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Research Perspectives on Eradication of Citrus Bacterial Diseases in Florida

Since September 1984, the citrus industry in Florida has been confronted with two bacterial diseases. A novel foliar disease, now known as citrus bacterial spot (CBS), caused by *Xanthomonas campestris* pv. *citrumelo* Gabriel (9; syn. = *X. c. citri* (Hasse) Dye group E), appeared first and was found predominantly in citrus nurseries on the rootstock Swingle citrumelo (*Citrus paradisi* × *Poncirus trifoliata*) (25). The disease has characteristic flat leaf, stem, and fruit lesions with necrotic centers and water-soaked margins (Fig. 1A and B). Beginning in the summer of 1986, Asiatic citrus canker caused by the group A strains of *X. c. citri* (9; syn. = *X. citri* (Hasse) Dowson) was discovered on leaves, stems, and fruit of mature trees at residences and orchards along the gulf coast of central Florida (25). Asiatic citrus canker is a well-known and widely distributed disease throughout southeastern Asia and the world. Young lesions on leaves, stems, and fruit are raised and pustular (Fig. 1C and D), but as lesions age they become corky and sunken in the center. The craterlike surface gives a rough feel to lesions on both sides of the leaf.

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Citrus canker was first introduced into Florida in the early 1910s. The introduction was traced to infected trifoliate orange seedlings imported from Japan for use as rootstocks (25). In 1915, a newly formed state regulatory board and the federal government initiated an eradication campaign in Florida. After $6 million had been spent for eradication, Florida was declared free from the disease in 1933.

Initially in 1984, the new nursery disease was thought by scientists at the Florida Department of Agriculture and Consumer Services, Division of Plant Industry (DPI), and the USDA Animal Plant Health Inspection Service (APHIS) to be a new form of citrus canker. This diagnosis triggered the implementation of the Citrus Canker Disease Action Plan developed in 1982 by USDA-APHIS in cooperation with citrus-producing states in the United States (25). The purpose of the plan was to ensure that federal and state regulators were prepared to eradicate citrus canker as soon as it reappeared in the United States. Eradication was to be accomplished by: 1) burning plants in a nursery where an infected plant was found, 2) destroying all trees with canker symptoms within orchards and defoliating surrounding trees, and 3) using fruit from diseased or exposed trees in orchards for processing only (25).

In the 4 years following the outbreak of CBS, state and federal regulators attempted to limit dissemination of the pathogen and reduce the number of future eradication sites. By 1991, over 20 million trees in more than 100 nursery and orchard locations had been destroyed at a cost of nearly $94 million (L. Hebb, DPI, and J. Thomas, USDA-APHIS, *personal communications*). During this process, nurseries were subjected to lengthy quarantines, planting of orchards was restricted, and citrus orchards had to be surveyed continually before movement of fruit was permitted. In addition, packinghouses were under compliance agreements to treat fruit with disinfestants and fruit shipment to citrus-producing states was not allowed. These measures were costly and inconvenient to growers, and markets for fruit were lost. There was a great deal of controversy over the quarantines and restrictions in the regulatory, industrial, and scientific arenas alike.

During that same period and even now, Asiatic citrus canker has been much more significant. Infections in orchards on the west coast of Florida (Fig. 2) were suppressed by removal of diseased trees and extensive defoliation of surrounding areas. However, additional trees with canker symptoms appeared in 1989, more than 2 years after eradication of the disease. These recurrences led to removal of all trees in affected blocks by 1990. Again, in January 1991, a few additional trees with citrus canker were found in an orchard some distance from the trees removed in 1989 and 1990. Because the origin of the new infections could not be determined, the pathogen was difficult to eradicate in these locations. Even more disconcerting was the discovery of a widespread major disease focus in a newly planted orchard in the very center of the citrus-growing area in fall 1990 (Fig. 2). Although this

isolated occurrence was linked with previous outbreaks on the west coast of Florida, it pointed to the possibility of other undiscovered disease foci and the need for continued vigilance.

In 1984, the U.S. Congress appropriated $1 million a year for 5 years of cooperative state and federal research on citrus canker. The University of Florida's Institute of Food and Agricultural Sciences (IFAS) received 75% of the yearly appropriation and the USDA Agricultural Research Service (ARS) received 25%. Broad areas of research that were defined included: 1) methods for detection and identification of *X. campestris* causing citrus canker and citrus bacterial spot to supplement visual diagnosis and pathogenicity tests; 2) biochemical, serological, and genetic characterization of strains of *X. c. citrumelo* compared with strains of *X. c. citri*; 3) susceptibility of *Citrus* species and relatives; 4) epidemiological research in nurseries in Florida and field research in Florida, Argentina, and Maryland; and 5) methods for eradicating and controlling the pathogen to minimize plant destruction. The research was intended to assist the DPI and APHIS in modifying policy and procedures in the Citrus Canker Disease Action Plan (16).

In September 1990, all regulations of the "Florida nursery strain of citrus canker" (i.e., CBS) were removed (7). This action was based on "recent scientific reports and articles as well as experience in Florida which indicate(d) that none of the various forms of Florida nursery disease causes a disease dangerous to citrus or other plants or fruit." This rule change released all areas of Florida from quarantine, except where foci of Asiatic citrus canker were detected during the previous 2 years, and relaxed restrictions on interstate movement of citrus fruit and plants.

Because the eradication program for the various forms of citrus canker and now the deregulation of CBS were based in part on the biology of the diseases and their causal bacteria, discussion of the perspectives that research conducted by IFAS and USDA-ARS afforded in the regulatory processes is pertinent.

