Temporal and Spatial Spread of Citrus Canker Within Groves

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ABSTRACT

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The spread of citrus canker, which is caused by *Xanthomonas campestris* pv. *citri*, was studied in Argentina. Disease gradients were obtained by plotting the proportion of diseased trees in individual rows or subplots of grouped rows versus distances from inoculum sources. The Gompertz transformation was used to linearize the gradients when plotted versus $\log_{10}$ (distance). In the regression equation for the linearized disease gradients, the slope b ranged from -0.2 to -4.13. Incidence of disease increased faster near inoculum sources; thus, disease gradients became steeper with time. The increasing steepness of the gradients was evident 40 mo after an inoculum source had been eradicated. Slope values were approximately the same for four scion-rootstock combinations at a given value of the *Y*-intercept (a). However, a and b increased slower in time on a resistant scion-resistant rootstock combination. Foci were detected by doublet analysis. Primary spread of canker resulted in a distribution of diseased trees that was sparse, but with a gradient. Diseased trees were not aggregated in the early spread. The later secondary spread was limited, and the incidence of disease increased faster near the inoculum sources. Aggregation of diseased trees then occurred.

Additional key words: *Citrus deliciosa, C. limon, C. sinensis, C. unshiu,* epidemiology, *Poncirus trifoliata.*

The production of citrus throughout the world is threatened by citrus canker, which is caused by *Xanthomonas campestris* pv. *citri* (Hasse) Dye. Symptoms of citrus canker are craterlike lesions which are surrounded by a yellow halo on stems, leaves, and fruit. The disease causes reduction of photosynthetic leaf area, defoliation, depreciation of fruit quality, and fruit drop (9).

Three strains (A, B, and C) of the pathogen have been described (12). The A-strain is the most virulent, has the widest host range, and is spreading fastest. In South America, canker caused by the A-strain was first observed in 1957 in Brazil (4), in 1967 in Paraguay (13), and in 1972 in Argentina (6).

The increase of citrus canker in a grove is not well understood. It was possible to study the spread of this disease in initial epidemic stages (at less than 5% incidence) because of the recent introduction of the A-strain of the citrus canker organism into Argentina. The advance of citrus canker in time and distance from known inoculum sources was followed without the interpretative problems associated with an endemic disease.

MATERIALS AND METHODS

Groves. The spread of citrus canker was studied in two citrus groves in the same citrus-growing region in Argentina. One grove was situated in the southeastern portion of Corrientes province, Departamento Juan Pujol. Trees in the monitored plots were planted in 1974 at a spacing of 7×7 m. All trees in adjacent plots of grapefruit and Navel orange were eradicated in December 1978 and June 1979, respectively, because of severe canker. Lemon trees in a neighboring grove to the north were heavily diseased throughout the experiment. The trees of grapefruit, Navel orange, and lemon were suspected as sources of canker-causing bacteria for the monitored plots. Three plots in the Corrientes grove were selected for intensive study (Fig. 1, Table 1). The scion types in each plot were either Navel (*Citrus sinensis* Osbeck), Satsuma (*C. unshiu* Markovitch), or Comun mandarin (*C. deliciosa* Ten.). All trees were on trifoliate (*Poncirus trifoliata* (L.) Raf.) rootstock.

The second grove was located in Entre Rios province, Departamento Federación, Colonia Ensanche. This grove was located about 70 km south of the Corrientes grove and more than 10 km from any other commercial citrus plantations. The grove was surrounded by commercial plantations of pines and eucalyptus. The citrus trees were planted in 1971 at a spacing of 8×4 m. Within this grove two plots of grapefruit trees (Fig. 2) were considered as possible sources of canker-causing bacteria for the monitored

