Differential Host Range Reaction of Citrus and Citrus Relatives to Citrus Canker and Citrus Bacterial Spot Determined by Leaf Mesophyll Susceptibility

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ABSTRACT

Gottwald, T. R., Graham, J. H, Civerolo, E. L., Barrett, H. C., and Hearn, C. J. 1993. Differential host range reaction of citrus and citrus relatives to citrus canker and citrus bacterial spot determined by leaf mesophyll susceptibility. Plant Dis. 77:1004-1009.

The leaf mesophyll susceptibility of 54 citrus species, cultivars, and relatives to *Xanthomonas campestris* pv. *citrumelo*, the cause of citrus bacterial spot, was evaluated in Hastings, Florida, during 1989 and 1990. A similar host range of 53 citrus species, cultivars, and relatives was tested in Beltsville, Maryland, during 1991 to compare their differential susceptibility to *X. c. citri*, which causes citrus canker, and to *X. c. citrumelo* by inoculations on foliage of the same trees in replicated field plots. Field-grown trees were pruned to stimulate synchronous leaf flush for inoculation by a modified pinprick method. Lesion size at 60 days (Hastings plots) or 45 days (Beltsville plots) postinoculation was used to quantify leaf mesophyll susceptibility. For *X. c. citrumelo* inoculations, lesion expansion was greatest on cultivars of trifoliate orange and trifoliate orange hybrids. Smaller lesions formed on *Citrus* spp. such as grapefruit, sweet orange, sour orange, mandarin, lemon, and their hybrids, with the exception of Key lime, which developed lesions similar to those formed on trifoliate hybrids. Susceptibility of most citrus types to *X. c. citri* was more general. Lesion sizes resulting from pinprick inoculations with *X. c. citri* were not significantly different among *Citrus* spp. and hybrids, indicating a general susceptibility of leaf mesophyll. Smaller lesions generally formed on citrus relatives, including some cultivars of trifoliate orange. Because pinprick inoculations cause wounds and open the leaf mesophyll to direct colonization by bacteria, this method bypasses stomatal infection and does not consider other factors that may affect field resistance.

Additional keywords: epidemiology, field inoculation

Since 1984, considerable attention has been given to two bacterial diseases of citrus. Over 70 outbreaks of citrus bacterial spot and 13 outbreaks of Asiatic citrus canker have been reported in Florida (15). Asiatic citrus canker caused by *Xanthomonas campestris* pv. *citri* is endemic in many citrus-growing areas around the world but is exotic to the United States (6). Prior to the recurrence of citrus canker in 1985, the disease was found in 1912 in the Gulf Coast states and was presumed to have been eradicated in Florida by the early 1930s (24,34). In contrast, citrus bacterial spot, caused by *X. c. citrumelo*, is known to occur only in Florida and was first encountered in a citrus nursery in central Florida in the fall of 1984. The two pathogens, *X. c. citri* and *X. c. citrumelo*, have been demonstrated to be genetically, physiologically, pathogenically, and serologically different (2,3,20). Strains of *X. c. citri* are genetically homogeneous, whereas strains of *X. c. citrumelo* are heterogeneous. Thus, the taxonomic validity of *X. c. citrumelo* as a new pathovar and the inclusion of this diverse group of strains in the same pathovar have been questioned (4,35). However, all *X. c. citrumelo* strains produce similar symptoms in the field, i.e., flat, spreading, water-soaked lesions that often become necrotic and are distinct from field lesions of *X. c. citri*, which are raised due to hyperplasia and hypertrophy (15).

Citrus canker is still considered a potentially destructive disease to U.S. citrus and therefore continues to be under an eradication program. In contrast, citrus bacterial spot is now believed to be an endemic disease and only a nominal nursery problem in Florida and has been deregulated by state and federal plant regulatory agencies, i.e., the Florida Department of Agriculture and Consumer Services, Division of Plant Industry (DPI), and the U.S. Department of Agriculture, Animal and Plant Health Inspection Service (USDA/APHIS) (15).

