Variation in Aggressiveness of *Xanthomonas campestris* pv. *citrumelo* Associated with Citrus Bacterial Spot in Florida Citrus Nurseries

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ABSTRACT

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Reactions on wound-inoculated detached leaves of Swingle citrumelo and Duncan grapefruit were used to characterize strains of *Xanthomonas campestris* pv. *citrumelo* associated with citrus bacterial spot (CBS) in Florida citrus nurseries and to distinguish these strains from *X. c. citri*, the cause of Asiatic citrus canker. Strains of *X. c. citrumelo* varied in aggressiveness based on the extent and persistence of water-soaking and the development of necrosis. Aggressiveness on detached leaves was correlated with that on wound-inoculated leaves in the greenhouse and field. Reactions on detached leaves developed rapidly and could be evaluated after 7 days, whereas 30 days were required for the development of lesions on attached leaves. In vitro inoculations distinguished the flat-spreading lesions of CBS from the erumpent, calluslike reaction produced by *X. c. citri*. In four nurseries, the incidence, severity, and spatial distribution of CBS was related to strain aggressiveness. Only the most aggressive strains were associated with natural spread, whereas less aggressive strains were evidently spread mechanically by nursery operations. In one nursery, where strains varied from weakly to moderately aggressive, aggressiveness differed among separate disease foci. Strains from 25 unrelated nursery infestations were evaluated, and the most aggressive strains occurred in only four nurseries. More than 75% of the nursery outbreaks were associated with Swingle citrumelo. This variety was more susceptible than Duncan grapefruit to the aggressive strain of *X. c. citrumelo* and less susceptible to *X. c. citri* in attached leaf tests. There were significant interactions of strains of *X. c. citrumelo* of different aggressiveness with the two citrus cultivars.

Additional keywords: disease eradication.

In 1984, a new foliar disease of citrus caused by *Xanthomonas campestris* pv. *citrumelo* (9) (syn. = *X. c. citri* group E) was identified in a central Florida citrus nursery (24). Since then, strains of *X. c. citrumelo* have been isolated from more than 30 nursery and orchard locations. The disease, termed citrus bacterial spot (CBS), is characterized by leaf lesions that are flat with a necrotic center and a water-soaked margin, which sometimes is surrounded by chlorosis (10,13,24). Asiatic citrus canker caused by *X. c. citri* (syn. = *X. citri* [9]), which was more recently found on mature trees in Florida, is characterized by erumpent, corky lesions on leaves, fruit, and stems (3,13,24). Cytopathological differences between CBS and citrus canker have been confirmed (6,21). Strains of *X. c. citrumelo* are serologically and genetically distinguishable from *X. c. citri* (1,8,9,14,15,24).

Pathogenicity of strains in a pathovar of *X. campestris* varies, due to interactions between strains and hosts (2,5,7,16,17). However, the leaf symptoms of CBS, as well as the incidence, severity, and distribution of the disease have been observed to vary widely on clonally propagated cultivars within and among nursery outbreaks (13). The most extensive outbreaks have been associated with grapefruit scions and the rootstock, Swingle citrumelo, and with leaf lesions that have necrotic centers with extensive and persistent water-soaking at the margin (11–13). Less severe outbreaks of CBS have been characterized by lesions that are more limited in size, dry in appearance, and lack persistent water-soaking. Likewise, strains of *X. c. citrumelo* isolated from different nurseries and lesion types are genetically heterogeneous (9,14,15) and exhibit a wide range of reactions on leaves after inoculation of grapefruit, Swingle citrumelo, and other citrus hosts (9,13, *unpublished data*).

The purpose of this study was to characterize the aggressiveness of strains of *X. c. citrumelo* within and among CBS outbreaks that vary with regard to the incidence, severity, and spatial distribution of the disease and to compare the reactions of strains of *X. c. citrumelo* with that of *X. c. citri* on Swingle citrumelo and grapefruit. Most of the earlier infestations of CBS were eradicated before epidemiological data and large numbers of strains

could be collected (12). Recently, more thorough scientific assessments before plant destruction have been allowed.