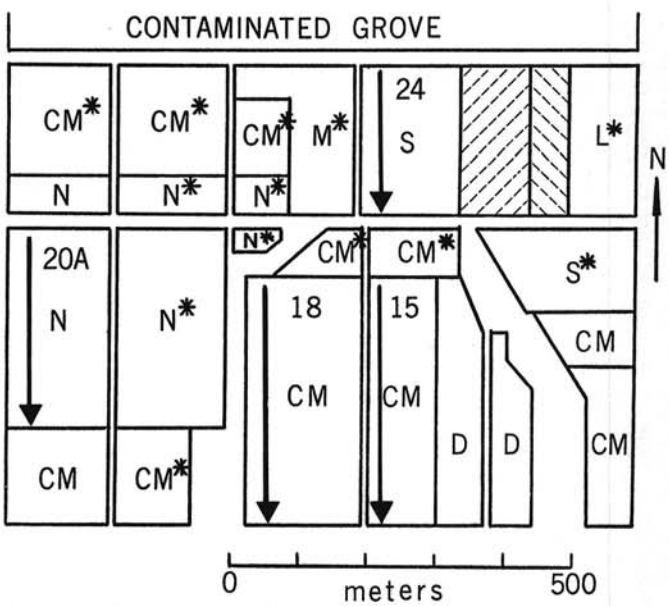

Fig. 1. Map of a citrus grove in Corrientes, Argentina, where canker spread was studied. The scion types in the plots were Comun mandarin (CM), Navel (N), Murcott (M), Satsuma (S), Dancy (D), and lime (L). Trees in all plots were on trifoliate rootstock. An asterisk identifies plots in which citrus canker was found in February 1979. All trees in a grapefruit plot ( ) and in a Navel orange plot ( ) were eradicated in December 1978 and in June 1979, respectively, because of severe canker. Spread of citrus canker was studied in plots 20A, 18, 15, and 24. The arrow marks the direction of the dominant gradient. The grove was surrounded by other citrus groves. Citrus canker was present in the contaminated neighboring grove on the north side throughout the experiment.

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plots. In one of the two grapefruit plots, canker lesions were found in 15% of the trees by December 1977 and in 17% of the trees in the other plot by May 1978. All trees in both plots were destroyed by burning soon after the discovery of canker. Contaminated packing boxes were suspected as the initial source of bacteria for the grove. Two plots in the Entre Rios grove were selected for intensive study (Fig. 2, Table 1). Trees in one plot were Valencia (*C. sinensis* Osbeck) scion on rough lemon (*Citrus limon* Burm.) rootstock, those in the other plot were Valencia scion on trifoliate rootstock. Four surveys for diseased trees had been made prior to this study. During those four surveys, the trees found to have canker lesions were eradicated.

Surveys. Four additional surveys were made between April 1979 and April 1980 in the Corrientes plots, and five surveys were made between May 1979 and April 1981 in the Entre Rios plots. The trees were less than 2.5 m high; this facilitated thorough inspection for canker lesions. The inspectors tried to restrict the spread of the disease with sanitation. Green branches with canker lesions were removed and placed in plastic sacks in which they were burned at convenient sites. Entire trees were not removed from any of the five plots after May 1978.

If a citrus canker lesion was found, the tree was considered diseased from that time onward and its location was noted on a map of the plot. The proportion of disease incidence (y), ie, the number of diseased trees divided by the total number of trees in a particular plot or row, was calculated.

Transformation. Citrus canker was assumed to be a compound interest disease, sensu Vanderplank (15). As such, linearization of the proportion of disease incidence versus time or versus distance from the inoculum source would ease epidemic analyses. Because the curve of the increase of citrus canker in time was asymmetrically sigmoidal (6), the logistic transformation did not provide the best statistical fit of the values. The Gompertz transformation (2) was used instead. The proportion of canker incidence was transformed by $Y = -\ln(-\ln(y))$ and plotted versus time or versus $\log_{10}$ (distance). A straight line was fitted through the gompit values (Y) by linear regression. The slope (k) of the regression obtained for Y versus time was the average epidemic rate. The slope (b) of the regression line obtained for Y versus $\log_{10}$ (distance) was the average decrease of disease incidence (transformed) in distance from the inoculum source.

Disease gradients were calculated from the transformed proportion of canker incidence in each row of citrus trees parallel to the suspected inoculum source versus $\log_{10}$ (distance) of the row to that source.

Doublet analysis. Aggregation of diseased trees was determined by doublet analysis (14) for each of the plots for each survey. The number of doublets expected to occur by chance was calculated by: $d_1 = \mu(\mu-1)/n$ (14) and by $d_2 = (n-r) \times d_1/(n-1)$ (5) in which d = the number of expected doublets, n = the total number of trees, r = the number of rows in the direction in which the doublets were counted, μ = the number of diseased trees, and $(n-r)/(n-1)$ = the correction factor for the lack of continuity between the parallel rows when concatenated to a single row. Aggregation of diseased trees was assumed if the number of observed doublets exceeded $d_2 + 2\sqrt{(d_2)}$ (5,14).