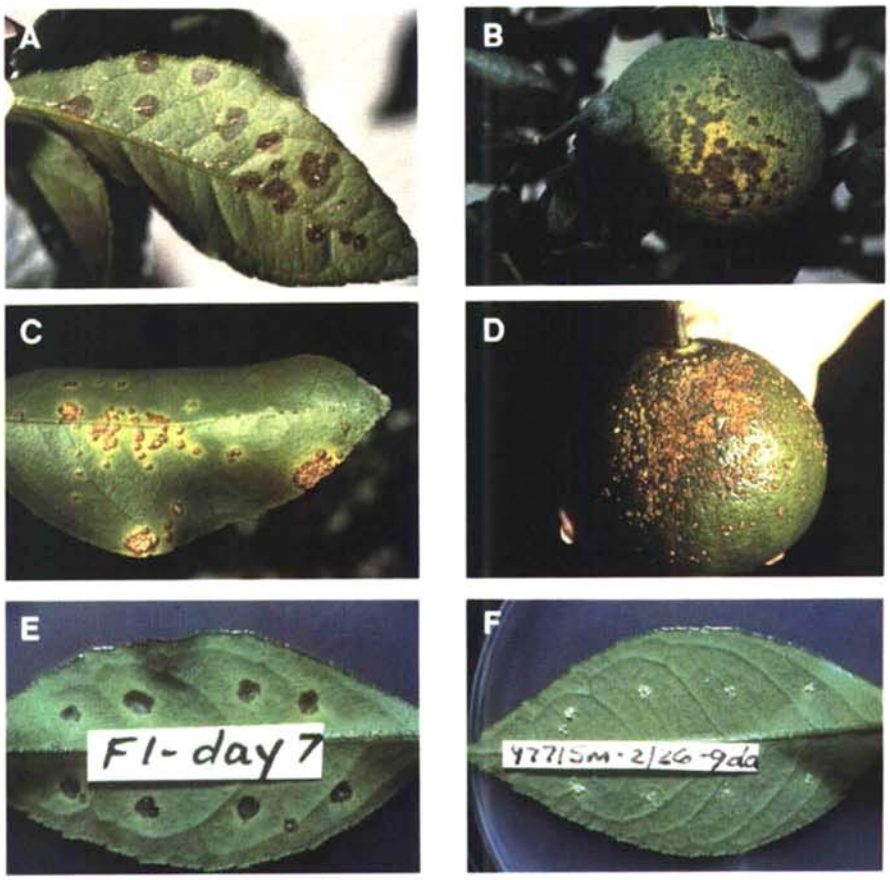


Fig. 1. (A) Lesions of citrus bacterial spot on a Swingle citrumelo leaf from a Florida nursery are flat with brown necrotic centers and water-soaked margins. (B) Lesions of citrus bacterial spot on a fruit of trifoliate orange (cv. Flying Dragon) from a Florida nursery have slightly raised to sunken necrotic areas with narrow water-soaked margins. (C) Lesions of Asiatic citrus canker on a red grapefruit leaf from a Florida orchard are raised and corky and surrounded by chlorosis. (D) Lesions of citrus canker on young red grapefruit in a Florida orchard are raised and corky. (E) Reaction of the aggressive strain F1 of *Xanthomonas campestris* pv. *citrumelo* on wound-inoculated detached leaves shows persistent water-soaking and indistinct necrosis. (F) Reaction of an Asiatic strain of *X. c. citri* shows a calluslike eruption of tissue and no water-soaking or necrosis.

Methods for Detection and Identification

Before regulatory actions could be completed, isolations of the bacteria from leaf lesions and pathogenicity tests on citrus were necessary to establish the nature of the disease. The semiselective medium KCB (containing kasugamycin, cephalexin, and chlorothalanil [Bravo]) proved very useful for isolation of xanthomonads in low numbers from lesions, plant surfaces, and soil (7,14,18,19).

A detached-leaf assay was developed for inoculation of citrus in containment facilities (9,14,17). This assay permitted rapid and accurate evaluation of bacterial strains. Lesions usually developed 10–14 days after inoculation of intact leaves in the greenhouse but after 7 days on detached leaves under artificial light. With the assay, the flat-lesion type of CBS could be readily distinguished from the erumpent, calluslike reaction of citrus canker (17) (Fig. 1E and F).

Inoculation of plants was necessary to determine pathogenicity, but tests were time-consuming. Consequently, faster detection techniques were developed to identify the bacterial strains in leaves and from culture media. Both *X. c. citrumelo* and *X. c. citri* were detected by immunofluorescence microscopy of bacteria from leaf extracts of symptomatic and asymptomatic citrus leaves (3). Polyclonal antisera labeled with tetramethylrhodamine isothiocyanate was made against several strains of *X. c. citrumelo* and a single strain of *X. c. citri*. The antisera to *X. c. citrumelo* reacted weakly or not at all with some strains from CBS outbreaks because of strain heterogeneity (2). With the antiserum prepared against an Asiatic strain, the A group of strains of *X. c. citri* were readily distinguished by immunofluorescence microscopy from the B, C, and D groups that cause cancrosis B on lemons, canker C, and bacteriosis on Mexican lime, respectively. However, the antiserum cross-reacted with some strains of *X. c. citrumelo* by enzyme-linked immunosorbent assay (4; E. L. Civerolo, *personal communication*).

Monoclonal antibodies (Mabs) were developed to distinguish strains of *X. c. citri* from those of *X. c. citrumelo* (1). The Mabs were used to group *X. c. citrumelo* strains that had been categorized as aggressive, moderately aggressive, or weakly aggressive by detached-leaf assay and other greenhouse and field inoculations (11,17). Strain-specific Mabs were potential probes for aggressive strains of *X. c. citrumelo* that did not cross-react with less aggressive or opportunistic

xanthomonads occurring in background microflora in Florida.

