Citrus Bacterial Canker Disease of Lime Trees in the Maldive Islands

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ABSTRACT

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A serious decline of lime (*Citrus aurantifolia*) trees was investigated on a number of islands of the Republic of Maldives in 1987. Surviving trees on the islands of the north and south Male' atolls had typical symptoms of citrus bacterial canker disease (CBCD) on branches, trunks, limbs, and fruit. On the southern islands of the Laam atoll, trees had severe symptoms of CBCD on all aboveground parts, including severe leaf drop and twig, branch, and trunk dieback, with death of some trees. *Xanthomonas campestris* pv. *citri* was isolated from many lesions on infected twigs, leaves, and fruit, and the strains were identified as the pathotype associated with Asian CBCD. Temperature, rainfall, and wind in the Maldives are optimum for CBCD development and could account for the rapid decline of lime trees on the Maldive Islands. This is the first report of CBCD in the Maldive Islands. The apparent rapid decline of lime trees is new in the epidemiology of CBCD.

In October 1987, the first author investigated the nature of an unknown decline of lime trees in the Maldive Islands (15). The decline was first noted in the late 1970s, and many of the lime trees on the islands of the Republic of Maldives had since died. Herath (9), in a comprehensive report on the status of agriculture in the Maldive Islands, suggested that the decline of lime trees was due to heavy rainfall in 1986 that predisposed trees to *Phytophthora* spp. (collar rot).

The small fruited lime (*Citrus aurantifolia* (Christm.) Swingle), commonly called the Mexican, West Indian, or Key lime in North America, has been grown in the Maldive Islands for many years. The fruit is a primary source of vitamin C and is used extensively as a condiment for fish, the main food staple of the islanders. Thus, the loss of these trees, limiting the availability of lime fruit, is a very serious problem.

This paper, the first report of citrus bacterial canker disease (CBCD) in the Maldive Islands, describes the epidemiology and presents recommendations for its control.

MATERIALS AND METHODS

Geographic area. The Republic of Maldives consists of over 1,200 coral islands within chains of 19 atolls lying southwest of the tip of India and extending 764 km to the equator (Fig. 1). Most homes on the inhabited islands had one to five lime trees, and the fruit was valued by their owners primarily for home use or as a cash crop for shipping to markets on the populated island of Male'.

All existing lime trees were examined on the islands of Male', Hura, Hinma Fushi, Bondos, and Karumba in the north Male' atoll and on Guhli and Maa Fushi in the south Male' atoll, as well as on three tourist islands. A visit was also made to the Laam atoll, which lies 290 km south of the Male' atoll and at 2° north latitude. The temperatures in the islands are warm and exceptionally uniform throughout the year, with monthly means ranging from 25.2–26.4 to 29.7–31.4 C. Rainfall from April through December ranges from 130.1 to 233.8 mm per month, and maximum wind velocities during the rainy period range from 16.0 to 26.8 m per second (Table 1).

Pathogen isolation and identification. Individual leaf, twig, and fruit lesions were aseptically excised, diced with a flame-sterilized scapel, and placed into 1 ml of sterile deionized water. After incubation for 20–30 min at room temperature, the pathogen was isolated directly by streaking a 10-μl aliquot of each suspension on the surface of Difco nutrient agar supplemented with 2% glucose and containing 16 μg/ml each of kasugamycin, cephalexin, and chlorothalonil (Bravo 75WP) (NGA/KCB). Plates were incubated at 27 C for 6 days. Cells from suspect yellow mucoid colonies were restreaked on Wakimoto's potato semisynthetic (Wkm) medium (11) and yeast extract-dextrose-$CaCO_3$ (YDC) medium (18) to determine culture purity and colony characteristics, respectively. In addition, the pathogen was indirectly isolated by semiselective enrichment in detached citrus leaf tissue. Leaf tissue disks (10 mm diameter) were aseptically excised from detached leaves of *C. aurantifolia* (clone H-7 grafted on *C. macrophylla* rootstock) grown in the greenhouse and previously surface-disinfested in 1% NaOCl for 2–3 min at room temperature. Each leaf tissue disk was wounded by puncturing the lower surface five times with a flame-sterilized insect mounting pin. The disks were then placed on the surface of 2 ml of 1% water agar in each well of a 24-well flat-bottom, polystyrene tissue culture plate (Corning No. 25820), and diced lesion suspension (10 μl) was placed on each needle puncture wound on the lower leaf surface. The plates were incubated in a continuously lighted incubator at 27–28 C. Bacteria were subsequently isolated from lesions and streaked on NGA/KCB after 10–14 days as described above.

