Susceptibility of Citrus Fruit to Bacterial Spot and Citrus Canker

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Florida Agricultural Experiment Station Journal Series No. R-01864.

This research was supported by USDA-ARS AAD/IFAS Cooperative Agreement No. 58-43YK-5-3 and 58-43YK-0-0008.

We appreciate the technical assistance of A. Dow and J. Bittle in Florida and C. Hurtado in Argentina and project supervision by L. W. Timmer in Argentina.

Accepted for publication 25 November 1991 (submitted for electronic processing).

ABSTRACT

Graham, J. H., Gottwald, T. R., Riley, T. D., and Bruce, M. A. 1992. Susceptibility of citrus fruit to citrus bacterial spot and citrus canker. Phytopathology 82:452-457.

A pressurized spray (1 g/mm^2) that water-soaked the rind of citrus fruit was used to obtain infection by *Xanthomonas campestris* pv. *citri*, *X. c. citrumelo*, and other *X. campestris* pathovars capable of infecting leaves of the citrus hybrid Swingle citrumelo (*Poncirus trifoliata* × *Citrus paradisi*). An aggressive strain of *X. c. citrumelo* readily infected fruit 20–40 mm in diameter, but fruit of smaller and larger diameters were not as susceptible. Marsh White and Marsh Red grapefruit cultivars developed larger lesions over a wider range of fruit sizes compared with Hamlin and Valencia sweet orange and Orlando tangelo. After 28 days, lesions caused by *X. c. citrumelo* strains did not expand further into rind tissue. Resistance of fruit to several strains of *X. c. citrumelo* and other pathovars of *X. campestris*, both of which produced small, discrete lesions, was confirmed by the inability of these strains to multiply in the rind tissue of Marsh White grapefruit. Nearly all strains of *X. c. citrumelo* were also incapable of sustaining growth and lesion expansion in leaf tissue of Ruby Red grapefruit and Swingle citrumelo; exceptions were aggressive strains, which produced expanding lesions on Swingle citrumelo. The relationship between fruit size and infection of citrus fruit cultivars by an Asiatic strain of *X. c. citri* was similar to that for *X. c. citrumelo*. Red Blush grapefruit was more susceptible to Asiatic citrus canker than Hamlin sweet orange, whereas Capurro mandarin was resistant. Unlike lesions produced by *X. c. citrumelo*, canker lesions continued to expand up to 106 days after inoculation of fruit 20–40 mm in diameter. Lesions did not expand on fruit >60 mm in diameter.

Economic losses from Asiatic citrus canker, caused by *Xanthomonas campestris* pv. *citri*, occur when lesions mar the appearance of the fruit or cause premature fruit drop (2). The same concerns were raised for citrus bacterial spot, caused by *X. c. citrumelo* (syn. = *X. c. citri* group E), when fruit of the rootstock trifoliate orange (*Poncirus trifoliata* (L.) Raf.) was found to be infected in a field nursery in 1985 (5). The lesions of Asiatic citrus canker and citrus bacterial spot superficially resembled one another, but were distinctly different (7). Canker lesions in Florida outbreaks in 1986 on Pineapple sweet orange and grapefruit varied from raised and corky on young fruit, to deeply sunken and craterlike, surrounded by a chlorotic halo on older fruit; i.e., if lesions developed when the fruit were young, the lesions continued to expand and envelope deeper tissues in the rind. In the field, lesions of citrus bacterial spot on Flying Dragon trifoliate orange fruit were slightly raised to sunken with water-soaked margins and chlorotic halos. No corky tissue developed, and the necrosis of the fruit rind remained superficial and did not extend below the epidermal cell layers (5,7).

Canker susceptibility of fruit correlates with that of leaves of citrus cultivars (2). Grapefruit and certain sweet orange cultivars are considered highly susceptible, whereas mandarins and their hybrids are less so. No systematic rating of the susceptibility of different citrus cultivars has ever been made, so rankings are based on field observations of naturally infected fruit.

Very early studies (4,12) indicated that citrus fruit 20–50 mm in diameter (depending on the cultivar) were susceptible, but that as fruit approached full expansion, they became resistant. For Satsuma mandarin in Japan, inoculations through stomata with *X. c. citri* at 10^8 colony-forming units (cfu)/ml resulted in infection from June to the middle of August; the most susceptible period occurred from mid-June to mid-July (14). Koizumi (10) referred to lesions that developed on young Satsuma mandarin fruit as "early infection type," where extensive bacterial growth in the fruit rind caused cell collapse and necrosis followed by cell proliferation and hypertrophy. The "late infection type" was characterized by small, greenish spots, a lack of bacterial proliferation, and development of necrotic tissue.

