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The effect of irrigation practices on the spatio-temporal increase of Asiatic citrus canker in simulated nursery plots in Reunion Island

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Abstract

Asiatic citrus canker is a potentially severe disease of several citrus species and cultivars in many tropical and subtropical areas. In such areas, infected nursery plants constitute an important source of primary inoculum for newly established citrus groves. The influence of overhead, drip, and mist irrigation systems on the development of Asiatic citrus canker was studied in simulated, Mexican-lime nurseries in Reunion Island. Overhead irrigation exacerbated the increase of disease incidence and severity caused by a streptomycin-resistant strain of *Xanthomonas axonopodis* pv. *citri*. The temporal development of Asiatic citrus canker for overhead irrigated nursery plots was best described by an exponential model, because disease incidence in these plots did not come close to an asymptote during the experimental period. This can be explained by the continuous production of new growth, susceptible to infection by *Xanthomonas axonopodis* pv. *citri*, and splash dispersal of *Xanthomonas axonopodis* pv. *citri* associated with overhead irrigation. Based on spatial correlation and spatio-temporal analyses, aggregated disease patterns were found irrespective of the irrigation system. In overhead-irrigated plots, the spread of *Xanthomonas axonopodis* pv. *citri* lacked directionnality. Rainstorms of short duration and high intensity were apparently associated with disease increase in drip-irrigated plots. There is a need to improve cultivation practices in Reunion Island citrus nurseries to minimize Asiatic citrus canker incidence in nurseries and to minimize the introduction of *Xanthomonas axonopodis* pv. *citri* to new groves.

Introduction

Asiatic citrus canker (ACC), associated with *Xanthomonas axonopodis* pv. *citri* (*Xac*), is a potentially severe disease of several citrus species, particularly cultivars and hybrids of sweet orange [*Citrus sinensis* (L.) Osb.], grapefruit [*C. paradisi* Macf.], and lime [*C. aurantifolia* (Christm.) Swing.] in tropical and subtropical areas. ACC decreases fruit quality, can drastically reduce crop yields by causing premature fruit drop on susceptible cultivars, and can induce severe defoliation in groves and nurseries. The disease is endemic throughout Southeast Asia, in many countries bordering the Indian Ocean, and in South America (Civerolo, 1984).

On Reunion Island, a French territory located in the Indian Ocean east of Madagascar, ACC was first reported in the 1970's (Brun, 1971), but it is likely that it was introduced much earlier. There are three main climatic seasons in Reunion. From May to September, a cool and dry weather prevails. Temperatures rise in October and the weather remains mostly dry until December. Hot and humid weather occurs from January to April. Numerous rainstorms, tropical storms, and hurricanes occur during the latter season and rapid epidemics of ACC are, as in many tropical areas, associated with such storms over the island and the disease is now considered to be endemic. The local citrus industry is essentially based on the use of mandarins and mandarin hybrids which constitute more than 80% of commercial citrus, and which are tolerant to ACC. However, there is a need and desire by growers to diversify commercial citrus cultivars in Reunion Island, most of which are considerably more susceptible to ACC than mandarins. Therefore, control of ACC is of great concern and diversification of citrus cultivars should be associated with a modernization scheme of cultural practices in local nurseries.

Leaf infections occur through stomata and wounds and foliage is most susceptible when leaves are 50–80% expanded (Gottwald and Graham, 1992). Rain associated with windspeeds $\geq 8\,\mathrm{m/s}$ can cause water congestion of leaf and green twig tissues (Serizawa et al., 1969; Serizawa and Inoue, 1975). Such congestion results in a continuous column of water from the leaf mesophyll through the stomata to the leaf surface and facilitates bacterial ingress (Graham et al., 1992).

In countries where ACC is endemic, occurrence of the disease and spread of the pathogen in nurseries is favored by cultural practices such as open-air nurseries, pruning, overhead irrigation, lack of windbreaks, etc. In such cases, disease control is difficult and even the use of larger quantities of antibacterial compounds such as copper-based bacteriocides and antibiotics do not provide satisfactory control (Gottwald and Timmer, 1994). Moreover, in these situations, there is the potential risk that bacterial strains resistant to these compounds could develop. Overt or latent leaf and twig infections on nursery plants constitute an important source of primary inoculum for subsequent grove infections. In tropical areas, production of *Xac*-free nursery plant material is therefore of great concern. For this purpose, a specific and sensitive immunocapture nested PCR-based assay for detection of *Xac* was developed (Hartung et al., 1996).

Dispersal of *Xac* in nurseries is due to splashing of rain or irrigation water splash or by wind-driven rains (Gottwald et al., 1988). Ecological and epidemiological studies on ACC in citrus nurseries have been mostly performed in Japan (Kuhara, 1978; Goto, 1992) and in subtropical to arid areas of Argentina (Danos et al., 1984; Gottwald et al., 1988; Gottwald et al., 1989; Gottwald et al., 1992b), and therefore, there is a need for data from tropical areas (Civerolo, 1994). Results from studies in Argentina nurseries showed that spread of *Xac* had very little directionality, which emphasized that overhead irrigation was more important than wind-driven rains in the dispersal of inoculum (Gottwald et al., 1989). Mechanical transmission of *Xac* has also been reported in commercial citrus nurseries (Gottwald et al., 1989).

Several models (e.g. exponential, monomolecular, logistic, Gompertz, and Weibull) have been used to describe temporal disease progress of ACC in Argentinian citrus nurseries over a period of more than 400 days, and Gompertz model gave the best overall fit. Slopes of disease gradients linearized with Gompertz model were related to the susceptibility of the cultivars to ACC (Gottwald et al., 1989). Disease gradients are known to often fluctuate over time, due to increases and decreases in disease incidence related to production of new noninfected foliage, infection of that new foliage, and subsequent severe defoliation induced by *Xac*. ACC was found to be strongly aggregated in nurseries. In nurseries of various cultivars in Argentina, Lloyd's index of patchiness, a measure of spatial aggregation, was high in the first stages of epidemics. Aggregation then decreased over time as secondary foci occurred and coalesced. Aggregation was similar among rows and across rows due to close spacing of the plants in the plots. Mechanical transmission produced a greater within-row than across-row aggregation (Gottwald et al., 1989). Spatio-temporal autoregressive integrated moving-average models (STARIMA) have been used to analyze the relationship between disease incidence and the spatial location of the diseased plants at time t to plants that become diseased over time. A STARIMA (l, m, p, q) model indicates that autocorrelations begin to decay after p-l lags in space and q-m lags in time (Reynolds and Madden, 1988). STARIMA (0, 4, 1, 1) is the model that described best the spatio-temporal disease increase in Argentinian citrus nurseries and demonstrated moderate, localized spatial aggregation that was maintained through multiple assessment dates (Gottwald et al., 1992b).