The host range of Asiatic citrus canker is fairly broad, consisting of most citrus species, citrus hybrids, and some but not all citrus relatives (24). It has been found on numerous hosts in Florida both during 1912–1930s and since 1985 (15). In contrast, citrus bacterial spot has been found on about 20 different hosts in Florida, but over 75% of the disease occurrences have been associated with Swingle citrumelo (*Poncirus trifoliata* (L.) Raf. × *Citrus paradisi* Macf.) (14,17). Swingle citrumelo is a relatively new rootstock that has been used commercially in the United States and elsewhere only in the past 18 yr. In epidemiological studies in the United States and in Argentina, the rate of disease progress of Asiatic citrus canker was somewhat lower for Swingle citrumelo than for sweet orange (*C. sinensis* (L.) Osbeck) or grapefruit (*C. paradisi*) plantings (10,12). In contrast, Swingle citrumelo was more susceptible to infection by *X. c. citrumelo* than grapefruit, and grapefruit was more susceptible than sweet orange (14,17,18). Although as many as 20 different cultivars of citrus have been reported as susceptible to *X. c. citrumelo*, most of these were infected by mechanical operations in nurseries that caused wounding of the young trees. Such wounding opens the mesophyll to infection of cultivars that otherwise have field resistance (15).

The most discriminating test for susceptibility of citrus to xanthomonad diseases has been the pinprick inoculation assay, in which the increases of lesion diameter and in vivo bacterial populations are followed over time (23,29,31). In studies of cultivar-specific interactions to infection by *X. c. citrumelo*, lesion expansion continued for over 40 days in Swingle citrumelo and *P. trifoliata* inoculated with the aggressive strain of *X. c. citrumelo*, whereas lesions expanded for only 10–20 days in all other cultivars and relatives tested (17). In vivo bacterial populations of *X. c. citrumelo* in these tests were maintained only in Swingle citrumelo and trifoliate orange, whereas *X. c. citri* bacterial populations continued to increase for up to 40 days in all citrus species and relatives tested (17).

Field host range studies with *X. c. citri* have been conducted in Japan, the Philippines, South America, and other places where the disease is endemic, but these studies have focused on commercial citrus species and relatives important in those regions (1,21–25,28,30–32,37). Susceptibility to *X. c. citri* is thought to be

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Accepted for publication 21 June 1993.

related to host age and maturity of foliar tissues (8,18,26,27,33,36,37). Previous host range studies with *X. c. citrumelo* are few and have been limited in numbers of commercial cultivars and citrus relatives (17).

The purpose of this study was first to establish the reactions of a broad range of citrus and citrus relatives to *X. c. citrumelo* under Florida conditions and then to compare the reactions of a similar range of citrus to both *X. c. citrumelo* and *X. c. citri* for the first time in field plots at the same location to determine similarities and differences between the two pathogens.

MATERIALS AND METHODS

Rootstock seed and budwood from citrus, citrus hybrids, and citrus relatives were obtained from the USDA-ARS Whitmore Foundation Farm in Leesburg, Florida. Rootstock plants and a few citrus relatives were grown from seed, whereas citrus cultivars, hybrids, and most citrus relatives were budgrafted onto potted Swingle citrumelo rootstocks. All greenhouse-grown potted plants were 12–24 mo old when transplanted to the field plots. The first field plot to test the susceptibility of a host range to *X. c. citrumelo* was planted at a disease quarantine field facility at the University of Florida Agricultural Research and Education Center in Hastings, Florida. Field plots were planted in June 1988 and consisted of approximately 10 plants of each of 54 hosts in a completely randomized design in 10 rows of approximately 50 plants each. Plants were 0.3 m apart within the row, and rows were separated by 0.75 m. Plants were allowed to establish for the remainder of the season, then cut back in the spring and summer of 1989 to promote two flushes of susceptible new foliage for two inoculation trials during 1989.