Different methods have been used to inoculate fruit with variable results. Fulton and Bowman (4) found that needle-pricking the rind ruptured oil glands and interfered with lesion development, although wounding the rind was useful in the study of the infection process. To obtain stomatal infections without wounding, they (4) swabbed the fruit surface with bacteria, whereas Koizumi (10) used a rubber-block press method to force bacteria through the stomata. Miller et al (13) used a pressurized spray at 6.65 kg/cm^2 and inoculum densities of 10^4 or 10^5 cfu of *X. c. citri* per milliliter to obtain lesions on fruit 40–120 cm in diameter (several citrus cultivars), but no correlations were made between fruit size and lesion development.

Because of quarantine restrictions on shipment of Florida fruit to other citrus-producing states, considerable attention has been focused on whether citrus fruit are susceptible to citrus bacterial spot. The purpose of this work was to determine whether citrus fruit cultivars are susceptible to citrus bacterial spot and if there is a relationship between fruit size and susceptibility to citrus canker. Tests with *X. c. citri* on field-grown fruit could not be conducted in Florida because of quarantine prohibitions. Therefore, we used the same parameters that produced infection with *X. c. citrumelo* to inoculate fruit of similar cultivars with *X. c. citri* in Argentina where citrus canker is endemic.

MATERIALS AND METHODS

Bacterial strains and plant material. A field strain of *X. c. citri* from Argentina (Table 1) was used for fruit inoculations conducted there. From 1984 to 1989, strains of *X. c. citrumelo* were isolated from nursery outbreaks of citrus bacterial spot by

the Florida Department of Agriculture and Consumer Services, Division of Plant Industry and by us (Table 1). The identification, pathogenicity, and host range of some of these strains were reported previously (6,8). Other *X. campestris* pathovars represented strains that either elicited symptoms on Swingle citrumelo or did not (9). Bacteria were suspended in a mixture of milk and glycerin and mixed with silica gel for storage at 5 C until tests were conducted (15).

Three-year-old citrus trees of the following cultivars were transplanted in March 1988 from containers onto raised soil beds in the quarantine field research facility at the University of Florida, Hastings Agricultural Research and Education Center: Marsh White and Marsh Red grapefruit (*Citrus paradisi* Macf.) on trifoliate orange rootstock (*P. trifoliata*), Orlando tangelo (*C. paradisi* × *C. reticulata* Blanco) on trifoliate orange, and Hamlin and Valencia sweet orange (*C. sinensis* (L.) Osb.) on sour orange (*C. aurantium* L.). Eight trees of each cultivar were located in a completely randomized design. Fruit inoculations began in April 1989.

Fruit inoculations with *X. c. citrumelo*. Preliminary inoculations in 1988 indicated that consistent infection could not be obtained by nonpressurized spray of the fruit surface with high concentrations of *X. c. citrumelo* and that the fruit rind had to be water-soaked during the inoculation process. Thus, a CO_2-driven pressurized sprayer with an adjustable nozzle was calibrated to water-soak fruit 20 mm in diameter. The force of the pressurized spray was ~1 g/mm^2 with the nozzle adjusted to treat a 1-cm-diameter area on the fruit surface.

Strain F1 of *X. c. citrumelo* (Table 1) was grown on nutrient-glucose agar overnight and washed from the plate with 0.075 M Na-K phosphate buffer (pH 7.0), and the bacterial suspension was adjusted turbidimetrically to 10^4, 10^6, and 10^8 cfu/ml immediately before treatment. Inoculum concentrations were confirmed by plating dilutions of the suspension on nutrient-glucose agar. Two separate blooms of all cultivars occurred in 1989. Fruit from the initial bloom reached 15 mm in diameter in early April, except for Orlando tangelo, which was smaller (~10 mm). The second bloom was treated in May after it reached at least 30 mm in diameter. Groups of at least 40 fruit were treated biweekly from the 15- to 30-mm-diameter stage to the 50- to 70-mm-diameter stage. The treatments were 10 ml of buffer only, 10^4 cfu/ml, 10^6 cfu/ml, and 10^8 cfu/ml in a pressurized spray; a nonpressurized spray of 10^6 cfu/ml was applied with an atomizer as a control. At 14 and 28 days after inoculation, fruit were rated for lesion development as follows: 0 = no lesions, 1 = discrete lesions within the water-soaked area (~1 cm diameter), 2 = coalesced lesions within the water-soaked area and discrete lesions outside of the area, and 3 = coalesced lesions inside and outside the water-soaked area.