RESULTS

Disease incidence and gradients. Initially, the proportion of disease incidence in the plots was low ($0.008 < y < 0.037$). The number of diseased trees decreased with distance from certain plot borders, ie, gradients existed. The gradients were always away from neighboring plots with more disease when these neighboring plots were located either north or south of the plot (Figs. 1 and 2). In both groves, rain storms with wind occur primarily from the north or

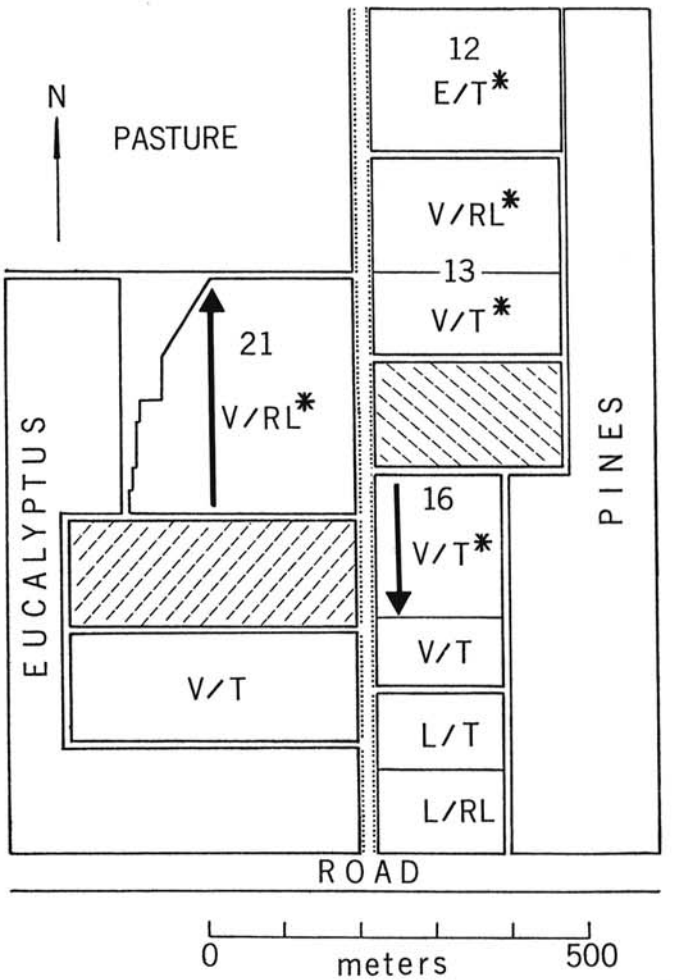
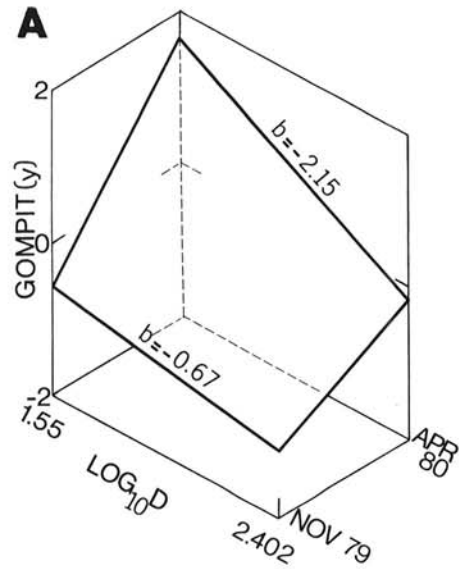


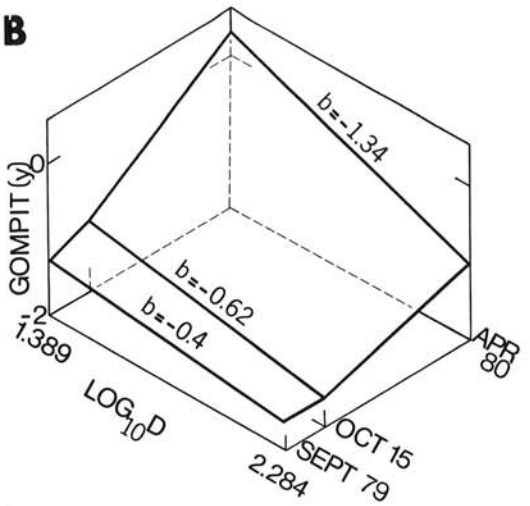
Fig. 2. Map of a citrus grove in Entre Rios, Argentina, where spread of canker was studied. The scion types in the plots were Valencia orange (V), Ellendale (E), and lemon (L). Rootstocks were rough lemon (RL) and trifoliate (T). An asterisk identifies plots in which citrus canker was found in May 1978. Two contaminated grapefruit plots were eradicated in December 1977 ( ) and in May 1978 ( ). Spread of citrus canker was studied in plots 21 and 16. The arrow marks the direction of the dominant gradient. On each side of the central road in the grove a single row of pine trees was planted in 1971 (·····). There were commercial plantations of eucalyptus at the south and west of the grove; and of pines at the east side.