MATERIALS AND METHODS

Bacterial strains. Strains of *X. c. citrumelo* were isolated from diseased leaves of several scion and rootstock varieties of *Citrus* and *Poncirus* and their hybrids located in 25 infested nurseries from 1984 to 1988 (Table 1). Nurseries were determined to be infested with CBS by the Florida Department of Agriculture and Consumer Services, Division of Plant Industry (DPI), and in most cases, the outbreaks were not related by plant movement between nursery locations. Most of the test strains were obtained from J. W. Miller of DPI. Strains of *X. c. citrumelo* were identified by DPI, E. L. Civerolo, USDA-ARS, Fruit Laboratory, Beltsville, MD, and the authors by two or more of the following tests: 1) pathogenicity on Duncan grapefruit after injection-infiltration of fully expanded immature leaves with 10^6 to 10^8 cfu/ml (determined spectrophotometrically), 2) detached leaf assay on Duncan grapefruit, Swingle citrumelo, or Mexican lime (*C. aurantifolia* (Christm.) Swingle), 3) hypersensitive reaction on tobacco leaves (23), 4) color and appearance of colonies on yeast extract-dextrose-calcium carbonate (YDC) agar (23), 5) xanthomonadin pigment assay (18), 6) enzyme-linked immunosorbent assay (4), and 7) other biochemical tests for *Xanthomonas* (23). Strains were mixed with silica gel, milk, and glycerin, and stored at −5 C until further tests were conducted.

Four nurseries, Frostproof, Ocoee, Lake Wales, and Venice (Table 1), were intensively sampled immediately before disease eradication in conjunction with an epidemiological evaluation (11). Diseased leaves were collected from different rows and distances within the row in the infested area of the nursery (11). Individual lesions from different leaves on a single plant were excised with a cork-borer, triturated in 0.075 M phosphate buffer (pH 7.0) plus 0.1% peptone (22), and shaken for 1 hr. From each sample, 0.1-ml aliquots of the wash suspension and tenfold serial dilutions were spread on single plates of KCB medium (nutrient agar 23.0 g/L, glucose 0.1 g/L [NGA], kasugamycin 16.0 mg/L, cephalexin 16 mg/L, chlorothalonil [Bravo 720] 12.0 mg/L). At least two individual colonies from a plate (lesion) were streaked on NGA and single colony isolations restreaked on NGA before tests were conducted.

Characterization of strain aggressiveness in a detached leaf assay. Aggressiveness of each strain was evaluated with a detached-leaf assay (9,12). Swingle citrumelo and Duncan grapefruit were used as the hosts because these cultivars were most commonly associated with nursery outbreaks of CBS (24, Table 1). Two-thirds to fully expanded, immature leaves from seedlings grown in the greenhouse were detached and separated into leaflets. Leaflets were surface disinfested in 70% ethanol for 1 min, rinsed twice with sterile-distilled water (SDW), and placed on 1.5% water agar plates with their adaxial surface exposed. Leaflets were punctured 10 times with a 26-gauge syringe needle.

Bacteria were grown overnight in nutrient-glucose-broth, pelleted by centrifugation, and resuspended in sterile-distilled water. Ten microliters of a bacterial suspension containing 10^8 cfu/ml (determined spectrophotometrically) was placed on each wound site. Uninoculated controls were treated with an equal volume of SDW. The treated detached leaves in petri dishes were incubated under fluorescent lights (60 μE sec^{-1} m^{-2}) for 14-hr photoperiods. Seven days after inoculation, an average disease severity rating (0–3) of the 10 sites on each leaf was made based on the criteria in Table 2. The detached-leaf assay was performed twice with one leaflet used per strain. The results of one test are reported.

TABLE 1. Aggressiveness of strains of *Xanthomonas campestris* pv. *citrumelo* from 25 nursery infestations of citrus bacterial spot from 1984 to 1988

Nursery location	Date of identification	Hosts[a]	Aggressiveness type[b]	Strains tested (no.)
Avon Park I	9/84	SC, CM, SO, WMG, RRG, PO, VO, NO, HO, MT	3	4
Haines City I	10/84	SC, TPG, RRG, WMG, ML	2,3	5
Valrico	4/85	SC	1	16
Haines City II	8/85	SC, CC, FD	2	6
Avon Park II	8/85	SC	1	3
Desoto City I	9/85	SC	2	16
Avon Park III	9/85	SC	1	6
Wahneta	10/85	SC, HO, VO	1	7
Zellwood	10/85	SC	1	4
Ft. Basinger	10/85	SC, VO	1	4
Desoto City II	12/85	SC	1	20
Dundee I	7/86	SC, WMG, RRG, NT, SO, CM	2	33
New Port Richey	9/86	ST, SO	1	7
Frostproof	9/87	HRG, RRG, PBO	2	66
Ocoee	10/87	SC, RRG, HO	3	26
Lake Wales	10/87	SC	1	60
Immokalee I	11/87	WMG, CC	2	6
Dade City I	11/87	SC, RRG	1	8
Dade City II	11/87	WMG, SO	2	12
Immokalee II	12/87	SC	2	8
Lithia	12/87	RRG, SO	1	14
Venice	2/88	SC	3	61
Lake Alfred	5/88	RRG, SC	1	3
Bartow	10/88	SC	1	3
Dundee II	10/88	RRG, CC, DT, VO	2	90

