

ECOLOGY, BEHAVIOR AND BIONOMICS

Geostatistical Characterization of the Spatial Distribution of *Xylella fastidiosa* Sharpshooter Vectors on Citrus

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Caracterização Geoestatística da Distribuição Espacial de Cigarrinhas Vetores de *Xylella fastidiosa* em Citrus

RESUMO - A distribuição espacial das espécies de cigarrinhas (*Dilobopterus costalimai* Young, *Acrogonia* sp. e *Oncometopia facialis* Signoret), vetoras da *Xylella fastidiosa*, agente causal da Clorose Variegada dos Citros, foi estudada com o uso da geoestatística. As avaliações foram feitas em um pomar comercial de laranja 'Pêra' (*Citrus sinensis* [L.] Osb.), objetivando estabelecer meios para melhor controle dos vetores e da doença. O monitoramento da ocorrência das cigarrinhas no pomar foi feito através de amostragens mensais, utilizando-se armadilhas adesivas amarelas de 3" x 5", distribuídas uniformemente em 50 pontos na área, dispostas em laranjeiras à altura de 1,5 m do solo e substituídas mensalmente. *Acrogonia* sp. foi a espécie prevalente nas amostragens. Os resultados possibilitaram ajustar modelos aos semivariogramas da distribuição espacial das três espécies no pomar estudado. Durante os três anos consecutivos de amostragem, as populações de *Acrogonia* sp., *D. costalimai* e *O. facialis* apresentaram modelos de distribuição agregada somente nos meses de verão, inverno e primavera, respectivamente, mostrando a necessidade de monitoramento constante desses vetores para reduzir a sua população em épocas favoráveis ao seu desenvolvimento. Através de parâmetros geoestatísticos foi possível calcular a área de agregação das cigarrinhas no pomar. A espécie *Acrogonia* sp. apresentou área média de agregação de 15.760 m², enquanto para *O. facialis* e *D. costalimai* foi possível constatar áreas médias de agregação de 11.555 m² e 10.980 m², respectivamente. Esses resultados indicaram que para um levantamento seguro de cigarrinhas é necessário pelo menos dispor de uma armadilha por hectare.

PALAVRAS-CHAVE: Clorose Variegada dos Citros, *Citrus sinensis*, semivariograma, krigagem

ABSTRACT - The spatial distribution of the three principal species of sharpshooter (*Dilobopterus costalimai* Young, *Acrogonia* sp. e *Oncometopia facialis* Signoret), vectors of *Xylella fastidiosa* causal agent of Citrus Variegated Chlorosis, was studied by using geostatistics. The evaluations were carried out in a commercial 'Pera' sweet orange (*Citrus sinensis* [L.] Osb.) grove budded on Rangpur lime (*Citrus limonia* Osb.) located at Bebedouro, São Paulo state, aiming to establish better means for vector and disease control. The sharpshooters were monitored using with 50 uniformly distributed 3" x 5" yellow sticky traps hanging down in branches at 1.5 m up to the ground, which were replaced monthly. *Acrogonia* sp. was the prevalent species sampled. The results were used to adjust population models to semivariograms of the spatial distribution of the three sharpshooters species in the grove. During the three consecutive years of sampling, the population of *Acrogonia* sp., *D. costalimai* and *O. facialis* showed an aggregated distribution only during summer, winter and spring, respectively, showing that intensive monitoring of these vectors is necessary to control their population in periods favorable to their development. The aggregation area of the sharpshooters within the orange grove was determined using geostatistic parameters. *Acrogonia* sp. showed a mean aggregation area of 15,760 m², whereas for *O. facialis* and *D. costalimai*, the corresponding areas were 11,555 m² and 10,980 m², respectively. Therefore, in order to obtain a reliable estimate of sharpshooter populations, at least one trap should be used per hectare.

KEY WORDS: Citrus Variegated Chlorosis, *Citrus sinensis*, semivariogram, kriging

The Brazilian citrus production is threatened by the Citrus Variegated Chlorosis (CVC), a bacterial disease that affects primarily sweet orange cultivars (Lima 1995). The disease was first observed in Argentina during the early 1980s and soon thereafter in Minas Gerais and São Paulo states in Brazil (Rossetti et al. 1990). More recently the disease has been confirmed in several other areas of Brazil (Laranjeira et al. 1998).