TABLE 1. Strains and pathovars of *Xanthomonas campestris* and their host origin

Strain ID (source)[a]	Pathovar	Host
82-1 (RES)	*alfalfae*	*Medicago sativa*
X6 (ELC)	*campestris*	*Brassica oleracea*
A1 (A)	*citri*	*Citrus paradisi*
F1 (ELC)	*citrumelo*	*Poncirus trifoliata* × *C. sinensis*
F6 (ELC)	*citrumelo*	*C. paradisi*
F100 (ELC)	*citrumelo*	*P. trifoliata* × *C. paradisi*
BHGN-5 (A)	*citrumelo*	*C. paradisi*
BHGN-60 (A)	*citrumelo*	*C. sinensis*
BRN-20 (A)	*citrumelo*	*P. trifoliata* × *C. paradisi*
LPN-1 (A)	*citrumelo*	*P. trifoliata* × *C. paradisi*
X151 (ARC)	*fici*	*Ficus benjamina*
X22J (DPI)	*maculifoliigardeniae*	*Gardenia* sp.
X45 (ATCC)	*phaseoli*	*Phaseolus vulgaris*
X198 (ARC)	Undetermined	*Strelitzia reginae*

[a]Sources of strains: A = authors; ARC = A. R. Chase, University of Florida, Apopka; ATCC = American Type Culture Collection; DPI = Division of Plant Industry, Gainesville, FL; ELC = E. L. Civerolo, USDA, Beltsville; RES = R. E. Stall, University of Florida, Gainesville.

Univariate analysis (SAS, Cary, NC) was used to establish the five size classes of fruit that were inoculated. Thus, the factorial design included three main effects: five cultivars, five inoculation treatments, and five size classes of fruit. For each size class, 10–40 fruit were rated for lesion development depending on the number of fruit that remained on the tree through the 28-day evaluation period. The significance of main effects and their interaction was examined by the general linear models (GLM) procedure. Fruit from the two blooms in 1989, treated separately, gave similar results. The results from the first bloom are reported.

Population dynamics of *X. campestris* strains in fruit and leaf tissue. In Hastings, fruit of Marsh White grapefruit were treated with a bacterial suspension of 10^8 cfu/ml in a pressurized spray when they reached ~30 mm in diameter. All strains listed in Table 1 but A1 were evaluated. Ten inoculation sites on individual fruit were sampled at 1, 14, 21, 28, 35, 56, and 98 days after treatment by excising a 10-mm-diameter area of the fruit epidermis with a cork borer. The rind tissue below the epidermis was removed, and lesioned epidermal tissue was macerated in 2 ml of Na-K phosphate buffer with a mortar and pestle. The macerated tissue was sonicated for 3 min and placed on a rotary shaker for 30 min. Serial dilutions of the macerated tissue were plated on kusagamycin-cephalexin-Bravo (KCB) selective agar (6,8). Bacterial populations were expressed as colony-forming units per square centimeter of fruit surface area. Areas under the population curve (AUPC) for each replicate inoculation were calculated with the data from the seven sampling times (GraphPad InPlot Software, San Diego, CA). The GLM procedure and Student-Newman-Keul's multiple range test were used to compare population development among strains. Lesions, when they developed, were rated as before.

Expanded, immature leaves on 3-yr-old trees of Swingle citrumelo (*P. trifoliata* × *C. paradisi*) and Ruby Red grapefruit were inoculated with the same strains used to treat fruit in the above test. Leaves were wounded by scratching the cuticle with a No. 3 insect-mounting needle, and the wound was swabbed with a cotton-tipped applicator soaked in a bacterial suspension of 10^8 cfu/ml. Ten inoculation sites on individual leaves were sampled at 1, 7, 14, 21, 28, 42, 63, and 103 days after treatment by excising a 10-mm-diameter area of the leaf with a cork borer. Leaf disks were individually ground in 1 ml of buffer, and serial dilutions of the buffer were plated on KCB medium. Bacterial populations were expressed as colony-forming units per square centimeter of leaf surface area. The AUPCs were used to compare population development among strains in leaves as described above for fruit. Lesions, when they developed, were repeatedly measured for 10 inoculation sites per strain at 20, 40, and 60 days after inoculation.

Fruit and leaves were inoculated with *X. campestris* in 1989 and 1990, with similar results of lesion rating and size each year. The 1990 results, which also included population development, are reported.

Fruit inoculations with *X. c. citri*. Field inoculations of fruit with *X. c. citri* were conducted at the experiment station of the Instituto Nacional de Técnologia Agropecúaria in Concordia, Entre Rios, Argentina. Asiatic strain A1 of *X. c. citri* was prepared for inoculation as described for *X. c. citrumelo*. In December 1989, 20 fruit each of Red Blush grapefruit, Hamlin orange, and Willow leaf mandarin (*C. reticulata*) were inoculated with a bacterial suspension of 10^6 cfu/ml in a pressurized spray as before; five size classes of fruit were used, beginning at the 30-mm-diameter stage through the 55- to 80-mm-diameter stage depending on cultivar. A pressurized spray with Na-K phosphate buffer was included as a control to detect damage to the fruit by the treatment. In 1990, 20 fruit of Red Blush grapefruit, Hamlin orange, and Capurro mandarin were treated with a bacterial suspension of 10^8 cfu/ml in a pressurized spray on five fruit size classes of fruit beginning at the 20- to 25-mm-diameter stage through the 55- to 80-mm diameter stage. Depending on the experiment, lesion development on fruit was rated at six or seven intervals up to 106 days after each inoculation. In the 1989 trial, lesions continued to expand in size; in 1990, a lesion rating of 4 was added to

account for increase in lesion size with time beyond the rating of 3. The factorial design included three cultivars, five size classes, and seven evaluations of lesion rating. Significance of main effects and their interactions was examined by the GLM procedure. The results of 1990 trial are presented because they included the revised lesion rating procedure.

