Analysis of Disease Progress of Citrus Canker in Nurseries in Argentina


First author: research plant pathologist, U.S. Department of Agriculture, Agricultural Research Service, Horticultural Research Laboratory, Orlando, FL 32803. Second and third authors: professor and postdoctoral research associate, University of Florida Citrus Research and Education Center, Lake Alfred 33850. We wish to thank S. Garran for project supervision; N. Timmer, A. Dow, J. Bittle, C. Hurtado, M. Scheiffer, V. Scheiffer, and V. Figueroed for data collection and technical assistance; and C. L. Campbell and L. V. Madden for consultation on statistical analysis. Supported in part by U.S. Department of Agriculture/OICD grant IR-AR-FL-137. Accepted for publication 16 June 1989 (submitted for electronic processing).

ABSTRACT


Three nursery plots of Duncan grapefruit, Pineapple sweet orange, and Swingle citruleno rootstock were established in Concordia, Entre Rios, Argentina, to study the temporal increase and spatial spread of citrus bacterial canker from a single focal point. Pectoral trees of each cultivar were inoculated with Xanthomonas campestris pv. citri, the causal agent of Asian citrus bacterial canker, and planted in the center of each plot. Disease increase over time was measured as either disease severity (proportion of leaves infected per plant) or disease incidence (proportion of plants infected). Exponential, monomolecular, logistic, Gompertz, and Weibull models were tested for appropriateness by nonlinear regression analysis. The Gompertz model was superior for describing increase in disease incidence and disease severity in all three citrus nurseries. The rate of disease increase was greater in the most susceptible host, Duncan grapefruit, than in less susceptible hosts, Pineapple orange or Swingle. Disease spread coincided with rain splash dispersal and a rapid increase in the apparent infection rate after windblown rainstorms. Rate of disease spread was independent of wind direction. Aggregation of diseased plants was observed in all three nurseries throughout the duration of the experiments. Aggregation of individuals appeared to be equivalent between and among nurseries, indicating that splash dispersal of inoculum was not impeded beyond a few meters. Secondary foci were established early in the epidemics and soon overcame the effect of the original focus of disease. The slope of linearized disease gradients, $[-\ln(-\ln(y))] = a + b \ln x$, where $y$ = disease incidence and $x$ = distance from the focus of infection in meters, fluctuated over time because of disease-induced defoliation of severely infected plants. Defoliation of more severely diseased plants near the focus subsequently resulted in positive disease gradient slopes for the susceptible Duncan grapefruit nursery as disease levels near the focus diminished.

Additional keywords: dispersion, isophat, quantitative epidemiology.

Xanthomonas campestris pv. citri causes Asian citrus bacterial canker (CBC, "citrus canker") in numerous citrus-producing countries worldwide (3,13-15). The disease causes erumpent lesions with chlorotic halos and water-soaked margins on fruit, foliage, and green wood (3). Fruit spotting reduces marketability, and the presence of the disease severely hampers inter- and intranational movement of citrus because of regulatory measures to preclude entry of the disease into canker-free areas (15). Severe CBC can reduce crop yield as well as quality. In mature trees with severe infections, defoliation and dieback of green wood may occur (8). Citrus nursery trees with CBC infections are rendered unmarketable if the disease is detected. Infected nursery trees are the primary source of pathogen dissemination over long distances (3,15).

Recent outbreaks of CBC on the Gulf Coast of Florida and of a bacterial spot disease of citrus caused by Xanthomonas campestris pv. citruleno pv. nov. in nurseries has resulted in the eradication of more than 22 million citrus trees and stimulated new research on CBC (6,7,10,22,23).

