GEOSTATISTICAL ANALYSIS OF SPATIAL DYNAMICS OF CITRUS VARIEGATED CHLOROSIS

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ABSTRACT

The spatial dynamics of Citrus Variegated Chlorosis (CVC) was studied in a five-year old commercial orchard of 'Valencia' sweet orange (*Citrus* sp.) trees, located in the northern region of the state of São Paulo, Brazil. One thousand trees were assessed in 25 rows of 40 trees, planted at 8 x 5 m spacing. Disease incidence data were taken beginning in March 1994 and ending in January 1996, at intervals of four to five months. Disease aggregation was observed through the dispersion index analysis ($I\beta$), which was calculated by dividing the area into quadrants. CVC spatial dynamics was examined using semivariogram analysis, which revealed that the disease was aggregated in the field forming foci of 10 to 14 m. For each well-fitted model, a kriging map was created to better visualize the distribution of the disease. The spherical model was the best fit for the data in this study. Kriging maps also revealed that the incidence of CVC increased in periods during which the trees underwent vegetative growth, coinciding with greater expected occurrence of insect vectors of the bacterium in the field.

Additional key words: CVC, *Xylella fastidiosa*, epidemiology.

RESUMO

Análise da dinâmica espacial da Clorose Variegada dos Citros através da Geoestatística

A dinâmica espacial da Clorose Variegada dos Citros (CVC) foi estudada em um pomar comercial de laranja (*Citrus sinensis*) 'Valência' de cinco anos de idade, localizada na região norte do Estado de São Paulo, Brasil. Foram avaliadas 1.000 plantas em uma quadra disposta em 25 ruas por 40 plantas por rua, plantada no espaçamento 8 x 5 m. As avaliações (presença ou ausência da doença) iniciaram-se em março de 1994 e estenderam-se até janeiro de 1996, em um intervalo de quatro a cinco meses entre uma e outra. A agregação da doença foi observada por meio da análise do índice de dispersão ($I\beta$), o qual foi calculado dividindo-se a área em quadrantes. A dinâmica espacial da CVC foi avaliada por análises de semivariogramas e apresentou padrão agregado da doença no campo formando reboleiras de 10 a 14 m. Para cada modelo ajustado, um mapa de krigagem foi gerado para uma melhor visualização da distribuição da doença. O modelo esférico foi o que melhor se ajustou aos dados. Observou-se também por meio dos mapas de krigagem que a incidência da CVC aumentou em períodos onde as plantas se encontravam em desenvolvimento vegetativo, coincidindo com maior ocorrência esperada de insetos vetores da bactéria no campo.

INTRODUCTION

The citrus industry in Brazil currently produces more than one third of the world's oranges (FAO, 1998). Several Brazilian states are engaged in citrus (*Citrus* sp.) production but São Paulo represents approximately 80% of the production and has the majority of the processing plants.

Presently, citrus production in São Paulo state is threatened by the Citrus Variegated Chlorosis (CVC), a bacterial disease that affects primarily sweet orange (*Citrus sinensis* L. Osbeck) cultivars (Lima, 1995). The disease was first observed in Argentina during the early 1980s and soon thereafter, in the Brazilian states of Minas Gerais and São Paulo (Rossetti *et al*., 1998). More recently the disease has been confirmed in several other areas of Brazil (Laranjeira *et al*., 1998). Pioneering studies in the early 1990s found xylem vessels plugged by bacteria (Chagas *et al*., 1992) that could be detected with serological methods (Beretta *et al*., 1991). The xylem limited gram-negative bacterium *Xylella fastidiosa* Wells, was then confirmed to be the causal agent of CVC by Chang *et al*. (1993) and Hartung *et al*. (1994).

The symptoms of CVC are foliar necrotic spots, leaf scorch, stunting of the tree, and reduction of fruit size and yield. Initially, symptoms are present in some branches but the entire canopy displays the disease overtime. The bacterium is spread from plant to plant by grafting with infected buds material and by sharpshooter leafhoppers (Hemiptera: Cicadellidae) (Roberto *et al*., 1996; Lopes *et al*., 1996). Control of CVC, by pruning of symptomatic branches is effective under certain circumstances (Rodas, 1994). This

control measure is normally costly and dependent on bacterium distribution in the tree (Coletta Filho *et al*., 2000) and on vector control.

