

Relationship of Leaf Surface Populations of Strains of *Xanthomonas campestris* pv. *citrumelo* to Development of Citrus Bacterial Spot and Persistence of Disease Symptoms

T. R. Gottwald, J. H. Graham, and S. M. Richie

Research plant pathologist, Agricultural Research Service, U.S. Department of Agriculture, 2120 Camden Rd., Orlando, FL 32803; professor, University of Florida, IFAS, Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred, FL 33850; and assistant professor, University of Central Florida, Department of Electrical Engineering, P.O. Box 25000, Orlando, FL 32816.

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ABSTRACT

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Fluctuations of leaf surface populations of *Xanthomonas campestris* pv. *citrumelo*, incidence of citrus bacterial spot, and cumulative rainfall adjusted for evapotranspiration were found to be interrelated based on analysis with the Fourier transformation. In cross-correlation analyses, recoverable leaf surface bacteria were correlated best with disease incidence that occurred 25 days later (range for $r = 0.86-0.87$; 19-32 days). Cumulative rainfall was correlated best with disease incidence that occurred 25 days later (range $r = 0.55-0.56$; 27-42 days). However, no clear relationship existed between cumulative rainfall and leaf surface bacteria. In a second experiment, rows of Swingle citrumelo seedlings in eight citrus nurseries were inoculated at one end with aggressive, moderately aggressive, or weakly aggressive strains of *X. c. citrumelo*. Rainstorms with windblown rain were simulated by spraying water at high velocity (24-32 m/s) over the inoculated plants toward the receptor plants down the rows. Leaf surface populations of *X. c. citrumelo* on receptor plants were recovered immediately after the simulated rainstorms. The initial populations were correlated ($r = 0.679-0.960$) with disease incidence assessments on these plants 21 days after the simulated rainstorm. The

slope of the bacterial deposition gradient was positively related to eventual disease development and negatively to strain aggressiveness (the steeper the gradient, the less the aggressiveness). In some of the nurseries, disease incidence decreased over time at approximately the same rate irrespective of bacterial strain aggressiveness. This decrease was due to the continued growth of plants, which added new healthy susceptible tissue; to the lack of disease development or spread; and to the disease-induced defoliation. In a third experiment, persistence of disease symptoms caused by each aggressiveness type was examined over time on Swingle citrumelo and Duncan grapefruit under simulated citrus grove conditions. Disease decrease was nearly linear. Rates (r) of disease decrease, from linearized-transformed data, were -0.0054 , -0.0061 , and -0.0067 for Swingle and -0.0067 , -0.0055 , -0.0018 for grapefruit, for aggressive, moderately aggressive, and weakly aggressive strains, respectively. Based on lack of persistence of disease symptoms (i.e., polycyclic disease development) in citrus groves, even on a susceptible host, and on the resistance of commercial scion cultivars, citrus bacterial spot in Florida should be considered a minor disease of citrus.

Xanthomonas campestris pv. *citrumelo* Gabriel (2) (syn. *X. c. citri* (Hasse) Dye group E) has been associated with over 64 outbreaks of citrus bacterial spot (CBS) in Florida since 1984 (5,6,9,11-13,24,25). Strains of *X. c. citrumelo* are genetically heterogeneous (2,3,17,19,20). These strains have been segregated into three aggressiveness categories (aggressive, moderately aggressive, and weakly aggressive) based on inoculation of attached and detached citrus leaves (6,12). Aggressive strains of *X. c. citrumelo* can also be further differentiated as a clonal group from the more heterogeneous, less aggressive strains by monoclonal antibody reaction patterns and by restriction fragment length polymorphism analysis (4,13,20).

Unlike Asiatic citrus canker, caused by *X. c. citri* group A, the compatible host-pathogen combination for CBS is restricted to only the aggressive group of strains and to cultivars with trifoliolate orange (*Poncirus trifoliata* (L.) Raf.) parentage (13,15). On Swingle citrumelo (*P. trifoliata* × *Citrus paradisi* Macfady), the aggressive strains form lesions that expand for several weeks and produce consistently high populations of bacteria in vivo and on the lesion surface in the field (1). By comparison, less aggressive strains form lesions of limited size on Swingle citrumelo and other citrus cultivars, and populations in and on lesions are variable and decline rapidly.

Based on analyses of populations associated with different host-bacterial strain interactions and of spatial and temporal data from Florida outbreaks of CBS, aggressive strains may be the only strains of *X. c. citrumelo* capable of natural spread under field conditions (1,13). In contrast, moderately aggressive and weakly aggressive strains have always been associated with mechanical dispersal (6,9,12).

Populations of *X. c. citri* and *X. c. citrumelo* decline on phylloplane surfaces, even on susceptible hosts, if they are not renewed by bacteria exuded from active lesions (1,29-31). Bacteria of both pathovars are susceptible to desiccation and xeric conditions, because these conditions cause accelerated death of bacterial populations on the leaf surface (29,30; T. R. Gottwald and J. H. Graham, unpublished). Although these pathogens may exist as casual or resident epiphytes, there is no evidence that they can act as an inoculum source for infection or play an active role in the spread of disease.

Local dispersal of *X. c. citri* from lesions between nursery plants has been associated with rain splash, whereas longer distance dispersal, such as between grove trees, has been associated with blowing rainstorms (8,10). Infection of citrus foliage by rain-splashed inoculum from citrus canker lesions required water-soaking of the foliage by wind speeds ≥ 8.0 m/s and inoculum concentrations of $\geq 10^5-10^6$ cfu/ml (26-28). In field, greenhouse, and growth chamber tests, strains of *X. c. citrumelo* often failed to produce disease when applied as a spray. Inoculum concentrations of about 10^6 cfu/ml combined with water-soaking of tissues by a pressurized sprayer were required for the consistent occurrence of infection (13).

The purpose of the present study was to determine if temporal relationships exist among rainfall, the dynamics of leaf surface bacteria, and eventual disease development in a citrus nursery situation; to examine deposition gradients of *X. c. citrumelo* strains of different aggressiveness due to isolated blowing rain-storm events and the relationship of the resulting leaf surface populations to eventual disease incidence; and to examine the persistence of CBS lesions when diseased plants were transplanted from citrus nursery to grove conditions.

MATERIALS AND METHODS

All field studies were conducted at the University of Florida, Agricultural Research and Education Center, in Hastings, Florida. At this location, remote from commercial citrus, a 5.7-ha quarantine field site was established for research with CBS (13).