[a] SC = Swingle citrumelo (*Poncirus trifoliata* × *Citrus paradisi*), CM = Cleopatra mandarin (*C. reticulata*), FD = Flying Dragon trifoliate orange (*P. trifoliata*), ML = Milam rough lemon (*C. jambhiri*), CC = Carrizo citrange (*C. sinensis* × *P. trifoliata*), SO = Sour orange (*C. aurantium*), WMG = White Marsh grapefruit (*C. paradisi*), RMG = Red Marsh grapefruit, HRG = Henderson Red grapefruit, RRG = Ruby Red grapefruit, TPG = Thompson Pink grapefruit, NO = Navel orange (*C. sinensis*), PO = Pineapple orange, HO = Hamlin orange, VO = Valencia orange, PBO = Parson Brown orange, ST = Sunburst tangerine (*C. reticulata* hybrid), NT = Nova tangerine, MT = Murcott tangerine, and DT = Dancy tangerine.

[b] Detached-leaf rating: 1 = weakly aggressive, 2 = moderately aggressive, and 3 = aggressive (see Table 2).

Differences in detached leaf ratings among strains for each host were evaluated by analysis of variance and Duncan's multiple range test. Interactions between location in the nursery or lesion of origin and strain aggressiveness were determined by the *F*-test.

Attached leaf inoculations. To determine if the detached-leaf assay correlated with more natural inoculation methods, an attached leaf assay similar to the detached-leaf assay was performed under greenhouse conditions at the DPI quarantine facility in Gainesville, FL. Strains F1 from the Avon Park I (DPI X84-3048), F6 from Haines City I (DPI X84-3401), and F100 from Desoto City II (DPI X85-12689) nurseries (Table 1) were compared because field symptoms and the severity of the epidemics in the nurseries indicated that the strains differed in aggressiveness. The reactions of strains of *X. c. citrumelo* were compared with those of a single strain of *X. c. citri* (DPI X86-9771) from the outbreak of Asiatic citrus canker in Manatee County, FL, and *X. c. vesicatoria* (XV1, J. B. Jones, University of Florida), a nonpathogen of citrus.

Attached leaves (two-thirds to fully expanded) were punctured four times on each side of the midrib. A 10-μl droplet of the test isolate was suspended from the adaxial surface of the leaf at each wound site. The reaction at each inoculation site was evaluated after 30 days by measurement of the diameter of the lesion that developed. For comparison of the two assays, lesions on attached leaves were rated as in the detached-leaf assay (Table 2) and the diameter of the lesions on detached leaves was measured. The attached leaf and detached leaf assays were performed twice with one and two leaves of each citrus variety per strain, respectively. The results of one set of detached- and attached-leaf assays conducted at the same time with the same source of leaves are presented. Detached-leaf ratings were compared with the lesion diameter on attached leaves by regression analysis. Host × strain interactions in the attached-leaf assay were identified by the *F*-test.

Field inoculations. The relative aggressiveness of strains F1, F6, and F100 was compared further in simulated nursery plots of Swingle citrumelo and Duncan grapefruit at a quarantine facility in Hastings, FL. Plots for inoculation with each strain consisted of four rows of 25 seedlings (20–30 cm tall) of each variety at a spacing of 10 cm within the row and 30 cm between the rows with a 10-m spacing between inoculated plots.

A suspension of 10^9 cfu/ml of each strain was prepared for inoculation as described above and mixed with Carborundum to serve as a wounding agent. This suspension was mechanically rubbed onto the upper and lower surface of all leaves on each plant. Seedlings of Swingle citrumelo and Duncan grapefruit were inoculated on 15 September 1988 and disease symptoms evaluated on 27 October 1988. In each plot of each variety and strain, four seedlings were selected at random from the row of inoculated plants for evaluation of disease incidence per plant (no. of leaves with lesions/total no. of leaves). The diameter (mm) of 25 lesions on randomly selected plants from each row was measured with a micrometer. For strain F100, the incidence of infected leaves per plant was less, so 15 lesions were measured. Detached-leaf ratings for these strains were compared with lesion diameters and disease incidence on field-inoculated leaves by regression analysis. Host × strain interactions were identified by the *F*-test.

Nursery survey. At least three strains of *X. c. citrumelo* from each of 25 nursery infestations (Table 1) were evaluated for aggressiveness with the detached-leaf assay. Duplicate or triplicate leaves of Swingle citrumelo, Duncan grapefruit, or Mexican lime were inoculated. An aggressiveness type was assigned to each nursery based on the average rating of all strains tested. Information reported by DPI (24) on the date of positive identification of CBS in the nursery and the varieties found to be infested is also presented (Table 1).