Because *X. c. citri* is a quarantined and exotic pathogen to the United States, field inoculations could not be conducted in Florida or other citrus-growing states. Therefore, host range comparisons between *X. c. citri* and *X. c. citrumelo* were conducted at Beltsville, Maryland, 1,100 km north of commercial citrus production in an environment not conducive for long-term survival or overwintering of the experimental plots. A similar host range as that used in Hastings was prepared for the comparison of *X. c. citri* and *X. c. citrumelo* strains. Plants were shipped to Beltsville, where they were established in a disease-containment field site on the grounds of the USDA-ARS Agricultural Research Center, approved by USDA/APHIS and the Maryland Department of Agriculture for the study of citrus canker. Plants were transplanted in early May 1991, and two plots were established, each consisting of approximately 10 plants of each host in a completely randomized design consisting of 10 rows of approximately 50 plants per row. Rows were 0.75 m apart, and plants were 0.3 m apart within rows.

Inoculum of bacterial strains of *X. c. citrumelo* [F1 (DPI X84-3048)], previously demonstrated to be an aggressive strain (13), and *X. c. citri* [MF23P] was prepared by suspending the bacteria harvested from 48-hr-old nutrient glucose agar cultures in sterile distilled water. The suspensions were adjusted spectrophotometrically to approximately 10^8 cfu of bacteria per milliliter of distilled water, and inoculum density was confirmed by plating on nutrient glucose agar. A set of tongs was fitted with two rubber stoppers, one of which had two rows of five insect pins inserted through it such that the points of the pins extended about 3.0 mm. When the tongs were closed, the pinpoints in this stopper pressed against the flat surface of the second stopper. Inoculations were performed by dipping the tip of the tongs with the rubber stoppers into a suspension of inoculum and clamping the rubber stoppers over a leaf. This resulted in two rows of five pinprick inoculations through each inoculated leaf blade. Five expanding leaves of each of approximately 10 plants per plot were inoculated by this method.

Lesion size at 28 and 60 days (Hastings plots) or 45 days (Beltsville plots) postinoculation was used to quantify mesophyll susceptibility. The average diameter of five lesions, selected at random, on each of the approximately 10 plants per plot was subjected to the general linear models procedure (Statistical Analysis System, SAS Institute, Cary, NC), and differences among means were examined by the Student-Newman-Kuel test for mean separation. Trials at both locations were repeated once.

RESULTS

As previously reported, lesions caused by *X. c. citrumelo* continued to increase over time from 20 to 40 days after inoculation, then leveled off for most species tested with the exception of trifoliate orange and trifoliate orange hybrids, for which lesions continued to expand up to 60 days (17). This was consistent with lesion expansion in field inoculations on a smaller host range of citrus and citrus relatives (17). Previous greenhouse × cultivar interaction studies with *X. c. citri* demonstrated that lesion expansion generally slows after 40 days postinoculation (17). Therefore, a 45-day assessment was used in the Beltsville host range tests to allow two replications of the test during a single season, whereas a 60-day assessment of the Hastings plots was considered to be superior for differentiation of lesion size associated with citrus bacterial spot alone.

Although identical host ranges were not tested in Hastings and Beltsville, those citrus types common to both sites were consistent in reaction. For both Hastings and Beltsville tests, lesions induced by *X. c. citrumelo* were generally largest on trifoliate orange (4.7–8.1 mm in diameter, with occasional lesions as large as 12 mm), followed by trifoliate orange hybrids (1.5–6.2 mm in diameter) (Tables 1 and 2). Not all trifoliate orange hybrids developed large lesions. For instance, in the Hastings test, two selections of Changsha × English Large trifoliate orange were significantly different in their responses to *X. c. citrumelo* (Table 1). A citrus relative, *Clausena lansium* (Lour.) Skeels, commonly called wampee, also developed large lesions (5.0–7.0 mm in diameter) (Table 2). Grapefruit and pummelo plants in general were moderately susceptible to *X. c. citrumelo*, with medium-sized lesions (1.2–3.4 mm in diameter). Other moderately susceptible species were Key lime and Rangpur lime, which developed medium-sized lesions (1.9–4.3 mm in diameter, with occasional lesions on Key lime as large as 8 mm). Most other citrus types tested, including oranges, mandarins, lemons, limes, and other citrus relatives, generally had small lesions (1.0–2.7 mm in diameter). Very small lesions developed on Etrog citron and calamondin. A single citrus relative, *Murraya paniculata* (L.) W. Jack, developed no lesions and appeared to be completely resistant to infection by *X. c. citrumelo* (Table 2).