Biological and environmental factors may affect development of diseases (Berger, 1988). The CVC epidemic develops in response to orchard management. If the disease spread is well characterized, this could aid in better understanding of the pathosystem and its control. Following these principles, Gottwald *et al*. (1993) reported that temporal progress of CVC is well represented by the Gompertz model and is associated with vectors of limited dispersion range. However, this report was based in a single epidemic where evaluations of disease were made once a year.

Later, Laranjeira *et al*. (1998) studied temporal and spatial progress of the disease in eleven blocks of sweet orange that were sampled bimonthly. The authors concluded that the Logistic model best represents the progress of CVC and that spatial distribution is patchy, which is consistent with low mobility and aggregation of the vectors. An important finding of these authors was that the greater and lesser progress of CVC was observed in winter-summer and autumn-winter seasons, respectively. However, statistics used by these authors as the variance/mean ratio and indices such as Taylor and Morisita are based on means and variances. These indices focus on the frequency distribution of samples (most quantify the relationship of the sample variance to the mean) but ignore the spatial location of samples (Farias *et al*., 2002a).

The analysis of spatial dependence among samples requires the use of geostatistics, which was first applied in South Africa by Krige (1951) to mining data. He concluded that it would not be possible to study variances in spatial dependence without considering the distance between samples and therefore applied the "Theory of Regionalized Variables" (Matheron, 1963) to the geological data.

Using geostatistics, it is possible to characterize spatial dependence of parameters and, in the case of CVC, to define the dynamics of the spatial patterns. This provides a means to make inferences regarding disease spread and future orchard management. Once the spatial dependence between observations has been identified by the use of a semivariogram, the process of kriging allows estimation of values for sites not sampled.

Therefore, the objective of this paper was to analyze CVC spatial progress using geostatistics to characterize the dynamics of the disease in a commercial orchard of 'Valencia' sweet orange in Northern São Paulo, Brazil.

MATERIAL AND METHODS

Statistical design and assessment of CVC incidence

The incidence and position of CVC affected plants were assessed visually in a five-year-old commercial orchard of 'Valencia' sweet orange trees, in the northern region of São Paulo, Brazil. One thousand plants were assessed in a block of 25 rows of 40 trees, planted at 8 x 5 m spacing. The assessments of the presence or absence of the disease were made every four to five months starting on March 1994 and ending in January 1996.

Aggregation analysis

Disease aggregation was evaluated through dispersion index analysis ($I\beta$) for each assessment (Gottwald *et al*., 1993), which was calculated by dividing the area into quadrants (2x2, 3x3 and 4x4) (Gottwald *et al*., 1996). The chi-square test ($P<0.05$) was used to test for goodness-on-fit, calculated by $I\beta$ (N_q - 1), where N_q represents the number of quadrants and N_q - *1* represents the degrees of freedom. If the values of $I\beta$ were not significantly different from 1 ($P>0.05$), then the pattern of disease trees was considered indistinguishable from random. A large (>1) $I\beta$ or a small P (<0.05) suggest rejection of H_0 (random pattern) in favor of H_1 (aggregated pattern of diseased trees) (Gottwald *et al*., 1996). The Beta-Binomial Distribution (BBD) software version 1.2 developed by L.V. Madden (Ohio State University, US) and G. Hughes (University of Edinburgh, England) was used for this analysis.

Geostatistical analysis

A standard geostatistical analysis includes exploratory data analysis, semivariogram analysis of the spatial structure, surface interpolation, and display of the results.

Semivariogram

The spatial dependence between neighboring samples/counts was measured with the semivariance (Vieira *et al*., 1983), estimated by:

$$\gamma^*(h) = \frac{1}{2\,N(h)} \sum_{i=1}^{N(h)} [Z(x_i) - Z(x_i + h)]^2$$

where $N(h)$ is the total number of pairs of disease counts which are separated by a distance h, and $Z(x_i)$ represents the observed values of the regionalized variable (presence or absence of the disease). The graph of $\gamma^*(h)$ versus the corresponding values of h, called a semivariogram, is a function of the distance h, and, therefore, depends on distance magnitude and direction. A mathematical equation of the semivariogram to express the spatial dependence among samples to allow estimation of values for unsampled locations. For properties that are spatially dependent, the increment $[Z(x_i)-Z(x_i+h)]$ is expected to increase with distance, up to some distance beyond which it stabilizes at a *sill* value, symbolized as C_1 and is numerically almost equal to the variance of the data. This distance is called the *range* (*a)* and represents the radius of a circle within which the observations are correlated. The semivariance value at the intercept to the $\gamma^*(h)$ axis is called *nugget effect* (C_0), and represents the variability at distances smaller than the minimum sampling distance.