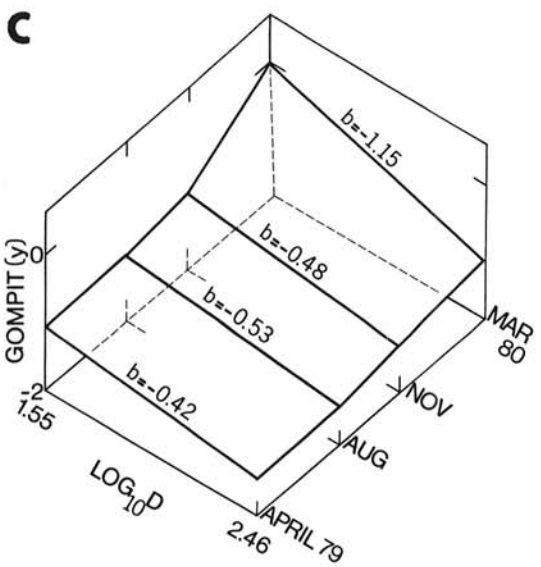
TABLE 1. Characterization of plots in two Argentinian citrus groves used to study spread of citrus canker

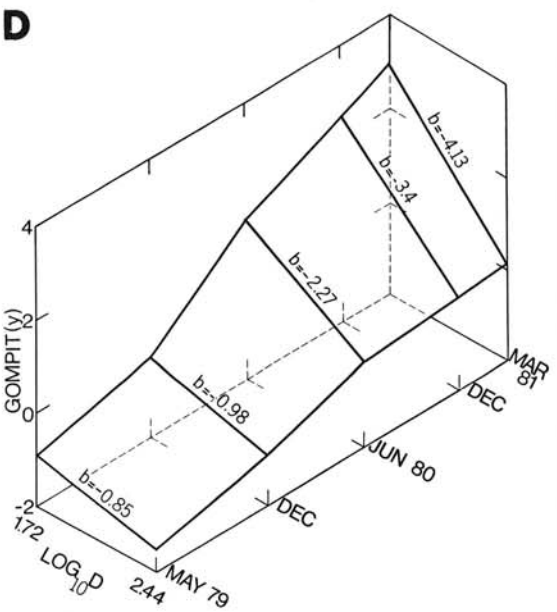
Province	Plot	Scion-rootstock combination[a]	Number of rows[b]	Tree spacing (m)		Distance of last row (m)[c]	Tree population
				N-S	E-W		
Corrientes	20A	Navel/T	42	7	7	291	966
	24	Satsuma/T	30	7	7	221	606
	15 & 18	C. mandarin/T	45	7	7	312	1,756
Entre Rios	21	Valencia/RL	36	8	4	308	2,634
	16	Valencia/T	24	8	4	188	922

[a] Rootstocks were trifoliate (T) and rough lemon (RL).
[b] Rows parallel to the inoculum source.
[c] Distance from the inoculum source.