Size of lesions induced by *X. c. citri* did not vary greatly among citrus types, and few statistical differences were detected (Table 2). In general, lesion size among citrus types ranged from 2.0 to 3.5 mm in diameter. Only three citrus relatives—*Fortunella margarita* (Lour.) Swingle, *M. paniculata*, and *Severinia buxifolia* (Poir.) Ten.—consistently demonstrated resistance with significantly smaller lesions (≤1.7 mm in diameter). As with *X. c. citrumelo* inoculations, only *M. paniculata* was completely resistant to *X. c. citri* and did not develop lesions.

DISCUSSION

The reaction of citrus cultivars, hybrids, and relatives to *X. c. citri* and *X. c. citrumelo* differed markedly. The host range of *X. c. citri* was broad, with few differences in response to pinprick inoculation, and was consistent with previous reports (1,22,23,25,28–30,32,36), whereas susceptibility to *X. c. citrumelo* (production of large lesions) was limited primarily to trifoliate orange, its hybrids, and a few other individual species (17,19). Of special interest was the susceptibility of Key lime to *X. c. citrumelo*; at times, lesions were similar in size to those on trifoliate orange. The genetics of Key lime are unknown, but it is thought to be a complex hybrid with an undetermined citrus relative such as *Poncirus*. If true, this would explain the

Table 1. Diameter of *Xanthomonas campestris* pv. *citrumelo* leaf lesions for a host range of 54 citrus species, hybrids, and relatives resulting from pinprick inoculations at Hastings, Florida