The pathogenicity of presumptive *Xanthomonas campestris* pv. *citri* (Hasse) Dye was determined by spray-inoculating attached leaves and young actively growing terminal twigs of *C. aurantifolia* (clone H-7 grafted on *C. macrophylla*) in the greenhouse with cell suspensions containing approximately 1–2 $\times 10^8$ cfu/ml. Whole detached leaves of *C. aurantifolia* were also inoculated, as described above, by applying 10-μl droplets of cell suspension inocula containing 10^6–10^8 cfu/ml to each of 10 needle puncture wounds on the lower leaf surface. Xanthomonadin pigment extraction and analyses were as previously described (18). The sensitivity of bacterial strains to citriphages CP1, CP2, and CP3 was determined as previously described (6). Indirect double-antibody sandwich enzyme-linked immunosorbent assays, using protein A-alkaline phosphatase conjugate, also were performed as previously described (4).

RESULTS

Observations of trees in the north and south Male' atolls. Meteorological data collected at Male' from 1967 through 1985 are presented in Table 1. The mean rainfall was 164.4 mm per month, the mean temperature was 25.7 C minimum

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and 30.3 C maximum, and the maximum wind velocity ranged from 10.3 to 26.8 m per second.

Most of the original lime trees that had lived for many years on the islands of the north and south Male' atolls had been killed since 1979. Apparently, trees died rapidly after the onset of infection. The few trees left had been selectively pruned and fertilized. No lime seedlings or trees were found growing wild. All trees were grown from seed, as seedlings in small containers or in the ground. Most of the seedlings were growing poorly and had chlorotic leaves and extensive defoliation. The coral soil in this area is highly alkaline and very low in certain macronutrients and micronutrients (15). The only two insect pests observed were an unidentified leaf miner species and blackfly (*Aleurocanthus woglumi* Ashby). These insects did not appear to cause appreciable damage to the lime trees and were probably not responsible for the decline, although leaf miner injuries apparently afforded ready entry for the CBCD bacteria and were loci for infection. No symptoms of infection with *Phytophthora* spp. or gumming were observed, and the roots of affected citrus trees did not show any *Phytophthora*-induced sloughing. No symptoms of citrus tristeza or greening disease were observed, and the respective aphid and psyllid vectors of the causal agents of these pathogens were not found. No symptoms of other known citrus virus or viruslike disease were observed.