RESULTS

Fruit inoculations with *X. c. citrumelo*. Initially, fruit of the five cultivars increased in size at a linear rate, and then rate of growth decreased as fruit approached full expansion (Fig. 1). Fruit of Marsh White and Marsh Red grapefruit were still expanding after 20 wk, but fruit growth of Valencia and Hamlin sweet orange and Orlando tangelo had slowed. This was expected because the grapefruit cultivars attained larger sizes than sweet oranges by the end of the season.

The pressurized spray treatment in the absence of bacteria rarely caused visible wounding of the epidermis of any of the cultivars when observed 28 days after treatment. Water-soaking by the pressurized spray treatment was variable on fruit less than 20 mm in size for all cultivars. The pressurized spray consistently produced water-soaking on fruit >20 mm diameter, although there was a tendency for water-soaking to decrease in extent with increase in fruit size.

Lesion development after inoculation with strain F1 of *X. c. citrumelo* was significantly ($P < 0.01$) affected by cultivar, inoculum density-inoculation method, and fruit size. There were highly significant ($P < 0.01$) interactions among these factors, as well. When applied in a nonpressurized spray at 10^6 cfu/ml, strain F1 did not cause water-soaking but occasionally produced infection of fruit. A few discrete lesions were observed on Hamlin and Valencia orange fruit of 20–30 mm diameter and on grapefruit and Orlando tangelo fruit of 20–40 mm diameter (Fig. 2).

Inoculum applied in a pressurized spray resulted in much more consistent lesion development and greater numbers of lesions than the nonpressurized spray. Pressurized spray of strain F1 at 10^4 cfu/ml produced few lesions on fruit in size class 1 (15–25 mm) except Valencia orange (Fig. 3A), but more consistently yielded lesions on size class 2 (26–40 mm) of all cultivars. Slight infection occurred on fruit size class 3 (41–55 mm) of all cultivars, but there were only a small number of fruit with lesions on sweet orange cultivars and Orlando tangelo above 55-mm fruit diameter. Lesions occurred on fruit of all size classes of grapefruit cultivars to 70 mm diameter.

Strain F1 at 10^6 and 10^8 cfu/ml in a pressurized spray consistently produced notable infection on fruit of all cultivars except for size classes 4 and 5 where lesions did not develop on Hamlin orange and Orlando tangelo (Fig. 3B and C). Except for Hamlin orange and Orlando tangelo, the average lesion rating on cultivars of size class 1 was less than 1. Fruit of size class 2 generally had the highest lesion ratings, but lesion development was less on larger fruit size classes. Decrease in lesion development with an increase in fruit size was more pronounced on orange than grapefruit cultivars and more apparent at 10^6 than at 10^8 cfu/ml of strain F1. Orlando tangelo responded like the oranges at the 10^6 cfu/ml inoculum level but like the grapefruit cultivars at 10^8 cfu/ml. In all treatments, lesions developed by 14 days and no further lesion expansion was observed after 28 days.

Population dynamics of *X. c. citrumelo* in fruit and leaf tissue. Inoculation of Marsh White grapefruit at the 30-mm-diameter stage with the aggressive strain F1 of *X. c. citrumelo* produced lesions that were rated ~3 in severity (Table 2). Lesions were coalesced within and outside of the water-soaked area by 14 days after inoculation. The other aggressive strain, BRN-20, and two moderately aggressive strains (F6 and BHGN-60) produced lesions that coalesced within the water-soaked area. Other *X. c. citrumelo* strains and noncitrus strains of *X. campestris* elicited small, discrete lesions within the inoculated area. Strains F100 of *X. c. citrumelo* and X6 of *X. c. campestris* did not give a reaction. Populations of all strains in the rind tissue on day 7 averaged 3.8 log cfu/cm^2, but as lesions developed at 14 and 21 days, populations decreased on the average to 1.4 log cfu/cm^2 (data not shown). None of the strains were recovered after 35 days except for strain F1, and this strain was not detected after 63 days. The AUPC was greatest for strain F1, intermediate for the other *X. c. citrumelo* strains, and lowest for noncitrus strains. The AUPC was highly correlated ($r = 0.90$, $P < 0.01$) with lesion rating when all strains were considered.

All strains except *X. c. campestris* X6 produced some necrosis on leaves of Swingle citrumelo and Ruby Red grapefruit. Noncitrus strains of *X. campestris* elicited only a slight response compared with *X. c. citrumelo* strains. In all cases, lesion expansion ceased by 20–30 days after inoculation except for the aggressive strains, F1 and BRN-20, of *X. c. citrumelo* that produced lesions on Swingle citrumelo that expanded up to 60 days (data not shown).