RESULTS

Characterization of strain aggressiveness. Uninoculated detached leaves of Swingle citrumelo and Duncan grapefruit placed on water agar remained healthy in appearance and free of contamination for at least 30 days. Inoculum or water placed on the wound sites either infiltrated through the wound to the water agar-leaf interface or remained on top of the wound for the duration of the test. Infiltration did not affect the development of the reaction. After 7 days, wound sites treated with water were about 0.5 mm in diameter surrounded by a narrow margin of tan tissue discoloration. The reaction after inoculation with *X. c. vesicatoria* was similar to the water-treated control except that the color of the affected tissue was darker (Table 3).

Strains F1 and F6 caused water-soaking after 2–4 days on both Swingle citrumelo and Duncan grapefruit. The extent and persistence of the water-soaking depended on strain aggressiveness (Table 3). Strain F1 was rated 3 because water-soaking was more extensive and persistent than for F6 (Fig. 1A and B). The significant difference between F1 and F6 in lesion diameter on detached leaves of Swingle citrumelo and Duncan grapefruit also reflected this (Table 3). Necrosis was indistinct within the water-soaked area 7 days after inoculation with F1 (Fig. 1A). Strain F6 was rated 2.0 because necrosis developed rapidly after water-soaking and completely surrounded the wound site by 7 days (Fig. 1B). Lesions produced by strain F100 had little water-soaking and necrosis was not extensive (Fig. 1C, Table 3). The reaction did not extend completely around the wound site on Duncan grapefruit, which further reduced the rating to 1.0.

The Asiatic strain of *X. c. citri* was not assigned a rating because the lesion differed so greatly from that of CBS (Fig. 1D, Table 3). The reaction resembled callus tissue, while water-soaking and necrosis were absent after 7 days. The lesion did not spread but was markedly erumpent. This was confirmed by the significantly smaller diameter of the reaction compared with strain F1 of *X. c. citrumelo* (Table 3).

Aggressiveness of the same strains was further compared in an attached- and detached-leaf assay. The reactions were evaluated with the rating scale used for the detached-leaf assay except that lesions required 30 days rather than 7 days to develop.

TABLE 2. Disease severity rating for the reaction of detached and attached citrus leaves after inoculation with strains of *Xanthomonas campestris* pv. *citrumelo*[a]

	Rating			
Symptom	No reaction 0	Weakly aggressive 0.5–1.5	Moderately aggressive 1.5–2.5	Aggressive 2.5–3.0
Water-soaking	...	Indistinct, +/− around wound, < 1 mm wide	Distinct from necrosis but limited, completely around wound, < 1 mm wide	Extensive, > 1 mm wide around wound
Necrosis	...	Distinct but limited, +/− around wound	Distinct from water-soaking, completely around wound	Extensive, indistinct from water-soaking

[a] Leaves were punctured in 8 or 10 locations and 10 μl of 10^8 cfu/ml is placed on each puncture wound.

On attached leaves, water-soaking and necrosis developed slowly and were not always in zones around the point of inoculation. The reaction of the aggressive strain F1 was characterized by mottling of necrotic tissue within an area of water-soaking (Fig. 1E). As in the detached-leaf assay, the diameter of the water-soaked areas on attached leaves of Swingle citrumelo was greater for strains F1 than for F6 (Fig. 1E and F; Table 3). On Duncan grapefruit, the rating and diameter of the lesions produced by strains F1 and F6 were not different in the attached-leaf assay (Table 3). The reaction of strain F100 on attached leaves of both cultivars was no greater than that caused by *X. c. vesicatoria* (Table 3), and water-soaking was absent (Fig. 1G). Among strains F1, F6, and F100 of *X. c. citrumelo* on Swingle citrumelo and Duncan grapefruit, the aggressiveness rating on detached leaves was significantly ($P \leq 0.001$) correlated ($r = 0.92$) with lesion diameter on attached leaves. However, there was a significant interaction ($P \leq 0.001$) of strains F1 and F6 with the two hosts on attached leaves that was not detected on detached leaves.

The attached-leaf reaction of Asiatic strain of *X. c. citri* was markedly different from that on detached leaves (Fig. 1D and H). The lesion was less erumpent and as extensive as strain F1 on Duncan grapefruit (Table 3). The center of the lesion was