Profiles of cellular fatty acids also were used to identify strains of *X. c. citrumelo* and to separate them from Asiatic canker strains. Software from the Microbial Identification System (Microbial ID, Inc., Newark, DE) was used to generate libraries of fatty acid profiles for each group of strains. Strains from each group were always identified correctly based on similarity indices >0.85 for profile comparisons (26).

A DNA-DNA hybridization probe was useful for specific detection of strains of *X. c. citrumelo* from lesions and leaf washes from asymptomatic plants (14). The probe was derived from a cloned fragment of DNA from another *X. campestris* pathovar so it was not always specific for strains of *X. c. citrumelo* (14). An essential limiting factor for clinical diagnosis was the requirement for ^{32}P labeling of the probe.

DNA fingerprinting also was used to identify bacterial strains. After restriction of DNA with *Eco*RI endonuclease and gel electrophoresis, the DNA patterns of A strains from throughout the world were found to be very similar. Strains from the cancrosis B group were also very similar to one another but were readily distinguished from the A group (22). In contrast, the strains of *X. c. citrumelo* showed a wide variety of genomic fingerprints that varied not only among separate outbreaks of CBS in Florida but also within a single nursery. This technique provided the first presumptive evidence that strains of *X. c. citrumelo* were not recently introduced into Florida but were representatives of an endemic bacterial flora of *X. campestris* (22).

Genomic fingerprinting using endonucleases that cut DNA of *X. campestris* infrequently and pulsed-field gel electrophoresis confirmed that *X. c. citrumelo* strains were heterogeneous compared with the Asiatic strains (Fig. 3). This technique was even more valuable for identifying closely related strains of *X. c. citri* and tracing their origin. The recent outbreak of Asiatic citrus canker in central Florida was presumed to be linked to disease foci on the west coast of Florida for two reasons: 1) There was movement of personnel between the two outbreaks and 2) the DNA fingerprints of strains from the two sources were identical. The DNA fingerprint of a strain from a separate, isolated occurrence of the disease in north Florida in 1985 was different (Fig. 3A).

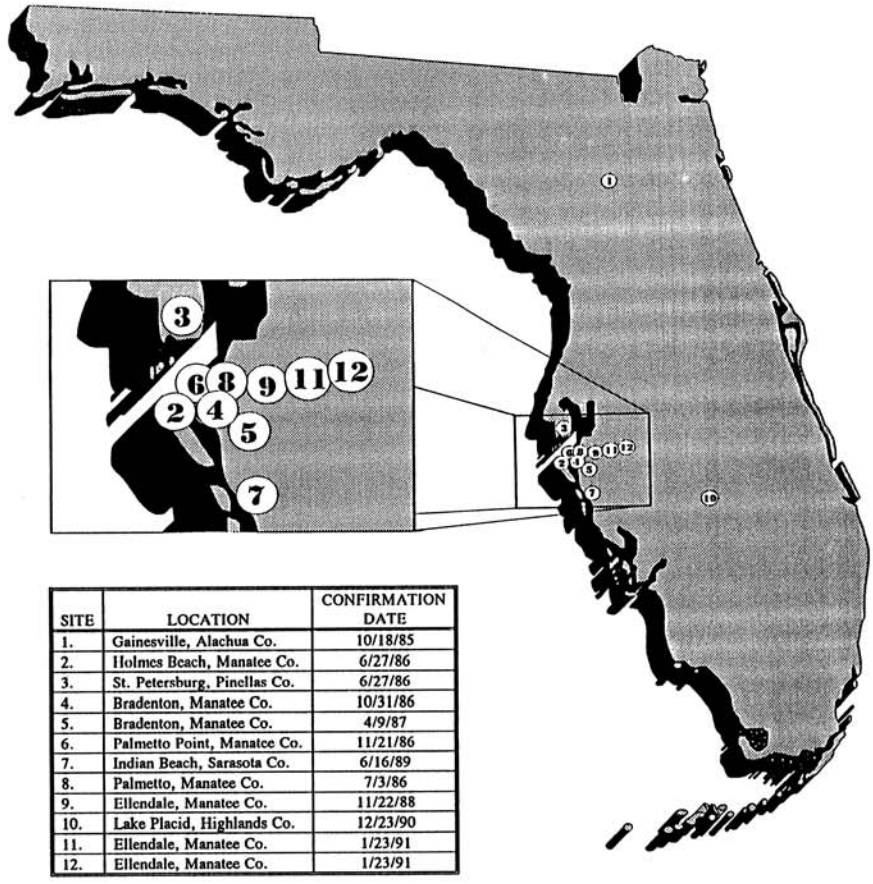
Comparison of *X. c. citri* and *X. c. citrumelo*

The apparent similarities between Asiatic canker and CBS at the initial nursery outbreak were striking. Although the leaf symptoms clearly differed, CBS and Asiatic citrus canker affected many of the same citrus cultivars under nursery conditions (25). In retrospect, the host range observed in the first outbreak was highly misleading because at the time the outbreak was discovered, the bacteria had been widely transmitted in the nursery. Presumably, this occurred by mechanical operations such as planting, leaf stripping, and budding of seedlings and topping of trees. These operations wounded leaves and stems and rendered most cultivars susceptible to infection. Furthermore, the initial outbreak was the first of only four occurrences of the most aggressive strains of *X. c. citrumelo* ever encountered (17). The over 50 additional finds of CBS were associated with weakly aggressive strains (17).

The appearance of the Asiatic strain in a limited geographic area on the gulf coast of Florida was not unexpected because canker-affected citrus was intercepted at ports of entry approximately 175 times between 1971 and 1983 by APHIS inspectors (27). The program outlined in the Citrus Canker Disease Action Plan, with recent modifications, continues to be appropriate for eradicating or suppressing apparently exotic strains of *X. c. citri*. In contrast, the origin and virulence of the strains of *X. c. citrumelo* that cause CBS presented unique problems for regulators and plant pathologists (16).