Dissemination of X. c. citri is directly related to wind-driven rain, especially when wind speeds exceed 8 m/sec (8,19,24,27). Bacteria exude from lesions when wetted and splash to nearby plants (21,24). Bacterial concentrations of $10^5$-$10^{10}$ colony-forming units (cfu)/ml have been collected from rainwater on foliage infected with X. c. citri, and bacteria of X. c. citri have been detected in rainwater up to 32 m from diseased foliage (13). High wind speeds during rain can cause water soaking and can facilitate entry of bacteria through stomatal openings into leaves. Lesion development occurs 7-10 days later (18).

In a previous study, rates of CBC disease increase $(k)$ in infested mature groves in Argentina, where nonpoint inoculum source were widespread, were calculated from linearized, Gompert transformed data to be $0.04-0.06, 0.1, 0.18$, and $0.13-0.24$/d from mandarin, satsuma, navel, and sweet orange, respectively (1,3,4). In a recent study, rates of CBC increase were determined from discrete foci of infection in new grove plantings to be $0.0$ and $0.09$/day for orange and grapefruit, respectively (8). Spread of the disease was directional with the highest rates predominant to the northeast in response to dissemination by wind-driven rain. Rates of disease increase are affected by scion-rootstock combinations (17).

Disease gradients of CBC in infested groves have been described previously. Slopes of Gompert $(y)$ versus $\ln \ln (x)$ linearized disease gradients, where $y = \text{disease incidence and } x = \text{distance in meters}$ ranged from $-0.21$ to $-4.13$; however, discrete initial foci inoculum were not determined (4). In a recent study in which foci were determined, slopes of similarly transformed disease gradients varied over time from $-0.713$ to $-1.237$ and $+0.0$ to $-1.856$ in orange and grapefruit new groves, respectively (13). This variation was the result of disease-induced defoliation and subsequent infection of regrowth in heavily infected trees near the foci. Disease gradients were flattest in the northwest (downwind) direction. Aggregation of diseased trees occurred through out the epidemic and was somewhat higher during the early stages of the epidemic (8).

Although some information is available on the epidemiology of CBC in orchards and new plantings, the spatial and temporal dynamics of citrus canker in citrus nurseries have not been described previously. Study of the epidemiology of CBC in citrus nurseries is important because movement of infected plant stock is the major means of long-distance disease dissemination. The potential of the disease in and from citrus nurseries is
concern both in the United States and abroad because of potential plant quarantines, embargoes, and loss of markets. The purpose of this study was to quantify the spatial and temporal progress of CBC in nurseries from a known focal source on citrus species of different susceptibility.

MATERIALS AND METHODS

Nursery plots of Pineapple sweet orange (Citrus sinensis (L.) Osb.), Duncan grapefruit (C. paradisi Macf.) and Swingle citrumelo (Poncirus trifoliate) (L.) Raf. × C. paradisi) were established at the Instituto Nacional de Tecnología Agropecuaria, an agricultural experiment station near Concordia, Entre Rios, Argentina, to evaluate the disease dynamics of CBC under field conditions. All plots were planted in rows 0.75 m apart with 5 cm between plants within rows, a common nursery spacing. Plants were grown in plastic bags to maintain free moisture and high humidity for approximately 48 hr and placed in a growth chamber at 25°C. Bins were removed, and symptoms allowed to develop for 15-30 days in a growth room before the plants were planted in the field nurseries. One inoculated plant was planted in the center of the center row of each plot to act as a disease point of disease within the plot.

Sampling design and analysis of temporal disease progression. Disease was assessed visually on every tree in each plot on every sample day. Assessments were made in intervals of 14 to 21 days during the growing season and 30 to 45 days during the winter. Disease progression was followed for 382, 383, and 468 days in the grapefruit, orange, and Swingle plots, respectively. Disease progression was described by the number of diseased leaves per tree. Disease incidence (proportion of trees expressing disease in the nursery as a whole). Disease progression models were tested on each plot as a whole over time and in four quadrants (northeast, southeast, southwest, and northwest) within each plot. The common corner of the quadrants was the central focal plant. The appropriateness of the nonlinear forms of the exponential, monomolecular, logistic, Gompertz, and Weibull models was examined for disease severity and disease incidence data for each nursery by nonlinear regression analysis (16,20,26,28). The appropriateness of each model was assessed by examining standard residual plots and tested by correlation analysis of observed versus predicted values. Models with the highest level of correlation were chosen as superior. Rate parameters of the Gompertz model (k) (shown later to be most appropriate) of disease progression of individual directional quarters within each nursery and (k) between nurseries were compared by an F-test for all quadrant combinations.