	Lesion diameter (mm)			
	Trial 1		Trial 2	
Group	28 days	60 days	28 days	60 days
Trifoliate orange (*Poncirus trifoliata* (L.) Raf.)				
English Large Flower	4.6 ab[y]	6.2 bcd	3.8 a	6.0 a
Kryder 43-3	4.4 abc	NT[z]	4.1 a	5.4 ab
Kryder 5-5	4.4 abc	9.0 a	3.5 ab	5.5 ab
Yamaguchi	3.2 b–f	5.7 b–e	4.0 a	5.4 ab
Ronnse	5.3 a	7.5 ab	3.8 a	5.6 ab
Large Flower	3.9 a–d	7.4 ab	3.8 a	5.5 ab
Flying Dragon	5.2 a	8.4 a	3.5 abc	5.3 ab
Argentina	4.4 abc	6.6 bc	3.4 abc	4.7 bc
Hybrids with trifoliate orange				
Pummelo hybrids (*Citrus grandis* (L.) Osbeck)				
Thong Dee × Pomeroy	4.1 abc	6.2 bcd	2.8 b–e	4.0 c–f
Nakon × Flying Dragon	3.3 b–e	4.9 c–g	3.2 a–d	4.7 bc
Siamese × Large Flower HRS-802	2.2 d–g	4.3 d–i	2.0 e–j	3.1 f–i
Grapefruit hybrids (*C. paradisi* Macf.)				
Swingle citrumelo seedling	2.8 c–g	5.5 c–f	2.3 d–h	3.7 c–g
Duncan × Gotha Road	2.1 efg	3.4 g–n	2.3 d–j	2.8 f–k
Sweet orange hybrids (*C. sinensis* (L.) Osbeck)				
Norton citrange	3.0 b–g	3.9 e–k	2.2 d–j	3.0 f–j
Carrizo citrange seedling	2.8 c–g	3.7 f–m	2.3 d–i	3.4 d–h
Troyer citrange	2.7 c–g	3.7 f–l	2.0 e–j	2.8 f–k
Flying Dragon × Succory	1.9 efg	2.4 h–n	1.5 f–j	1.6 j–m
Mandarin hybrids (*C. reticulata* Blanco)				
Changsha × English Large HRS-899	2.3 d–g	4.4 d–h	3.3 abc	4.5 bcd
Changsha × English Large HRS-809	1.0 g	1.8 j–n	1.6 f–j	1.7 i–m
Changsha × English Small HRS-801	2.7 c–g	3.1 g–n	2.5 c–g	2.7 f–k
Sunki × Benecke HRS-812	2.0 efg	2.8 g–n	2.6 b–f	3.3 d–h
Other hybrids				
Citrumelo 80-9 × Succory sweet orange - 1169	1.4 efg	2.9 g–n	1.4 g–j	1.5 klm
Citrumelo 80-9 × Succory sweet orange - 119	1.7 efg	2.9 g–n	1.7 e–j	2.4 g–m
Mandarins				
Changsha	1.0 g	1.5 k–n	1.3 g–j	1.3 klm
Sunki	1.2 fg	1.7 j–n	1.3 g–j	1.5 klm
Kawano Wase	2.1 efg	2.6 h–n	1.2 hij	1.3 klm
Cleopatra seedling	1.6 efg	2.1 h–n	1.3 g–j	1.7 j–m
C. tachibana (Mak.) Tan.	1.4 efg	1.7 j–n	1.5 f–j	1.7 i–m
Clementine	1.2 fg	1.4 lmn	1.1 hij	1.3 klm
Dancy	1.4 efg	1.9 j–n	1.2 hij	1.2 lm
Mandarin hybrids				
Orlando tangelo	1.1 fg	2.0 j–n	1.4 g–j	1.6 j–m
Sunburst tangerine	0.9 g	1.2 mn	1.0 j	1.0 m
Temple tangor	1.3 efg	1.8 j–n	1.2 hij	1.0 m
(King × Changsha) × Satsuma	1.4 efg	2.1 h–n	1.6 f–j	1.7 i–m
Fortune × Encore - 65	1.5 efg	2.1 h–n	1.5 g–j	1.7 i–m
Fortune × Encore - 70	1.5 efg	2.7 g–n	1.4 g–j	1.5 klm
Grapefruits				
Duncan seedling	1.6 efg	2.1 i–n	1.1 hij	1.2 lm
Marsh	1.9 efg	2.9 g–n	1.4 g–j	2.2 h–m
Foster (irradiated) - 51	1.5 efg	2.8 g–n	1.6 f–j	2.6 g–l
Ruby Red	2.1 efg	3.4 g–n	1.5 f–j	2.1 h–m
Ray Ruby	1.9 efg	2.6 h–n	1.5 f–j	2.0 h–m
Sweet oranges				
Hamlin	1.1 g	1.8 j–n	1.0 j	1.0 m
Valencia	1.0 g	1.2 n	1.0 ij	1.1 lm
Navel	1.3 efg	2.2 h–n	1.2 hij	1.3 klm
Pineapple	1.3 efg	2.2 h–n	1.5 f–j	1.0 m
Succory	1.1 g	1.6 k–n	1.3 g–j	1.3 klm
Lemons and limes				
Rough lemon (*C. limon* (L.) Burm. f.) seedling	1.4 efg	1.5 k–n	1.3 g–j	1.4 klm
Vangasay rough lemon	1.2 fg	1.4 lmn	1.1 hij	1.2 lm
Volkamer lemon (*C. limon* (L.) Burm. f.) seedling	NT	NT	1.1 hij	1.4 klm
Key lime (*C. aurantifolia* (Christm.) Swingle)	NT	NT	2.2 d–j	4.3 b–e
C. macrophylla Wester seedling	NT	NT	1.5 g–j	2.6 f–l
Sour oranges				
Sour orange (*C. aurantium* L.) seedling	1.1 fg	1.5 k–n	1.3 g–j	1.3 klm
Smooth flat seville	1.6 efg	2.1 h–n	1.5 f–j	1.7 i–m
Other citrus hybrids				
Eremocitrus glauca (Lindley) Swingle × Shamouti Med Sweet Orange	1.9 efg	2.3 h–n	1.9 e–j	2.3 g–m

[y] Values followed by the same letter are not significantly different at $P = 0.05$ by the Student-Newman-Kuel multiple range test for variability.
[z] NT = not tested.