That the nursery strains of CBS, unlike the Asiatic strains of *X. c. citri*, were pathologically variable was not realized until large numbers of strains from several CBS outbreaks were collected and compared. The detached leaf analysis provided a rapid, easy method to measure strain aggressiveness based on the extent and persistence of water-soaking and development of necrosis on wounds after 7 days (17; Fig. 1E). Reactions on attached leaves in the greenhouse and field, which required at least 30 days to develop fully, confirmed the differences in aggressiveness found in vitro (17).

The strains of *X. c. citrumelo* interacted differently from the strains of *X. c. citri* with *Citrus, P. trifoliata*, and their hybrids (19). Rates of lesion expansion were compared on different cultivars after pinprick inoculation of attached leaves in the greenhouse and field (19). Trifoliate orange hybrids, including Swingle citrumelo, were susceptible to the most aggressive strains of



SITE	LOCATION	CONFIRMATION DATE
1.	Gainesville, Alachua Co.	10/18/85
2.	Holmes Beach, Manatee Co.	6/27/86
3.	St. Petersburg, Pinellas Co.	6/27/86
4.	Bradenton, Manatee Co.	10/31/86
5.	Bradenton, Manatee Co.	4/9/87
6.	Palmetto Point, Manatee Co.	11/21/86
7.	Indian Beach, Sarasota Co.	6/16/89
8.	Palmetto, Manatee Co.	7/3/86
9.	Ellendale, Manatee Co.	11/22/88
10.	Lake Placid, Highlands Co.	12/23/90
11.	Ellendale, Manatee Co.	1/23/91
12.	Ellendale, Manatee Co.	1/23/91

Fig. 2. Outbreaks of Asiatic citrus canker began with an isolated instance in north Florida in October 1985 (site 1). Since June 1986, several outbreaks have been confirmed on the west coast of the state in dooryard plantings (sites 2–7) and orchards (sites 8, 9, 11, and 12). In October 1990, the disease was discovered in a newly planted orchard in south-central Florida (site 10); this outbreak was apparently related to those on the west coast.

X. c. citrumelo, but other citrus cultivars were not susceptible. The aggressive strains elicited much greater expansion of lesions on Swingle citrumelo than did the moderately or weakly aggressive strains. Differences in lesion development among strains and cultivars were confirmed by measuring development of internal and external populations of bacteria associated with lesions in the greenhouse and field (5,19). Populations of the aggressive strains increased or were maintained in leaves of Swingle citrumelo but decreased or varied in leaves of grapefruit. Unlike Asiatic strains of *X. c. citri*, which affected trifoliate and citrus cultivars alike, the aggressive strains of *X. c. citrumelo* affected only trifoliate orange and its hybrids (19). The moderately and weakly aggressive strains of *X. c. citrumelo*, even though isolated from citrus, did not multiply in any of the cultivars tested, which suggested these strains should not be classified as *X. c. citrumelo*.

Additional evidence that moderately and weakly aggressive strains were not *X. c. citrumelo* was based on genomic, serological, and biochemical analyses of strain groups. The aggressive strains of *X. c. citrumelo* were highly related to one another by restriction fragment length polymorphism (RFLP) analysis of genomic DNA, Mab serogroup associations, and profiles of cellular fatty acids (11,23,26). The clonal nature of this group is analogous to the Asiatic strains worldwide and cancrosis B strains from Argentina, which showed only a limited number of RFLPs within each group of strains (8,23). The less aggressive strains did not fit this pattern; they fell into several Mab serogroups (11) and were heterogeneous by RFLP (8,23), fatty acid profile (20), and DNA fingerprinting analyses (Fig. 3B). Paradoxically, the less aggressive strains, though genetically variable according to these analyses, were highly related to each other and the aggressive strains by DNA-DNA reassociation of their total DNA (6). Thus, the strains associated with CBS probably belong to the same DNA homology group (30) even though they are genetically variable.

Gabriel et al (9) proposed that the pathovar *citrumelo* include all the aggressiveness types from CBS despite the variation they found among these strains in RFLPs and pathogenicity on detached citrus leaves. However, at least some of these strains were highly related to *X. campestris* pathovars that attack legumes, e.g., *X. c. alfalfae* (9). Graham et al (20) proposed that weakly aggressive strains might constitute several different pathovars that parasitize citrus only under conditions in citrus nurseries. When they compared weakly aggressive citrus strains with pathogenic *X. campestris* from noncitrus hosts in Florida, approximately 20% of the strains from other plants caused necrosis of wounded detached citrus leaves. These noncitrus strains elicited necrotic spots when sprayed onto immature foliage of Swingle citrumelo. In leaves, the strains multiplied and reached populations as high as those attained by weakly aggressive strains from citrus. Other noncitrus pathovars of *X. campestris* that did not elicit leaf necrosis failed to multiply in citrus leaves (nonspotting strains). In RFLP and fatty acid profile analyses, noncitrus strains that induced leaf necrosis were moderately related to each other and to the weakly aggressive strains from citrus, but nonspotting strains were not related to strains from citrus. Moreover, citrus leaf-spotting strains from noncitrus hosts were indistinguishable from citrus strains by DNA-DNA reassociation (Table 1 [6]) and fatty acid profile and RFLP analyses (20).

On the basis of this experimental evidence, strains causing CBS in nurseries appear to originate from a microflora of genetically related *X. campestris*, perhaps on vegetation in and around nurseries. In this population are strains that are weakly parasitic or pathogenic on citrus and on other noncitrus hosts in Florida. The existence of weakly aggressive strains with apparently a broad host range does not correspond well with the pathovar concept based on host specificity. These findings illustrate fundamental weaknesses of the "host from which first isolated concept" for definition of pathovars. New unique strains of *X. campestris* on a host such as citrus cannot be readily classified because possible relationships to other pathovars are uncertain. Thus, the debate surrounding the classification of nursery strains first as *X. c. citri* group E and later as *X. c. citrumelo* (9) is by no means resolved (30,33).