A comparison of the semivariogram parameters for different situations can provide important information on the corresponding spatial distribution. For instance, the ratio between $C_0/C_0 + C_1$ (k parameter) provides an estimation of the amount of randomness that exists in the data at increments smaller than the sampling distance (Farias *et al*., 2002b). The exponential and spherical models were tested using

theoretical properties and the semivariogram parameters were fitted considering the model that gave the best coefficient of determination (R^2). The validation of models was conducted analyzing the *k* parameter (Journel & Huijbregts, 1978).

Kriging

One may often be interested in going beyond modeling the spatial structure, such as when values for unsampled locations must be estimated to build a detailed, precise map of the variable under study. In this case, it is necessary to interpolate between the sampled points. If an estimation, z^*, is to be made for any location, as a linear combination of the neighboring measured values (x_0), the equation is:

$$z^*(x_0) = \sum_{i=1}^{N} \lambda_i \, z(x_i)$$

where *N* is the number of measured values $z(x_i)$ involved in the estimation, and λ_i are the weights associated with each measured value. If the spatial correlation expressed through the semivariogram is used to define the weights, $\lambda_{i,}$ then the estimation process is called kriging. This estimation is unbiased and has minimum variance (Deutsch & Journel, 1992).

The semivariogram analysis was performed with GEOSTAT (Vieira *et al*., 1983). Models were fit to the semivariograms and the data were kriged, based on these models, to determine spatially related patches of data. The kriged estimates were imported into SURFER software version 6.04 (Golden Software, 1996) to produce contour maps.

RESULTS AND DISCUSSION

An aggregated disease pattern was indicated by dispersion index analysis ($I\beta$) for all quadrants evaluated (Table 1). $I\beta$ values were statistically greater than 1 ($P<0.05$) for most of the samplings, indicating an aggregated pattern of diseased plants as previously described by Gottwald *et al*. (1993) and later by Laranjeira *et al*. (1998). This pattern was not restricted to the first sampling (Mar/94) (Table 1), when the disease incidence was 11% (Table 2), indicating that at a low incidence of CVC, the spatial distribution of diseased plants was random. Subsequently, an aggregated pattern of the disease was detected for all the remaining sampling periods ($I\beta > 1$ e $P \leq 0.05$).

After two years, 82% of the trees showed symptoms of CVC (Table 2). This rapid increase in disease incidence was due to the absence of vector control in the area. Highest increases in disease incidence were observed between August 1994 and January 1995, and also between September 1995 and January 1996. Both periods coincided with peak sharpshooter vector incidence in spring and summer seasons (Roberto *et al*., 1996). These results are supported by those of Garcia Junior *et al*. (1997), who reported that the population of sharpshooter vectors increases after the beginning of the rainy season in the spring, reaches a peak in the summer and autumn and decreases rapidly going into the winter.

Omnidirectional semivariograms (the spatial continuity is the same in all directions) were calculated resulting in a set of distances and number of pairs as: 5.00 m (1,950 pairs), 8.95 m (5,664 pairs), 11.85 m (5,548 pairs), 16.41 m (10,830 pairs), 20.65 m (12,156 pairs), 24.86 m (10,254 pairs), 27.53 m (13,180 pairs), 31.83 m (16,280 pairs), 35.47 m (17,158 pairs), 39.98 m (21,400 pairs), 43.86 m (19,070 pairs), 47.75 m (18,612 pairs), 51.25 m (23,464 pairs), 56.12 m (23,464 pairs), 60.04 m (19,260 pairs), and 64.24 m (30,166 pairs). The number of pairs greatly exceeded 30, the minimum necessary for semivariogram calculation (Guerra, 1988).