Comparison of leaf swab vs. leaf wash assays. Because individual young citrus seedlings were assayed repeatedly for leaf surface bacterial populations, a nondestructive method for estimating surface populations was required to avoid defoliating entire plants and removing potentially epidemiologically important inoculum. The accuracy of the leaf swab technique for estimating leaf surface bacterial populations was compared with the traditional leaf wash technique. An aqueous suspension of *X. c. citrumelo* strain F1 was adjusted spectrophotometrically to 10^8 cfu/ml and sprayed with a handheld tank sprayer until runoff onto 20-cm-tall Swingle citrumelo seedlings in a citrus nursery plot during the late afternoon. The leaf surface populations of bacteria from individual trees were sampled by both leaf swab and leaf wash techniques at four different times: early morning when dew was still on the plants; immediately after dew had dried; 8 h after dew had dried; and early morning of the second day, 24 h after the first sampling. For each plant assayed, a cotton swab was moistened with 0.075 M phosphate buffer (pH 7.2, containing 3.5 g of KH_2PO_4 and 5.8 g of Na_2HPO_4 per liter). The entire surfaces of 10 leaves from each plant were swabbed. Leaf surface bacteria were assayed by similar swab samples collected from each of 20 plants for each treatment. The cotton tip was placed in a vial containing 5 ml of phosphate buffer and maintained at 5 C until plating. For leaf washes, 10 leaves were harvested from each of 20 plants and placed in a 125-ml flask containing 20 ml of phosphate buffer and stored at 5 C. Within 24 h, vials containing swabs and flasks containing leaves were sonicated for 3 min to dislodge the bacteria; they were then shaken for 30 min. The resulting suspension was plated in a dilution series on semiselective KCB medium consisting of 23 g of nutrient agar, 16 mg of kasugamycin, 35 mg of cephalixin, and 12 mg of chlorothalonil (Bravo 720, Ferment ASC Co., Mentor, OH) per liter as previously described (22). Plates were incubated for 3–5 days at 27 C. The resulting leaf surface populations were reported as colony-forming units per square centimeter of leaf surface area. The average leaf surface area of 10 typical Swingle citrumelo leaves that varied little in size on nursery plants with adequate moisture and nutrition (about 97.2 ± 1.1 cm², leaf area \pm SD) was used. The average was determined with a leaf area meter (Model LI-31000, Li-Cor, Inc. Lincoln, NE). Leaf surface bacterial populations as detected by leaf swab and leaf wash techniques were compared by Student's *t* test.

Initial study of the relationship between leaf surface bacterial population dynamics and disease incidence in a nursery. This experiment was conducted during 1987–1988 in a single plot consisting of 11 rows of 96 plants per row of 1-yr-old Swingle citrumelo seedlings. The source plant in the middle row was inoculated, while still in the greenhouse, with strain F1 (Florida Department of Agriculture and Consumer Services, Division of Plant Industry [DPI] X84-3048) of *X. c. citrumelo* by atomizing the foliage with a suspension containing $\sim 10^8$ cfu/ml. The plant was covered with a plastic bag for ~ 48 h in a disease-containment greenhouse, then was uncovered and allowed to develop symptoms for ~ 30 days. The source plant was transplanted to the field plot and served as the point source in the late spring of 1987. Disease and leaf surface populations of strain F1 in proximity

to the source plant were assessed biweekly for 15 mo. To identify contamination by strains other than F1 in the plots, individual colonies were inoculated periodically from leaf assays into greenhouse plants to test for pathogenicity. On each sampling date, all plants in the nursery were assessed for disease incidence, defined here as the number of symptomatic leaves divided by the total number of leaves per plant.

Leaf surface populations of plants surrounding the central source plant were assayed with the leaf swab technique. Foliage was swabbed when wet from dew or rain. As the bacteria spread away from the focus, the number of assay plants was increased such that the area assayed always exceeded the area where bacteria were detected previously by 10–20 plants. Pathogenicity of field-isolated bacteria was checked periodically by selecting individual colonies from the semiselective media, suspending the bacteria in sterile distilled water, adjusting the suspension to about 10^8 cfu/ml, and inoculating surface-sterilized detached citrus leaves as previously described (12).

Bacterial dispersal and disease development. Five and three citrus nursery plots were established in 1989 and 1990, respectively; each plot consisted of nine rows of Swingle citrumelo with 65 plants per row. Plant spacing was 1 m between rows and 23 cm between plants within the row; rows were oriented east-west in the general direction of the prevailing winds. The first six plants at the west end of each row were inoculated with either strain F1 (DPI X84-3048), F6 (DPI X84-3401), or F100 (DPI X85-12689), which represented aggressive, moderately aggressive, and weakly aggressive strains of *X. c. citrumelo*, respectively (6,12). Source plants were inoculated by mixing a bacterial suspension of 10^8 cfu/ml of the required strain (adjusted spectrophotometrically) with 0.5–1.0 g of Carborundum per liter of inoculum and atomizing the suspension onto the foliage of plants until runoff. The moistened plants then were rubbed with a leather-gloved hand to cause small wounds. Disease symptoms were allowed to develop for 30 days.

For the simulation of rainstorms with high winds, a mistblower (Hardi Mini/max Model DK2600, Handi Glastrup, Inc.), with all the nozzles adjusted horizontally, was driven from south to north along the west end of each plot 30 days after inoculation. The mistblower discharged nonchlorinated, well water. Tractor speed was adjusted so that the spray impacted each nursery row for ~ 60 s as it blew across the diseased source trees toward the nondiseased trees. Wind speeds were measured within the rows at varying distances from the sprayer with a thermoanemometer (Alnor Model 8525, Alnor Instrument Co., Skokie, IL).

Disease assessments and assays of leaf surface bacteria. Assays for leaf surface bacteria were conducted immediately, as well as 20, 40, and 80 days after the simulated blowing rainstorms in 1989 and 21, 42, 63, and 84 days after the simulated blowing rainstorms in 1990. The third inoculum source plant and plants at 0.2, 0.9, 2.0, 3.7, 6.6, 10.0, and 13.5 m from the sixth inoculum source plant, in rows 3, 5, and 7, were sampled by the swab method. Assessments of disease incidence were made on all plants in each plot on 0, 20, 40, and 80 days after the simulated rainstorms in 1989 and 0, 21, 42, 63, and 84 days after the simulated rainstorms in 1990.