Spatial-lag autocorrelation analysis is based on Modjeska and Rawlings model for correlation analysis of uniformity data (Modjeska and Rawlings, 1983; Gottwald et al., 1992c). It has been used to assess the spatial relationship among ACC-infected trees in a Florida citrus grove and to interpret sequential data sets related to other diseases of citrus over time such as citrus scab, greening, and tristeza (Gottwald et al., 1992a; Gottwald, 1995). Geostatistics, a technique that quantifies the spatial dependency of sample points with one another, has been extensively used for numerous studies in geology, geography, agronomy and, interestingly, entomology and plant pathology (Matheron, 1971; Viera et al., 1983; Chellemi et al., 1988; Isaaks and Srivastava, 1989; Lecoustre et al., 1989; Schotzko and O' Keeffe, 1989; Schotzko and O' Keeffe, 1990; Webster and Oliver, 1990; Williams et al., 1992; Gottwald et al., 1995). Results are often presented as semivariograms, that can be used to evaluate spatial dependency, which is a measurement of the variation of regionalized variables among samples given by the sum of squared differences between all sample pairs for each distance, *h*, in the data matrix. Semivariograms can be calculated for specific directions to test for anisotropy, an indication of directional dissemination. Semivariance can be fitted to several spatial models to determine the structure of the spatial data and the spatial relationships among plants over spatial lags. Recently, geostatistics have been used for spatio-temporal analyses of epidemics for which diseased plants were given a value representing the length of time over which they have been infected (van de Lande, 1993; Gottwald et al., 1996). This approach is useful for analyzing relationships between diseased plants over a period of several assessment dates.

The purpose of this study was to the examine temporal, spatial, and spatio-temporal development of ACC in simulated nurseries in Reunion Island outside of the hurricane season and to comparatively evaluate the influence of various irrigation systems on dispersal of *Xac*. This information will be used to develop nursery citriculture practices to minimize ACC increase and spread in nurseries and thereby limit the introduction of ACC to new citrus plantings via infected and/or contaminated nursery plant materials.

Materials and methods

Plot design and experimental conditions

At the beginning of June 1994, 16 plots were established at CIRAD experiment station in Bassin Plat, Saint Pierre, Reunion, at an elevation of 150 m (Table 1). Each plot consisted of 240 Mexican lime seedlings in black plastic containers arranged in eight rows of 30 plants per row. Plants were placed 0.2 m apart within and across rows. Plants were examined individually to ensure the absence of visual symptoms of ACC prior to placement in the plots.

Inoculum source plants were prepared by wound inoculating 5% (12 plants per plot) of the nursery plants using a bacterial suspension containing approx. 10^8 cfu/ml, prepared from strain C40S, a virulent spontaneous mutant resistant to streptomycin sulfate (Vernière, 1992). Each inoculated plant had 10 leaf lesions resulting from wound inoculation distributed on five leaves. Inoculated plants were placed in a growth chamber at 30 °C and 95% RH for optimal symptom expression. Lesions were three weeks old when plants were placed in the experimental plots and were either randomly placed in the plot or were grouped upwind depending on the plot design (Table 1). All plots were separated from each other by walls consisting of polyethylene plastic sheets to avoid movement of inoculum between plots. Ten plots were oriented with their rows parallel to the prevailing southeast wind direction. Six plots were oriented perpendicularly to the prevailing winds and the other plots to evaluate the possibility of human spread during disease assessments (Table 1). Plots were subjected to one of three irrigation systems, overhead, mist, or drip irrigation. The three irrigation systems were adjusted such that plants in each plot received equal amounts of water. Each plant received approx. 1.3 l of water three times a week until the end of October and then 1.3 l four times a week there after.

Climatic data including minimum, maximum, and average temperature; minimum, maximum, and average humidity, rainfall, windspeed, and wind direction were recorded hourly during the experiment (Figure 1).

Citrus leaf miner (*Phyllocnistis citrella*) is known to be an ACC-aggravating factor (Goto, 1992). Although it has now been reported in Reunion Island (Quilici et al., 1995), leaf miner was not present in the plots at the time of the experiment.

Assessment of disease

To evaluate the influence of the three irrigation systems on ACC development, the study was conducted during dry, i.e., the non-rainy season. Disease assessments were made weekly from June 26, 1994 until December 5, 1994 on all plants from all plots. The 20 weekly assessment dates were occasionally delayed one to two days if rain occurred. Thus, disease records were always done on dry plants to minimize the probability of human dispersal of the bacteria. For disease severity assessments the following rating scale was used: 0 = healthy plant, 1 = 1 to 10 canker-like lesions/plant, 2 = 11 to 20, 3 = 21 to 30, $n = 10 * (n - 1) + 1$ to $10 * n$ canker-like lesions. The maximal disease severity score recorded during the experiment was 19. Disease incidence was estimated as the proportion of diseased plants per plot.

Reisolation from lesions

At the end of the experiment, 100 lesions from each plot were randomly collected, except for a few plots

Table 1. Plot design and nonlinear exponential regression analysis with fixed y-min. of disease incidence of Asiatic citrus canker in simulated nurseries

Plot	Irrigation system	Orientation	Inoculum	Rate (k)	Standard error of k	Correlation observed vs. predicted[a]
C	Overhead	SE/NW	Grouped	0.0110	0.000325	0.982
N	Overhead	SE/NW	Grouped	0.0110	0.000389	0.962
H	Overhead	SE/NW	Random	0.0110	0.000502	0.955
K	Overhead	SE/NW	Random	0.0110	0.000368	0.973
E	Mist	SE/NW	Grouped	0.0055	0.000579	0.967
M	Mist	SE/NW	Grouped	0.0043	0.000188	0.974
G	Mist	SE/NW	Random	0.0055	0.000288	0.960
L	Mist	SE/NW	Random	0.0055	0.000474	0.958
D	Drip	SE/NW	Grouped	0.0086	0.000214	0.980
A	Drip	SE/NW	Random	0.0055	0.000258	0.906
I	Overhead	NE/SW	Random	0.0110	0.000276	0.986
J	Overhead	NE/SW	Random	0.0110	0.000337	0.986
B	Mist	NE/SW	Random	0.0055	0.000686	0.969
O	Mist	NE/SW	Random	0.0038	0.000261	0.911
F	Drip	NE/SW	Random	0.0055	0.000619	0.970
P	Drip	NE/SW	Random	0.0100	0.000291	0.944

All plots were established with inoculum present from the onset. Therefore, the amount of initial inoculum and the date of first infection were fixed values.
[a]Correlation coefficients (r^{*2}) of predicted values against observed.

in which there were fewer than 100 lesions. In such cases, all lesions were collected. Each lesion was excised from the leaf blade and individually blended in 1 ml of Sigma™ 7–9 pH 7.2 buffer (Sigma Chimie, Saint Quentin Fallavier, France) using a Ultraturax T25 homogenizer (Janke & Kunkel, IKA labortechnik, Germany). Fifty µl of the homogenized suspension were streaked on LPGAT media (yeast extract 7 g, pastone 7 g, glucose 7 g, agar 15 g, distilled water 1000 ml, Tilt™ (propiconazole 500 g/l – Ciba-Geigy, Switzerland 40 µl/l, pH 7.2) and LPGATS media (LPGAT supplemented with 50 mg/l streptomycin sulfate).