For the first five sampling dates the semivariograms were fitted to the spherical model (Figures 1A, B, C, D and E) and revealed an average range of around 12.00 m. There was a strong indication that the diseased plants were initially randomly distributed in the orchard. The parameters of semivariograms were fitted according to the model that gave the best coefficient of determination (R^2), which in most cases was high (Table 2). These models were validated through the relation $C_0/C_0 + C_1$ (*k*), in which we can see that for this disease, this index varied from 0.60 to 0.75 (Table 2). These results confirm those of Journel & Huijbregts (1978) who concluded that values above 0.80 indicate that the

TABLE 1 - Index of dispersion ($I\beta$) analysis for Citrus Variegated Chlorosis for three quadrant sizes in a 'Valência' (*Citrus sinensis*) orchard

Quadrat size	df [b]	Date of assessment [a]						
		Mar/94	Aug/94	Jan/95	May/95	Sep/95	Jan/96	May/96
2 x 2	239	1.064 (0.235)	1.241 (0.006)	1.401 (0.000)	1.230 (0.009)	1.241 (0.006)	1.344 (0.000)	1.297 (0.001)
3 x 3	103	1.128 (0.175)	1.437 (0.002)	1.611 (0.000)	1.363 (0.008)	1.387 (0.006)	1.820 (0.000)	1.372 (0.007)
4 x 4	59	1.032 (0.406)	1.315 (0.053)	1.932 (0.000)	1.707 (0.003)	1.910 (0.000)	3.700 (0.000)	2.790 (0.000)

[a]: Index of dispersion ($I\beta$) and associated probability (*P*) values for the indicated quadrant sizes for 'Valencia' sweet orange infected with CVC. Values presented for each assessment date are $I\beta$ (=observed variance/binomial variance) and *P*-value. *P* values were calculated by comparison of df x $I\beta$ with the chi-squared distribution. Values of $I\beta$ not significantly different from 1 ($P>0.05$) indicate that the pattern of diseased trees is indistinguishable from random. A large (>1) $I\beta$ and a small *P* (≤ 0.05) suggest rejection of H_0 (random pattern) in favor of H_1 (aggregated pattern of diseased trees).
[b]: df: degrees of freedom.

TABLE 2 - Citrus Variegated Chlorosis incidence, semivariogram parameters fitted to the models, coefficient of determination (R^2) and *k* parameter for CVC geostatistical analysis in a 'Valencia' (*Citrus sinensis*) orchard

Assessment date	Disease incidence [a]	Semivariogram Parameters			Range area (m^2) [b]	Plants/ range [c]	R^2	*k* [d]
		C_0	C_1	*a* (m)				
Mar/1994	0.11	0.06	0.04	10.00	314.16	8.00	0.81	0.60
Aug/1994	0.16	0.08	0.05	12.00	452.39	11.00	0.80	0.61
Jan/1995	0.30	0.13	0.08	13.00	530.92	13.00	0.83	0.62
May/1995	0.40	0.16	0.07	13.00	530.92	13.00	0.73	0.69
Sep/1995	0.50	0.18	0.06	14.00	615.75	15.00	0.80	0.75
Jan/1996	0.82	0.14	0.00	0.00	---	---	---	1.00

[a] Proportion of diseased plants in the plot.
[b] Area calculated by πr^2, where π=3.14 and r = *a*.
[c] Range area/plant area (8 x 5 m = 40 m^2).
[d] $C_0/C_0 + C_1$ ratio.