Analysis of spatial disease progression. Aggregation of diseased plants was determined by ordinary runs analysis for each sampling date within and across rows (17). Aggregation was assessed as the proportion of rows and across-row nursery segments with significant (P = 0.05) clustering. Aggregation of diseased plants was examined using Lloyd’s index of patchiness (LIP) (27). Three nurseries were planted in a rectangular lattice. Therefore, a nursery was divided into quadrants consisting of five plants in a row. This quadrat size was chosen because the space occupied by five plants down the row was equivalent to the space between rows. This quadrat size thus squared the rectangular lattice of plants.

Aggregation of diseased plants also was examined by the use of disease isopaths maps. Maps were generated for each nursery for dates corresponding to times early in the development of the epidemic, just after the epidemic had entered a rapid disease increase stage, and at the termination of the epidemic, that is, the last day that data were collected. Contour lines were generated corresponding to 0.05 increments of disease severity. To visualize disease severity, three-dimensional response surface maps were generated for the same data as isopaths maps. These were examined to determine the intensity of disease in secondary foci and else-

Fig. 1. A and B, Disease incidence of citrus canker over time compared with rainfall. C and D, Change in the rate of disease incidence and disease severity, respectively. DGN = Duncan grapefruit nursery; PON = Pineapple orange nursery; SWN = Swingle rootstock nursery. Arrows indicate rainstorms associated with wind in excess of 8 m/sec. DY/DT = the change in disease incidence over time. DZ/DT = the change in disease severity over time.

Vol. 79, No. 11, 1969 1277
where within each plot, in relationship to the focus of disease. In addition, they were examined to better understand the directionality of disease spread within and across rows and spatially within the matrix of the nursery plots.

Disease gradient analysis was accomplished by subjecting the spatial arrangement of disease severity assessments to analysis using the GRADCALC program (9). The program calculates the distance from the central focal plant to every other plant in the spatial matrix of the nursery. It then was used to examine weighted means of disease severity, that is, average disease severity divided by the total number of plants falling within 0.5-m-wide concentric bands around the focus. The output data of disease severity versus distance by sampling date was used to generate response surfaces directly. Disease gradients were further analyzed by linear regression of Gompertz-transformed disease regressed on the natural logarithm of the distance from the focal tree.

RESULTS

Analysis of temporal disease progress. Initially disease incidence increased slowly in the three nurseries. Disease occurrence on plants beyond the focal plant was first detected 18, 46, and 46 days after placement in the field for Swingle, grapefruit, and orange nurseries, respectively. In all three nurseries, the proportion of diseased plants remained low for a long period of approximately 300-320 days for grapefruit and orange nurseries and approximately 400 days for the Swingle nursery (Fig. 1). Rain also did not appear to have an effect on disease increase. However, the absolute rate of disease increase rose noticeably a few days after blowing rainstorms with high winds (indicated by arrow in Fig. 1A and B). Of the nonflexible models evaluated (exponential, monomolecular, Gompertz, and logistic), the Gompertz model was judged to be the most appropriate for describing disease progression (either as disease incidence or disease severity) all three nurseries based on residual plot analysis and correlation of observed versus predicted values. However, the exponent and logistic models also gave acceptable fits of disease progression data over time (Table 1). In the case of the grapefruit nursery plot, the Gompertz, exponential, and logistic models were nearly equally appropriate in describing the increase of disease proportion over time. Disease severity more accurately reflects the dynamics of disease progression in the field than did disease incidence measurements. Disease-induced defoliation lowered disease severity on individual plants, whereas disease incidence (+/− disease) remained unchanged. Disease incidence all reached an asymptote in all three nurseries toward the end of the epidemics, whereas no asymptote was reached when disease was measured as disease severity (Fig. 1C and D).