Table 2. Diameter of *Xanthomonas campestris* pvs. *citri* and *citrumelo* leaf lesions for a host range of 53 citrus species, hybrids, and relatives resulting from pinprick inoculations at Beltsville, Maryland

	Lesion diameter (mm)			
	X. c. citri		*X. c. citrumelo*	
Group	Trial 1	Trial 2	Trial 1	Trial 2
Trifoliate orange (*Poncirus trifoliata* (L.) Raf.)				
Chinese	2.6 a–e[y]	2.8 a–f	8.1 b	6.1 a
Large Flower	1.8 e	1.4 g	5.8 c	5.5 a
Flying Dragon	2.3 a–e	2.0 c–g	9.4 a	5.8 a
Argentina	2.0 de	1.7 efg	7.5 b	5.0 a
Small Flower	2.6 a–e	2.6 a–f	7.3 b	5.5 a
Hybrids with trifoliate orange				
Grapefruit hybrids (*Citrus paradisi* Macf.)				
Swingle citrumelo seedling	3.0 a–e	2.6 a–f	5.0 cde	3.8 b
Citrumelo 80-9	2.4 a–e	2.2 b–g	4.1 efg	3.0 bcd
Sweet orange hybrids (*C. sinensis* (L.) Osbeck)				
Carrizo citrange seedling	3.2 a–d	2.1 c–g	4.0 e–h	2.5 b–e
Troyer citrange	3.3 ab	2.8 a–e	4.5 def	3.7 bc
Mandarins (*C. reticulata* Blanco)				
Tankan	2.9 a–e	2.3 a–g	0.9 nop	2.3 b–f
Ponkan	2.8 a–e	3.1 a–d	1.9 j–o	0.7 e–h
Sunki	2.0 cde	2.0 d–g	1.6 k–p	2.1 c–g
Cleopatra	2.5 a–e	2.3 a–g	2.0 i–o	1.4 d–h
Clementine	2.9 a–e	3.1 a–d	1.8 j–o	1.5 d–h
Dancy	3.2 a–d	2.6 a–f	1.3 k–p	0.7 e–h
Owari satsuma (*C. unshiu* (Mak.) Marc.)	2.0 de	2.9 a–d	1.2 k–p	1.3 d–h
Mandarin hybrids				
Orlando tangelo	2.9 a–e	3.2 abc	1.1 l–p	1.6 d–h
Minneola tangelo	3.1 a–d	3.4 ab	1.5 k–p	1.5 d–h
Sunburst tangerine	3.3 ab	3.5 a	1.5 k–p	0.7 e–h
Sun Shu Sha Kat	2.4 a–e	2.4 a–f	1.1 l–p	1.1 d–h
Robinson	3.5 a	3.1 a–d	1.3 k–p	1.4 d–h
Nasnaran	2.4 a–e	2.7 a–f	1.9 j–o	2.3 b–f
Murcott	2.9 a–e	2.2 b–g	1.6 k–p	1.6 d–h
Ambersweet Orange (tangor)	2.9 a–e	2.5 a–f	1.0 m–p	0.2 fgh
Temple tangor	3.0 a–e	2.9 a–e	1.8 j–o	1.5 d–h
Ellendale tangor	2.9 a–e	2.7 a–f	1.8 j–o	1.6 d–h
