.i./L) is under way.

Additional key words: citrus bacterial canker disease, citrus disease

In November and December 1981, symptoms of a new leaf- and twig-spotting disease were observed on Mexican lime trees (*Citrus aurantifolia*) near Tecomán, Colima, Mexico (4,15). Lesions appeared generally similar to those of citrus bacterial canker disease caused by *Xanthomonas campestris* pv. *citri* (*X. c. citri*), and subsequently, these bacteria were isolated from foliar dew and diseased tissue. Because of differences between observed symptoms and those of known forms of citrus canker, the disease is currently referred to as "citrus bacteriosis" (CB). Incomplete knowledge about the etiology of CB and infrequent isolation of *X. c. citri* from infected tissue have commercial implications for the entire Mexican citrus industry. Internal quarantines regulating the movement of fruit and vegetative material of Mexican and Persian (*C. latifolia*) limes have been in effect since 1982. Importation of certain citrus fruits into the United States has been prohibited since 1982 and has been periodically suspended from suspect geographical areas until deemed safe.

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Distribution. CB is currently known to occur only in the subtropical citrus-growing areas along the Pacific Coast of Mexico (Fig. 1). Confirmed infestations are known in the states of Jalisco, Colima, Michoacan, and Guerrero. In addition, suspected CB lesions have been observed on Mexican lime trees in northern Oaxaca. Prior to the implementation of quarantine restrictions, nursery plants from Colima with suspected CB were destroyed in the states of Nayarit and San Luis Potosi. The disease appears to be centered near Tecomán, Colima. Thirty thousand hectares of Mexican lime groves were surveyed in Colima in 1983. CB occurred in 21,000 ha, of which 4,500 ha were considered to contain severely affected trees (7). CB was not detected during surveys of citrus in the Mexican Gulf Coast states in 1983–1985.

Symptomatology. Mexican lime is the principal host of CB, although lesions were identified visually and serologically (enzyme-linked immunosorbent assay using antiserum of *X. c. citri* A strain) on other citrus, including Persian lime and grapefruit, growing near Mexican lime groves with CB symptoms (J. J. Stapleton and E. L. Civerolo, *unpublished*). Disease symptoms are evident throughout the year; however, incidence and severity appear to increase during the dry season (October through June) and decrease after the rainy season growth flushes (July through September) (J. J. Stapleton and P. Perez Serrato, *unpublished*). Dew often occurs during the dry season. Lesions on Mexican lime begin as small, yellowish, raised, water-soaked areas on succulent leaves, twigs, and stems. On leaves, lesions enlarge and become tan to brown raised pustules surrounded by distinct chlorotic halos 1–4 mm in diameter (Fig. 2A,B). Well-developed lesions become concave and craterlike on the upper leaf surface and convex on the lower surface. Lesions become necrotic and corky and may crack. CB lesions develop only in succulent tissue and cease to expand as tissue becomes lignified (3,8). Leaves with lesions clustered near the margin may become deformed or torn. Severe

infections may result in slight defoliation and occasionally twig death. CB lesions have not been observed on fruit to date. CB symptoms are not identical to those of known forms of citrus canker (3).

Causal agent. Five of six aqueous extracts (2) of naturally occurring lesions on Mexican lime leaves reacted positively in indirect $F(ab')_2$-based enzyme-linked immunosorbent assays (10) using rabbit antisera prepared against intact cells of two strains (XC62 and XC69) of *X. c. citri* (Table 1). The mean A_{405nm} values for the lesion tissue preparations ranged from three to 16 times greater than those for similar preparations of healthy tissue. Five bacterial isolates from CB lesions from Tecomán were identified as *X. campestris* on the basis of reactions to the following tests: Gram reaction; flagellum configuration; acid production from arabinose, cellobiose, glucose, mannose, sucrose, and trehalose; utilization of L-asparagine as sole carbon and nitrogen source; catalase reaction; starch hydrolysis; protein digestion; urease production; gelatin liquefaction; indole production; H_2S production aesculin hydrolysis; pigment properties; fatty acid analysis; and serological relationships to A- and B-type reference strains of *X. c. citri* (5,14) (Table 2). These bacteria contained a water-insoluble yellow pigment that was extracted with methanol. The relative mobilities of these pigments from CB isolates on silica gel 60 thin-layer chromatography sheets, as well as visible light (350–550 nm) absorption characteristics, were identical to those of *X. c. citri* (strain XC62). Artificial leaf inoculations were made by wounding leaves with a wire brush or needle and swabbing with inoculum-saturated cotton (about 10^8 cells per milliliter). Inoculated leaves were enclosed in plastic bags containing small amounts of water. After inoculation, these bacteria caused chlorosis, rapid water-soaking, and lesions with raised tissue in the center (Fig. 3). These lesions were similar to naturally occurring CB lesions. No lesions developed on control leaves similarly inoculated with *Corynebacterium fascians* or mock-inoculated with sterile distilled water. Lesion development on wound-inoculated leaves was dependent on leaf tissue age. Since the bacteria were pathogenic on citrus, they are provisionally identified as *X. c. citri* pending additional information. The relationship of these bacteria to other known *X. c. citri* pathotypes is under investigation.