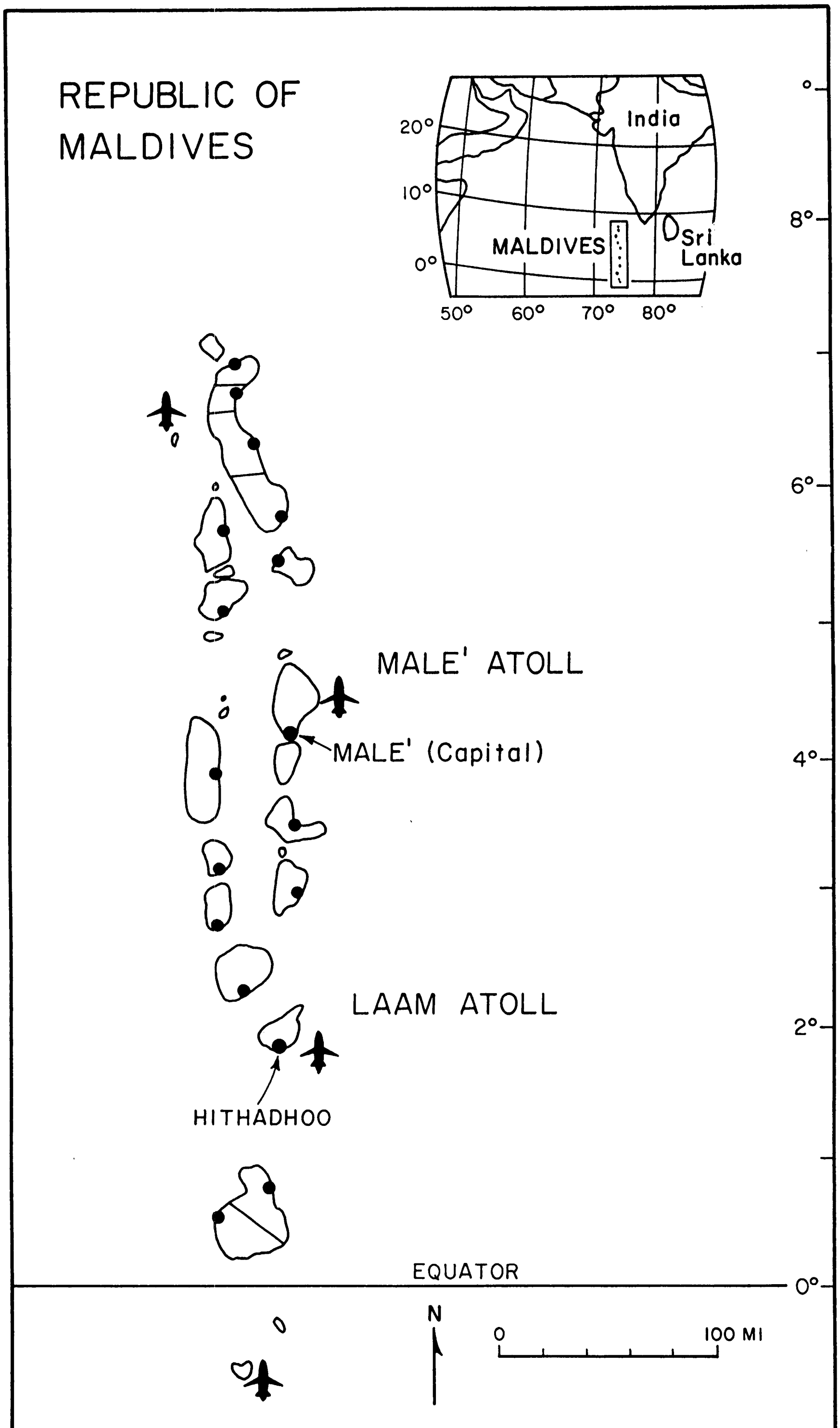


Fig. 1. The Republic of Maldives is divided into 19 districts representing atolls or parts of large atolls. Of the approximately 1,200 individual coral islands in the republic, only about 200 are inhabited. The islands are located within a string of atolls extending from the southwest tip of India to the equator.

Lesions similar to those of CBCD occurred on leaves of many lime trees on the islands of Male', Hura, Hinma Fushi, and Maa Fushi but were not observed on the young trees on Guhli, Bondos, and Karumba, all in the Male' atoll. The few older trees remaining on the island of Male' had CBCD-like lesions on fruit, leaves, twigs, and branches, including extensive lesions on the main trunk. One such tree in Male' had a moderate set of fruit and had been cared for, pruned, and fertilized by the owner. Leaves, however, showed many lesions typical of CBCD infection (Fig. 2A). Stem and branch lesions were severe (Fig. 2B), although lesions on the larger branches and main trunk were moderate. Fruit also had typical CBCD lesions (Fig. 2C). Samples of leaves, twigs, and fruit with lesions were collected from this tree for culture and isolation of possible pathogenic agents.

An older tree on Maa Fushi Island in the Male' atoll also had severe dieback, chlorosis, many small leaves, and extensive cankerous lesions on the trunk and branches (Fig. 2D). This tree, however, had no fruit. CBCD-like lesions also occurred on leaves, stems, and fruit of citron seedlings on the islands of Maa Fushi, Hura, and Hinma Fushi. The leaves and stems of some small sour orange seedlings on these islands had no CBCD lesions, however.

Many fruit sold at the main market on the city island of Male' had lesions similar to those shown in Figure 2C. On the island of Karumba, two healthy 8-yr-old, closely planted lime trees were found. They were unusually large, had abundant fruit, and had no CBCD-like lesions. Leaf miner and blackfly were present but appeared to have little effect on tree vigor. These trees gave some hope that lime cultivation could be reestablished on these islands.