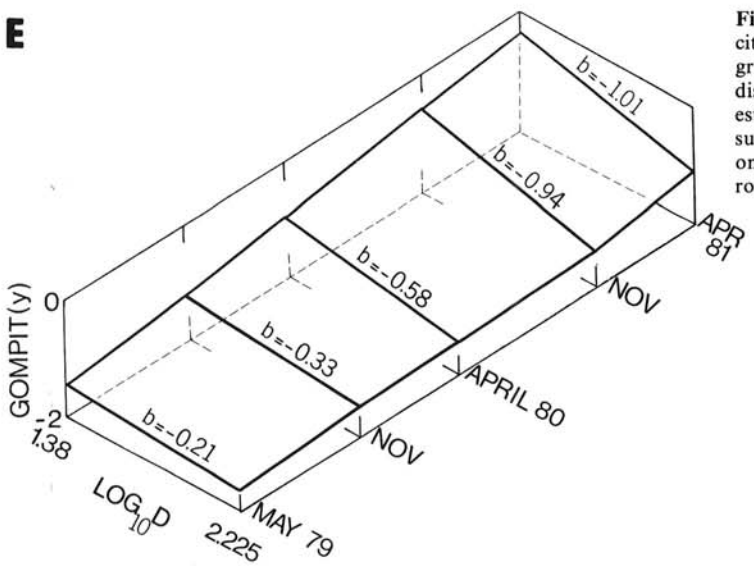


Fig. 3. Response surfaces obtained from the transformed proportion (y) of citrus canker incidence ($Y = -\ln(-\ln(y))$) in each of four subplots of grouped rows parallel to the inoculum source. Each subplot was at a median distance D from that source. The slope b of the regression line was an estimate of the average rate of disease decrease with distance. Response surfaces are for: **A,** Navel; **B,** Satsuma; or **C,** Comun mandarin scions (all on trifoliate rootstock); and for Valencia scions on **D,** rough lemon rootstock or **E,** trifoliate rootstock.

south.

During the early stages of the epidemic, in many rows there were no diseased trees. Because the logarithm of zero cannot be taken, the transformed disease proportions (y) for rows with no diseased trees could not be regressed on $\log_{10}$(distance). The problem of zero values of disease incidence in individual rows was overcome by assigning contiguous rows into subplots. In each subplot there were at least six rows parallel to the inoculum source. With this larger population of trees, there was more likelihood that one or more trees would be diseased. Zero values of y were thus avoided, and disease gradients could be calculated for most plots in all surveys.

Disease gradients in time. The change of the disease gradient in time for each of five plots was graphed as a response surface (Fig. 3). The incidence of citrus canker increased faster in time near the inoculum source in all five plots; therefore, the disease gradients became steeper with time. The incidence of canker close to the inoculum source increased faster for Navel (Fig. 3A) than for Satsuma (Fig. 3B) or for Comun mandarin (Fig. 3C); and faster for Valencia scion on rough lemon rootstock (Fig. 3D) than for Valencia scion on trifoliate rootstock (Fig. 3E).

The slopes (b) of the disease gradients were plotted versus time and regressions for the changes of gradients over time were obtained. The slopes of disease gradients increased over time for all plots. The steepness of the disease gradients for Navel increased twice as fast as those for Satsuma, and three times as fast as those for Comun mandarin. The change in gradients with time was four times faster for Valencia scion on rough lemon rootstock than for Valencia scion on trifoliate rootstock. Thus, the steepness of disease gradients increased in time faster on the susceptible scion type or on the susceptible citrus combination of scion and rootstock.

The correlation of Y-intercept (a) versus the slope (b) for the linearized disease gradients was determined in four plots. Values for Navel were not included because gradients were available from only two of the three surveys ($y < 0.007$ for first survey). For the other four citrus combinations, the values of b were highly correlated ($P > 0.01$) with the values of a (Table 2). Therefore, if the incidence (a) of canker at 1 m from the inoculum source was known, the gradient b could be predicted. Representative gradients for selected values of a on each citrus combination are given in Table 2. Approximately the same slopes of disease gradients were obtained for all four citrus combinations at a given value of a.

Doublet analysis. A range of disease proportions at which aggregation occurred was identified in each subplot for each survey. Aggregation of diseased trees was found when the disease proportion (y) in the subplot was ~0.31 on Navel, from 0.27 to 0.43 on Satsuma, and from 0.08 to 0.58 on Valencia scion on rough lemon rootstock. For Comun mandarin and for Valencia scion on trifoliate rootstock, aggregation of diseased trees occurred only in a few subplots. The disease proportion in the subplots in which aggregation occurred was always at $y > 0.07$. In the Valencia plots, there was more aggregation of diseased trees in the direction in which trees were spaced 4 m apart compared to the direction in which trees were 8 m apart.

DISCUSSION

The spread of citrus canker based on incidence of diseased trees was quantitatively determined in time and distance with large populations of trees. Early in the epidemic, gradients were observed, although diseased trees were sparsely distributed in the grove and some diseased trees were distant (>300 m) from the suspected source. Diseased trees were not aggregated in these early surveys. Thus, disease found in the first surveys was most likely caused by primary spread (disease caused by inoculum from an external initial source).

In subsequent surveys, the slopes for disease gradients became steeper and diseased trees tended to be aggregated. When the linearized disease gradients were steeper than approximately $b = -0.7$, secondary spread (disease caused by inoculum produced within a plot) seemed to become dominant over primary spread.