Populations of bacteria were higher and survived longer in leaf tissue of Swingle citrumelo and Ruby Red grapefruit than in fruit rind tissue of Marsh White grapefruit as reflected by higher AUPC values (Table 2). Populations of several strains declined to nondetectable levels by 63 days; exceptions were the aggressive strains, F1 and BRN 20, in Swingle citrumelo and grapefruit, and the moderately aggressive strains F6, BHGN-60, and BHGN-5 in grapefruit (data not shown). The AUPC was highly correlated with lesion diameter for Swingle citrumelo

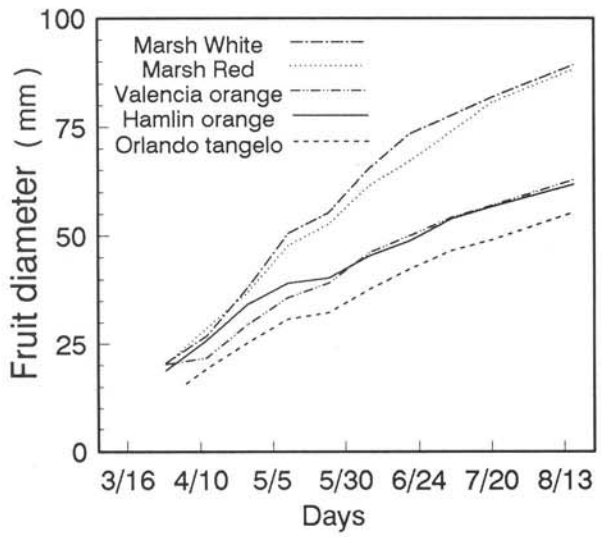

Fig. 1. Growth of fruit of citrus cultivars used for inoculation with *Xanthomonas campestris* pv. *citrumelo* in Hastings, FL, in 1989.

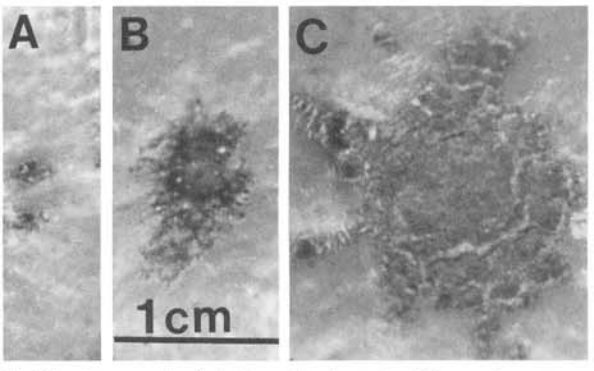

Fig. 2. Orlando tangelo fruit inoculated at the 30-mm-diameter stage with a pressurized spray (1 g/mm^2) of *Xanthomonas campestris* pv. *citrumelo* at: **A,** 10^4 cfu/ml to give a rating of 1 = discrete lesions within the water-soaked area; **B,** 10^6 cfu/ml to give a rating of 2 = coalesced lesions within the water-soaked area; and **C,** 10^8 cfu/ml to give a rating of 3 = coalesced lesions inside and outside the water-soaked area. Lesions that develop 28 days after inoculation are slightly raised, necrotic spots. The necrosis remains superficial and does not extend below the epidermal cell layers of the fruit rind.

($r = 0.92$, $P < 0.01$) and Ruby Red grapefruit leaves ($r = 0.86$, $P < 0.01$).

Fruit inoculations with *X. c. citri*. The relationship between fruit size class and rating of citrus canker lesions on Red Blush grapefruit, Hamlin orange, and Capurro mandarin was analogous to the infection of similar cultivars with the aggressive strain of *X. c. citrumelo* (Figs. 3 and 4). There were highly significant ($P < 0.01$) effects of cultivar, fruit size, and time after inoculation on lesion development. Initially, *X. c. citri* gave disease ratings after 18 days similar to those of *X. c. citrumelo*. However, canker