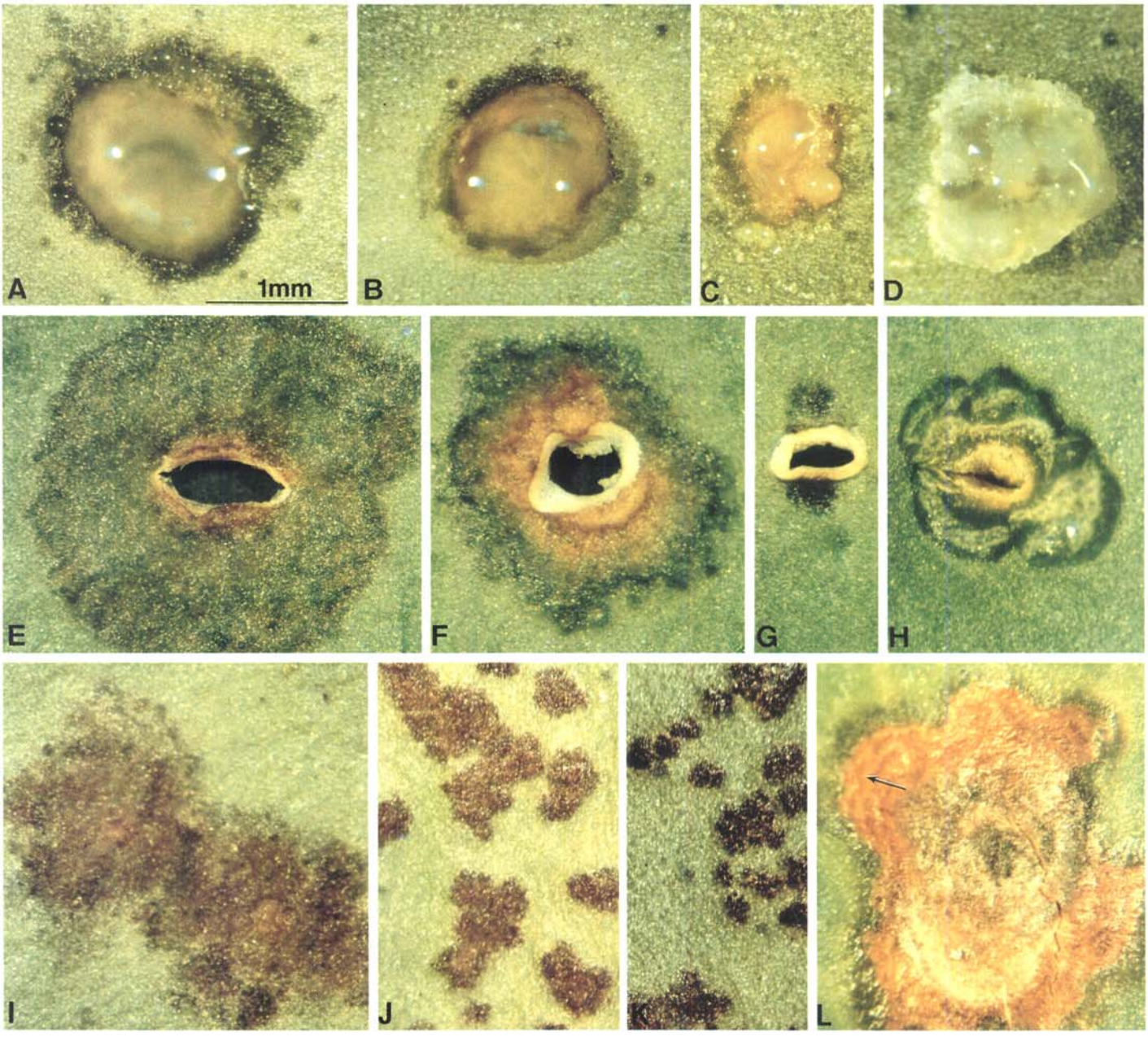

Fig. 1. Reactions on wound-inoculated detached leaves of Swingle citrumelo in vitro and attached leaves in the greenhouse and the field following inoculation with strains F1, F6, and F100 of *Xanthomonas campestris* pv. *citrumelo* from citrus bacterial spot and one strain of *X. c. citri* from Asiatic citrus canker. **A–D,** Lesions on detached leaves 7 days after inoculation. A, Aggressive strain F1 with persistent water-soaking and indistinct necrosis; B, moderately aggressive strain F6 with distinct necrosis and limited water-soaking; C, weakly aggressive strain F100 with very limited necrosis and no water-soaking; D, Asiatic strain of *X. c. citri* with a calluslike eruption of tissue and no water-soaking or necrosis. **E–H,** Lesions on attached leaves 30 days after inoculation. E, Aggressive strain F1 with mottling of necrotic tissue within the area of water-soaking; F, moderately aggressive strain F6 with a discrete zone of necrosis with limited water-soaking at the perimeter; G, weakly aggressive strain F100 with slight necrosis at the wound site; H, Asiatic strain of *X. c. citri* with erumpent, but extensive necrosis surrounded by a water-soaked margin. **I–K,** Lesions on field leaves 45 days after inoculation. I, Aggressive strain F1 with indistinct water-soaking within necrotic areas; J, moderately aggressive strain F6 with discrete areas of necrosis and limited water-soaking at the perimeter; K, weakly aggressive strain F100 with limited necrosis and no water-soaking; **L,** lesions from Ocoee field nursery (Table 1) associated with aggressive strains have concentric rings (arrow) where extension of the lesion ceased then resumed. All magnifications are the same. Refer to the scale in panel A.

tan-brown and surrounded by a wide water-soaked margin (Fig. 1H).