Temporal analyses

The temporal evolution of disease incidence in each plot was graphed and graphs were examined visually to determine the shape of the curve and thus which temporal models to test. Linear, exponential, and Gompertz models were first tested by linear regression using the SAS PROC GLM procedure (SAS Institute, Inc., Cary, North Carolina, USA: version 6.04). Because the exponential model was overall the most appropriate model by linear regression, a nonlinear exponential model was fitted to all temporal data sets (SAS NLIN procedure using the DUD option). The appropriateness of the exponential model was verified by examination of the residual plots and by correlation analysis of observed vs. predicted values. Exponential rates of disease increase (k) were compared by t-test to evaluate the influence of irrigation systems on disease development.

Spatial analyses

For spatial analysis the (x, y) spatial location as well as the disease severity value for each plant on each assessment date was used as input data. The strength and directionality or orientation of aggregation among quadrats of various sizes containing *Xac*-positive trees in each plot were examined with spatial autocorrelation analysis using the LCOR2 software (Gottwald et al., 1992c). The program calculated proximity patterns of positively correlated lag positions (SL+), from which the following measurements of spatial patterns were calculated: (i) size and shape of core and reflected clusters of SL+. A core cluster = a group of significant, positively-correlated ($p = 0.05$), spatial lag distance classes that form a discrete and contiguous group with the origin (i.e., lag [0, 0]) of the autocorrelation proximity pattern. A reflected cluster = a discrete group of two or more significant positive lag distance positions discontinuous with the origin and/or core cluster;

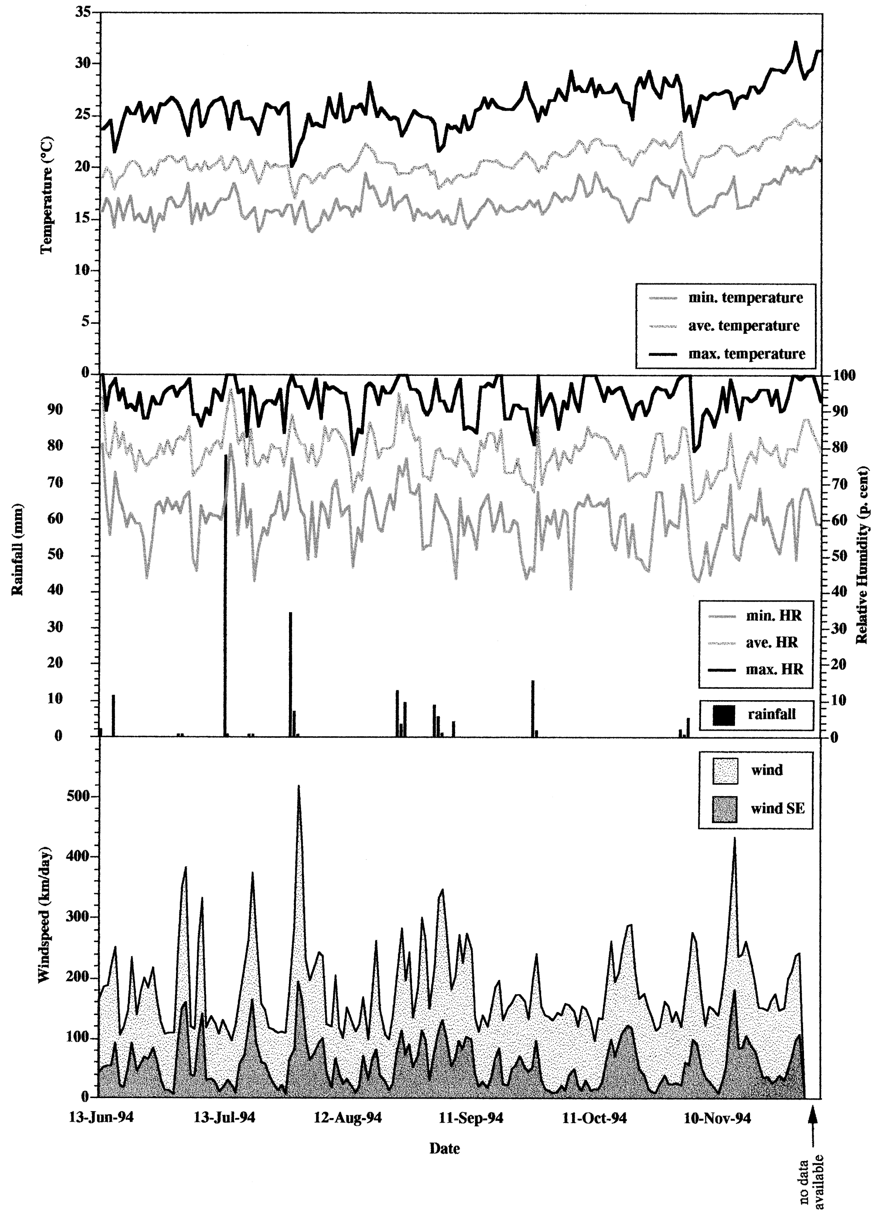

Figure 1. Meteorological conditions at the CIRAD experiment station in Bassin Plat, Saint Pierre, Reunion, at an elevation of 150 m.

(ii) strength of aggregation = a measure of the saturation of the core clusters with significantly positive lags, i.e., the proportion of lag positions within the extents of the cluster that were significantly positive; (iii) row effects = the number of SL+ distance positions within the first row (within) or within the first column (across) of the autocorrelation proximity pattern which are contiguous with the origin; (iv) edge effects = the proportion of the distal most lag positions in the proximity pattern that are SL+; v) strength of non randomness = the proportion of (SL+) + (SL−) positions within the proximity matrix (Gottwald et al., 1992c; Gottwald, 1995).

Spatio-temporal analyses

Semivariograms were used to examine the spatial relationships between *Xac*-diseased plants over time.