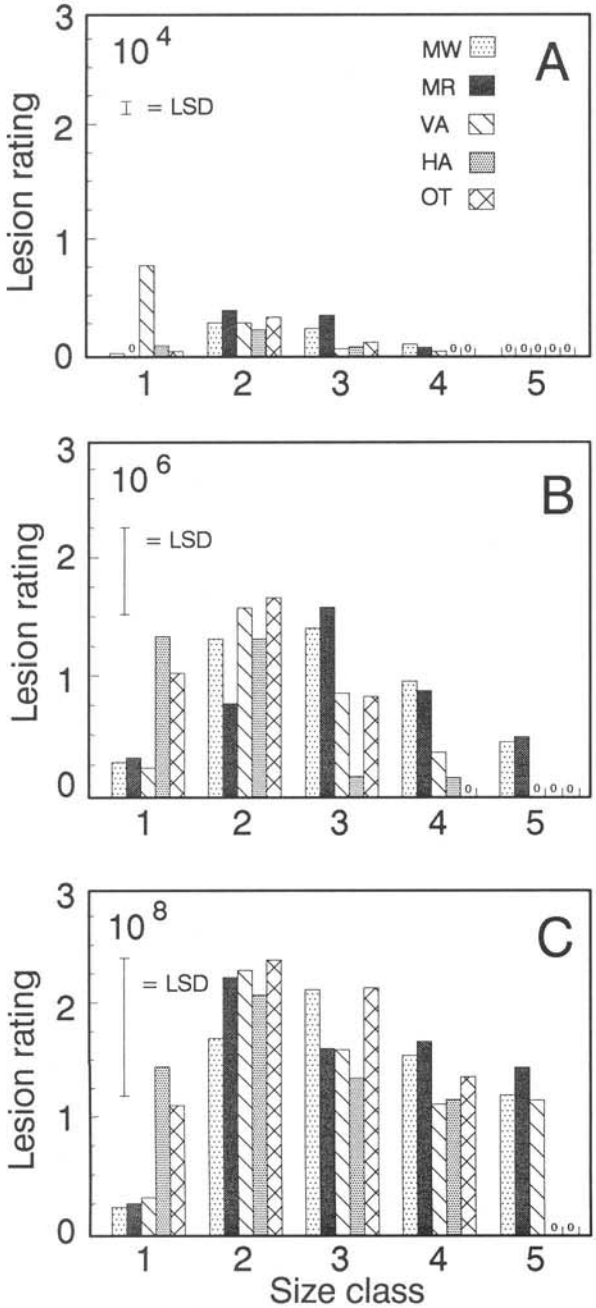

Fig. 3. Effect of fruit size on susceptibility of citrus cultivars to citrus bacterial spot 28 days after inoculation with *Xanthomonas campestris* pv. *citrumelo* at **A,** 10^4; **B,** 10^6; and **C,** 10^8 cfu/ml in Hastings, FL, in 1989. Fruit size classes were 1 = 15–25 mm, 2 = 26–40 mm, 3 = 41–55 mm, 4 = 56–70 mm, 5 = >70 mm diameter. Citrus cultivars were Marsh Red grapefruit (MR), Marsh White grapefruit (MW), Hamlin sweet orange (HA), Valencia sweet orange (VA), and Orlando tangelo (OT). Lesion ratings were: 0 = no lesions, 1 = discrete lesions within the water-soaked area, 2 = coalesced lesions within the water-soaked area, and 3 = coalesced lesions inside and outside of the water-soaked area. Ten to 40 fruit were rated per size class of each cultivar. Bars represent the LSD at $P < 0.05$.