Fig. 2. Three-dimensional response surface representations of citrus canker disease development and spread in three citrus nurseries on the indicated days postinoculation. Note disease incidence of focus (peak) compared to height of peaks of secondary foci. DGN = Duncan grapefruit nursery; PON = Pineapple orange nursery; SWN = Swingle rootstock nursery.
The Weibull model with the location parameter (a) fixed at 1 gave the best overall fit to disease progress data among all models and all plots tested. Coefficients of determination ($R^2$) of observed versus predicted values ranged from 0.991 to 0.998 (Table 1). However, even when b and c parameters were restricted to small-range estimates, the full Weibull model with a fixed at 0 did not consistently converge. Because the long lag period of low disease intensity at the beginning of the epidemic may have adversely affected the Weibull model performance, one or more of the data points corresponding to the beginning of the epidemic were eliminated. However, the Weibull model still did not converge. Parameter b, the scale parameter, was inversely related to the rate of disease increase. The b parameters were all high, ranging from 331 to 544 days, indicating a slow rate of disease progress of CBC in nurseries compared to other disease situations previously described (20). Parameter c, the shape parameter, was an indication of the inflection point of the rate of disease increase and normally ranges from 1.8 to 9.2 for most disease situations (20,26). Parameter c ranged from 9.89 to 15.09 and indicated that an inflection point of the disease progress curve occurred relatively late in the epidemic.

Because of the lack of convergence by the full Weibull model, the Gompertz model was generally accepted as the preferable model to describe CBC disease increase over time. However, it was recognized that the exponential and logistic models also were appropriate to describe CBC disease increase in most cases.

Gompertz rate parameters (k) for disease incidence and disease severity for all three nurseries are given in Table 1. Rates of disease increase between nurseries were compared via the $t$-test (Table 2). The rate of disease increase in the Swingle compared to orange nurseries was not significantly different; however, all other combinations of comparisons between nurseries demonstrated significantly different rates of disease increase. Citrus canker progressed slowest in the Swingle nursery and fastest in the grapefruit nursery, although these epidemics were not concurrent.

The Gompertz rate of disease increase also was examined in quadrants within each nursery plot. There were no statistical differences in $k$ among the quadrants within each of the three nurseries except in a single case (Table 3). Here the northeast

### TABLE 2. Comparison of slopes from Gompertz model with $t$-tests for disease incidence and disease severity between nurseries* infected with citrus canker

<table>
<thead>
<tr>
<th>Plot</th>
<th>Disease incidence*</th>
<th>Disease severity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PON</td>
<td>2.394**</td>
<td>2.987**</td>
</tr>
<tr>
<td>SWN</td>
<td>3.449**</td>
<td>3.839**</td>
</tr>
</tbody>
</table>

*Grapefruit nursery (GDN) vs. Pineapple sweet orange (PON), GDN vs. Swingle citrusmelo nursery (SWN), and PON vs. SWN had 22, 32, and 32 degrees of freedom, respectively.

**Disease incidence = proportion of diseased plants; disease severity = number of diseased leaves per total number of leaves per plant.