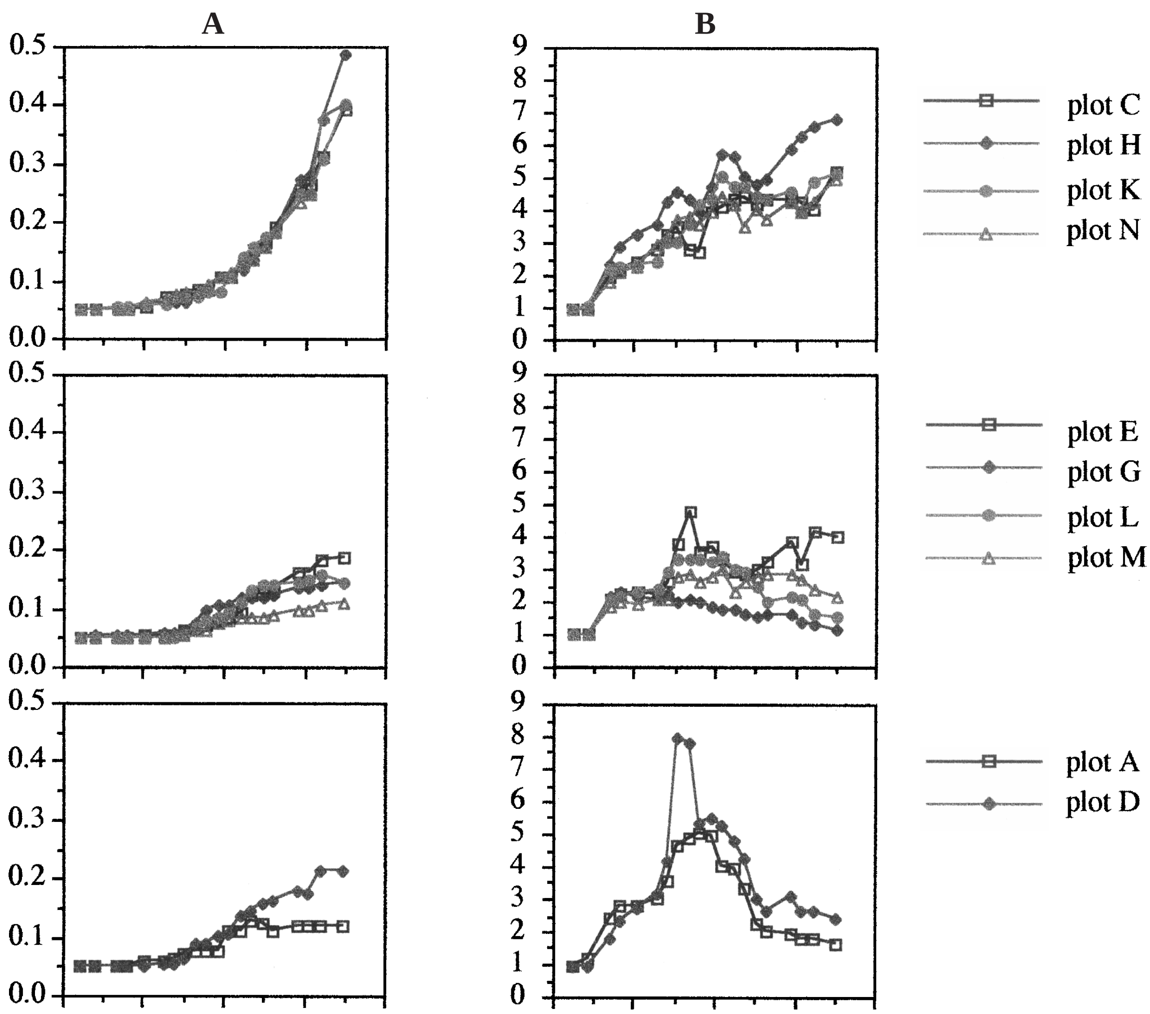

Figure 2. Progress curves for disease over time. A: Temporal evolution of disease incidence (DI); B: Temporal evolution of disease severity (DS) among focal plants.

To accomplish this, individual nursery plants were assigned a temporal value calculated as the number of days infected relative to the final assessment date of the experiment. Thus, a plant was quantitatively weighted more heavily if it became infected earlier as opposed to later in the epidemic. The spatial semivariograms were plotted by considering the spatial distribution of these temporal values. Thus, while traditional semivariance analyses are performed at a discrete point in time, this method takes into account the temporal dynamics of the data as well as its spatial distribution. The analyses were performed using GS + /386 software (release 2.3, Gamma Design Software, Plainwell, MI – USA) for 0° relative to the axis of the rows with a tolerance of ±180°. Thus all data in the matrix are considered relative to each point and the analysis is refered to as omnidirectional. Subsequent analyses were also performed for 0°, 45°, 90°, and 135° relative to the rows in each plot with an angle of tolerance of ± 90°. Adjusted semivariance g(h)/s (where g(h) is the semivariance and s the sample variance) was plotted versus distance. Linear, linear-with-sill, exponential, spherical, and Gaussian transitional models were fitted to adjusted semivariance versus distance by means of a nonlinear regression analysis done by a subroutine of GS+/386. Two groups of models can be distinguished e.g. with or

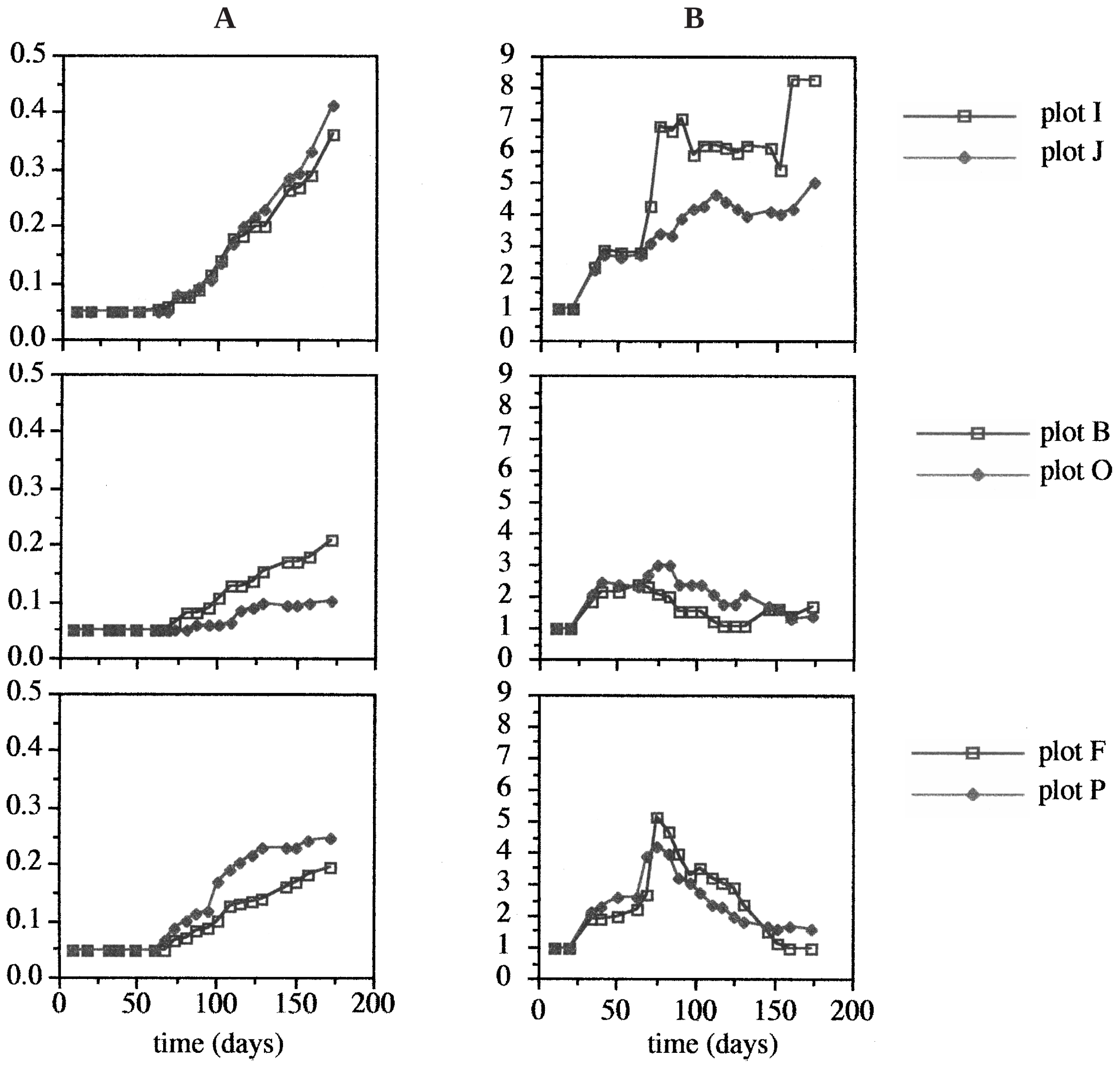

Figure 2. Continued.