Calculations of bacterial dispersal gradients and disease gradients. For the 1989 and 1990 nurseries, where blowing rainstorms were simulated, subsets from each plot were utilized to calculate disease gradients and bacterial dispersal gradients. These subsets consisted of only those plants between the inoculum source and the farthest plant on which either leaf surface populations were detected or symptoms eventually developed. Disease gradients were calculated via a modified Gregory model as $\ln(\text{disease incidence} + 0.01)$ vs. $\ln(\text{distance in meters from the inoculum source})$ (18). Bacterial dispersal gradients were calculated as $\ln(\text{colony-forming units per square centimeter of leaf surface area})$ vs. $\ln(\text{distance in meters from the inoculum source})$.

Dynamics of disease incidence, leaf surface bacterial population, and rainfall. Average disease incidence and average leaf surface bacterial population curves were compared directly. The cumu-

lative amount of rain was adjusted by subtracting an arbitrary "decay" factor of 0.25 cm per day to account for evaporation (the amount is arbitrary because evapotranspiration was not measured at the site, but it is realistic for this area of Florida) and to more accurately reflect the amount of available moisture. The adjusted cumulative rain was then compared to average disease incidence and average leaf surface bacterial population to determine if any relationship existed among these factors.

Relationships among these parameters were compared with an integral transformation known as the Fourier transform and by using the SAWCAD computer program (Surface Acoustic Wave Computer Aided Design, Solid State Devices and Systems Laboratory, Department of Electrical Engineering, University of Central Florida, Orlando). The transform equation takes the form:

$$S(f) = \int_{-\infty}^{\infty} S(t)e^{-j2\pi ft} dt$$

in which t = time (in days), S = frequency (1/days), and $j = \sqrt{-1}$ (a constant from complex number theory). The Fourier transform or Fourier integral is an arbitrary integral that maps time (t) to the frequency (f) domain and is commonly used to analyze electronic, acoustic, and other types of signals. With this analytical technique, an arbitrary set of temporal data or a time domain function may be expressed in terms of a set of complex unchanging coefficients multiplied by a set of known functions of time. The function that results from the Fourier transform calculation is continuous and represents a "signature" of the signal (= data set). For the Fourier transform, the set of known functions is the complex exponential function with a continuous range of periods. This set was chosen, because the complex exponential function was always a solution to a system described by a linear differential equation with constant coefficients. When two sets of data are transformed and normalized, and the magnitude of the coefficients are compared, a measure of the similarity of the two data sets is obtained by comparing their signatures (23,32).

The temporal relationships among cumulative rainfall, populations of leaf surface bacteria, and disease incidence were examined by "cross-correlation" analysis of the 395-day data set. To accomplish this, a computer program was written in the "C" language. The program interpolated values between temporal sampling points by assuming a linear continuum between points. The time scale of one of the data sets to be correlated was adjusted by offsetting the time scale by -394, -393, -392 . . . +392, +394 days, whereas the time scale of the second data set to be correlated was left unadjusted. Thus, all possible temporal offsets were analyzed by correlation. The results were plotted graphically, and the temporal offset with the highest positive correlation coefficient (peak of the plot) was used to estimate the temporal lag between the two data sets being compared.

Change in disease incidence and persistence of symptoms in new groves. A new grove plot (plot 19), with three rows of Duncan grapefruit and three rows of Swingle citrumelo planted on a 4.4- × 5.9-m spacing with nine trees or replications per row, was established. The first six trees in each row were inoculated with F1, F6, or F100 by the Carborundum method described above. Visual assessments of disease incidence were made 19, 54, 96, and 124 days post-inoculation.

Two additional grove plots, one of Red Grapefruit (plot 16) and one of Pineapple Sweet Orange, *Citrus sinensis* (L.) Osbeck (plot 17), were established. Each plot consisted of 11 rows of 11, 2-yr-old trees on a 4.4- × 5.9-m spacing. The center tree (row 5, tree 5) in each plot was inoculated with aggressive isolate F1 as above, and all trees were assayed for CBS disease incidence on several occasions over two growing seasons.

RESULTS

Comparison of leaf swab vs. leaf wash assays. Student's t test analysis of populations of bacteria, detected by the leaf swab and the leaf wash techniques, indicated no significant difference between the two assays in estimating surface populations (Table 1). Both techniques lead to the recovery of nearly the same mean

population levels, and standard errors of the means were of similar magnitude.

Initial nursery disease spread study. Little disease occurred during the first season; however, leaf surface bacteria were detected on plants immediately surrounding the inoculum focus by early spring of the second season, about 180 days post-inoculation. No nonpathogenic xanthomonads were recovered by leaf swabbing or were confirmed by detached leaf inoculations. Surface populations and disease were detected up to 2.28 and 1.98 m from the inoculum source, respectively. However, fewer plants with leaf surface populations of *X. c. citrumelo* than plants that eventually developed disease symptoms were detected.

Curves of average disease incidence and average recovered leaf surface population level of *X. c. citrumelo* appeared to be related (Fig. 1B). When the data from the progress of disease incidence of surface bacterial populations and adjusted cumulative rainfall were normalized to adjust for amplitude, the Fourier signature curves were similar in general shape and trends (Fig. 1C). Increases and decreases in bacterial populations on leaf surfaces were followed by the same general trends in disease incidence a few days later. Bacterial populations and disease incidence increased substantially in early spring of the second season (180-225 days post-inoculation). This period corresponded to several spring rain showers. Leaf surface populations and disease incidence decreased somewhat during midsummer, then increased again in the fall (Fig. 1A,B).

Signature curves of Fourier-transformed data of disease incidence, surface bacterial populations, and cumulative rain aligned well and were of similar magnitude (Fig. 1C). The peaks nearer the origin indicate curve characteristics that occurred less frequently and over longer time periods, whereas those further removed from the origin, on the X-axis, occurred with greater frequency and over shorter time periods. The amplitude of the peaks indicates the relative influence of the data curve trend to the overall shape of the resulting signature curve. When the duration of time over which the epidemic was monitored is considered, the less frequent characteristics of higher magnitude in the data curves (such as those represented by the first two to three peaks from the origin of the signature curve) contribute the most influence to the dynamics of the data curves. Thus, the first two to three peaks of the signature curves represent the major upward and downward trends in all three data sets. Fourier-transformed curves for surface bacterial populations and disease incidence were similar in amplitude and phase for the first two peaks, indicating a commonality in the major trends of the two curves. Cumulative rainfall also aligned for the first two peaks; however, the second and third peaks were considerably lower in amplitude.