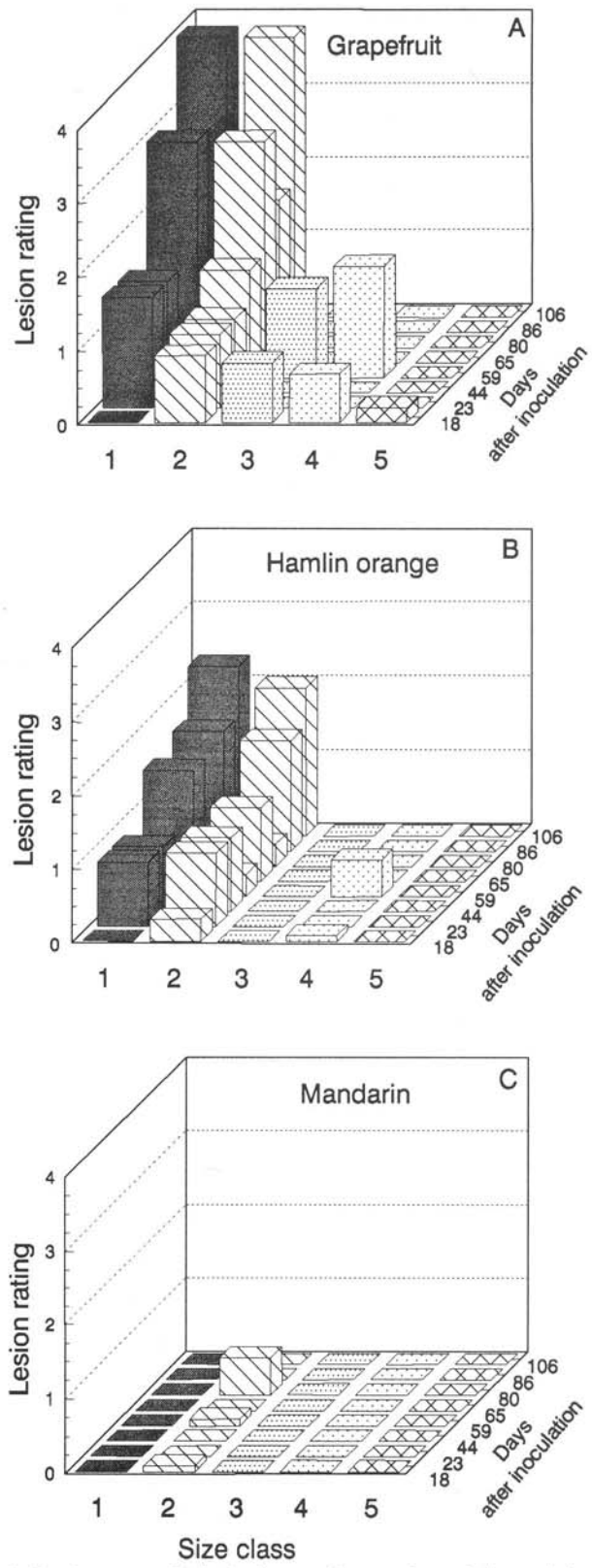

Fig. 4. Development of Asiatic citrus canker on three cultivars of citrus fruit at different stages of growth in Concordia, Argentina in 1990–91. **A,** Red Blush grapefruit; **B,** Hamlin orange, and **C,** Capurro mandarin were inoculated with 10^8 cfu/ml of *Xanthomonas campestris* pv. *citri* and lesion development was followed up to 106 days after inoculation. Fruit size classes were: 1 = 20–25 mm, 2 = 26–35 mm, 3 = 36–40 mm, 4 = 41–60 mm, 5 = > 60 mm diameter. Lesion ratings were: 0 = no lesions, 1 = discrete lesions within water-soaked (WS) area, 2 = coalesced lesions within the water-soaked area, 3 = coalesced lesions inside and outside of the water-soaked area, and 4 = expansion of lesions beyond the 3 rating with time after inoculation. Twenty fruit were rated per size class of each cultivar.

lesions continued to increase in extent due to additional necrosis in the adjacent rind tissue. Lesion rating on Red Blush grapefruit exceeded 3 by 106 days after inoculation of fruit in the lower size classes (Fig. 4A). When lesions formed on fruit of larger size classes, they did not expand as much with time. Compared by cultivars, grapefruit had lesions on all size classes of fruit, and lesion severity on the smaller size classes of fruit was consistently greater than on Hamlin orange (Fig. 4A and B). Only one Hamlin orange fruit larger than 35 mm diameter developed lesions. Lesion development was limited to the size class 2 of Capurro mandarin, and the lesions produced within the water-soaked zone were very limited in size and number (Fig. 4C).

DISCUSSION

Inoculation of citrus fruit with a suspension of an aggressive strain of *X. c. citrumelo* in a nonpressurized spray failed to consistently produce water-soaking of the rind tissue and infection of fruit. A pressurized spray reproducibly water-soaked the rind tissue and, when used with high inoculum levels, produced infection of citrus fruit with most of the *X. campestris* strains whether or not the strain originated from citrus. Thus, water-soaking of tissue was apparently a prerequisite for bacterial penetration of the epidermis and infection as demonstrated for leaves (7; T. R. Gottwald and J. H. Graham, *unpublished data*).

The *X. campestris* strains that caused lesions on fruit were previously characterized as able to grow in leaves and cause lesions on Swingle citrumelo (9). Strain X6 of *X. c. campestris*, which was previously reported to be unable to cause necrosis on leaves (9), was also unable to infect fruit. In the present study, strain X45 of *X. c. phaseoli* was found to cause necrosis on leaves and fruit, although it was not reported to do so on leaves in the previous study (9). Fruit were most readily infected by strain F1 of *X. c. citrumelo* when the diameter was 20–40 mm but fruit became resistant as they expanded further. Fruit smaller than 20 mm diameter were inconsistently infected. Strains of *X. campestris*, excluding *X. c. citri*, that elicited lesions on young citrus fruit were incapable of maintaining population growth or producing lesions that expanded with time on citrus fruit or grapefruit leaves. By comparison, the aggressive strains F1 and BR-20, which were pathogenic on Swingle citrumelo leaves (6,8), grew in leaves and induced lesion expansion for several weeks after wound inoculation. Trifoliate orange, the susceptible parent of the hybrid Swingle citrumelo, is the only cultivar for which infection of fruit in the field has been observed (5). Unfortunately, trifoliate orange and Swingle citrumelo were not sufficiently precocious such that young trees could be established under quarantine and fruit produced for inoculations in the field. Based on the relationship between susceptibility of fruit and leaves to *X. c. citri*, we would predict that fruit of these cultivars are as susceptible as their leaves to the aggressive strains of *X. c. citrumelo*.