CVC affects all commercial orange varieties budded on all existing rootstocks. It is caused by a bacteria which is limited to the xylem, *Xylella fastidiosa* Wells (Chang et al. 1993). This disease reduces sweet orange (*Citrus sinensis* [L.] Osb.) fruit size and production (Rossetti et al. 1990).

This bacteria is disseminated by contaminated plant material and by sharpshooters of the subfamily Cicadellinae (Hemiptera: Cicadellidae). Among the vectors of *X. fastidiosa* on citrus, the three most abundant in São Paulo state are *Dilobopterus costalimai* (Young), *Acrogonia* sp. and *Oncometopia facialis* (Signoret) (Lopes et al. 1996, Roberto et al. 1996). The first symptoms of CVC appear six months after inoculation by infective insects. Recently, eight more species of sharpshooters have been identified as vectors of CVC: *Plesiommata corniculata* (Young), *Bucephalagonia xanthophis* (Berg), *Sonesimia grossa* (Signoret), *Homalodisca ignorata* (Melichar), *Ferrariana trivittata* (Signoret), *Macugonalia leucomelas* (Walker), *Parathona gratiosa* (Blanchard) and *Acrogonia virescens* (Metcalf) (Roberto & Yamamoto 1998).

Conventional statistics describes the distribution of an insect population as aggregated, uniform or random, ignoring the spatial distribution of the sampling stations (Farias et al. 2001). Even though the numerical distribution of a population can be classified according to statistical indexes, based on means, variances and frequency distributions (variance/mean ratio, Taylor, Lloyd, Morisita indexes, k parameter of the negative binomial distribution, etc.), these techniques do not allow us to correlate sample data with their spatial location (Ellsbery et al. 1998). Spatial attributes in sample data require us to use another kind of statistics, known as Geostatistics, which was first mentioned in South Africa, when Professor D. G. Krige in 1951, working with data on gold concentrations, concluded that it was not possible to make sense of the variances unless the distance between sampling points was taken into consideration (Farias et al. 2002b). Matheron (1963), based on these observations, developed a theory which he called the Theory of Regionalized Variables, which contains the fundamentals of Geostatistics.

Geostatistical methods have been utilized to characterize the spatial distribution of insects by entomologists who study population dynamics (Ellsbery et al. 1998, Darnell et al. 1999, Barrigossi et al. 2001). Geostatistical methodology applied to Entomology is primarily based on the work of Liebhold et al. (1993) and Roberts et al. (1993).

Behavioral factors, or any other kind inherent to insect vectors can be better correlated with the dissemination and development of disease if the spatial distribution of the sampling stations is taken into account. Despite the importance of sharpshooters in the dissemination of CVC, there is little information on their spatial distribution during

the different seasons. Studies of distribution models of these insects would contribute to epidemiological studies and to sharpshooter management in the groves.

Thus, this work was an attempt to determine the spatial distribution of sharpshooter (*Acrogonia* sp., *O. facialis* e *D. costalimai*) samples, using geostatistical analysis, in a commercial sweet orange grove in São Paulo state, aiming to establish better means of vectors and disease control.

Materials and Methods

The spatial distribution of the sharpshooters *Acrogonia* sp., *D. costalimai* and *O. facialis* was studied in a commercial 'Pera' sweet orange grove (*C. sinensis* [L.] Osb.), budded on 'Rangpur' lime (*C. limonia* Osb.). The 2,600-tree grove was five years old, planted in a 7 x 4 m spacing, located at Bebedouro, São Paulo state.

Sampling. Sharpshooters were monitored in the grove by sampling, using 3" x 5" yellow sticky cards (Olson Products Inc., Ohio, USA), uniformly distributed in the area, i.e., covering the whole grove and placed in a same distance of each other, hanging down in branches located in the northern side of the plants, at 1.5 m over the ground. The traps were replaced monthly, as described by Roberto et al. (1997). The number of individuals of each of the three species of sharpshooters trapped per card was record every 15 days, from December 1996 to December 1999. The data were then grouped in 3-month intervals, corresponding to the seasons: December-February (summer), March-May (autumn), June-August (winter) and September-November (spring), in order to determine the seasons of the year that the sharpshooters are more prevalent in the grove, for better population of these vectors control.