Once the similarity among the dynamics of surface bacterial populations, disease incidence, and rainfall had been established,

TABLE 1. Paired Student's t test comparison of washing and swabbing methods for recovering *Xanthomonas campestris* pv. *citrumelo* leaf surface bacteria^a

Treatment	Log cfu/cm ² of leaf surface area		t Statistic
	Swab ± SE	Wash ± SE	
1	0.0602 ± 0.0044	0.0616 ± 0.0068	-0.1754
2	0.0441 ± 0.172	0.0449 ± 0.270	-0.2222
3	0.0385 ± 0.0033	0.0362 ± 0.0020	0.6036
4	0.0509 ± 0.005	0.0407 ± 0.0068	1.1683

^aPlants were spray-inoculated with 10⁸ cfu/ml of bacteria until run-off in late afternoon and were allowed to dry. Ten leaves were collected from each of 20 plants for each treatment. Leaves were washed in 0.075 M phosphate buffer and dilution-plated. From each of these plants, 10 leaves were swabbed, and the swabbed sample was dilution-plated. Treatments were as follows: sampled the following morning when dew was still on plants; sampled the following morning 1 h after dew had dried; sampled the following afternoon 6 h after dew had dried; and sampled the second morning when dew was still on plants. The t statistics for 19 df are 2.093 and 2.861 for $\alpha = 0.05$ and 0.01, respectively.

the correlation of the curves at a range of temporal offsets was examined to determine the temporal difference between each pair of curves. In cross-correlation analyses, the highest correlation coefficients occurred with very little difference among the coefficients over a range of dates (Fig. 1D). The highest correlation between recoverable leaf surface bacteria and disease incidence occurred when disease assessments were correlated with surface bacterial populations recovered 25 days earlier (range for $r = 0.86-0.87$; 19–32 days). Cumulative rainfall was best correlated

with disease incidence that occurred 25 days later (range for $r = 0.55-0.56$; 27–42 days). The relationship between cumulative rainfall and leaf surface bacteria was less clear. The highest correlation of cumulative rainfall was with leaf surface bacteria that occurred 26 days before rainfall ($r = 0.67$). The more pertinent correlation was cumulative rainfall with leaf surface bacteria that occurred more than 100 days after rain events.

Simulation of blowing rainstorm events. Wind speeds created by the mistblower diminished rapidly for the first 0.5 m, then gradually became undetectable with a thermoanometer at ~6 m (Fig. 2). In general, leaf surface populations of *X. c. citrumelo* and plants that eventually became diseased were restricted to the first 1.8 m from the inoculated edge of the nursery. However, diseased plants occasionally developed as far as 4.1 m from the nursery edge for the 1989 and 1990 plots, respectively. Wind speeds at 1.8 and 4.1 m from the source were ~9.5 and 5.0 m/s, respectively (Fig. 2). Leaf surface populations of bacteria detected immediately after the spread event were generally well-correlated with eventual levels of disease incidence for both the 1989 and 1990 nursery plots. Correlations were occasionally better for earlier dates of disease assessment than for later dates for the 1989 plots, but were more uniform across time for the 1990 plots (Table 2).

Gradients of disease that resulted from blowing mist events in 1989 nursery plots tended to flatten slightly over time after the rain events for both aggressive F1 and moderately aggressive F6 strains (Fig. 3A,B,D,E). Gradients did not flatten as much in control plot 10, which did not receive blowing mist, and in plot 7, where blowing mist was applied to plants inoculated with the moderately aggressive strain F6 (Fig. 3B,D). No disease developed for the weakly aggressive isolate F100 beyond the inoculated source plants, even with the mistblower treatment in the 1989 nursery plots (Fig. 3C). Disease decreased after inoculation in all 1989 nurseries at similar rates over time, whether or not the nursery was exposed to blowing mist (Fig. 3K–O). Slightly higher levels of disease occurred on plants that were assayed for surface bacterial populations by the leaf swab technique than on unsampled plants.

When the experiment was repeated in 1990, CBS disease gradients that resulted from mistblower events did not change over time for either aggressive F1 or moderately aggressive F6 strains (Fig. 4A,B). As in the 1989 tests, no disease gradient was detected in the weakly aggressive F100 plot (Fig. 4C). In the 1990 nursery plots, disease incidence did not decrease over time as it did in 1989, but rather increased slightly (Fig. 4G–I). As in the 1989 plots, slightly higher levels of disease were recorded on plants assayed by the leaf swab technique.