Disease gradients obtained for incidence of citrus canker became steeper with time. This change was contradictory to all previous reports. Gregory (7) provided examples showing that disease gradients commonly flatten in time, presumably from secondary spread of the pathogen and from background contamination. However, MacKenzie (11) did not observe a flattening of disease gradients for stem rust of wheat even when disease severity exceeded 50%. Berger and Luke (3) reported that when gradients were plotted with Gregory's model ($\log_{10}[y]$ versus $\log_{10}$[distance]), the slopes would flatten in time because $\log_{10}(y)$ did not linearize sigmoidal disease progress curves. Thus, alternate transformations were proposed.

The increase in steepness of gradients over time may have been from the earlier dominance of secondary spread over primary spread on the trees in rows that were proximal to the initial inoculum source. Trees at considerable distance (>300 m) from the source were infected in the primary spread. These distant, infected trees would result in a flat gradient. Secondary spread would be mostly to neighboring trees which results in a steep gradient. The resulting gradient for the plot was then an average of the primary and secondary gradient, with the latter becoming dominant as the epidemic advanced.

In some plots, *X. campestris* pv. *citri* was most likely disseminated from neighboring plots to the monitored plots during the years of the surveys. This influx may have contributed to the increase in steepness of the gradients in time, but the effect could not be partitioned in our analyses. However, for two plots, the suspected sources were eradicated 1 yr before the survey was begun. In these latter plots, the gradients still became steeper up to 40 mo after the eradication of the initial inoculum source.

X. campestris pv. *citri* may be a 'resident' (8) for a long time on citrus trees in a symptomless association. In Florida, trees sometimes did not develop canker lesions until 2 yr after they were removed from contaminated nurseries (1). The disease that would eventually occur from resident bacteria could also increase the steepness of the gradient since the likelihood of trees having resident bacteria would be greater on trees proximal to the source.

Vanderplank (16, page 135) wrote that "the greater, the horizontal resistance, the lower the infection rate, the steeper the gradient, and the more compact the epidemic's front will be." This means that resistance in the host affects the disease gradients. MacKenzie (11), Berger and Luke (3), and Luke and Berger (10) could not differentiate cultivar resistances by disease gradients. For citrus canker, the gradients (slopes b) were nearly identical among citrus types at any given incidence of disease close to the inoculum source. Therefore, gradients per se were not directly affected by

TABLE 2. Regression values for slopes (b) versus Y-intercepts (a) of the linearized disease gradients of citrus canker in Argentina

Citrus combination[a]	Surveys (no.)	Regression coefficient for b vs. a	R^2	Y-intercepts (a)[b]				
				−1	0	1	2	3
Satsuma/T	3	−0.33	0.98**[c]	−0.33[d]	−0.66	−0.99	−1.32	−1.64
C. mandarin/T	4	−0.34	0.98**	−0.24	−0.57	−0.92	−1.26	−1.60
Valencia/RL	5	−0.34	0.98**	−0.21	−0.55	−0.89	−1.24	−1.58
Valencia/T	5	−0.37	0.98**	−0.25	−0.63	−1.01	−1.38	−1.76

[a] Rootstocks were trifoliate (T) and rough lemon (RL).
[b] The Y-intercept is the transformed proportion (gompit Y) of canker incidence at 1 m from the inoculum source.
[c] **Significant at $P = 0.01$.
[d] Estimates of b for given Y-intercept.

host resistance to canker.

MacKenzie (11) pointed out that the *Y*-intercept (*a*) and not the slope (*b*) of the disease gradient was associated with a reduced rate of spread of *Puccinia graminis* on wheat. MacKenzie made no interpretations as to the source of variations of *a*. It was obvious that, in the linearized disease gradients for citrus canker, the rate of increase of *a* versus time was determined by the rate (*k*) of disease increase in time. The time that *a* reached a certain value was determined by citrus type (6). Thus, the slower increase in slope *b* of the disease gradient on a resistant citrus type compared to a susceptible citrus type was dependent upon the changing levels of *a*, and *a* in turn was determined by rate *k*.

Citrus canker is very difficult to control solely by sanitation. In Argentina, the rate of canker spread was faster in a plot with a greater percentage of diseased, but eradicated, trees than in comparable plots with fewer diseased, but eradicated, trees (6). Even though diseased green branches were removed during each of our surveys, this pruning did not slow the spread of the disease. With the capability of the bacteria for rapid spread in a grove, and this spread coupled with an unnoticeable resident phase, it is not surprising that canker is considered an imminent threat to citrus in the United States (12).

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