### TABLE 3. Comparison of slopes from Gompertz model with $t$-tests for disease incidence and disease severity between quadrants within nurseries* infected with citrus canker

<table>
<thead>
<tr>
<th>Nursery</th>
<th>Disease incidence*</th>
<th>Disease severity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>SE</td>
<td>2.066</td>
<td>1.337</td>
</tr>
<tr>
<td>SW</td>
<td>2.747*</td>
<td>0.836</td>
</tr>
<tr>
<td>NW</td>
<td>0.586</td>
<td>1.335</td>
</tr>
<tr>
<td></td>
<td>2.018</td>
<td>0.239</td>
</tr>
<tr>
<td></td>
<td>1.410</td>
<td>1.775</td>
</tr>
<tr>
<td>PON</td>
<td>0.385</td>
<td>0.222</td>
</tr>
<tr>
<td>SW</td>
<td>1.477</td>
<td>0.222</td>
</tr>
<tr>
<td>NW</td>
<td>1.146</td>
<td>0.217</td>
</tr>
<tr>
<td></td>
<td>0.662</td>
<td>1.412</td>
</tr>
<tr>
<td></td>
<td>1.407</td>
<td>0.221</td>
</tr>
<tr>
<td>SWN</td>
<td>0.879</td>
<td>0.250</td>
</tr>
<tr>
<td>SW</td>
<td>1.460</td>
<td>0.796</td>
</tr>
<tr>
<td>NW</td>
<td>1.516</td>
<td>0.853</td>
</tr>
<tr>
<td></td>
<td>0.006</td>
<td>1.027</td>
</tr>
<tr>
<td></td>
<td>1.514</td>
<td>0.040</td>
</tr>
</tbody>
</table>

*Grapefruit nursery (GDN), Pineapple sweet orange nursery (PON), and Swingle citrusmelo nursery (SWN) had 22, 22, and 42 degrees of freedom, respectively.

### TABLE 1. Nonlinear regression analysis of disease incidence and disease severity of citrus canker in citrus nursery plantings in Argentina over time

<table>
<thead>
<tr>
<th>Model</th>
<th>Nursery</th>
<th>Parameter</th>
<th>Estimate</th>
<th>Asymptotic standard error</th>
<th>Asymptotic 95% confidence interval</th>
<th>$R^2$ of correlation of observed vs. predicted values</th>
<th>Asymptotic standard error</th>
<th>Asymptotic 95% confidence interval</th>
<th>$R^2$ of correlation of observed vs. predicted values</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>Orange</td>
<td>r</td>
<td>0.01577</td>
<td>0.00014</td>
<td>0.00155 - 0.00160</td>
<td>0.978</td>
<td>0.01120</td>
<td>0.00040 - 0.01032</td>
<td>0.01210</td>
</tr>
<tr>
<td>E</td>
<td>Orange</td>
<td>k</td>
<td>0.00079</td>
<td>0.00000</td>
<td>0.00078 - 0.00083</td>
<td>0.790</td>
<td>0.00117</td>
<td>0.00039 - 0.00201</td>
<td>0.888</td>
</tr>
<tr>
<td>E</td>
<td>Orange</td>
<td>c</td>
<td>0.0127</td>
<td>0.0002</td>
<td>0.01167 - 0.01317</td>
<td>0.825</td>
<td>0.00147</td>
<td>0.00044 - 0.00217</td>
<td>0.974</td>
</tr>
<tr>
<td>E</td>
<td>Orange</td>
<td>b</td>
<td>0.1635</td>
<td>0.0004</td>
<td>0.1551 - 0.1721</td>
<td>0.968</td>
<td>0.00502</td>
<td>0.00020 - 0.00300</td>
<td>0.980</td>
</tr>
<tr>
<td>G</td>
<td>Orange</td>
<td>r</td>
<td>0.0293</td>
<td>0.0007</td>
<td>0.02823 - 0.03237</td>
<td>0.994</td>
<td>0.00096</td>
<td>0.00043 - 0.00867</td>
<td>0.996</td>
</tr>
<tr>
<td>G</td>
<td>Orange</td>
<td>k</td>
<td>0.0315</td>
<td>0.0003</td>
<td>0.03233 - 0.03433</td>
<td>0.994</td>
<td>0.00096</td>
<td>0.00043 - 0.00867</td>
<td>0.996</td>
</tr>
<tr>
<td>G</td>
<td>Orange</td>
<td>b</td>
<td>0.3592</td>
<td>0.0007</td>
<td>0.3533 - 0.3650</td>
<td>0.994</td>
<td>0.00096</td>
<td>0.00043 - 0.00867</td>
<td>0.996</td>
</tr>
<tr>
<td>W</td>
<td>Grapefruit</td>
<td>r</td>
<td>0.3310</td>
<td>0.0005</td>
<td>0.3246 - 0.3375</td>
<td>0.994</td>
<td>0.3907</td>
<td>0.3874 - 0.3930</td>
<td>0.999</td>
</tr>
<tr>
<td>W</td>
<td>Grapefruit</td>
<td>k</td>
<td>0.1272</td>
<td>0.0003</td>
<td>0.1227 - 0.1317</td>
<td>0.994</td>
<td>0.1209</td>
<td>0.1197 - 0.1313</td>
<td>0.999</td>
</tr>
<tr>
<td>W</td>
<td>Grapefruit</td>
<td>c</td>
<td>0.0108</td>
<td>0.0003</td>
<td>0.0104 - 0.0112</td>
<td>0.994</td>
<td>0.0109</td>
<td>0.0105 - 0.0114</td>
<td>0.999</td>
</tr>
<tr>
<td>W</td>
<td>Grapefruit</td>
<td>b</td>
<td>0.4288</td>
<td>0.0005</td>
<td>0.4227 - 0.4334</td>
<td>0.994</td>
<td>0.5436</td>
<td>0.5380 - 0.5492</td>
<td>0.999</td>
</tr>
</tbody>
</table>