Lesions on Carborundum-inoculated plants in the field resembled reactions on attached leaves (Fig. 1E–G, I–K). Strain aggressiveness on Swingle citrumelo based on lesion diameter and the incidence of diseased leaves per plant after 6 wk was similar to that in the detached- and attached-leaf assays (Tables 3 and 4). The aggressiveness of strains on Duncan grapefruit in the field was the same as on attached leaves in that the lesions of strains F1 and F6 did not differ in diameter or incidence. Again, there was a significantly different ($P < 0.001$) interaction of the aggressive and moderately aggressive strains on the two cultivars. Overall, when all strains on Swingle citrumelo and Duncan grapefruit were considered, there were significant correlations between detached-leaf assay rating and lesion diameter ($r = 0.84$) and disease incidence ($r = 0.86$) in the field. Because the aggressive and moderately aggressive types could be distinguished on detached leaves of Swingle citrumelo more readily, this cultivar was used for further evaluation of strains.

The detached-leaf assay was used to characterize a large number of strains of *X. c. citrumelo* from the Frostproof, Ocoee, Lake Wales, and Venice nurseries (Tables 1 and 5). The leaf symptoms of CBS differed within and among these nurseries. In the Lake Wales nursery, most lesions were associated with thorn punctures that occurred when seedlings were bundled before planting in beds for budding. The lesions were uniformly small (1–2 mm diameter) with a brown necrotic center and no water-soaking at the margin of tissue discoloration. There was a yellow halo >1 mm wide surrounding the necrotic area. Of the 60 strains examined, more than half resulted in a very slight or no reaction on detached leaves (Table 5). For the remainder of the isolates, the rating varied to a maximum of 1.5, and the reaction resembled that of the weakly aggressive strain F100 (Fig. 1C). There was no significant effect of location in the row or bed in the nursery on strain aggressiveness.

Lesions in the Frostproof nursery were variable in size and many were associated with wounds resulting from hedging of the foliage. The necrotic area in the center of lesions ranged from 1 to 7 mm in size and was reddish-brown in color. A water-soaked margin around the necrosis was sometimes present but was <1 mm wide. Some lesions became brittle and cracked, which resulted in a shot-hole. In the detached-leaf assay, the ratings for the strains varied from 0.5 to 2.0 (Table 4). Most strains were of the moderately aggressive type and their reactions resembled strain F6 (Fig. 1B). There was a highly significant ($P < 0.0001$) effect of location in the nursery on strain aggressiveness but no effect of the source of strain from lesions within a location.

In comparison with the Frostproof nursery, the lesions observed in the Ocoee and Venice nurseries were uniform in size and appearance. The necrotic area was 5–7 mm in diameter, gray-brown, and was surrounded by an extensive gray water-soaked margin of 1 mm or more in width. In the Ocoee nursery, older lesions on the lowest leaves on the stem were characterized by concentric rings that were apparently caused by cessation and resumption of lesion extension (Fig. 1L). In contrast to the Lake Wales and Frostproof nurseries, strains from the Ocoee and Venice nurseries were uniformly aggressive in the detached-leaf assay (Table 5). The reaction was characterized by extensive water-soaking with indistinct necrosis like that of strain F1 (Fig. 1A).

Nursery survey of strain aggressiveness. Strains of *X. c. citrumelo* in the 25 independent nursery outbreaks were predominantly of the less aggressive types (Table 1). Aggressive strains were associated with four nurseries, whereas the moderately aggressive strains were found in nine nurseries and the weakly aggressive strains in 13 nurseries. Haines City I nursery was associated with both moderately and highly aggressive strains. All three aggressiveness types were found on Swingle citrumelo (Table 1). The weakly aggressive type was associated with Swingle citrumelo in all but two nurseries. The moderately aggressive type was found on grapefruit varieties as often as on Swingle citrumelo. In Haines City II, the moderately aggressive strains were primarily associated with Flying Dragon trifoliate orange (12). All of the field infestations were discovered during the period from August through December, except the Venice and Lake Alfred outbreaks, which occurred in February and May, respectively. The two other exceptions, Valrico and Dundee, were greenhouse nurseries.