TABLE 2. Area under the population curve[y] (AUPC) and lesion development for strains of *Xanthomonas campestris* inoculated into fruit rind tissue of Marsh White grapefruit and leaf tissue of Ruby Red grapefruit and Swingle citrumelo in Hastings, FL, in 1990

	Fruit		Leaves			
			Grapefruit		Swingle citrumelo	
Strain ID[w]	Lesion rating[x] (0–3)	AUPC	Lesion diam. (mm)[y]	AUPC	Lesion diam. (mm)	AUPC
F1	3	332 a[z]	4.4 d	476 b	5.8 a	514 a
F6	2	178 b	7.0 a	585 a	3.6 b	209 b
BHGN-60	2	148 bc	6.6 b	656 a	3.1 b	199 b
BRN-20	2	140 bc	5.0 c	635 a	6.2 a	455 a
LPN-1	1	139 bc	4.7 cd	372 c	3.0 b	218 b
BHGN-5	1	112 cd	3.1 e	446 b	2.0 c	223 b
F100	0	31 e–g	2.0 f	161 d	2.0 c	102 c
X45	1	78 de	3.1 e	165 d	0.8 d	213 b
X198	1	64 e	2.5 f	153 d	0.5 d	40 cd
X151	1	52 ef	3.0 e	66 e	0.6 d	6 d
82-1	1	39 e–g	2.5 f	17 e	0.5 d	3 d
X22J	1	34 e–g	2.5 f	197 d	0.4 d	54 cd
X6	0	3 fg	0.0 g	6 e	0.0 d	1 d
Noninoc.	0	0 g	0.0 g	0 e	0.0 d	0 d

[y] AUPC over 98 days for fruit and 103 days for leaves.
[w] Description of strains in Table 1.
[x] Lesion ratings were made 28 days after inoculation of 30-mm-diameter fruit as follows: 0 = no lesions, 1 = discrete lesions within the water-soaked area, 2 = coalesced lesions within the water-soaked area, and 3 = coalesced lesions inside and outside of the water-soaked area.
[y] Evaluated 60 days after inoculation.
[z] Values followed by unlike letters are significantly different at the $P < 0.01$ level according to Student-Newman-Keul's multiple range test.

All tested citrus fruits were susceptible to the Asiatic strain of *X. c. citri*. As previously reported (2,4,12,14), grapefruit was substantially more susceptible than sweet orange, whereas mandarin was almost completely resistant. Furthermore, the relationship between stage of fruit expansion and the development of resistance to infection was verified (4,10,12,14). Fruit of 20–40 cm diameter were much more susceptible to Asiatic citrus canker than larger size classes of fruit. In contrast to the lesions produced by *X. c. citrumelo* and noncitrus strains of *X. campestris*, which failed to expand beyond 28 days after inoculation, lesions caused by *X. c. citri* expanded up to 106 days on fruit 20–40 cm in diameter. This type of lesion development is similar to the early infection type associated with continual growth of *X. c. citri* in the fruit rind described by Koizumi (10).

The relationship between fruit size and susceptibility to infection was consistent across cultivars for *X. c. citrumelo* and *X. c. citri*. That is, lesion severity was greater on grapefruit than on sweet orange cultivars, and lesion development decreased with an increase in size of fruit for all cultivars. We believe these relationships were similar, in part, because the processes of water-soaking and bacterial ingress through stomata in the epidermis were the same for *X. c. citri* and *X. c. citrumelo*. Very young fruit (< 20 mm diameter) were not as susceptible because stomata were not yet fully open at this stage (1). On rapidly expanding fruit (20–40 mm diameter), more stomata in the fruit rind were open (1), which allowed for maximum water-soaking and bacterial ingress; hence, more extensive lesions developed. Beyond the 40-mm-diameter stage, stomata may have continued to develop but at a slower rate, commensurate with decreased rate of fruit growth (see Fig. 1). Also, waxes build up on the fruit surface at this stage of fruit development (3), which might affect the wetability, and consequently, water-soaking of the tissue. We observed that as long as the fruit was expanding, water-soaking by the pressurized spray and infection by *X. c. citrumelo* occurred.

Grapefruit remained susceptible to infection for a longer period than oranges because the fruit expanded for several weeks beyond the time when orange fruit growth slowed. Expansion stage is the most important factor affecting water-soaking, bacterial ingress, and lesion development on leaves of grapefruit (7; T. R. Gottwald and J. H. Graham, *unpublished data*). The same relationship appears to apply for fruit infection, albeit over a period of months for expansion of fruit rather than weeks for expansion of leaves. Thus, protection of fruit against *X. c. citri* with bactericides, especially grapefruit, requires repeated treatment through the summer months as fruit continue to expand (11). Protection of leaf flush for a period of weeks in the spring is a much more practical and effective approach to reduce inoculum density of *X. c. citri* and minimize subsequent exposure of fruit throughout the summer months (16).

The demonstration that commercial citrus fruit cultivars are highly resistant to development of citrus bacterial spot was, in part, responsible for the deregulation of the disease in Florida in 1990 (7). This terminated a USDA-mandated quarantine that required statewide inspections of fruit in orchards and disinfestation of fruit in packinghouses before shipment (7). To date, commercial fruit cultivars have never been found to be infected by *X. c. citrumelo* in Florida. Nevertheless, fruit shipped to markets in California and Arizona still require treatment. Based

on our findings that strains of *X. c. citrumelo* do not survive until harvest in artificially inoculated fruit, we believe treatment of fruit to eradicate the bacterium is unnecessary.

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