Control. Control measures currently implemented include an internal quarantine (9), prohibition of lime imports into the United States, and a spray campaign using copper oxychloride (2.5 g a.i./L) to reduce inoculum, especially in Colima (1). In addition, evaluation of alternative spray regimes, pesticides, and chemical defoliants, as well as cultural control methods, are under way (11,13).

DISCUSSION

Citrus canker is a threat to citriculture worldwide. For this reason, CB should be contained while its etiology and epidemiology are under investigation. The symptomatology of CB is different from and apparently less severe than known forms of citrus canker. It is significant that no symptoms have yet been observed on fruit, even on heavily infected trees. Furthermore, tree vigor and yield were not shown to be adversely affected. Although bacteria identified as *X. c. citri* have been recovered from CB lesions, their isolation has been infrequent and inconsistent. Strains of *X. c. citri* associated with the cancrosis B form of citrus bacterial canker disease also are infrequently isolated on standard bacteriological media (16). It has been difficult to reproduce field symptoms precisely by artificial inoculation. Although lesions on artificially inoculated leaves are generally similar to naturally occurring CB lesions, they are not always identical. This may be due to as yet unknown specific environmental requirements for infection and symptom

Fig. 1. Map of Mexico showing citrus-growing areas with citrus bacteriosis infestations.

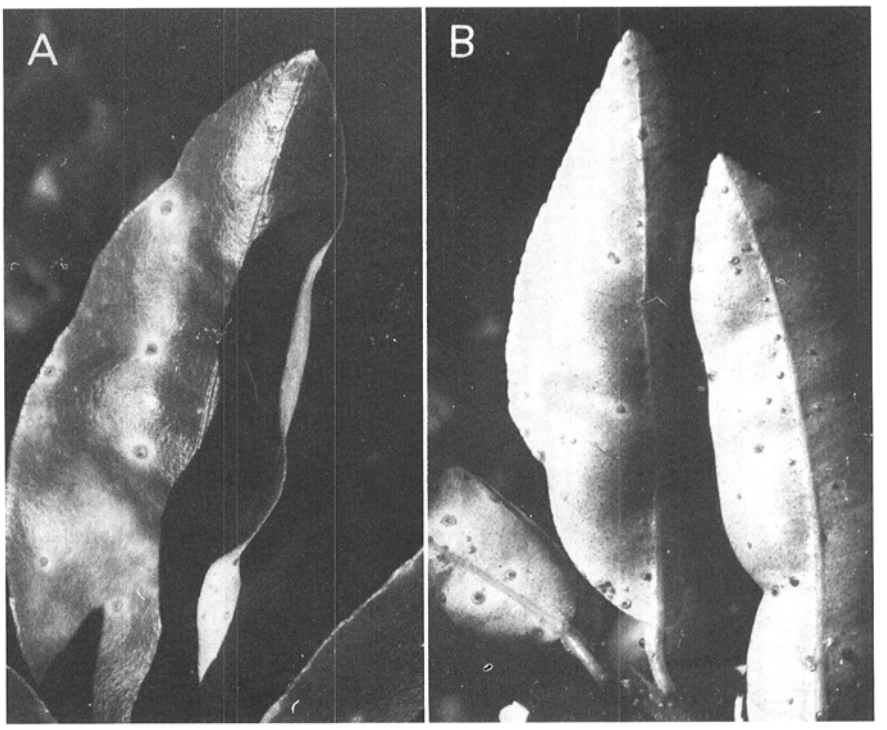

Fig. 2. Citrus bacteriosis symptoms on **(A)** upper surface and **(B)** lower surface of naturally affected Mexican lime leaves.