DISCUSSION

Reactions on wound-inoculated detached leaves in vitro were used to characterize strains of *X. c. citrumelo* associated with

TABLE 3. Comparison of detached and attached leaf reactions on Swingle citrumelo and Duncan grapefruit after inoculation with strains of *Xanthomonas campestris* pv. *citrumelo* (F1, F6, and F100), *X. c. citri*, and *X. c. vesicatoria*

Strain	Detached leaf[w]		Attached leaf[w]	
	Rating (0–3)[x]	Lesion diameter (mm)	Rating (0–3)[x]	Lesion diameter (mm)
Swingle citrumelo				
F1	3.0	1.2 a[y]	3.0	2.6 a
F6	2.0	0.8 b	2.5	1.7 b
F100	1.5	0.7 b	0.5	0.6 c
X.c. citri	NR[z]	0.9 b	NR	1.6 b
X.c. vesicatoria	0.5	0.6 c	1.0	0.7 c
Duncan grapefruit				
F1	3.0	1.2 a	2.5	1.8 a
F6	2.0	0.8 b	2.5	2.0 a
F100	1.0	0.5 c	1.0	0.6 b
X.c. citri	NR	1.0 b	NR	2.0 a
X.c. vesicatoria	0.5	0.6 c	0.5	0.9 b

[w] Readings 7 days after inoculation for detached leaves and 30 days for attached leaves.

[x] 0 = no reaction; 0.5–1.5 = weakly aggressive; 1.5–2.5 = moderately aggressive; 2.5–3.0 = aggressive (see Table 2).

[y] Mean separation of strains by Duncan's multiple range test. Means followed by unlike letters differ significantly at $P \leq 0.01$.

[z] NR = no rating; canker reactions were not comparable to CBS reactions (see Fig. 1).

TABLE 4. Severity and incidence of citrus bacterial spot on Swingle citrumelo and Duncan grapefruit in simulated field nurseries in Hastings, FL, after inoculation with *Xanthomonas campestris* pv. *citrumelo* strains of different aggressiveness

Strain	Swingle citrumelo		Duncan grapefruit	
	Lesion diameter (mm)	Incidence[y] (%)	Lesion diameter (mm)	Incidence[y] (%)
F1	2.38 a	52.3 a[z]	1.71 a	40.2 a[z]
F6	1.72 b	34.6 b	1.74 a	37.8 a
F100	0.89 c	19.0 c	0.65 b	9.6 b

[y] Number of infected leaves per total number of leaves per plant.

[z] Mean separation of strains by Duncan's multiple range test. Means followed by unlike letters differ significantly at $P \leq 0.01$.

TABLE 5. Aggressiveness of *Xanthomonas campestris* pv. *citrumelo* strains associated with citrus bacterial spot from four Florida citrus nurseries rated on detached leaves of Swingle citrumelo

Rating[b]	Population (%)[a]			
	Lake Wales ($n = 60$)	Frostproof ($n = 66$)	Ocoee ($n = 26$)	Venice ($n = 61$)
0	35.1	0	0	0
0.5	28.3	6.1	0	0
1.0	18.3	24.2	0	0
1.5	18.3	39.4	0	0
2.0	0	30.3	0	0
2.5	0	0	3.8	0
3.0	0	0	96.2	100

[a] Percentage of total isolates (n) tested from each nursery with indicated rating.

[b] 0 = no reaction; 0.5–1.5 = weakly aggressive; 1.5–2.5 = moderately aggressive; 2.5–3.0 = aggressive (see Table 2).

CBS in Florida citrus nurseries. Strains varied from aggressive to weakly aggressive, based on the extent and persistence of water-soaking and the development of necrosis 7 days after inoculation of puncture wounds. Aggressiveness on detached leaves in vitro was correlated with that on wound-inoculated attached leaves in the greenhouse and seedlings in the field. While lesions on attached leaves more closely resembled the symptoms observed in the field, reactions took longer to develop on attached leaves than on detached leaves. Koizumi (20) used detached leaves to study the relationship between growth of *X. c. citri* and symptom development. The Asiatic strain multiplied more rapidly in detached leaves than in attached leaves of *C. natsudaidai*. Water-soaking developed after 3–4 days on detached leaves, but not until 10 days on attached leaves.

The detached-leaf assay allowed for rapid evaluation of strains and was conducted in containment facilities where the use of intact plants for inoculation was prohibited by quarantine regulations. Detached leaves were also useful in clearly distinguishing the flat lesion type of CBS from the erumpent, calluslike reaction of citrus canker. The eruption of tissue observed in the detached leaf assay is similar to the hypertrophy and proliferation of cells after pin-prick inoculation of detached leaves with *X. c. citri* (19,21).