without a sill. A linear and horizontal plot indicates a random distribution without any spatial correlation. A linear but not horizontal plot indicates a moderately aggregated distribution. A parabolic shape indicates a strongly aggregated distribution. There are three main parameters in a semivariogram, the localized discontinuity or nugget (C_0), the sill ($C_0 + C$), and the range of spatial dependency (A_0). The localized discontinuity is related to the y-intercept, which is an approximation of the amount of aggregation (the lower the y-intercept, the higher the aggregation). It measures also the amounts of microdistributional and random variation as well as the measurement errors and estimates the proportion of the total variation which is below the sampling scale. The sill is the point along the y-axis where the semivariance no longer increases. The range of spatial dependency is the distance on the x-axis where the sill is reached and measures the spatial dependency among nursery plant samples. Anisotropy can be concluded when directional semivariograms diverge from one another over distance. In that case, it is therefore dependent on the direction of the sample pairs.

Results

Xac strains isolated from lesions within the experimental plots were tested for antibiotic sensitivity. For the majority of plots, 100% of cultures obtained were resistant to streptomycin, and therefore similar to strain C40S which was used for inoculations of the focal plants. Exceptions to this were strains isolated from plots D, H, and K from which 99, 98, and 90% of isolates recovered had streptomycin resistance, respectively. Thus, it was concluded that new infections were predominantly the result of the spread of C40S from inoculated plants. It was not known whether wild-type strains (i) resulted from spread of exogenous inoculum, (ii) were present from the beginning of the experiment as asymptomatic populations, or (iii) resulted from a sub-population of the C40S inoculated strain which had reverted from the induced streptomycin resistant phenotype.

Disease incidence progress curves are shown in Figure 2A. In a preliminary analysis of disease progress data by linear regression, the exponential model was the most appropriate for 12 of 16 plots (data not shown). For the other four plots the Gompertz model was the most appropriate, however, the exponential model also provided acceptable descriptions of these data sets with coefficients of correlation of observed versus predicted values > 0.96 in all cases. Consequently, a nonlinear exponential model was used to further analyze the data (Table 1). Exponential rates of disease increase were calculated and compared via paired t-test. Plots could be separated into groups based on statistical differences among their exponential rates of disease increase (k) (Figure 3). All plots with overhead irrigation were in one group with the highest k value. Generally, there were no distinct differences in k between plots watered by mist or drip irrigation. Interestingly, most plots with drip or mist irrigation were in three groups with low k (*a* to *c*), but two plots with drip irrigation either grouped with some plots with overhead irrigation (plot P – group *e*) or constituted a separate group of intermediate k (plot D – group *d*) (Figure 3). The highest disease incidence values over time for plots D and P were 0.212 and 0.248, respectively, whereas the mean disease incidence value for plots in group *c* was 0.172. The difference between the disease incidence observed in plots D and P vs. the disease incidence in other plots with mist or drip irrigation was not related to plant vigor, which was estimated by measurements of weight and of height of plants from top to collar (data not shown). Furthermore, plots D and P were not neighbored by plots with higher disease levels and, therefore, it is unlikely that they received inoculum from neighboring plots. Therefore, we assume that there was no interplot interference. The spatial position of the inoculated seedlings plants in the plots had no apparent effect on rates of disease increase.

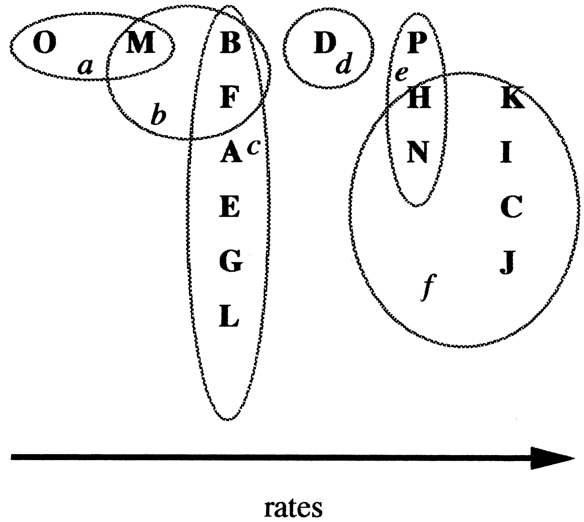

Figure 3. Study plots grouped by exponential rates (k) of disease increase. Plots in the same circle were not statistically different according to paired t test ($p = 0.05$). Italicized letters refer to groups discussed in the text. All plots with overhead irrigation (C, H, I, J, K and N) were in one group with the highest k value. Most plots with drip or mist irrigation were in three groups with low k (*a* to *c*), but two plots with drip irrigation either grouped with some plots with overhead irrigation (plot P – group *e*) or constituted a separate group of intermediate k (plot D – group *d*).

The evolution of disease severity over time on inoculum source plants is presented in Figure 2B. Generally, there was a slight increase of disease severity from the beginning of the experiment until 77–84 days. After this time, there was a defoliation of diseased leaves which resulted in a decrease in overall disease severity. In one case (plot D), a large increase of disease severity was observed from day 77 to 84. Plant material, at a growth stage susceptible to ACC, was recorded on most inoculated plants in this plot two to three weeks prior to the increase in disease severity. This increase of disease severity was followed by a drop of infected leaves one or two weeks after lesions became visible. Disease severity ratings are presented at various times after the experiment started on Table 2. There was no relationship between the irrigation method and disease