CBCD-like lesions occurred on many of the young and most of the remaining older lime trees observed on the islands of the north and south Male' atolls. A few of the young seedlings were free from CBCD-like lesions, whereas others had abundant lesions.

Observations of trees on the Laam atoll. On the southern islands of the Laam atoll, circumstances and timing were fortuitous for viewing a newly developing CBCD-like epidemic affecting all of the lime trees. The lime trees on Hithadhoo Island began declining about 1980–1982, and those in the villages on Isdhoo Island began declining about 1984. On these islands of the Laam atoll, the ravaging effects of CBCD on trees 20–50 yr old and older were striking. This active, highly visible destruction was not as dramatically apparent on trees observed in the north and south Male' atolls, since nearly all of the older trees on those islands had been destroyed about 8–10 yr previously.

The residents of the village of Hithadhoo described hundreds of lime trees with abundant fruit overhanging the streets 5–7 yr ago. Only a very few older trees were left, however, and these had extensive CBCD-like lesions on the main trunk, limbs, branches, and leaves, with much dead wood and falling leaves in evidence. One such dying tree was in the main courtyard of the village (Fig. 2E and 2F).

In villages on Isdhoo Island, the ravages of the epidemic that began in 1984 were visible. Mature trees were dying, and symptoms of CBCD were evident on leaves, twigs, small and large limbs, and trunks of all these dying trees, without exception. No healthy trees were seen in any of the villages visited on the Laam atoll. Also, all of the young lime seedlings and some of the young sour orange seedlings had typical CBCD lesions. All newly fallen leaves had CBCD-like lesions covering up to 50% of the leaf area (when lesions cover 10–20% of the leaf area, 80–100% of the leaves will drop [7]). Extensive CBCD-like lesions girdled the twigs and, combined with extensive loss of leaves, eventually killed the branch. Fruit, when present, were small and had brown, crater-centered lesions characteristic of CBCD (Fig. 2C).

On all of the islands visited, the appearance of citrus canker lesions was associated with the death and destruction of the lime trees. These lesions were not seen before the trees began to decline. Observations of CBCD-affected older trees in the villages of the Laam atoll presented clear diagnostic evidence that CBCD was the probable cause of the rapid decline and death of lime trees on the islands of the north and south Male' atolls and was probably responsible for the decline of lime trees reported on other islands in the Maldives.

Pathogen isolation and identification. Yellow, mucoid, xanthomonad-like bacterial colonies developed on Wkm, YDC, and NGA/KCB media from aqueous extracts of all leaf and twig lesions from *C. aurantifolia* collected in the Maldives. No other bacteria or fungi were readily and routinely isolated from or associated with these preparations. Strains originating from selected, single, well-separated colonies in primary isolation plates produced xanthomonadin pigment based on thin-layer chromatography and UV-absorption characteristics (18). These were indistinguishable from known strains of *X. c.* pv. *citri*, including the neopathotype strain XC115 (NCPPB 409).

Selected bacterial strains isolated from the Maldives citrus samples caused typical erumpent CBCD lesions on leaves and twigs from *C. aurantifolia* trees similar to those caused by the known strains of *X. c.* pv. *citri*, XC62 and XC115. In addition, the Maldives citrus bacterial strains also caused extensive development of erumpent, whitish, friable calluslike tissue at needle puncture wounds on detached *C. aurantifolia* leaves and leaf tissue disks similar to the reaction produced by *X. c.* pv. *citri* strains XC62 and XC115. In contrast, several *X. campestris* pathovars other than *X. c.* pv. *citri* produce limited responses consisting of water-soaking, chlorosis, tissue discoloration, and necrosis (Civerolo, *unpublished*).

Selected bacterial strains isolated from diseased Maldives citrus were susceptible to lysis by citriphages CP1 and CP2 but not by CP3 (2,3,6). This pattern of citriphage typing is similar to that of *X. c.* pv. *citri* strains associated with the Asiatic form of CBCD (2,3). However, some of the Maldives citrus bacterial strains tested were resistant to lysis by all three citriphages.