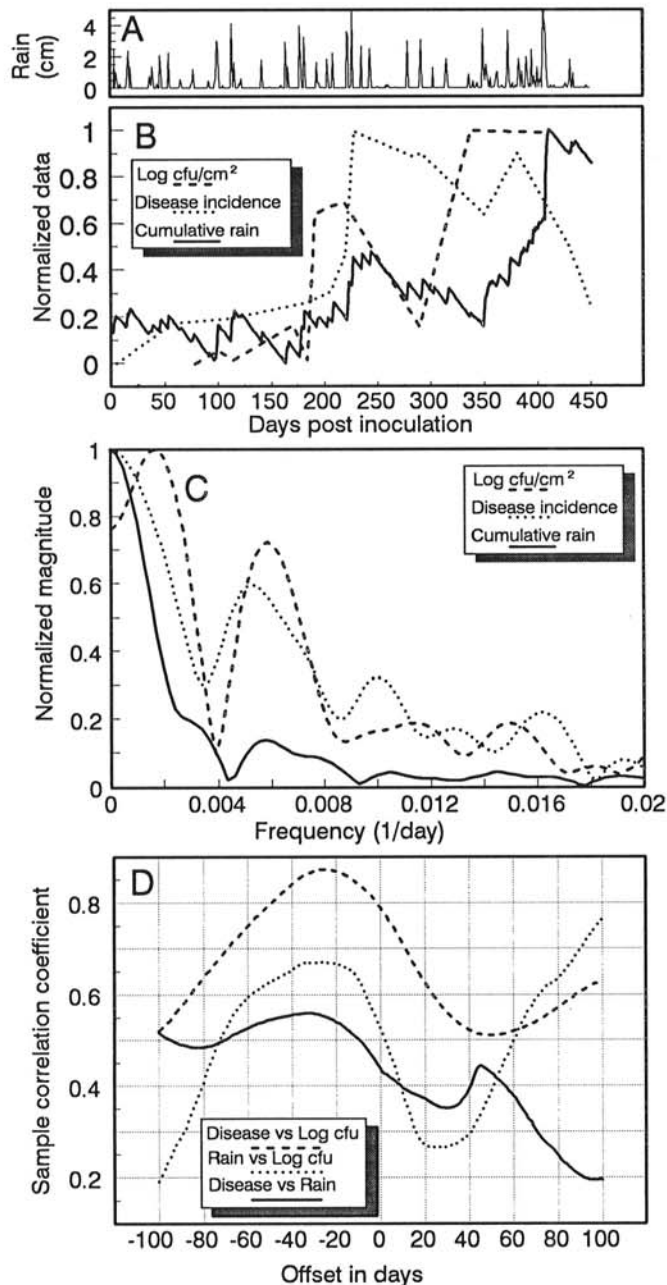


Fig. 1. The relationship between average leaf surface population concentration of *Xanthomonas campestris* pv. *citrumelo* and average disease incidence of citrus bacterial spot (measured as the number of leaves with symptoms/total number of leaves per plant) over time in a Swingle citrumelo citrus nursery. **A**, Rainfall in plot at Hastings, Florida. **B**, Normalized leaf surface population (log colony-forming units per square centimeter), disease incidence, and cumulative rainfall with a -0.25 cm per day adjustment to account for evapotranspiration. **C**, Fourier-transformed data: peaks at the same point on the X-axis indicate similar trends of the curves. Peaks of similar magnitude indicate common influences. **D**, Results of cross-correlation analysis of all combinations of leaf surface bacterial populations, disease incidence, and cumulative rainfall adjusted for evapotranspiration.

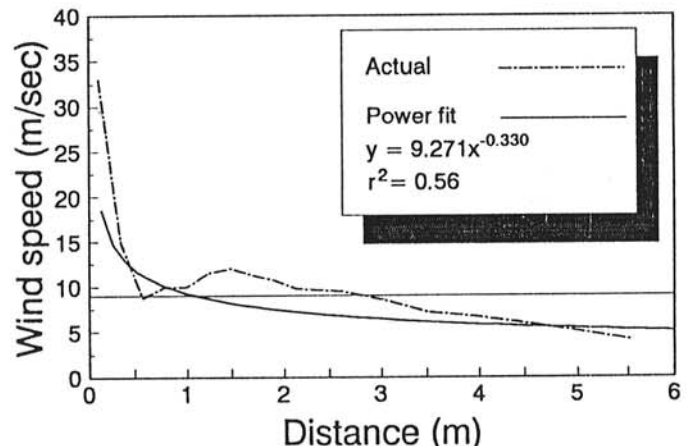


Fig. 2. Wind speeds generated at different distances from a mistblower driven down citrus nursery rows. The mistblower was utilized to simulate a blowing rainstorm. The horizontal line at 8 m/s represents the reported threshold for successful windblown infection of citrus foliage with citrus canker bacteria.

Bacterial deposition gradients established after the initial spread event were directly related to aggressiveness of the strain of *X. c. citrumelo*; the more aggressive the strain, the flatter the gradient. Simulations of blowing rain by a mistblower created similar gradients in 1989 plots 6 and 14 and 1990 plot 6, where the aggressive strain F1 was tested (Figs. 3F,J; 4D). The same strain was associated with a very steep deposition gradient of bacteria in control plot 10, in which no blowing mist was utilized (Fig. 3I). For both years, the deposition gradients of recoverable bacteria associated with the moderately aggressive strain F6 were steeper than those associated with F1 (Figs. 3F,G; 4D,E). In 1989, bacteria were detected for the weakly aggressive strain F100

beyond the inoculated source plants (Fig. 3H), but a very steep deposition gradient was detected in the 1990 F100 plot (Fig. 4F).

Disease spread and persistence in new groves. In plot 19, disease incidence decreased in both Swingle citrumelo and grapefruit plants regardless of strain aggressiveness (Fig. 5). Although all inoculations were made at approximately the same bacterial concentration of *X. c. citrumelo*, aggressive (F1) and moderately aggressive (F6) strains initially resulted in considerably higher levels of disease incidence than the weakly aggressive strain (F100) did. Decrease in disease incidence was nearly linear for all strains, regardless of cultivar. Low levels of disease for all isolates in both cultivars were reached by late fall (6 September, day 124).

TABLE 2. Correlation of leaf surface populations of strains of *Xanthomonas campestris* pv. *citrumelo*, which had different levels of aggressiveness and were recovered immediately after a simulated blowing rainstorm, with subsequent disease incidence^a

Nursery plot	Strain ^b	Correlation coefficients for nurseries in 1989				Correlation coefficients for nurseries in 1990				
		1 (6/21) ^c	20 (7/11)	40 (8/1)	76 (9/6)	1 (7/24)	21 (8/14)	42 (9/4)	63 (9/25)	84 (10/16)
6	F1	0.928	0.853	0.880	0.823	0.813	0.836	0.841	0.850	0.866
7	F6	0.921	0.798	0.818	0.798	0.849	0.856	0.841	0.857	0.829
8	F100	0.854	0.828	0.828	0.827	0.773
10	F1	0.888	0.941	0.960	0.768					
14	F1	0.836	0.885	0.679	0.733					

^aAll nursery plots except plot 10 were subjected to mistblower events in an attempt to disseminate bacterial inoculum from diseased plants on the edge of the plot. Plot 10 served as a nonevent control to estimate bacteria dissemination in the absence of simulated windblown rain.

^bF1 = aggressive strain, F6 = moderately aggressive strain, and F100 = weakly aggressive strain of *X. c. pv. citrumelo*.

^cDays postevent (date).

^dNo leaf surface bacteria detected and no disease symptoms developed.