Table 2. Disease severity ratings of plots at three dates during the experiment

Irrigation system	Plot	Assessment date		
		August 29	September 5	December 5
overhead	C	3.50 (0.52)[a]	2.83 (0.46)	5.25 (1.08)
"	N	3.75 (0.62)	3.83 (0.44)	5.00 (0.73)
"	H	4.58 (0.75)	4.42 (0.79)	6.83 (1.09)
"	K	3.08 (0.31)	3.58 (0.50)	5.17 (1.13)
"	I	6.75 (0.94)	6.58 (0.75)	8.25 (0.77)
"	J	3.42 (0.47)	3.33 (0.50)	5.00 (1.24)
drip	D	8.00 (1.43)	7.83 (1.31)	2.50 (0.54)
"	A	4.67 (0.58)	4.92 (0.61)	1.67 (0.26)
"	F	5.17 (1.60)	4.67 (1.52)	1.00 (0.17)
"	P	4.25 (1.44)	4.00 (1.46)	1.58 (0.53)
mist	E	3.75 (0.85)	4.75 (0.76)	4.00 (0.97)
"	M	2.83 (0.39)	2.58 (0.42)	2.17 (0.42)
"	G	2.00 (0.43)	2.08 (0.48)	1.17 (0.21)
"	L	3.33 (0.75)	3.33 (0.40)	1.50 (0.26)
"	B	2.08 (0.34)	2.00 (0.30)	1.67 (0.28)
"	O	3.00 (0.49)	3.00 (0.48)	1.42 (0.29)

[a]Disease severity = mean of 12 values. Standard errors are given under brackets.

severity prior to the beginning of disease induced defoliation. At the end of the experiment (Dec. 5, 1994), disease severity ratings in plots with overhead irrigation were higher than ratings in all other plots with either mist or drip irrigation (Table 2).

Aggregation of disease was demonstrated with spatial autocorrelation analysis by the presence of core clusters of significantly positive spatial lags (SL+) (Table 3). For plots with grouped initial inoculum, a greater aggregation of *Xac*-infected plants occurred within row than across rows from the beginning of the trial due to the experimental design. Within-row aggregation remained greater in plots with drip or mist irrigation, due to limited change in disease incidence in these plots, and thus the initial spatial organization of disease in the plots did not change greatly over time. In plots with overhead irrigation, there was a greater increase in disease incidence over time. At the end of the experiment, aggregation of diseased plants within row and across rows was similar (Table 3). Spatial autocorrelation analyses indicated that reflected clusters were few or absent and their size was small, i.e., no more than two SL+, suggesting that relationships among individual foci did not exist or were not strong. The presence of edge effects was rare indicating little or no effect of inoculum exogenous to the plots. The strength of non-randomness was low in all cases indicating that although there were areas of aggregation near the points of inoculum, these composed only a small proportion of the plot.

For most plots with initial inoculum randomly placed, aggregation of diseased plants within row and across rows was similar for all assessment dates, with the exception of plot G for assessments after November 21, 1994, for which aggregation of diseased plants within row was greater than across rows. However, for plot G, disease incidence did not increase much (from 0.136 to 0.142) between this date and the previous assessment date. The similarity in aggregation of diseased plants within rows versus across rows for all NE/SW oriented plots with drip or mist irrigation, indicated that directionality of dispersal, when it occurred, was probably associated with natural environmental events and could not be explained by human-induced spread caused during disease assessments.

Parameters of the spatio-temporal semivariograms obtained for each plot are presented in Table 4. Due to the experimental design, plots C, D, E, M, and N were initiated with aggregated placement of inoculum. The shape of the spatio-temporal semivariograms indicated that this initial aggregation was maintained and often accentuated over time. The range of spatial dependency (A_0) for these plots was 2.05, 0.83, 0.97, 0.76, and 1.84 m, respectively (Table 4). The range of spatial dependency was greatest for plots with overhead irrigation (range 0.69–2.06 m, mean = 1.68 m), indicating extensive aggregation over distance which was maintained through time. In contrast, for the rest of the plots the range of spatial dependency ranged from 0.46 to 0.97 m, also indicating aggregation which was maintained through time but was less extensive over distance. Aggregated spatio-temporal patterns were also recorded for all other plots. Semivariance curves were most often best fitted by the spherical model, with the exception of plot O, which had the lowest rate of disease increase, and for which the linear model was superior, and for plot G, for which none of the models used gave an acceptable fit. Due to the experimental design, anisotropy was detected for all plots with grouped initial inoculum. For most other plots, with the exception of plots G and O which both had atypical semivariograms, the absence of anisotropy indicated a lack of directionality in the spread of *Xac.*

Discussion

In many tropical areas where ACC is endemic, infected nursery plants constitute an important source

Table 3. Spatial autocorrelation analysis and associated statistics of selected plots for Asiatic citrus canker

Plot	Irrigation/ inoculation[a]	Date[b]	Disease incidence[c]	Significant lags[d]		Strength of aggregation[e]	Core cluster size[f]	Size of non core[g]	Total number of clusters[h]	Effects[i]			Strength of non-randomness[k]
				SL+	SL−					Within row	Across row	Edge	
A	D/R	26 Jun.	0.050	2	0	0.00	0	—	2	0	0	0.00	—
		4 Aug.	0.059	5	0	0.00	0	2	3	0	0	0.00	—
		22 Aug.	0.063	2	0	0.50	1	—	2	0	0	0.00	0.015
		29 Aug.	0.072	3	0	0.33	1	—	3	0	1	0.00	0.015
		5 Sep.	0.077	4	0	0.25	1	—	4	0	1	0.00	0.015
		26 Sep.	0.109	6	0	0.33	2	—	5	0	1	0.00	0.022
		3 Oct.	0.113	4	0	0.50	2	—	3	0	1	0.00	0.022
		10 Oct.	0.127	7	0	0.43	3	2	4	1	1	0.00	0.030
		17 Oct.	0.122	4	0	0.25	1	2	3	0	1	0.00	0.015
		24 Oct.	0.113	4	0	0.25	1	2	3	0	1	0.00	0.015
		5 Dec.	0.121	5	0	0.40	2	2	3	1	1	0.20	0.022
D	D/G	26 Jun.	0.050	8	0	1.00	8	—	1	4	1	0.00	0.067
		16 Aug.	0.054	7	0	1.00	7	—	1	4	1	0.00	0.059
		29 Aug.	0.063	7	0	1.00	7	—	1	4	1	0.00	0.059
		5 Sep.	0.090	8	0	1.00	8	—	1	4	1	0.00	0.067
		19 Sep.	0.104	9	0	1.00	9	—	1	4	1	0.00	0.074
		26 Sep.	0.108	9	0	1.00	9	—	1	4	1	0.00	0.074
		3 Oct.	0.135	10	0	1.00	10	—	1	5	1	0.00	0.081
		10 Oct.	0.144	10	0	1.00	10	—	1	5	1	0.00	0.081
		17 Oct.	0.158	10	0	1.00	10	—	1	5	1	0.00	0.081
		24 Oct.	0.162	10	0	1.00	10	—	1	5	1	0.00	0.081
		7 Nov.	0.181	11	3	1.00	11	—	1	4	2	0.00	0.089
		14 Nov.	0.176	12	11	1.00	12	—	1	5	2	0.00	0.096
		5 Dec.	0.212	14	22	1.00	14	—	1	5	2	0.00	0.111
P	D/R	26 Jun.	0.050	2	0	0.00	0	—	2	0	0	0.00	—
		22 Aug.	0.068	4	0	0.00	0	—	4	0	0	0.00	—
		29 Aug.	0.090	4	0	0.00	0	—	4	0	0	0.25	—
		5 Sep.	0.103	3	0	0.00	0	—	3	0	0	0.00	—
		12 Sep.	0.116	3	0	0.00	0	—	3	0	0	0.00	—
		19 Sep.	0.120	2	0	0.00	0	—	2	0	0	0.00	—
		26 Sep.	0.173	3	0	0.67	2	—	2	1	1	0.00	0.022
		3 Oct.	0.191	4	0	0.50	2	—	3	1	1	0.00	0.022
		10 Oct.	0.204	6	0	0.67	4	—	3	1	3	0.00	0.037
		17 Oct.	0.217	8	0	0.50	4	—	5	2	1	0.00	0.037
		24 Oct.	0.230	5	0	0.80	4	—	2	2	1	0.00	0.037
		21 Nov.	0.244	3	0	1.00	3	—	1	1	1	0.00	0.030
		5 Déc.	0.248	5	0	0.80	4	—	2	1	1	0.00	0.037