Grapefuits				
Duncan seedling	2.9 a–e	3.0 a–d	2.8 h–l	2.4 b–e
Marsh	2.7 a–e	2.7 a–f	2.5 i–n	2.1 c–g
Ruby Red	2.7 a–e	2.8 a–e	2.8 h–k	2.0 c–h
Ray Ruby	2.8 a–e	3.1 a–d	2.6 i–m	2.1 c–g
Pummelos (*C. grandis* (L.) Osbeck)				
Thong Dee	2.2 b–e	3.0 a–d	2.4 i–o	2.0 c–g
Nakon	3.1 a–d	3.2 a–d	2.7 h–l	1.0 e–h
Sweet oranges				
Hamlin	2.9 a–e	3.1 a–d	1.2 k–p	0.9 e–h
Pera	2.8 a–e	3.0 a–d	1.4 k–p	0.6 e–h
Shamouti	2.6 a–e	2.3 b–g	0.8 op	0.8 e–h
Valencia	2.9 a–e	3.2 abc	1.1 l–p	1.4 d–h
Navel	3.4 ab	2.9 a–d	1.6 k–p	1.2 d–h
Pineapple	3.3 abc	3.0 a–d	0.8 nop	1.1 d–h
Lemons and limes				
Eureka lemon (*C. limon* (L.) Burm. f.)	3.3 ab	2.3 b–g	1.5 k–p	1.0 e–h
Persian lime (*C. aurantifolia* (Christm.) Swingle) hybrid	2.0 de	2.2 b–g	1.1 l–p	1.1 d–h
Key lime (*C. aurantifolia* (Christm.) Swingle)	2.8 a–e	2.4 a–f	3.4 f–i	2.2 b–f
Rangpur lime (*C. limonia* Osbeck)	2.7 a–e	2.9 a–d	3.2 g–j	1.9 c–h
Volkamer lemon (*C. limon* (L.) Burm. f.) seedling	2.6 a–e	2.5 a–f	0.7 op	0.3 fgh
C. macrophylla Wester seedling	1.8 e	2.3 a–g	1.5 k–p	0.8 e–h
Sour oranges				
Sour orange (*C. aurantium* L.) seedling	3.2 a–d	2.4 a–f	1.3 k–p	0.6 e–h
Gou Tao (*C. aurantium* L.) hybrid	2.7 a–e	2.4 a–g	1.7 j–o	1.5 d–h
Other citrus species and relatives				
*C. micropter*a Wester	2.2 b–e	2.0 d–g	1.5 k–o	0.4 e–h
Etrog citron 861 (*C. medica* L.)	1.0 f	2.3 a–g	NT[z]	0.9 e–h
Nagami kumquat (*Fortunella margarita* (Lour.) Swingle	0.7 fg	1.4 g	1.7 j–o	0.6 e–h
Orange jessamine (*Murraya paniculata* (L.) W. Jack)	0.0 g	0.0 h	0.0 p	0.0 h
Chinese box orange (*Severinia buxifolia* (Lam.) Jack)	0.2 g	0.0 h	1.7 j–o	1.9 c–h
Calamondin (*C. reticulata* var. *austera* (?) × *Fortunella* sp.)	2.2 b–e	1.6 fg	0.8 nop	0.1 gh
Wampee (*Clausena lansium* (Lour.) Skeels)	2.3 a–e	2.2 c–g	5.4 cd	5.6 a