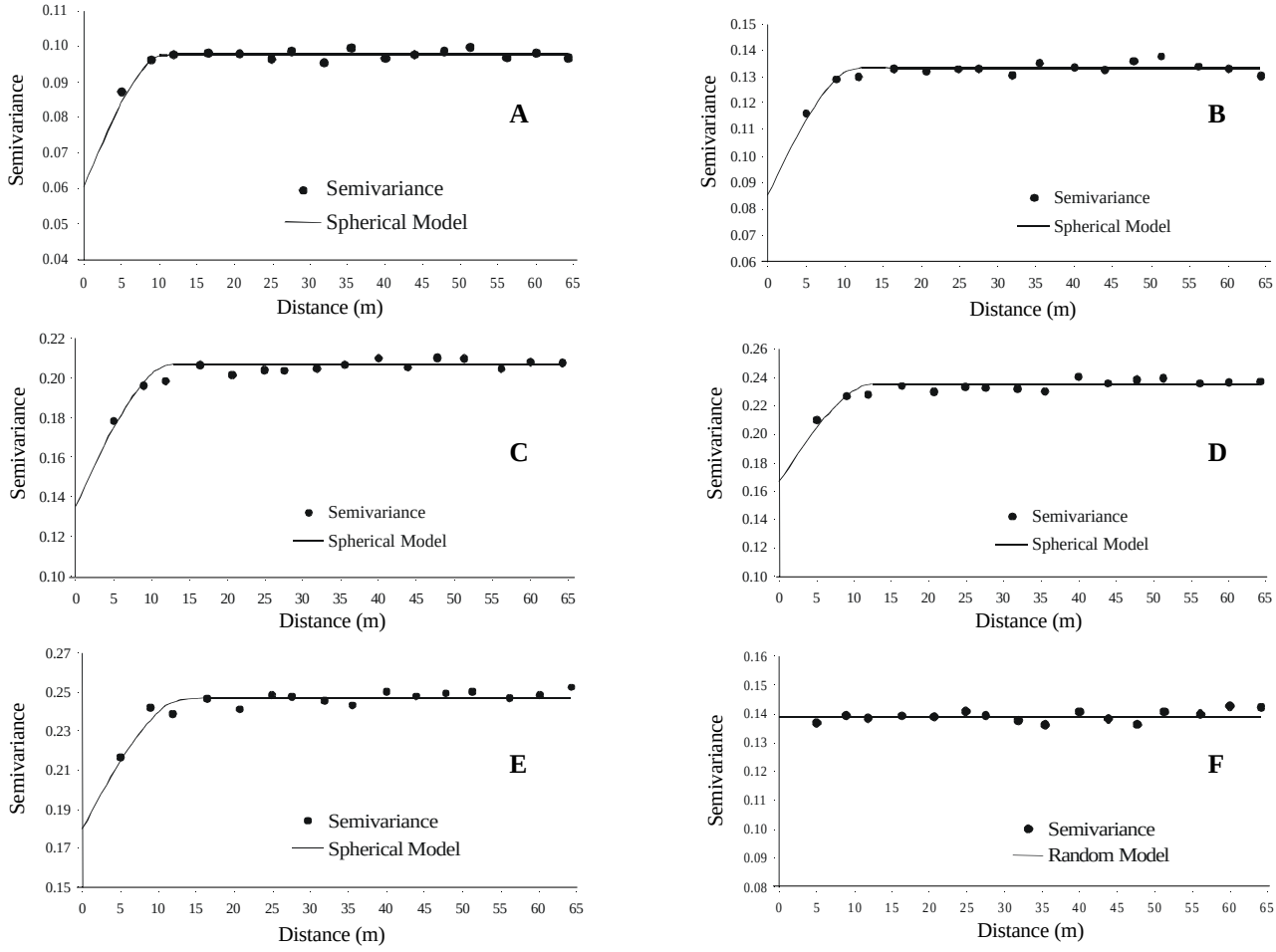

FIG. 1 - Semivariogram of CVC incidence in a 'Valencia' (*Citrus sinensis*) orchard. A: March/94; B: August/94; C: January/95; D: May/95; E: September/95; F: January/96.

phenomenon being studied is tending towards randomness, with no relation between samples. The *k* = 1.00 from the sampling made in January 1996, indicates that the disease was already uniformly distributed in the area, being represented by the random model (Figure 1F).

This spatial distribution pattern supports the probability that the disease was initially randomly distributed in the orchard due to planting of contaminated young plants. These young trees supplied inoculum to sharpshooter vectors, which then infected neighboring plants. This finding supports that of Gottwald *et al*. (1993) who concluded that the spatial dynamic of the disease is associated with vectors of limited

dispersion range.

The area of the range varied from 314.16 m^2 to 615.75 m^2 and the number of trees within this area, varied from eight to 15, respectively, over a 20-month period. The rapid progress of the disease shows the importance of periodic assessments to detect new symptomatic plants in the field and the necessity for prompt removal of the diseased trees to reduce sources of inoculum.

Semivariograms were subjected to kriging to construct contour maps of incidence of CVC (Figure 2A, B, C, D and E). Kriging was not done to samples of January 1996 (Figure 2F) due to lack of spatial dependency (Figure 1F).

The distribution of CVC in the orchard was initially random (Figure 2A) but after six months disease individuals were clustered as the disease incidence increased (Figure 2B). The periods of greatest increase in CVC incidence coincided with the population peak of sharpshooter vectors (Figure 2D and E). The orchard was almost completely diseased in a period of approximately two years (Figure 2F).