H	O/R	26 Jun.	0.050	5	0	0.00	0	—	5	0	0	0.00	—
		25 Jul.	0.054	6	0	0.00	0	—	6	0	0	0.00	—
		4 Aug.	0.059	6	0	0.19	1	—	6	0	0	0.00	0.015
		16 Aug.	0.063	7	0	0.14	1	—	7	0	0	0.00	0.015
		5 Sep.	0.077	6	0	0.19	1	—	6	1	0	0.00	0.015
		12 Sep.	0.090	6	0	0.19	1	2	5	1	0	0.00	0.015
		19 Sep.	0.108	4	0	0.25	1	2	3	1	0	0.00	0.015
		26 Sep.	0.112	4	0	0.25	1	2	3	1	0	0.00	0.015
		3 Oct.	0.121	3	0	0.33	1	2	2	1	0	0.00	0.015
		10 Oct.	0.135	5	0	0.20	1	2	4	1	0	0.00	0.015
		17 Oct.	0.157	4	0	0.25	1	2	3	1	0	0.00	0.015
		24 Oct.	0.184	3	0	0.33	1	—	3	1	0	0.00	0.015
		7 Nov.	0.273	4	0	0.75	3	—	2	1	1	0.00	0.030
		14 Nov.	0.282	5	0	0.60	3	—	3	1	1	0.00	0.030
		21 Nov.	0.376	6	0	0.50	3	2	3	1	1	0.00	0.030
		5 Dec.	0.488	8	0	0.38	3	2	4	1	1	0.00	0.030
L	M/R	26 Jun.	0.050	4	0	0.00	0	2	3	0	0	0.00	—
		29 Aug.	0.055	4	0	0.00	0	—	4	0	0	0.25	—
		5 Sep.	0.068	2	0	0.00	0	—	2	0	0	0.00	—
		12 Sep.	0.082	2	0	0.00	0	—	2	0	0	0.00	—
		19 Sep.	0.087	2	0	0.00	0	—	2	0	0	0.00	—
		26 Sep.	0.096	2	0	0.00	0	—	2	0	0	0.00	—
		3 Oct.	0.109	2	0	0.00	0	—	2	0	0	0.00	—
		10 Oct.	0.132	3	0	0.00	0	—	3	0	0	0.00	—
		17 Oct.	0.141	2	0	0.00	0	—	2	0	0	0.00	—
		7 Nov.	0.146	1	0	0.00	0	—	1	0	0	0.00	—
		14 Nov.	0.150	2	0	1.00	2	—	1	1	1	0.00	0.022
		21 Nov.	0.160	3	0	0.67	2	—	2	1	1	0.00	0.022
		5 Dec.	0.146	2	0	1.00	2	—	1	1	1	0.00	0.022

[a] For Irrigation: D = drip, M = mist, and O = overhead. For inoculation procedure: R = random and G = group.
[b] Assessment dates are included only if there was a change in disease incidence form the previous assessment date.
[c] Disease incidence = the number of *Xac*-positive trees divided by the total number of trees per nursery plot.
[d] Significant lags = the number of $[x, y]$ lag distance positions within the proximity pattern with autocorrelations significantly greater (SL+) or significantly less (SL−) than expected.
[e] Strength of aggregation = an estimate of the density of the core cluster, calculated as the proportion of SL+ within the area circumscribed by the outer row and outer column of the core cluster within the proximity pattern.
[f] core cluster size = the number of SL+ contiguous with the origin of the proximity matrix that form a discrete group.
[g] Size of non core clusters = the number of SL+ that form a discrete group within the proximity matrix but which are discontiguous with the core cluster.
[h] Total number of clusters = the number of discrete groups of SL+ within the proximity pattern that are discontinuous with the core cluster.
[i] Row effects = the number of SL+ distance positions within the first row (within) or within the first column (across) of the autocorrelation proximity pattern which are contiguous with the origin. Edge effects = the proportion of the distal most lag positions in the proximity pattern that are SL+.
[j] Strength of nonrandomness = the proportion of (SL+) + (SL−) positions within the proximity matrix.

Table 4. Spatio-temporal semivariance analysis and associated statistics for Asiatic citrus canker

Plot	Irrigation/ inoculation[a]	Best model[b] $- r^2$	Localized discontinuity[c] (C_0)	Sill[d] ($C + C_0$)	Range of spatial dependency[e] (A_0)
C	O/G	$S - 0.793$	$1 \cdot 10^{-3}$	1.35	2.05
N	O/G	$S - 0.860$	$1 \cdot 10^{-3}$	1.34	1.84
H	O/R	$S - 0.818$	0.43	1.03	0.76
K	O/R	$S - 0.793$	0.63	1.13	2.06
I	O/R	$S - 0.908$	0.35	0.99	0.87
J	O/R	$S - 0.523$	0.25	1.01	0.69
E	M/G	$S - 0.798$	$1 \cdot 10^{-3}$	1.22	0.97
M	M/G	$S - 0.728$	$1 \cdot 10^{-3}$	1.19	0.76
G	M/R	no fit	—	—	—
L	M/R	$S - 0.414$	0.41	1.01	0.52
B	M/R	$S - 0.349$	0.49	1.02	0.46
O	M/R	$L - 0.945$	0.48	—	—
D	D/G	$S - 0.845$	$1 \cdot 10^{-3}$	1.17	0.86
A	D/R	$S - 0.530$	0.33	0.97	0.62
F	D/R	$S - 0.256$	0.30	1.00	0.47
P	D/R	$S - 0.441$	0.50	1.00	0.66

[a] For Irrigation: D = drip, M = mist, and O = overhead. For inoculation procedure: R = random and G = group.
[b] Transitional models were fitted to adjusted semivariance $\gamma(h)$/s vs. distance by means of a nonlinear regression analysis. S = spherical; L = linear. r^2 = coffecient of regression.
[c] *y*-intercept, an indicator of aggregation (the lower the *y*-intercept, the higher the aggregation).
[d] Sill = the point along the *y*-axis where the semivariance no longer increases.
[e] Range of spatial dependency (RSD) = distance from the origin to the point on the *x*-axis where the sill is reached. It measures the spatial dependency among samples.