Antigenic preparations (aqueous cell suspensions heated in a boiling water bath for 30–60 min) of selected bacterial strains isolated from diseased Maldives citrus and *X. c.* pv. *citri* strains XC62 and XC115 were evaluated by indirect EIA using polyclonal antibodies prepared against *X. c.* pv. *citri* strain XC62 (4). The reactions (A_{410nm}) with preparations from the Maldives and citrus XC115 strains were quantitatively indistinguishable from each other and from a similar preparation of the homologous *X. c.* pv. *citri* strain XC62. Reactions of similar preparations from other *X. campestris* pathovars (pv. *pruni* strain XP1 and pv. *campestris* strain B24) were quantitatively distinguishable, with A_{410nm} values less than one-half that of the preparation from the homologous strain XC62.

DISCUSSION

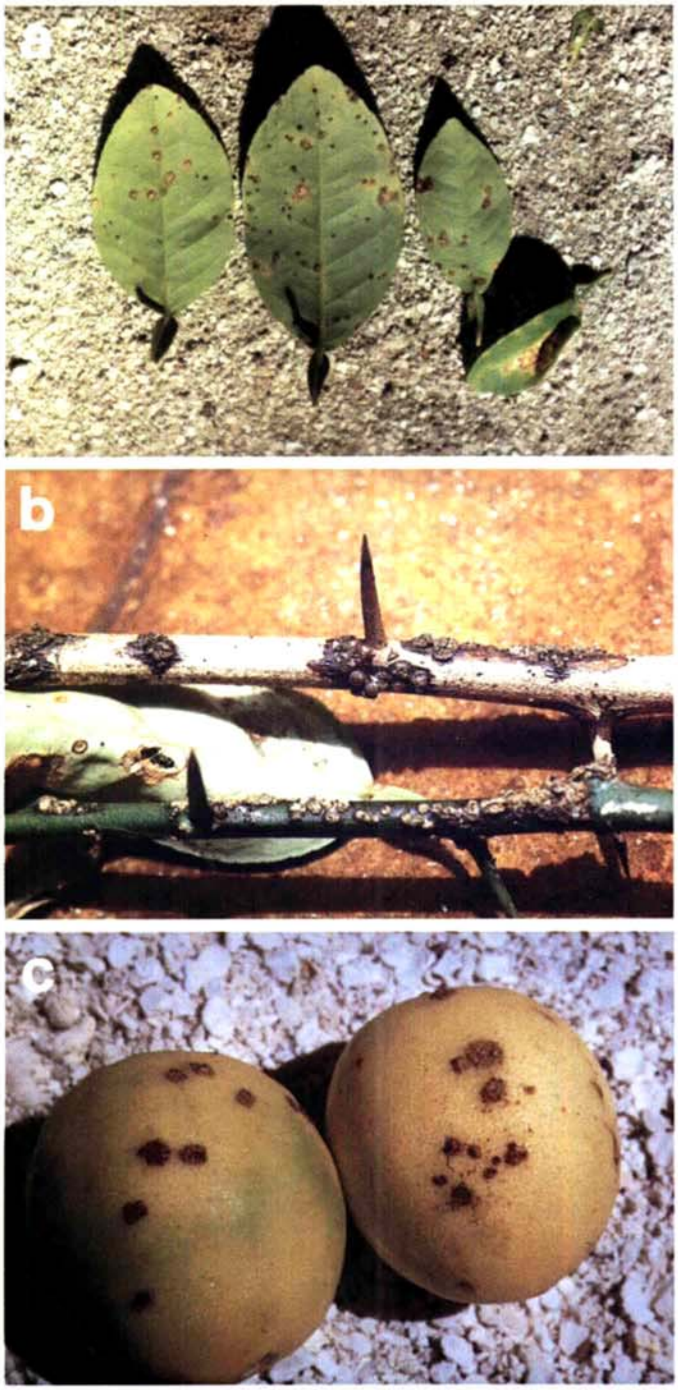
Conclusions. On the basis of these results collectively, we conclude that *X. c.* pv. *citri* was isolated from and is associated with the lesions on leaves and twigs of *C. aurantifolia* trees in the Maldives. In addition, CBCD is the cause of the decline of *C. aurantifolia* trees in the Maldives. Furthermore, these strains of *X. c.* pv. *citri* are similar to those associated with the Asiatic form of CBCD. Further characterization of the Maldives strains of *X. c.* pv. *citri* with respect to cultural, morphological, physiological, and biochemical characteristics, as well as plasmid DNA content and genomic DNA organization by restriction fragment length polymorphism, is in progress.