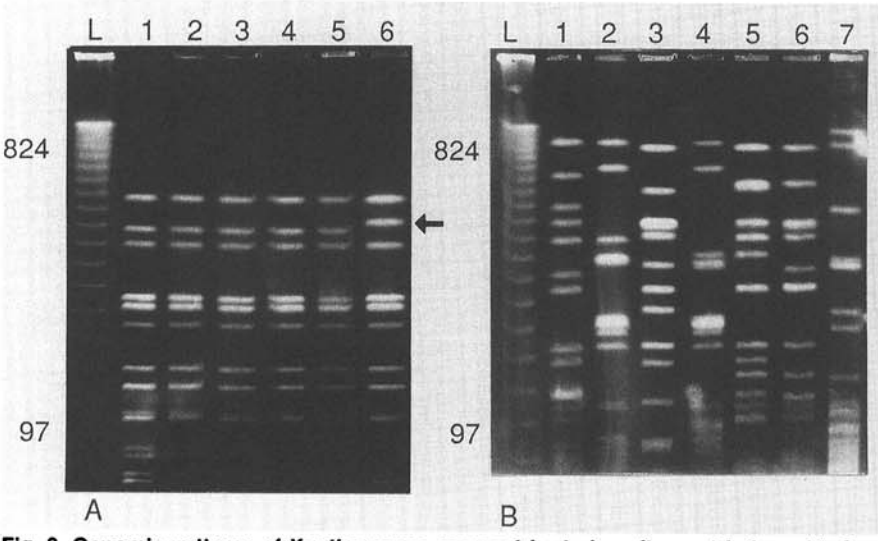


Fig. 3. Genomic patterns of *Xanthomonas campestris* strains after restriction with *Spe* I endonuclease and separation by pulsed-field gel electrophoresis: (A) Lanes 1, 2, and 3 are DNA patterns of *X. c. citri* from three disease foci of Asiatic citrus canker in a citrus orchard in central Florida in 1990; lane 4 is an identical pattern for a strain from an adjacent dooryard presumed to be the source; lane 5 is an identical pattern for a strain from an outbreak on the west coast of Florida in 1986; and lane 6 is a slightly different pattern (arrow) for a strain from an apparently unrelated outbreak of citrus canker in north Florida in 1985. L = lambda phage size markers in kilobases. (B) DNA patterns of moderately aggressive strains of *X. c. citrumelo* from several outbreaks of citrus bacterial spot in seven different nurseries in Florida indicate genomic heterogeneity compared with Asiatic strains.

Epidemiology in Nurseries and Orchards

Foci of disease and mode of spread from foci were determined from analyses of the spatial distribution in Florida nurseries. Unfortunately, these analyses were not possible until almost a year after eradication in nurseries began. The most valuable information was lost because trees in the first outbreak of the disease were eradicated before data could be collected, and in the second most extensive nursery outbreak in August 1985, information on distribution of diseased trees was obtained during the few days between disease confirmation and tree destruction (14). Thereafter, more time was permitted in nurseries to map the spatial distribution of CBS and to collect strains of different aggressiveness for evaluation of mode of spread (12,16,17).

Strict quarantine measures and the perceived risk of exposing the citrus industry to the pathogen also held up approval of field research on CBS in Florida until October 1986. This was more than 2 years after the initial outbreak of CBS, and the majority of the over 20 million nursery and newly planted orchard trees had already been destroyed. In the meantime, research was conducted with Asiatic citrus canker in

Argentina and at the USDA-ARS Foreign Disease and Weed Research Laboratory in Frederick, Maryland, under strict quarantine measures for containment of the Asiatic strain. In 1987, when the research protocols were deemed sufficient to contain the organisms, the quarantine facility was relocated to the Beltsville Agricultural Research Center. These locations afforded very limited opportunity for side-to-side comparison of the development of Asiatic citrus canker and CBS to ascertain the relative importance of CBS. Attempts to generate epidemics of both diseases in nurseries of susceptible hosts were limited by the short growing season and dry summers from 1986 to 1988.

Field research with CBS was finally begun in 1987 in Hastings, Florida, about 100–150 km north of the citrus production area. Nursery simulations of bacterial spread were initiated by placing a diseased seedling in the center or a line of diseased trees along the edge of stands of vigorously growing Swingle citrumelo and grapefruit seedlings. When mechanical transmission of the bacteria was avoided, very little movement of bacteria from the inoculated plants or disease spread was observed over a 2-year period (16).

Meanwhile, there were only two nursery outbreaks of CBS in Florida in 1986 because planting of the primary host, Swingle citrumelo, was prohibited from November 1985 through February 1986. When propagation resumed, the number of new infestations in nurseries rose to eight in 1987 (16). With more opportunity to study the spatial distribution of CBS in nurseries, it became apparent that mechanical spread of the bacterium within rows by planting, budding, and trimming operations was primarily responsible for the pattern of spread, particularly of weakly aggressive strains (12,17). In contrast, a 1988 outbreak of the aggressive type of *X. c. citrumelo* was clearly associated with spread by windblown rain across nursery rows. At this point, only four outbreaks of the aggressive strains were known (12,17). Nevertheless, strain aggressiveness and mode of dissemination of the bacterium appeared to be linked (12,17).