The increase in CVC incidence was very rapid, emphasizing the need for intensive inspection for disease symptoms to reduce initial inoculum. Failure to follow this

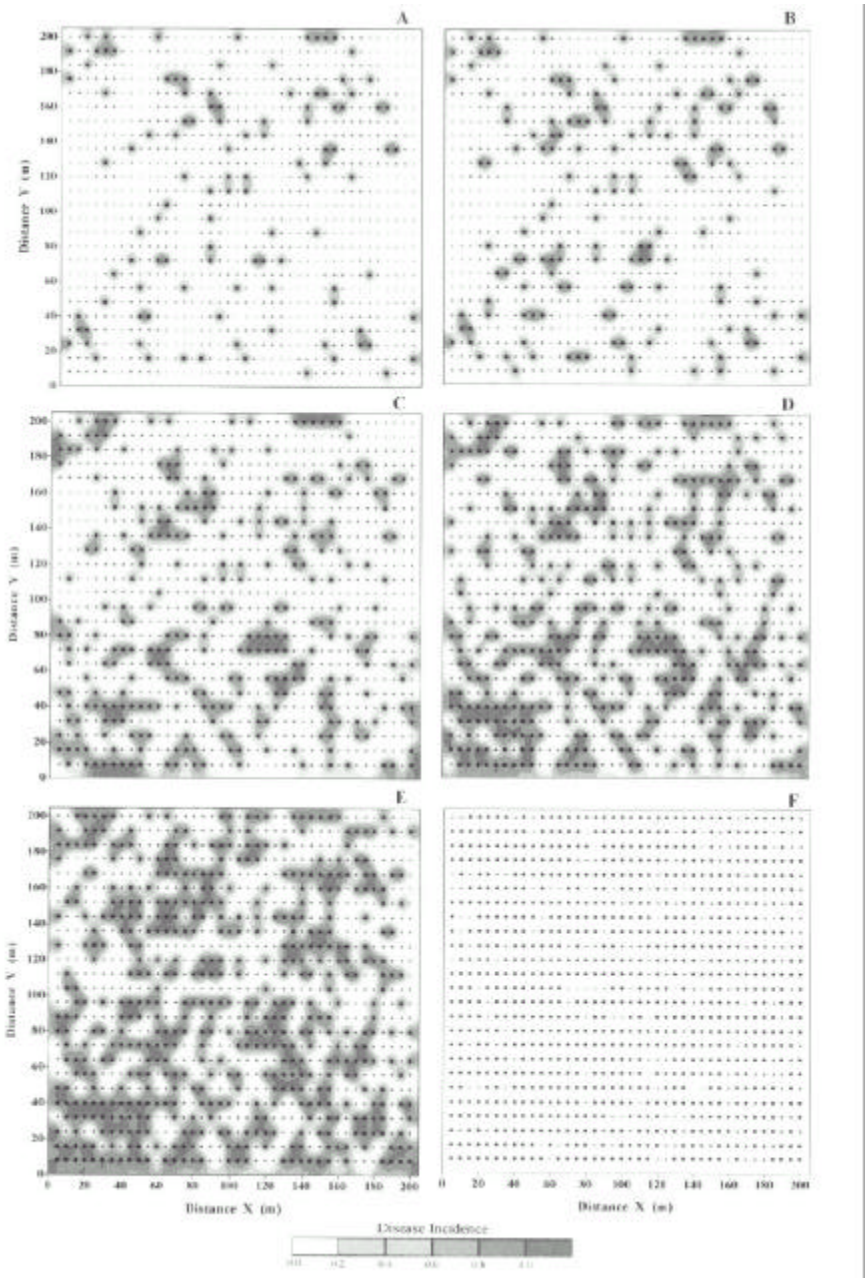

FIG. 2 - Kriging maps of the distribution of CVC incidence in a 'Valencia' (*Citrus sinensis*) orchard. A: March/94; B: August/94; C: January/95; D: May/95; E: September/95; F: January/96. (presence (●) and absence (+) of CVC).

practice means growers will face increasing risks of CVC losses in exposed orchards.

The highest peaks of disease incidence were observed in periods extending throughout spring and summer seasons in that location. However, symptoms are more likely to be detected during the winter season due to the near absence of new flushes that tend to cover symptomatic leaves from last season (Lima, 1995).

To summarize: the disease distribution was initially random in the grove and then aggregated within foci at less than 10-14 m apart. This confirms the role of sharpshooter vectors in causing a limited bacterial dispersal range (Gottwald *et al*., 1993). The rate of disease spread is predicted to be proportional to the number of initial foci and occurrence and efficiency of vectors. The current attempts of the Brazilian citrus industry to grow and maintain young plants that are CVC free are essential to reduce the causal bacterium.

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01153