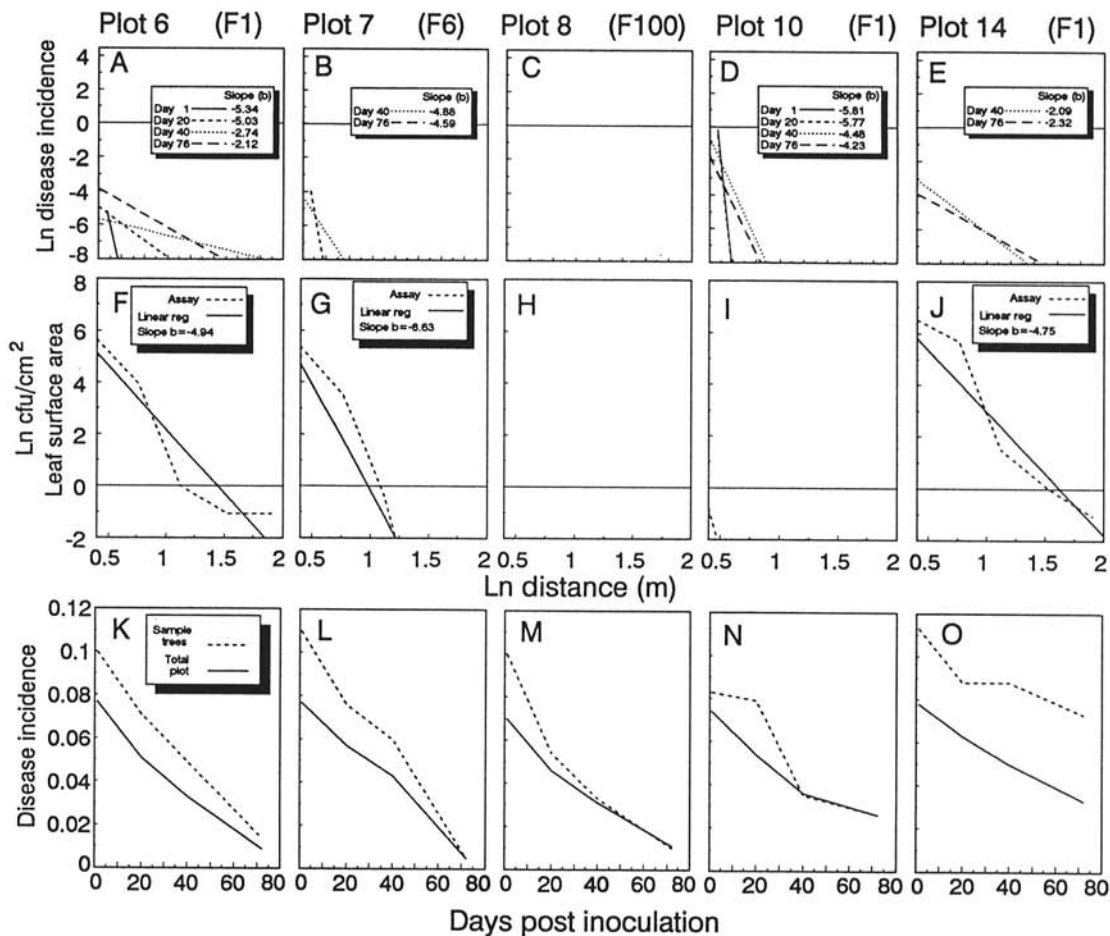


Fig. 3. A-E, Disease incidence gradients (percentage of leaves infected per plant) and F-J, leaf surface bacterial gradients over distance from an inoculum source in a 1989 simulated blowing rainstorm experiment. K-O, Disease incidence in each of the plots over time is given as the average disease incidence of either the total plot (solid line) or the predetermined sample trees (dashed line). The rainstorm was simulated with a mistblower. Plots 6, 7, and 8 were treated with the mistblower. Plot 14 was a replicate of plot 6 and was also treated with the mistblower. Plot 10 was an untreated check (i.e., no mistblower treatment). Plots were inoculated with either aggressive (F1), moderately aggressive (F6), or weakly aggressive (F100) strains of *Xanthomonas campestris* pv. *citrumelo*, the citrus bacterial spot pathogen (indicated at the top of each column of graphs).

Rates (r) of linearized disease decrease for F1, F6, and F100 were -0.0054 , -0.0061 , and -0.0021 for Swingle citrumelo and -0.0067 , -0.0055 , and -0.0018 for grapefruit, respectively. For plots 16 and 17, disease did not spread from the central inoculated tree to any of the receptor trees in either plot. By the end of the second season, disease on the inoculated trees had diminished to undetectable levels.

DISCUSSION

The Fourier transformation was extremely useful for comparison of the components that make up the curves of average disease incidence, average surface bacterial population, and adjusted cumulative rainfall and helped to confirm that the perceived visual similarities among the curves were real. Major peaks and valleys of all three transformed curves, which represent major trends in the nontransformed curves, occurred at similar locations along the ordinate (at close to the same frequency, indicating that the same physical and biological phenomena that contributed to the dynamics of one curve, likely contributed to the others as well). Thus, the factors that influence the behavior of the data or the data itself are composed of trends (constituent functions) with similar occurrences (periods). Therefore, the curves were considered to be similar and most probably influenced by many of the same contributing factors (23,32). For the signature curves of transformed data shown, a close association existed between

leaf surface bacterial population dynamics and subsequent disease development. Because the transformed cumulative rainfall curve and the other two curves, especially the surface bacterial population curve, were similar, rainfall was likely a major contributing factor to the dynamics of both surface bacterial populations and eventual disease incidence. Another way to interpret the transformed curves is that if rates of change of the curves are similar, they will have similar position of peaks on the X-axis (frequency axis). The more similar the original curves are, the more equivalent the amplitude of the corresponding peaks of the Fourier transformed curves. Thus, from the analysis of Fourier-transformed data, cumulative rainfall was closely associated with the dynamics of both leaf surface populations of *X. c. citrumelo* and disease incidence.