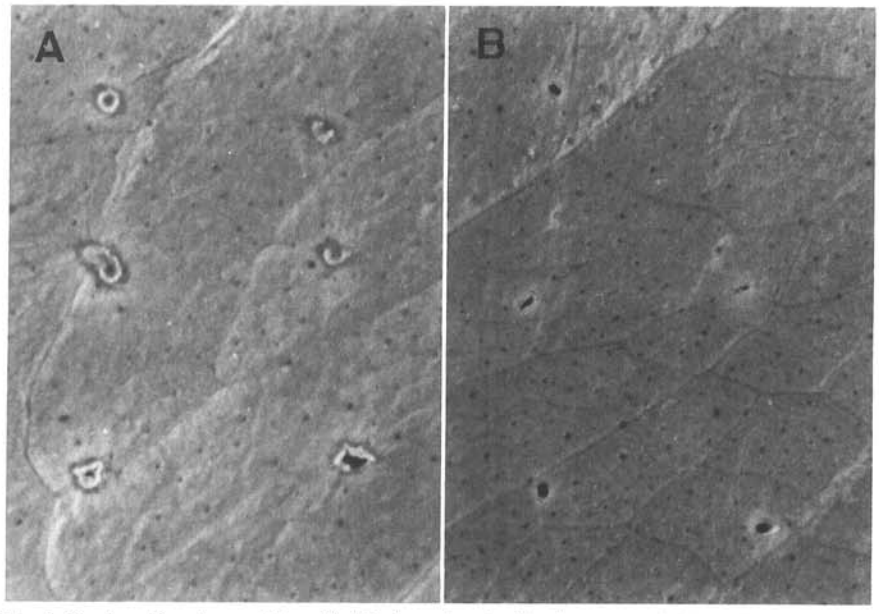

Fig. 3. Mexican lime leaves **(A)** artificially inoculated with citrus bacteriosis strain T20 and **(B)** inoculated with sterile distilled water.

Table 1. Indirect $F(ab')_2$-based double-antibody sandwich enzyme-linked immunorbent assay (EIA) of extracts of naturally occurring leaf lesions from citrus bacteriosis-affected Mexican lime trees in Colima, using rabbit antisera prepared against two strains of *Xanthomonas campestris* pv. *citri*[a]

EIA	Antigen	A_{405nm} Anti-XC62	A_{405nm} Anti-XC69
1	Leaf lesion extract		
	1	0.621 ± 0.055	···
	2	0.106 ± 0.078	···
	3	0.323 ± 0.051	···
	Healthy leaf tissue	0.108 ± 0.045	···
2	Leaf lesion extract		
	4	0.208 ± 0.041	0.458 ± 0.029
	5	0.271 ± 0.005	0.187 ± 0.081
	6	0.430 ± 0.020	0.457 ± 0.016
	Healthy leaf tissue	0.026 ± 0.003	0.029 ± 0.013

[a] Trapping antibody was 5 (anti-XC62) and 20 (anti-XC69) μg $F(ab')_2$ protein per milliliter EIA coating buffer. Detecting antibody was 5–20 μg $(NH_4)_2SO_4$ precipitated immunoglobulin protein per milliliter PBS-Tween. Conjugate was 1:1,000 dilution of Protein A-alkaline phosphatase (Zymed Laboratories) in PBS-Tween. Each value is the mean A_{405nm} ± SD of the reactions in 200 μl in each of three replicate wells in Immulon II microtiter plates (Dynatech Laboratories, Inc.).

Table 2. Indirect $F(ab')_2$-based enzyme-linked immunosorbent assay (EIA) using rabbit anti-*Xanthomonas campestris* pv. *citri* (strain XC62) serum

Antigen[a]	Mean A_{405nm} ± SD[b]	Ht/Hm[c]
T20	0.498 ± 0.024	0.27
T21	0.170 ± 0.013	0.09
T22	0.518 ± 0.010	0.28
T23	0.376 ± 0.016	0.21
T24	0.490 ± 0.011	0.27
XC62[d]	1.815 ± 0.050	1.00
EH1[d]	0.097 ± 0.013	0.05
None	0.074 ± 0.007	···

[a] Aqueous pure-culture cell suspensions in sterile distilled water, heated in a boiling water bath for 30 min, and adjusted to 0.1 A_{620nm} in EIA coating buffer.

[b] Mean A_{405nm} ± SD of contents of six replicate wells after 90 min at room temperature.

[c] Ratio of absorbance of heterologous (Ht) antigen preparation to absorbance of homologous (Hm) antigen preparation.

[d] Strain XC62 of *X. campestris* pv. *citri*; strain EH1 of *Erwinia herbicola*.

development. In addition, the possible involvement of other citrus phylloplane microorganisms cannot be precluded (6,12). Control by copper oxychloride sprays in Colima has not been completely successful (11,13), possibly because of the relative insensitivity of the pathogen(s) to the compound, the large area of Mexican lime groves involved, and the habit of Mexican lime trees to flush frequently and sporadically throughout the year. These sporadic flushes make scheduling of sprays very difficult. We hope that through the combined efforts of agencies of the Mexican Secretary of Agriculture and Water Resources (Dirección General de Sanidad Vegetal, Instituto Nacional de Investigaciones Agrícolas, and several fruiticulture agencies) as well as the USDA and other cooperators, CB will be rapidly characterized and controlled.

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