Based on the association, isolation, and positive identification of *X. c.* pv. *citri* from lesions on twigs, leaves, and fruit of lime trees and the apparent absence of other known destructive citrus diseases, we conclude that CBCD is responsible for the death and destruction of the lime trees on the Maldive Islands. However, the epidemic destruction of the lime trees in the Maldives by *X. c.* pv. *citri* within 3–4 yr after onset of the disease is new in the epidemiology of CBCD. The optimum temperature for *X. c.* pv. *citri* growth and infection is 25–30 C (12,13). These are the precise minimum and maximum temperatures recorded in the Maldive Islands, with very little month-to-month variation.

X. c. pv. *citri* is spread primarily by wind-driven rain (2,14,16). The rate of infection rises sharply as the wind velocity increases above 6.5 m/sec (16).

Table 1. Meteorological data for Male', Republic of Maldives, 1967–1985

Month	Rain (mm)	Temperature (C)		Wind speed (m/sec)		
					Maximum	
		Maximum	Minimum	Average	1986	1987
January	69.2	29.7	25.5	5.40	11.3	19.0
February	57.8	30.1	25.7	4.48	11.8	10.3
March	87.3	30.9	26.0	3.55	20.1	12.3
April	130.1	31.4	26.4	4.02	15.4	22.1
May	233.6	30.9	26.4	5.30	20.6	18.0
June	162.1	30.5	25.9	5.10	23.6	26.8
July	173.2	30.3	25.6	4.84	18.5	16.0
August	184.2	30.1	25.6	4.58	23.6	22.1
September	225.3	30.1	25.3	5.35	24.2	20.6
October	233.8	30.0	25.3	5.05	20.6	...
November	204.8	29.9	25.3	4.43	23.1	...
December	211.3	29.7	25.2	4.79	17.5	...
Total	1,972.7					
Mean	164.4	30.3	25.7	4.74	19.2	18.6