Once commonality of the shapes, and hence the dynamics, of the data curves was established by the Fourier transformation without reference to temporal position, the temporal offset of one data curve to another was examined by cross-correlation analysis. The relationship between disease incidence and cumulative rainfall was further substantiated by the cross-correlation analysis. Disease incidence was best correlated throughout the epidemic with cumulative rainfall levels that occurred about 33 days before. Cumulative rainfall was associated with disease dynamics, as would be expected with a rain splash disseminated pathogen. For instance, an upward trend in cumulative rainfall from days 175 to 225 preceded an increase in disease incidence from days 200 to 225 post-inoculation. However, the highest correlation between recoverable leaf surface bacteria and adjusted cumulative rainfall occurred when surface populations followed

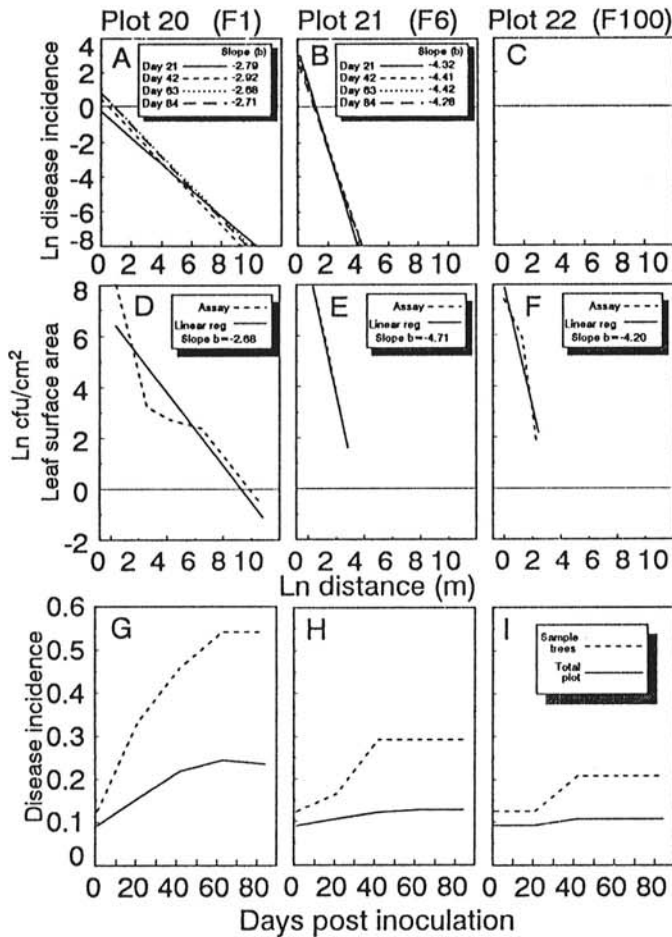


Fig. 4. A-C, Disease incidence gradients (percentage of leaves infected per plant) and D-F, leaf surface bacterial gradients over distance from an inoculum source in a 1990 simulated blowing rainstorm experiment. G-I, Disease incidence in each of the plots over time is given as the average disease incidence of either the total plot (solid line) or the predetermined sample trees (dashed line). The rainstorm was simulated with a mistblower. Plots were inoculated with aggressive (F1), moderately aggressive (F6), or weakly aggressive (F100) strains of *Xanthomonas campestris* pv. *citrumelo*, the citrus bacterial spot pathogen (indicated at the top of each column of graphs).

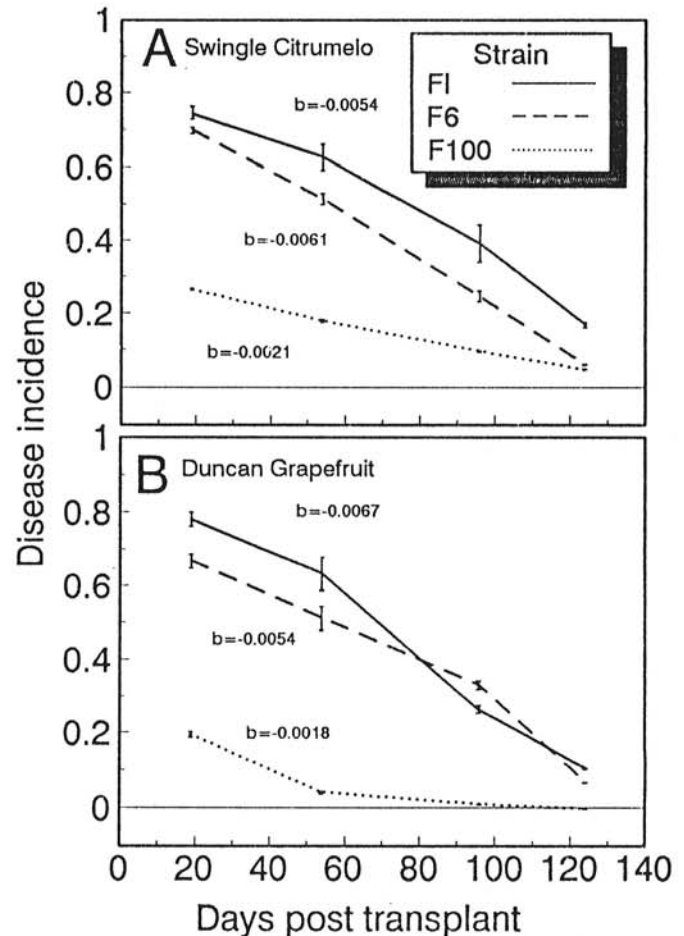


Fig. 5. Average disease decrease of citrus bacterial spot when infected nursery plants are transplanted to a grove configuration. Plants were infected in the nursery with either aggressive (F1), moderately aggressive (F6), or weakly aggressive (F100) strains of *Xanthomonas campestris* pv. *citrumelo*. All curves were nearly linear. Slopes (b) for linear regressions of each curve are presented nearest to the line indicated.

rainfall by more than 100 days. Because rainfall should have a more immediate effect on surface bacteria, such as by causing lesions to exude bacteria, promoting growth of surface bacteria, or washing off bacteria, this statistical relationship is at best questionable. In fact, the major upward trends in cumulative rainfall during days 200–225 and 350–407 corresponded to a leveling off of surface bacterial populations. One explanation might be that heavy rainfall may actually have stabilized the resident surface populations during these periods by diluting or washing off surface bacteria at nearly the same rate that bacteria were increasing or that additional bacteria were being deposited. Rainfall could also have altered the amount of nutritionally beneficial leachates on the leaf surface or prompted competitive microbes. Upward trends in surface bacteria were observed when rainfall was more moderate or intermittent. Thus, although moisture obviously affects the survival and dynamics of leaf surface bacterial populations as indicated by the Fourier analysis results, adjusted cumulative rainfall was not as related to the dynamics of surface bacterial populations as it was to disease progress as indicated by cross-correlation analysis. Although all three curves were similar, a biologically sound correlation of temporal offset between cumulative rainfall and surface bacterial populations was not apparent. The lack of correlation may be due to the more erratic nature within the cumulative rainfall data over time.