In 1989, field studies were conducted in which windblown rain was simulated by spraying water at high velocity (24–32 m/sec) over Swingle citrumelo plants inoculated with strains of different aggressiveness toward receptor plants down the rows. Populations of bacteria on the leaf surface were monitored by swabbing the leaves immediately after spraying and periodically thereafter. The slope of the bacterial deposition gradient was related positively to eventual disease development and negatively to strain aggressiveness (the steeper the gradient, the less the aggressiveness). The incidence of diseased leaves on the receptor plants was correlated well with detection of surface bacterial populations immediately after spraying, but populations then decreased rapidly to nondetectable levels. Disease incidence in the nurseries decreased with time regardless of strain aggressiveness because of: 1) continual production of new leaves, 2) lack of disease spread to the new leaves, and 3) disease-induced defoliation. In Beltsville, CBS induced more defoliation on inoculated nursery trees than did citrus canker (T. R. Gottwald and E. L. Civerolo, *unpublished*).

In Hastings, CBS did not spread in newly established orchards and incidence of diseased leaves decreased in a nearly linear mode irrespective of strain aggressiveness. The lack of disease progress confirmed that grapefruit and sweet orange cultivars were resistant and that CBS posed no threat to orchards. CBS was limited even when aggressive strains occurred on susceptible Swingle citrumelo.

Asiatic citrus canker spread rapidly and extensively in nurseries and orchards in Argentina (13,15). In nursery plots, spread and severity of disease were greater on susceptible grapefruit than on less susceptible sweet orange and Swingle citrumelo (15). The exponential rate of disease progress reflected the continual development of susceptible leaf flushes under nursery conditions. Disease spread was independent of wind direction because splash dispersal and rapid development of secondary foci of disease prevailed. In contrast, windblown rain was responsible for highly directional spread from a disease focus in simulated orchards (13). Subsequent spread from secondary foci was rapid and in many directions. The rate of disease progress was decreased by periodic disease-induced defoliation, which exceeded 90% on highly susceptible grapefruit trees.

The relative potential for spread of the Asiatic strain and the different aggressiveness types of *X. c. citrumelo* were confirmed by measuring the concentration and duration of bacterial exudation from leaf lesions (5,29). Young citrus canker lesions rapidly exuded up to 10^5–10^6 cfu per lesion within hours after wetting, but older lesions exuded 10^2–10^3 cfu per lesion (29). Lesions of CBS yielded fewer bacteria than did lesions of citrus canker. The aggressive strains of *X. c. citrumelo* had higher internal and external populations of bacteria on susceptible Swingle citrumelo than did other combinations of less aggressive strains and nonsusceptible cultivars (5). The higher availability of bacteria from lesions probably explained why *X. c. citri* had a greater ability to spread than *X. c. citrumelo* and why natural spread in Florida nurseries occurred only with the aggressive strains of *X. c. citrumelo* on Swingle citrumelo (5,29). In the field, populations of bacteria in lesions of CBS were indicative of external leaf populations and, therefore, were predictive of the ability of a given strain to spread on a host (5).

In Argentina, lemons are affected by cancrosis B caused by group B strains of *X. c. citri* as well as by citrus canker caused by A group strains. The host range of B strains is primarily limited to lemons and limes, and, on the basis of greenhouse inoculations, the strains are considered less aggressive than A strains on citrus hosts (10). Nevertheless, the disease progress of the A and B strains on Lisbon lemon is comparable (T. R. Gottwald and L. W. Timmer, *unpublished*) (Fig. 4A). This finding confirms the observation that cancrosis B causes substantial disease loss on lemons in affected orchards in Argentina and also emphasizes that comparative study of the epidemiology of these strains in the field is the most definitive approach to determining the relative importance of each disease. Because side-by-side studies of CBS and Asiatic canker were never allowed in Florida, the lack of spread by *X. c. citrumelo* in the first 2 years of field trials was difficult to explain.

Susceptibility of Foliage and of Fruit

Because rain driven at wind speeds exceeding 8 m/sec is essential for spread of the bacteria (13,15), windbreaks effectively reduce the probability of

Table 1. Similarity values generated by DNA reassociation analysis for strains of *Xanthomonas campestris* that produce lesions on citrus

Strains of *X. c. citrumelo*[a]	Percent reassociation with pathovars			
	X. c. maculifoliigardeniae X22j	Undetermined X198[b]	*X. c. fici* X151	*X. c. alfalfae* 82-1
F1	72 (4.1)[c]	89 (4.7)	90 (3.0)	88 (1.4)
F6	68 (1.1)	86 (3.5)	91 (2.9)	80 (12.3)
F100	76 (4.8)	90 (4.1)	87 (2.9)	83 (0.8)

[a] F1 = highly, F6 = moderately, and F100 = weakly aggressive (17).
[b] Isolated from *Strelitzia reginae*.
[c] Standard error of the mean in parentheses.

canker infection. In a recent outbreak of Asiatic citrus canker, bacteria are believed to have spread by windblown rain from diseased dooryard trees 200 m across an open pasture into an adjoining orchard of young trees. This event probably occurred during a heavy thunderstorm in August 1989, 3–5 months after the orchard was planted, when large flushes of young, susceptible tissue were being produced. The time of leaf flush is important because expanding leaf tissue is very susceptible to infection. As leaves reach full expansion, they become significantly more resistant (Fig. 5); this effect is only partially overcome by increasing the wind pressure on the leaf surface.

When leaves of different cultivars were 50–70% expanded (the stage most prone to water-soaking), the number of lesions that formed after treatment with a wind pressure of 1.0 g/mm^2 did not differ appreciably among cultivars (Graham and Gottwald, *unpublished*); that is, bacteria entered leaves of cultivars of differing susceptibility with equal ease. Subsequent differences in the rate of lesion expansion reflected the resistance factors in the mesophyll tissue that affected bacterial multiplication, because lesion development and bacterial growth in lesions were well correlated among cultivars (19; Graham and Gottwald, *unpublished*).