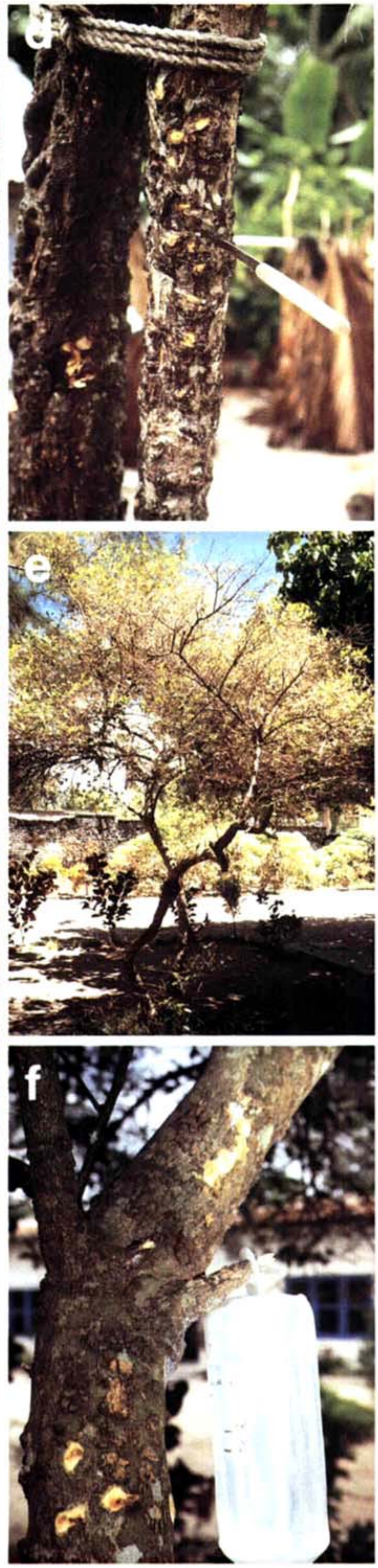


Fig. 2. (A) These lesions of citrus bacterial canker disease (CBCD) on leaves are typical of symptoms found throughout the islands visited. *Xanthomonas campestris* pv. *citri*, Asian strain, was isolated from such lesions. **(B)** Typical lesions of CBCD found on twigs and stems of lime trees on most of the islands visited. **(C)** Lime fruit from a tree in Male' showing typical craterlike lesions associated with CBCD, Asian strain. **(D)** Typical severe lesions of CBCD found all along the main trunks of a dying lime tree. **(E)** This dying lime tree in the center of the main court in Hithadhoo on the Laam atoll is one of the few trees remaining on the island after the CBCD epidemic. **(F)** These severe lesions of CBCD on the trunk of the tree shown in **E** are relatively recent and were apparently formed after the onset of the CBCD epidemic.

Winds above 6.5 m/sec promote thorn wounds and also disperse the bacteria to a greater extent. In Japan, citrus canker becomes prevalent when annual precipitation exceeds 125 mm per month (14). During 1967–1985, rainfall in the Maldive Islands averaged 130.1–233.8 mm per month during April–December. For these same 9 mo, the highest mean maximum temperature was 31.4 C and the lowest, 25.2 C. Maximum wind speeds during April–December 1986 ranged from 15.4 to 24.2 m/sec and during April–September 1987, from 16.0 to 26.8 m/sec. These conditions were exceptionally favorable for pathogen spread, infection, and disease development.

Lime is one of the most susceptible citrus species to *X. c.* pv. *citri* and is sensitive to the Asiatic form of CBCD (2,17). Thus, optimum temperatures, moisture, and wind for pathogen spread, infection, and disease development throughout most of the year combined with the high susceptibility of *C. aurantifolia* trees to CBCD appear to be responsible for the rapid spread and extensive destruction of lime trees by *X. c.* pv. *citri.*

Recommendations for the reestablishment of citriculture in the Maldive Islands. A preliminary survey has been made by radio contact with responsible individuals on 115 islands representing all of the atoll districts in the Republic of Maldives. Infection of lime trees was reported from 80 of the islands; trees on the remaining 35 islands are apparently free from infection. No atoll or district was found with all of their islands free from infected trees. A more detailed survey is now in progress. An immediate survey is needed to determine the distribution of CBCD on all of the inhabited islands in the republic. Dhagathi Island in the Ari atoll and six islands in the northern Shaviyani atoll are free from CBCD, and the trees and fruit production are normal. Visits were also made to six islands in the northern Shaviyani atoll, and all lime trees observed were also free from CBCD. Quarantine regulations against importation of trees or fruit into these islands must be established and implemented. CBCD has been reported from other islands in the Pacific (10) and is endemic in all citrus-growing areas of India (and probably in Sri Lanka). In Rajasthan, India, acid lime is more severely affected than lemon, pummelo, sour orange, mandarin, and grapefruit, and the CBCD bacteria are present throughout the year (1).

Continuation of the disease survey and establishment of rigid quarantine regulations for all of the islands in the Republic of Maldives are strongly recommended. These measures must be coupled with an educational program in many forms to teach inhabitants the danger of bringing in infected trees or fruits.

The finding of the two 8-yr-old healthy seedling trees on the tourist island of Karumba suggests that lime cultivation can be reestablished on islands where the disease occurred. The two healthy trees were reported to have been planted after all of the original trees were destroyed about 10 yr ago when the island was inhabited. Very few trees remain on islands ravaged by CBCD. These could readily be destroyed and a fallow period of 3–6 mo enforced, with no new plantings of citrus allowed during this period. Fulton (5) and Graham et al (8) have shown that populations of citrus canker bacteria decline relatively rapidly in the soil and on lesions of defoliated leaves and dropped fruit. The pathogen may survive in soil for about 2–8 wk or longer, depending on moisture and temperature. Development of a large-scale nursery operation to grow high-quality seedlings rapidly for replacing lost trees has been recommended (15), as well as a long-range cultivar testing program for evaluating new citrus cultivars and rootstocks.

Without eradication, quarantine, and educational programs, growth of lime trees on the islands of the Maldives will not be possible, considering the ideal environmental conditions for the growth and spread of the CBCD pathogen with its high potential for destruction. Because of the geographic isolation of these islands, however, eradication efforts should be successful.

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