*Model parameters were estimated by nonlinear regression of the integrated equations $y = y_0 e^{-x}$, $y = 1 - (1 - y_0)e^{-x}$, $y = 1/(1 + e^{-x})$, and $y = 1 - e^{-(x - c)}$ for the exponential (E), monomolecular (M), logistic (L), Gompertz (G), and Weibull (W) models, respectively, where $r$ and $k$ are rate parameters, $y$ is disease measured as incidence or severity, and $t$ is time in days. For the Gompertz model, $B = -\ln(y_0)$.

*Gompertz model, $a$ was fixed at 1 to estimate $b$ and $c$ parameters because the model does not converge with all three parameters when they are undefined.

*aSignificantly different at $P = 0.05$. Ho: $b_1 = b_2$, Ha: $b_1 \neq b_2$ where $b_n$ is the slope value of the Gompertz model.

*Coefficients of determination of predicted values against observed values to examine appropriateness of models.
quadrant of the grapefruit nursery had a significantly higher $k$ compared with the southwest quadrant of the same nursery.

**Analysis of spatial disease progress and aggregation.** The threedimensional response surfaces of disease incidence showed that secondary foci developed soon after disease spread was detected in each nursery (Fig. 2A, D, and G). Peaks of disease incidence of these secondary foci equal to or exceeding that of the focal plant occurred simultaneously with disease spread (Fig. 2A, D, and G). The dominance of the focus was lost early in the epidemic compared with developing secondary foci (Fig. 2B, C, E, F, H and I). Based on analysis of isopaths maps of disease severity, dispersion of inoculum emanating from the focus appeared to be nondirectional and secondary foci coalesced as the epidemic progressed through time (Fig. 3).

Infected plants were highly aggregated throughout the epidemics in all three nurseries, as determined by the significant departure from randomness in an ordinary runs analysis (Fig 4A and B). In general, aggregation increased in all three nurseries.