Throughout the world, Asiatic citrus canker is economically important because fruit lesions downgrade the appearance of fruit and, when severe, cause premature fruit drop (25,27). The same concerns were raised for CBS when fruit of the rootstock trifoliate orange (cv. Flying Dragon) were found diseased in a field nursery (14,16). As with leaf lesions, the fruit lesions of citrus canker and CBS superficially resembled one another. However, lesions of CBS had slightly raised to sunken necrotic areas with water-soaked margins and chlorotic halos (Fig. 1B). Necrosis on trifoliate orange fruit remained superficial and did not expand into the adjacent rind. Unlike citrus canker, CBS was never encountered on commercial citrus cultivars in orchards.

The susceptibility of fruit and leaves to Asiatic citrus canker is similar on the basis of field observations but not on the basis of systematic comparison after fruit inoculation. We found that fruit were most susceptible when 2–4 cm in diameter and became more resistant as size increased, but water-soaking and lesion formation occurred as long as the fruit were still expanding. CBS lesions, once developed, did not expand further into the rind tissue. Similar inoculations with group A strains of *X. c. citri* in Argentina, however, produced lesions that continued to expand laterally and into the rind tissue. Because *X. c. citrumelo* is unable to form lesions without water-soaking, and to multiply in rind tissue and cause lesion expansion, we consider fruit of all commercial citrus cultivars to be resistant to CBS.

Control Measures

Wind-driven rain and water-soaking of tissue are essential for dissemination and ingress of bacteria and for epidemic development of citrus canker. The most effective method of disease control in Japan and Argentina has been the use of windbreaks. Copper sprays can be used to reduce the inoculum levels on the leaf surface (28). In the absence of windbreaks, copper applied at 1-month intervals slightly reduced canker spread during windblown-rain events, but copper did not affect canker spread and disease severity when used in conjunction with windbreaks (L. W. Timmer and T. R. Gottwald, *unpublished*) (Fig. 4B). This frequency of sprays apparently did not adequately protect the continuously emerging susceptible flush leaves (28).

In Florida, several protocols were developed to address different requirements of the quarantine program. Prescribed treatments to disinfest fruit

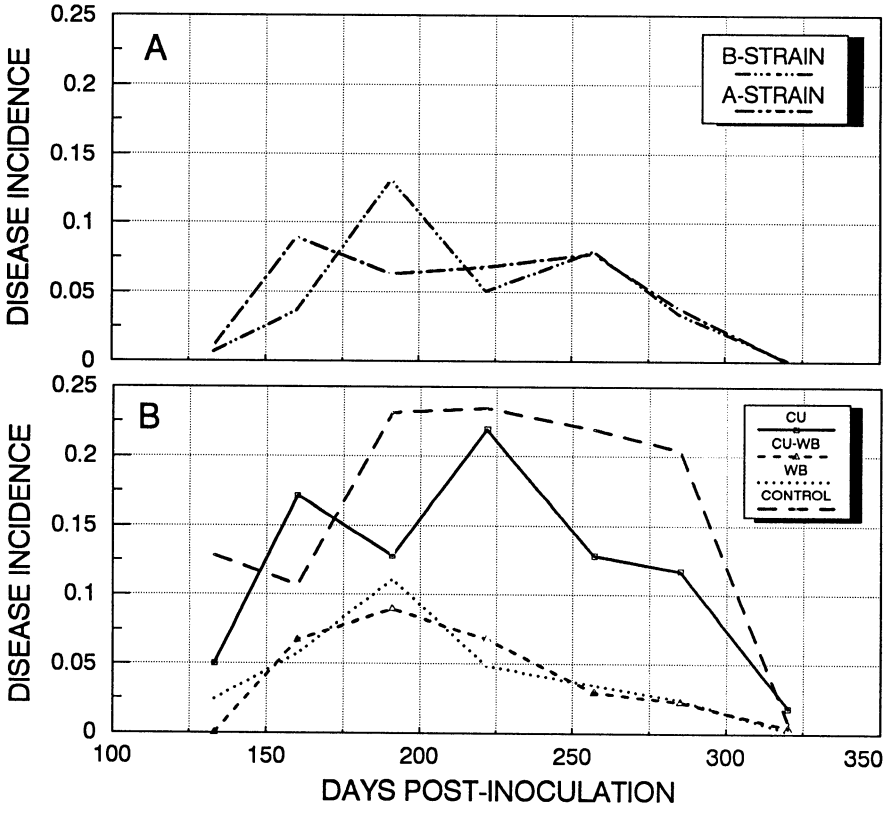


Fig. 4. Area under the disease progress curve (AUDPC) for: (A) Asiatic citrus canker (1,094) and B cancrosis (1,035) on Lisbon lemon trees in Argentina and (B) Asiatic citrus canker on Duncan grapefruit with and without applications of copper bactericide (CU) and/or windbreaks (WB); CU = 2,644, CU-WB = 904, WB = 886, control = 3,380. Rows of lemon trees and seedlings of Duncan grapefruit were inoculated at one end with group A or B strains of *Xanthomonas campestris* pv. *citri*. The spread of disease down the rows was followed for 320 days by measuring the incidence on each plant (percentage of leaves infected per plant).