Because aggressiveness of *X. c. citrumelo* could be rapidly characterized, we were able to evaluate several hundred strains from nursery outbreaks. A larger number of strains was evaluated in four nurseries that differed in the incidence and severity of symptoms and spatial distribution of CBS (11). Strains from the Lake Wales nursery gave no reaction or were weakly aggressive. Although the percentage of symptomatic plants at this location was higher than for other outbreaks (11), this was apparently a result of distribution of infested seedlings when they were lifted from the seedbed and transplanted. The incidence of lesions on individual plants was very low and the lesions were small and lacked water-soaking. The distribution of the disease in the Frostproof nursery was also indicative of mechanical spread of the bacterium from disease foci in two rows of older nursery trees by hedging activities (11). The strains ranged from weakly to moderately aggressive and the aggressiveness varied among locations in the rows. The separate discrete foci of the disease identified in this location (11) may have been associated with independent populations of strains. The outbreak was also characterized by lesions that varied in size and degree of necrosis and water-soaking, although no difference in aggressiveness among lesions within a location was detected.

In contrast to the nurseries with strains of lesser aggressiveness, in the Ocoee and Venice nurseries lesions that were larger and more uniform in size and the associated strains were uniformly aggressive in the detached-leaf assay. In the Ocoee nursery, spread resulted from mechanical transmission down the rows by budding activities from Swingle citrumelo that was infested at time of transplant (11). In the Venice nursery, the aggressive strain was secondarily spread from disease foci across nursery rows, presumably by windblown rain (11). This type of spread was not observed in the two nurseries associated with less aggressive strains, although spread across rows from a disease focus was observed in a previously studied (12) outbreak of moderately aggressive strains at Haines City II (Table 1). This situation was unique in that spread probably was from tall 8-yr-old trees of Flying Dragon trifoliate orange across adjacent rows of Swingle citrumelo. Thus, the movement of the bacterium from the disease focus was downwards, as well as, across the rows.

The capability of the aggressive strain F1 to spread by windblown rain from a diseased focal tree was evaluated in simulated nurseries in Frederick, MD, in 1985 (10) and in Beltsville, MD, and Hastings, FL, in 1987 and 1988 (unpublished data). During a wet, rainy summer in 1985 in Frederick, MD, the bacteria were detected 10 m from the disease focus (10); however, in the drier years of 1987 and 1988, the bacteria were detected only within a few meters of the infection focus.

Although the potential of the aggressive strains to spread in nursery situations is still uncertain, the occurrence of outbreaks of these strains has been infrequent compared with the less aggressive strains. Movement of less aggressive strains in the nursery evidently occurred by mechanical transmission of the bacterium during transplanting, trimming, and budding activities (11). Natural spread of these strains across nursery rows was not observed. We do not believe that the less aggressive strains pose a risk if outplanted into mature citrus groves. The ability of the aggressive strains to spread in newly established grove plantings and to establish on leaves and fruit of mature trees is under investigation at the quarantine field facility at Hastings.

More than 75% of the nursery outbreaks were associated with Swingle citrumelo. Disease occurred on this rootstock in all four nurseries with the aggressive strains, five of nine of the nurseries with the moderately aggressive strains, and 11 of 13 nurseries with the weakly aggressive strains. The timing of the outbreaks was consistent from year to year. Most of the field infestations were discovered during and after the summer rains from August through December. Two of the exceptions were greenhouse operations, where a conducive environment for disease development occurred independently from the season of the year.

Swingle citrumelo appears to be more susceptible to CBS than grapefruit, which is known to be highly susceptible to Asiatic citrus canker (3). Although Swingle citrumelo had slightly less extensive lesions after inoculation with *X. c. citri*, the reactions of the aggressive strain on attached leaves of Swingle citrumelo exceeded that on Duncan grapefruit in the greenhouse and the field. The aggressive and moderately aggressive strains produced equally extensive reactions on attached leaves of Duncan grapefruit but not on Swingle citrumelo. Thus, significant host × strain interactions occurred with CBS as observed for other *Xanthomonas* diseases (5,7,16,17).

Because Swingle citrumelo was associated with the majority of CBS infestations, the planting and movement of this rootstock was restricted from November 1985 until February 1986 (24). When this was done, the number of nursery infestations dropped from nine in 1985 to two in 1986. When propagation of Swingle citrumelo resumed, the number of nursery infestations rose to eight in 1987. At least three possibilities exist: 1) Swingle citrumelo is more susceptible to CBS than other citrus cultivars and effectively traps strains of *X. campestris* out of the environment, 2) Swingle citrumelo harbors the bacteria in the seed source trees and/or the seed, or 3) Swingle citrumelo is one of the most commonly grown rootstocks in Florida (25) and the association with CBS is spurious.

Since 1984, the incidence, severity, and distribution of CBS in Florida citrus nurseries has been generally related to the aggressiveness of the strains isolated. The ability to determine disease severity from epidemiological data combined with rapid identification of strain aggressiveness in the detached-leaf assay is significant in the assessment of the need for eradication. We believe the weakly and moderately aggressive strains pose no threat to the citrus industry. The aggressive strains that apparently can be naturally spread from disease foci in nurseries will require further evaluation before their epidemiological significance can be fully assessed.

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