![Diagram](image)

**Fig. 3.** Isopathic contour maps of citrus canker disease severity on the indicated days postinoculation. Note pattern of dissemination, especially in A, associated with splash dispersal of inoculum, establishment of secondary foci, and eventual coalescence of foci. DGN = Duncan grapefruit nursery; PON = Pineapple orange nursery; SWN = Swingle rootstock nursery. The focal plant is indicated by a triangle, and every fourth line (i.e., disease incidences 0.2, 0.4, 0.6, 0.8, 1.0) is dashed.
Analysis of disease gradients. The use of alternatives to the Gregory model for disease gradients has been proposed. Often the same transformation used in the temporal model of choice also has been used to describe the associated disease gradients (2,4,5,8,10,11). Because the Gompertz model was deemed the most appropriate to describe the temporal disease increase of CBC in citrus nurseries, the model, \(-\ln(-\ln(y)) = a + b \ln(x)\), similar to one previously described (4), was used to describe CBC disease gradients, where \(y\) = disease incidence and \(x\) = distance from the focus of infection (Fig. 4D).

Nontransformed data from the GRADCALC program described above were plotted by date to examine the gradient shapes before linearizing the data (Fig. 5). Disease severity fluctuated at and near the focus over time. The disease severity of plants, within the distances of 0-0.5 and 0.5-1.0 m from the focus, decreased twice during the duration of the epidemic in all three nurseries (denoted by arrows in Fig. 5). The effect of this decrease was the lowering of the slope of the linearized gradients (Fig. 4D). Thus, the slopes did not merely flatten over time as expected but fluctuated over time.

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Fig. 4. A and B, Proportion of aggregation of citrus canker-diseased plants over time resulting from ordinary runs analysis. Note that greater aggregation appears to predominate across rows. C, Lloyd's index of patchiness expressed over time. D, Slopes of disease gradients of three nurseries affected with citrus canker over time. Slopes were generated by regressing \(-\ln(-\ln(y)) = a + b \ln(x)\), where \(y\) = disease severity, \(x\) = distance from the focus in meters, and \(b\) = slope of the gradient. Note fluctuations in gradients associated with disease-induced defoliation including positive slope for Duncan grapefruit nursery in later stages of epidemic development. DGN = Duncan grapefruit nursery; PON = Pineapple orange nursery; SWN = Swingle rootstock nursery.
DISCUSSION

Citrus canker disease increase was slow initially in all three nurseries studied. Although minimal disease spread was seen very early in all three epidemics, rains during this period generally were not associated with high wind speeds and temperatures were low for long periods. The abrupt upward inflection in the rate of disease increase immediately following day 319 for grapefruit and orange nurseries and day 398 for the Swingle nursery was directly related to rainstorm events associated with high winds. The association of windblown rain with the spread of CBC has been noted previously (8,24,25). We feel that disease severity as estimated in this study provided more information than disease incidence. Citrus nursery plants, once infected, often caused some of their foliage with symptoms to abscise. The result was that disease severity decreased in those plants while disease incidence remained the same. This decrease in severity was observed in all three nurseries (Fig. 5). Thus, disease severity better reflected the natural fluctuation of CBC within nurseries. In addition, disease progress curves of disease severity never reached asymptotes during the duration of the observed epidemics whereas disease progress curves of disease incidence did (Fig. 1C and D).

The lack of asymptotes associated with disease severity better reflects the continuing increase in disease during the time citrus plants normally remain in nursery situations.

The Gompertz model was superior for describing both disease severity and disease incidence over time. As described above, the full (three-parameter) Weibull model did not converge consistently for all three nurseries in this case (27). Disease did not appear to approach an asymptote during the period of time the nursery plots were studied. The duration of the studies was equivalent to or exceeded the duration that citrus plants are normally allowed to remain in a nursery. Under nursery situations, citrus plants flushed and grew almost continuously and new susceptible tissues were added continuously to the pathosystem. Thus, susceptible tissue never became limiting. The monomolecular, logistic, Gompertz, and Weibull models all take into account that susceptible tissue becomes limiting as the epidemic progresses. The exponential model, however, does not deal with limited susceptible tissue. Therefore, the simplest model, the exponential, also described CBC adequately in nurseries. The exponential model actually was more appropriate than the Gompertz model when disease values were transformed and the model was fitted by linear regression analysis (Gottwald, unpublished data).