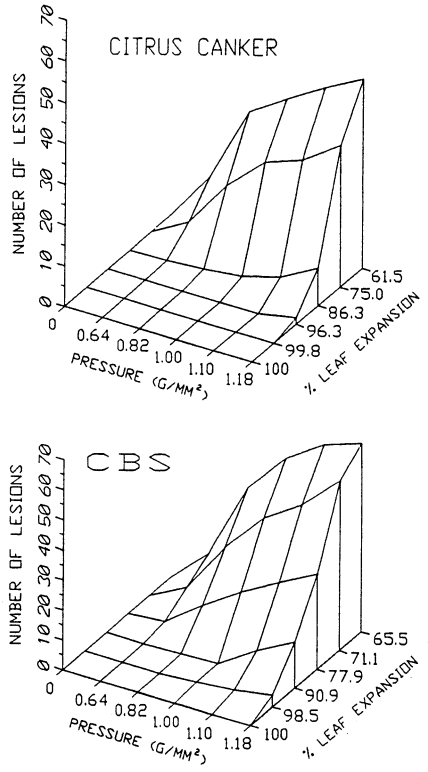


Fig. 5. Response of lesion development by *Xanthomonas campestris* pv. *citri* (Asiatic citrus canker) and *X. c. citrumelo* (citrus bacterial spot) to wind pressure and proportion of leaf expansion (a measure of leaf maturity) of Duncan grapefruit.

in the packinghouse before shipment were a 2-minute dip in chlorine (200 μg/ml at pH 7.0), a 1-minute dip in 2.0% sodium-*o*-phenylphenate (SOPP), or a 45-second wash with a soap formulation of SOPP (3). In mature groves, the diseased leaves and stems on trees were killed with the herbicide diquat. When all green tissue was temporarily removed, the pathogen had no opportunity to infect. Replanted trees require 5 years to reestablish, but a defoliated tree can regain its yield potential in 2–3 years (24). In blocks of heavily diseased trees, however, outbreaks occurred more than 2 years after defoliation, and the blocks were eventually eradicated.

Survival of bacteria in infested leaf debris and saprophytic activity in soil have been studied (18). In Argentina, the Asiatic strain of *X. c. citri* was recovered up to 4 months after leaf fall, but soil treatments, including burial of leaves, reduced survival significantly (21). In Maryland, both *X. c. citri* and *X. c. citrumelo* were recovered from soil beneath diseased trees, but removal of the inoculum source led to a demise of soil populations within days (18). Thus, eradicated sites could be replanted a relatively short time after removal of diseased plants.

Significance of Research and the Current Situation

Initially, regulations to deal with CBS were based on the presumption that the disease was similar to Asiatic citrus canker and that both diseases would behave in Florida as the Asiatic strain does elsewhere. These assumptions were questioned (31,32) and reevaluated as research information on CBS and regulatory experience increased (16). When even the most virulent form of *X. c. citrumelo* was found not to be a threat to commercial citrus cultivars, CBS was deregulated. The disease will probably continue to appear in citrus nurseries in Florida where Swingle citrumelo is grown. Excessive fertilization and irrigation produce large amounts of susceptible tissue and environments that are highly favorable for bacterial infection. With further investigation, CBS may well be found in other humid citrus-growing areas of the world, since it apparently is caused by a wide variety of weakly parasitic xanthomonads in Florida. The nursery strains of *X. campestris* are pathologically and genetically distinct from the groups of *X. c. citri* that cause citrus canker (6). The classification of nursery strains as *X. c. citrumelo* and the term "citrus bacterial spot" to distinguish the disease from citrus canker diseases have been widely, though not universally, accepted. Much more needs to be learned about the origin of strains in nurseries because there is still uncertainty about their relationship to other pathovars of *X. campestris*.

Asiatic citrus canker is obviously a far more important disease problem than CBS. Although occurrences of canker in Florida have been effectively suppressed, the recent outbreaks in the central and west coast areas of the state suggest that more canker may again appear. Citrus canker is not the killer disease it was sometimes portrayed as, especially early in the campaign to eradicate CBS. Citrus canker is a leaf, stem, and fruit spotting disease of importance in the humid subtropics, where inoculum levels can build up on abundant new growth. In these areas, wind-driven rain can spread the bacterium and initiate new disease foci. In Florida, where sufficient inoculum has built up on leaves, fruit spotting of grapefruit and early season oranges has been observed in mature orchards. Citrus canker could cause significant losses on these cultivars in some years, but losses of more resistant Valencia orange and processed fruit would be minimal. Dry weather usually prevails during the period of spring flush in Florida and would tend to limit the buildup of inoculum for early infection of fruit. Frequent summer flushes of leaves and severe thunderstorms, on the other hand, could lead to significant spread of citrus canker and infection of expanding fruit. Grapefruit, which has a long period of fruit development, would be particularly prone to infection throughout the summer.

At present, efforts to suppress citrus canker are justified as long as the areas affected are limited and the costs of the program are not excessive. Even if the campaign of the early 1900s did not completely eradicate the disease, citrus canker was absent for 50–75 years in Florida orchards. If the current eradication effort fails, the program to suppress disease spread would at least delay the time when routine control measures might be needed in most citrus orchards.

In the early stages of the eradication program for CBS, nurserymen and growers were reimbursed from federal and state funds for a portion of the value of the plants destroyed. Since the end of 1988, compensation has been limited, and now losses are not being compensated. Over 60 lawsuits were filed against the DPI in an attempt to recover losses beyond the presumptive value of the destroyed trees. The first of these suits were found in favor of the plaintiffs. In response, the Florida legislature passed a bill in 1989 that provides administrative funding for nurserymen and growers to claim the commercial value of trees plus legal costs incurred. Subsequently, the Florida Supreme Court upheld the constitutionality of the legislation on presumptive value.

Despite repeated calls by other citrus-producing states for stricter quarantine measures aimed at reducing importation of fresh fruit from Florida, citrus canker probably would not be a significant disease problem elsewhere in the United States. Conceivably, citrus canker could be of some consequence in Texas for fresh market grapefruit, the principal product of the citrus industry there. However, most of the rains in south Texas occur in late summer, and citrus canker would be only a sporadic problem. The probability that citrus canker would be of significance in California or Arizona citrus is even lower, since most of the rainfall occurs when temperatures are too low for disease to develop.

Citrus canker will continue to plague the citrus industry in Florida in the near future and the long run. Research information and the experience of regulatory agencies with the disease have led to more rational approaches to control the disease. At this point, we feel citrus canker and CBS have been placed in much better perspective among the many other problems facing the citrus industry in Florida.

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