Geostatistical Analysis. A standard geostatistical analysis includes exploratory data analysis, semivariogram analysis of the spatial structure, surface interpolation, and display of the results. The spatial dependence between neighbouring samples/counts was measured with the semivariance (Vieira et al. 1983), estimated by:

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

where $N(h)$ is the total number of pairs of sharpshooter counts separated by a distance h and $Z(x_i)$ represents the observed values of the regionalized variable (number of sharpshooters collected). A graph of $\gamma^*(h)$ versus the corresponding values of h , called a semivariogram, is a function of the distance h , and, therefore, depends on both its magnitude and direction. A mathematical model equation needs to be fitted to the semivariogram so that the expression of the spatial dependence can be used on the estimation of values for unsampled locations. For properties that are spatially dependent, it is expected that the increment $[Z(x_i) - Z(x_i + h)]$ will increase with distance, up to some distance beyond which it stabilizes at a *sill* value, which has a symbol C_f , and is numerically almost equal to the variance of the data. This distance is called *range* (a) and represents the radius of a circle within which the

observations are so similar that they are correlated. The semivariance value at the intercept to the $\gamma(h)$ axis is called *nugget effect* (C_0) and it represents the variability at spaces smaller than the minimum sampling distance. Therefore, the higher the value of the nugget effect with respect to the *sill*, the less spatial dependence the variable has. A comparison of the semivariogram parameters for different situations can provide important information on the corresponding spatial distribution. For instance, the $C_0/C_0 + C_1$ ratio (k parameter) provides an estimation of the amount of randomness that exists in the data at spaces smaller than the sampling distance (Farias *et al.* 2002a). The semivariograms were fitted according to the model which gave the best coefficient of determination (R^2).

The determination of the distance between traps was made for a general direction, that is, adopting a tolerance angle of 90° . This means that the distances between the trapping stations were determined in all directions within the space (grove).

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 values of the weights, λ_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

Population dynamics of *X. fastidiosa* vectors seems to be different during the different seasons of the year (Fig. 1), but a remarkable pattern of sharpshooter occurrence could not be identified during the three years of evaluation. *Acrogonia* sp. showed population peaks in spring during all three years and low population on autumn and winter seasons; a similar trend was observed for *D. costalimai* during the summer months and this vector was less prevalent than *Acrogonia* sp.

In São Paulo, most citrus vegetative growth occurs during spring and summer, which is when highest temperatures and rainfall occur (Tubelis 1995). The tendency of higher incidence of these two sharpshooter species during these seasons in the grove is probably due to the greater availability of water for the trees, and since these are sap sucking insects these insects could prefer trees that are not under water stress, as indicated by Roberto & Busoli (1999).