of primary inoculum for new citrus groves (Civerolo, 1984). Wind-driven rains generated by climatic events, such as rainstorms, tropical storms, and hurricanes, are highly efficient means for the spread of *Xac* in nurseries (Serizawa et al., 1969; Gottwald et al., 1989; Gottwald et al., 1992b). However, based on data collected in Argentina nurseries, there was a year-round, low, and regular increase of incidence and severity of the disease (Gottwald et al., 1988; Gottwald et al., 1989; Gottwald et al., 1992b). This was explained by short distance water splash of *Xac* due to overhead irrigation or gentle rains when associated with high densities of plants and constant availability of *Xac*-susceptible plant tissue. It is likely that some cultural practices in the nursery such as overhead irrigation favors splash dispersal of *Xac*. Epidemiological studies on *Xac* have been predominantly conducted in Argentina and Japan (Goto, 1992; Stall et al., 1993), and because climatic conditions prevailing in these countries, especially during winter, are appreciably different of those in tropical countries, there is a need for quantitative epidemiological studies of this disease in tropical areas (Civerolo, 1994).

The influence of three irrigation systems on the progress and spread of ACC in simulated nurseries was examined. The initial development of ACC in all plots was low, indicating that there is a requirement for inoculum build-up prior to extensive spread of *Xac*. The most appropriate general model for disease increase of ACC in the plots studied was the exponential model. Disease levels in many of these plots did not reach an asymptote during the period when the experiment was performed. Therefore, the logistic and Gompertz models, which are asymptotic, were not needed. The experiments were conducted over a 6 month-period, typical of the length of time citrus plants remain in local nurseries prior to use for new grove establishment. The steady increase of disease incidence in the plots and lack of an asymptote during this period can be explained by the continuous availability of growth flushes which are susceptible to ACC and a regular redistribution of *Xac* associated with overhead irrigation. For overhead irrigation plots, the results of the present study are consistent with those obtained previously in Argentina (Gottwald et al., 1989).

Exponential rates of disease increase were statistically higher in plots with overhead irrigation than in other plots, the only exception being plot P with drip irrigation, which was not different from two out of six plots with overhead irrigation. Also, the higher exponential rate of disease increase, *k*, in overhead-irrigated plots was not related to a difference of the vigor of plants in these plots. Drip or mist irrigation limits the development of ACC compared to overhead irrigation which causes splash of inoculum. Rates of disease increase, *k*, were 0.011/day for overhead irrigated plots in the present study, slightly less than, but consistent with, those obtained in Argentina for sweet orange (0.0158), grapefruit (0.0166), and Swingle citrumelo (0.0147) overhead irrigated nurseries (Gottwald et al., 1989).

Spatial and spatio-temporal analyses indicated aggregated patterns of ACC associated with all irrigation methods. These aggregated patterns were the result of foci of disease established at the beginning of the experiment and were maintained and enlarged throughout the duration of the experiment. This is consistent with previously published studies with ACC in nurseries in Argentina (Gottwald et al., 1989; Gottwald et al., 1992b). No hurricanes, tropical storms or rainstorms with winds, occurred during the experiment. Therefore, it is likely that the spread of *Xac* was mainly related to splash dissemination of inoculum by overhead irrigation and/or natural rainfalls. In previous studies, water splash spread of *Xac* in nurseries has been shown to lack directionality (Gottwald et al., 1989). For the present study, in most cases, the shape of core clusters found in spatial autocorrelation analysis and the absence of anisotropy as determined by semivariance analysis, indicated the lack of directionality of the spread of *Xac* during the experiment, consistent with splash dispersal of inoculum. Anisotropy was indicated only in plots with grouped initial inoculum and, in such cases, was regarded as due to the experimental design. Spatial autocorrelation also failed to detect significant edge effects, which was interpreted to indicate that inoculum was predominantly contributed from the foci within the plot and a general lack of intraplot interference. The maintenance and expansion of existing foci was further substantiated by the paucity of noncore clusters of significant size. This was also indicative of limited disease gradients that remained tightly associated with the initial foci.

Incubation period is known to be dependent on temperature, growth stage of plant material and amount of inoculum available (Civerolo, 1984). Koizumi (1976) conducted spray inoculations of several citrus cultivars at a susceptible growth stage with a suspension of $\sim 2 \cdot 10^8$ *Xac* cfu/ml. Inoculated plants that were kept in a growth chamber at a constant 21 °C or in a greenhouse whose mean temperature was approx. 20 °C developed canker lesions 17–21 days after inoculation whatever the host genotype. During the present study, major increases in disease over time in drip irrigation plots were noted at 77, 105, and 112 days. Since drip irrigation does not cause foliar wetting, it is hypothesized that those increases in disease incidence were related to natural rainfalls. Meteorological events which could be related to the observed higher exponential rates of disease increase are listed in Table 5. Three significant rainfalls occurred which were followed by increases in disease incidence. Mean temperatures during the month following these rainfall events were around 20 °C. The post-rain-event latent period was approx. 30 days, which is slightly longer than what was expected. Rains of high intensity are known to favor splash dispersal of plant pathogens (Madden, 1997). Rains of short length are the most efficient ones for dispersal of *Xac* (Serizawa, 1981). Rainfalls occurring on June 16 and July 13 did not lead to any increase of disease incidence, probably because they occurred early in the experiment, and insufficient inoculum had built-up prior to these rains for spread of *Xac* to have occurred. It is also possible that rains on July 13 induced a washing off of inoculum (Table 5).

Traditionally, nurserymen in Reunion Island grow citrus in polyethylene plastic bags and water by overhead irrigation. Infected nursery plants are believed to be the main source of primary inoculum in newly established citrus plantings (Vernière, 1992). Thus, control of ACC in nurseries would potentially reduce initial inoculum and thereby improve disease control in new planting situations. Based on the results of the present study, less ACC would develop with alternative irrigation practices such as drip or mist irrigation. Alternative irrigation practices integrated into a modern scheme with other cultural practices should minimize increase of ACC and spread within Reunion Island citrus nurseries and thus reduce the initial inoculum transferred to new groves.

Table 5. Occurrences of high intensity rains during the experiment in relation to potential spread of *Xac* in drip irrigation plots

Date[a]	Related meteorological event	Rain intensity (mm)	Rain duration (min)	Estimated latent period (days)	Mean temp. (°C)[c]	Max. temp. (°C)[c]	Min. temp. (°C)[c]
Aug. 29	Jul. 29 – 9 am	12	35	30	19.9	27.6	14.3
Sep. 26	Aug. 24 – 7 pm	9	15	31	19.8	28.0	14.5
Oct. 3	Sep. 2 – 7 pm	9	10	29	20.1	28.0	14.5
	Jun. 16 – 9 pm[b]	9	40				
	Jul. 13 – 6/10 pm[b]	62	420				

[a] Date when higher rate of disease increase was observed on.
[b] Meteorological events whose characteristics potentially could have caused a spread of *Xac* but which did not contribute to an increase in disease.
[c] Temperatures during the latent period.

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