[y] Values followed by the same letter are not significantly different at $P = 0.05$ by the Student-Newman-Kuel multiple range test for variability.
[z] NT = not tested.

similar responses of Key lime and trifoliate hybrids to *X. c. citrumelo*. Large differences also existed among trifoliate hybrids. Since the two Changsha × English trifoliate orange hybrids had significantly different lesion sizes 60 days after inoculation with *X. c. citrumelo*, resistance to *X. c. citrumelo* may be inherited quantitatively. Quantitative reactions of several citrumelo (*P. trifoliata* × *C. paradisi*) selections to the aggressive strain of *X. c. citrumelo* have previously been demonstrated by pinprick inoculation in the greenhouse (5). The greater susceptibility of trifoliate orange and its hybrids is consistent with the observation that most extensive field nursery outbreaks of the aggressive strain of *X. c. citrumelo* were associated with Swingle citrumelo (7,11,13).

The broad generalized host range of *X. c. citri* has been demonstrated in numerous field trials in the Orient and South America where the bacterium is endemic (21,23,25,30–32,36) and is in sharp contrast to that of *X. c. citrumelo*. Lesions caused by *X. c. citri* develop a hypertrophic and hyperplastic proliferation of cells resulting in a raised callus on the leaf surface, whereas those caused by *X. c. citrumelo* are more spreading, flat, and sunken (15,24). The general susceptibility of several citrus cultivars and species to *X. c. citri* has also recently been shown by nonwounding, stomatal inoculation (8,19). This phenomenon has been described as mesophyll susceptibility (19,33).

Field susceptibility to *X. c. citri* varies widely among citrus types, yet host range studies involving inoculation directly into the mesophyll tissues often result in susceptibility of cultivars that show general field resistance. Apparently, field resistance to *X. c. citri* is directly related to tissue juvenility and wounding (19,26,27,36,37). Because pinprick inoculation causes wounds and opens the leaf mesophyll to direct colonization by bacteria, the method bypasses stomatal infection and does not consider other factors that affect field resistance (31). For example, citrus cultivars and species with greater frequency, size, and duration of leaf flushes are more field-susceptible to *X. c. citri* than less vigorous cultivars or those whose foliage matures more rapidly (1,19,22,37).

Leaf age greatly influences water congestion of tissues and penetration by *X. c. citri* and *X. c. citrumelo* for Duncan grapefruit, but this was not correlated with stomatal size, structure, or number (8,19). It has recently been shown that immature tissue of foliage that is two-thirds to fully expanded is the most susceptible to both *X. c. citri* and *X. c. citrumelo* infection because of its ease of water congestion compared with mature tissue (8). Thus, although pinprick inoculation indicated little difference in susceptibility to *X. c. citri* between grapefruit cultivars vs. mandarin and trifoliate hybrids, differences in field susceptibility are easily demonstrable. Grapefruit, which is generally the most susceptible to *X. c. citri* in the field, is vigorous and has numerous large flushes that remain immature for several weeks, whereas trifoliates and mandarins (with the exception of Temple tangor), which have general field resistance, flush less frequently and less extensively and mature rapidly.

Epidemics of citrus canker occur on field-susceptible cultivars when leaf flushes coincide with storm conditions that are ideal for spread of *X. c. citri* and infection by rainwater congestion of immature tissues (19). Yet, *X. c. citri* infections on trifoliates and mandarins do occur when pruning or mechanical wounding opens the mesophyll to bacterial penetration (31). In Asia, infections caused by *X. c. citri* often occur in conjunction with leafminer damage on moderately resistant hosts, such as mandarin. The insect larva carries the bacterium on its body as it forms galleries in the leaf blade, causing numerous mesophyll infections of mature foliage normally resistant to infection (6).

The host range of the aggressive strain of *X. c. citrumelo* is quite unlike that of any of the *X. c. citri* groups because of its preference for *Poncirus* sp., its hybrids, and only a few other citrus relatives. Citrus bacterial spot is a nursery disease, and *X. c. citrumelo* populations in lesions apparently decrease under grove conditions (9). Commercially important scion cultivars are not affected by *X. c. citrumelo* in groves (15). *X. c. citrumelo* can affect the success of bud grafting by causing necrosis of the buds, but when the grafted buds have taken and the rootstock foliage is removed, it is no longer a commercial problem (16; R. E. Stall, *unpublished*). The vigor of susceptible rootstocks does not appear to be adversely affected by infection with *X. c. citrumelo*. Conversely, Asiatic citrus canker, caused by *X. c. citri* group A, is a problem in commercial groves in many parts of the world as well as in nurseries and has a very broad host range, as described above (6,24). *X. c. citri* groups B and C, which cause false canker in Argentina and Brazil, have more limited host ranges and are restricted primarily to lemon and lime hosts (15,24). The differential host ranges of *X. c. citri* and *X. c. citrumelo* based on mesophyll reactions in addition to differences in symptomatology further substantiate that although both pathovars attack some of the same citrus hosts and belong to the species *X. campestris*, the diseases caused by each are quite different.

ACKNOWLEDGMENTS

We wish to thank D. J. Hutchison, for consultation on the host range, and M. Bruce, T. Riley, T. Gouin, A. Dow, and P. Bell, for technical assistance.

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