The effect of meteorological events was not taken into account in the development of temporal models, yet the influence of blowing rainstorms on disease increase was apparent. Thus, acceptance of the appropriateness of any of the models suggested here should be viewed as preliminary until enough data can be collected to allow the development of more sophisticated temporal models that take into account host growth and major meteorological events.

Gompertz rates of disease increase were significantly different between grapefruit versus Swingle and grapefruit versus orange, but not between Swingle versus orange nurseries (Table 2). These results were consistent with previously reported susceptibility of these cultivars to CBC (1,6,8,19). Grapefruit has long been recognized as one of the most highly susceptible citrus species.

Rates of disease increase generally were not dependent on direction (Table 3). Previous studies in new grove situations indicated a direct association between disease spread in space and disease increase in time with wind direction as the result of severe rainstorms (8). The apparent lack of effect of windblown rain on directionality of spread reported here, except possibly for the grapefruit nursery, and resulting rate of disease increase can best be explained by the geometry of citrus nurseries versus that of citrus groves. In grove situations, trees are normally planted 4-6 m apart in a square or rectangular lattice pattern. Grove trees are also at least 1 m tall. Raindrops hitting a lesion oozing bacteria of X. c. citri would, of course, fragment into smaller droplets. Such droplets containing inoculum usually would not reach even the closest neighboring tree in the lattice because of the distance between individuals. However, during rainstorms with high winds, these small droplets would be carried predominantly downwind to neighboring trees (8). Infected grove trees one more meters tall also raise the origin of inoculum above boundary layer of air close to the ground and into the turbulent air layers where dissemination and diffusion of inoculum to eddies are possible. Nursery trees, especially when young, usually only a few centimeters tall and thus can be affected at some degree by the stagnant boundary air layer. This bound effect is intensified by the sheltering of numerous other individuals in close proximity. Splash dispersal of inoculum to nearby individuals is much more probable because of the close nursery lat spacing. Rains without wind are much more common in central Argentina and, although not as efficient at causing infection, are quite likely to disperse inoculum to nearby individ

![Fig. 5. Nontransformed disease gradients of citrus canker in nurseries over time. Note fluctuations in disease severity near the focus (ar in all three nurseries resulting from disease-induced defoliation.](image-url)

= Duncan grapefruit nursery; PON = Pineapple orange nursery; = Swingle rootstock nursery.
in a nursery. Hence, in nurseries splash dispersal and the movement of secondary foci in close proximity to the original point where wind dissemination of inoculum of *X. campestris pv. citri* Directional spread due to wind dissemination was not significant but might have occurred by splash dispersal and the early appearance of secondary foci. This effect also is seen in estimates of aggregation of these foci. Aggregation between nursery rows was stronger or stronger than within rows. Apparently splash dispersal between and within rows is nearly equivalent because the spacing of individuals (Fig. 4A and B). Isolates of disease severity provided an excellent means for studying the spatial location, size, and intensity of secondary foci as the coalescence of foci over time (Fig. 3). Most foci seemed to develop within about 3-4 m of the focus and then spread further from there. Comparison of adjacent maps of disease severity and disease incidence over time also can be observed. As described previously, disease severity in more heavily infected individuals is due to disease-induced defoliation (8). Isolates maps have been used to assess the spatial distribution of movement of foci and disease severity at different scales. The occurrence of disease severity at different scales seems to be caused by the number of foci that begin to coalesce. The disease-induced defoliation caused fluctuations in disease severity that were caused by the presence of disease in the immediate area. This may complicate further analysis of the spatial distribution of disease severity in the area. The disease severity at different scales appears to be inherent to citrus canker epidemics (8).

**LITERATURE CITED**