In the initial study of pathogen spread in the nursery, disease incidence was most highly correlated with bacterial populations 25 days earlier (i.e., fluctuations in surface populations preceded fluctuations in disease incidence by ~25 days). Fluctuations in disease incidence were preceded by similar fluctuations of cumulative rainfall by ~33 days. The relationship of leaf surface bacteria to disease development is uncertain. Higher surface populations often preceded disease development and could have directly contributed to disease. Alternatively, leaf surface populations recovered in this study were low ($\leq 10^3$ cfu/cm²) and may not have contributed directly to disease. Such low numbers of surface bacteria have been shown to contribute little to disease for other bacterial pathosystems (21). If this second interpretation was the case, then leaf surface bacteria were either exuded and splashed from existing lesions on nearby plants or were residual populations that were indicative of fluctuations of relatively short-lived inoculum. In this experiment, there were numerous plants that developed disease but from which no surface bacteria were detected. This is likely due to the short-lived nature of *X. c. citrumelo* on leaf surfaces (30; and J. H. Graham and T. R. Gottwald, unpublished) and/or to levels of surface bacteria below the detection threshold of 10^3 of the leaf swab assay. Plants, on which leaf surface populations of *X. c. citrumelo* were recovered, were occasionally noted, but the plants never developed disease. Thus, surface bacteria may not necessarily contribute directly to disease but can exist as casual leaf surface inhabitants (21).

Although the conclusion would seem intuitive, this is the first time that dissemination of populations of *X. c. citrumelo* and disease development have been shown to be related to both rainfall (preliminary nursery pathogen spread study) and windblown rain (nurseries with simulated windblown rain events via mistblower). Wind speeds in excess of 8 m/s are reported to be necessary for infection of citrus foliage by *X. c. citri* (26,27). Disease development of CBS at 1.8 m from the inoculum source (the front of the disease gradient) corresponded to a similar threshold wind speed (9.5–12.0 m/s), although infected plants were occasionally detected as far as 4.1 m from the nursery edge, where the wind speed was about 5 m/s. Thus, CBS and citrus canker may have similar wind speed thresholds for infection from inoculum carried by windblown rain. In several inoculation experiments on fruit and foliage, gentle sprays of inoculum rarely lead to disease. Waterborne inoculum must be driven with enough force to cause water-soaking of host tissues for disease to develop (16).

Although several methods have been tested, the leaf swab technique was an efficient, accurate, and nondestructive means

to estimate leaf surface populations of *X. c. citrumelo*. Recovery of bacteria was most consistent in early morning, while dew was still on the plants, or immediately after rainstorms, when the foliage was still wet. When wet foliage is sampled, recovered bacterial populations could be composed of epiphytic bacteria and bacteria exuded and splashed from lesions of symptomatic leaves to the foliage being sampled. Sampled plants had a slightly higher disease incidence. All strains of *X. c. citrumelo* are easily transmitted mechanically (6). Therefore, simply handling the plants being assayed could have caused transmission of the disease. Alternatively, swabbing may have caused microwounds on the leaf epidermis and/or introduced bacteria into the wounds or stomatal openings.

Disease gradients and leaf surface bacteria gradients that resulted from simulated rainstorms were directly related to aggressiveness of *X. c. citrumelo* strain. For the aggressive strain, bacterial dispersal and development of disease occurred over the greatest distance. The aggressive strain most consistently produced the highest leaf bacterial populations and largest lesions on Swingle citrumelo as compared to the moderately and weakly aggressive strains in previous nursery tests (1). Internal populations in lesions were indicative of bacterial populations exuded onto the lesion surface and available for spread by rain splash events (1,15,30). Thus, the ability of the different aggressiveness types to spread from an inoculum source has been directly related to their ability to produce bacteria from lesions (1). Furthermore, these results substantiate previous observations in which natural spread of CBS was found to occur only in nurseries infected with aggressive strains of *X. c. citrumelo* (6,12).

In 1989 nursery plots, disease decreased rapidly after initial disease development from mistblower dissemination of the pathogens (Fig. 3K–O). This decrease was due to the combined effects of lack of secondary spread and infection and the dilution effect of rapidly growing plants, which added new healthy tissue and resulted in decreased disease incidence ratings in subsequent assessments. In 1990 nurseries, disease increased slightly, then leveled off (Fig. 4G–I). This increase was most likely due to the slow development of subclinical infections from mistblower dissemination (not from secondary spread) combined with a lack of continued growth of host plants late in the season. The plants used in the 1990 nursery experiments were older and flushed only at the time of inoculation. Thus, they had less new tissue and did not dilute the effects of a static disease incidence level, whereas the plants used in the 1989 experiments were younger and flushed almost continually. The effect of the addition of new host tissue, which changed disease ratings of individual plants, was also seen in the change of disease gradients over time in the 1989 plots (Fig. 3A–E), whereas no appreciable change in disease gradients occurred over time in the 1990 plots, in which no disease spread was coupled with no new host tissue development (Fig. 4A–C).

In prior studies, CBS declined within a single season with no recurrence in successive seasons in nursery trees outplanted in grove configurations and spacing (6). In the present study, disease decreased on all new grove transplants regardless of host susceptibility or strain aggressiveness. In plots 16 and 17, where *X. c. citrumelo* aggressive strain F1 was inoculated to the control tree in each plot, disease did not spread to adjacent trees, and eventually all CBS lesions were lost as older leaves senesced and abscised. The decrease was most likely due to the more harsh, open, and dry environment of a grove compared with the more closed and humid conditions within stands of closely spaced nursery trees. In addition, disease-induced defoliation is common for plants infected with *X. c. citrumelo*, even when disease incidence is low. The addition of new, nondiseased foliage stimulated by disease-induced defoliation causes a further reduction in disease incidence. These factors result in a significant decrease in disease incidence and eventual disappearance of disease within a few months of transplanting to the grove. In other studies, populations of moderately aggressive and weakly aggressive strains of *X. c. citrumelo* were relatively short-lived and/or erratic in CBS lesions on all citrus hosts tested in the greenhouse and field. Populations of the aggressive strain remained consistently

high only in the susceptible hosts (1,15). However, under grove conditions, disease caused by the aggressive strain on susceptible Swingle citrumelo decreased at nearly the same rate as on less susceptible Duncan grapefruit.

Based on the limited aggressiveness of strains of *X. c. citrumelo*, lack of natural spread of all but rarely encountered aggressive strains, incompatibility with commercial citrus scion cultivars, and decreasing disease incidence when diseased nursery trees are transplanted to grove conditions, CBS should be considered a citrus nursery problem of less severity than several other citrus nursery diseases.

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