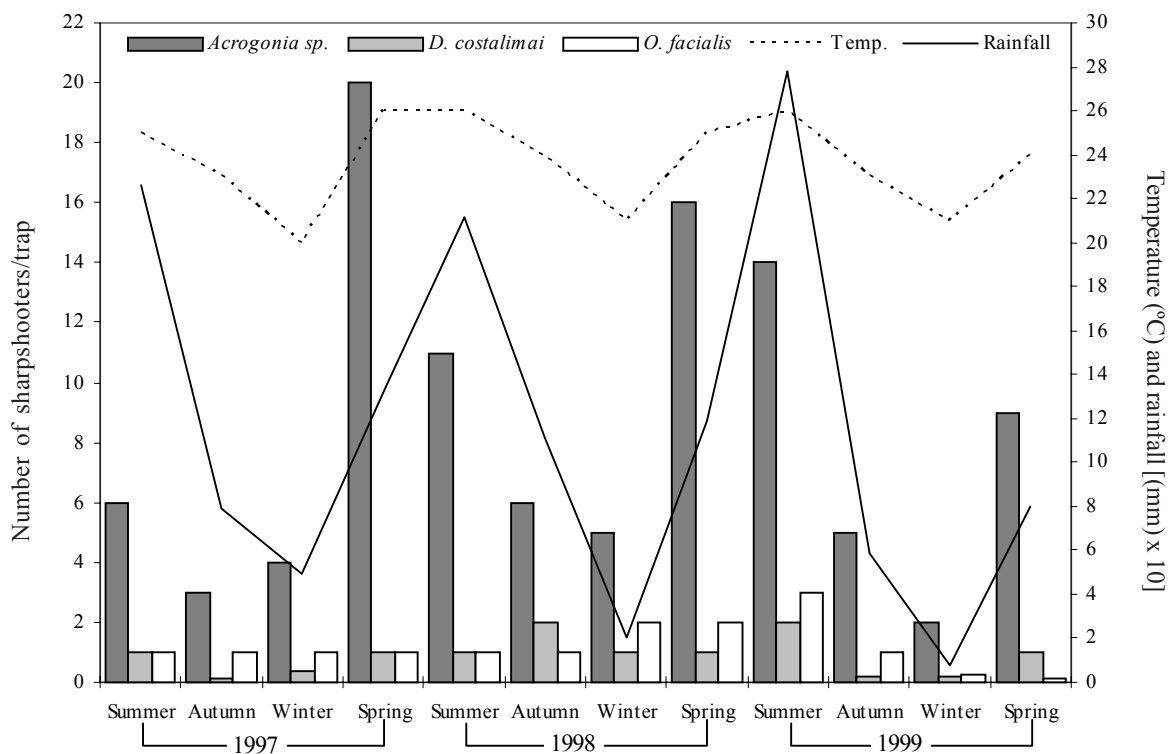


Figure 1. Population dynamics of *Acrogonia* sp, *D. costalimai* and *O. fascialis* in a sweet orange grove from December 1996 to November 1999. Bebedouro, SP.

Laranjeira *et al.* (1998) determined that the progress rate of CVC, i.e., the proportion of new diseased plants, is five times higher on spring and summer than on autumn and winter seasons. Thus, the authors predict that on spring and summer seasons, where new flushes and lack of water stress occur, there are favorable conditions for frequent feeding of vectors, and as a result, higher number of well-succeeded infections.

The food preference of *O. facialis* for older woody stems, different from the two other species, which prefer tender young stems (Gravena *et al.* 1998), may explain its lower abundance when host plant growth occurs.

The mean annual populations of the three sharpshooter species are presented in Fig. 2. The mean populations of *Acrogonia* sp. increased each year, from about six sharpshooters per trap in 1997 to more than 14 in 1999 (an increase of over 100%). The standard deviation also increased annually along with the means, as a consequence of increased aggregation. The other species of sharpshooters (*D. costalimai* and *O. facialis*) showed similar means, with a slight decline in 1999.

Considering these observations, the management of sharpshooters using chemical products should be initiated on the beginning of citrus growth season in order to reduce the vector populations and as a consequence, to reduce the disease dissemination. A similar procedure has been used to control the greening, a vascular disease disseminated by psylla vectors in South Africa (Buitendag & Naudé 1992).

Among the thirty six semivariograms calculated for the three years of sampling for the various species and seasons,

mathematical models were developed for nine of these graphs characterizing aggregation in the spatial distribution (Table 1, Fig. 3). The remaining semivariograms did not fit to any model, which means that the distribution was at random in these cases (random model).

The spherical model best adapted to the sharpshooter counts per trap for *Acrogonia* sp. and *D. costalimai*, in summer and spring, respectively, while the spatial distribution of *O. facialis* in winter corresponded to a Gaussian model (Table 1). These models were validated through the relation $C_0 / C_0 + C_1(k)$, which varied from 0.02 to 0.28 for the three species. These results confirm those of Journel & Huijbregts (1978) who concluded that values below 0.80 indicate that the phenomenon being studied is aggregated, with strong relation between samples.

By using the range of models (a) it was possible to calculate the aggregation area of the sharpshooters in the grove. *Acrogonia* sp. showed a mean aggregation area of 15,760 m². For *O. facialis* this area was 11,555 m², while for *D. costalimai* it was 10,980 m² (Table 1). These results indicate that in order to obtain a reliable estimate of sharpshooter populations, at least one trap should be placed per hectare.

Aggregation within the spatial population distribution of *Acrogonia* sp. was found during the summer season of the three years, and was characterized by semivariograms fitted to spherical models (Fig. 3A-C), with a variation in the limits of the dependency strip of 60 to 85 m (Table 1). The contour maps of the population densities interpolated by normal kriging showed aggregation areas within the grove, with

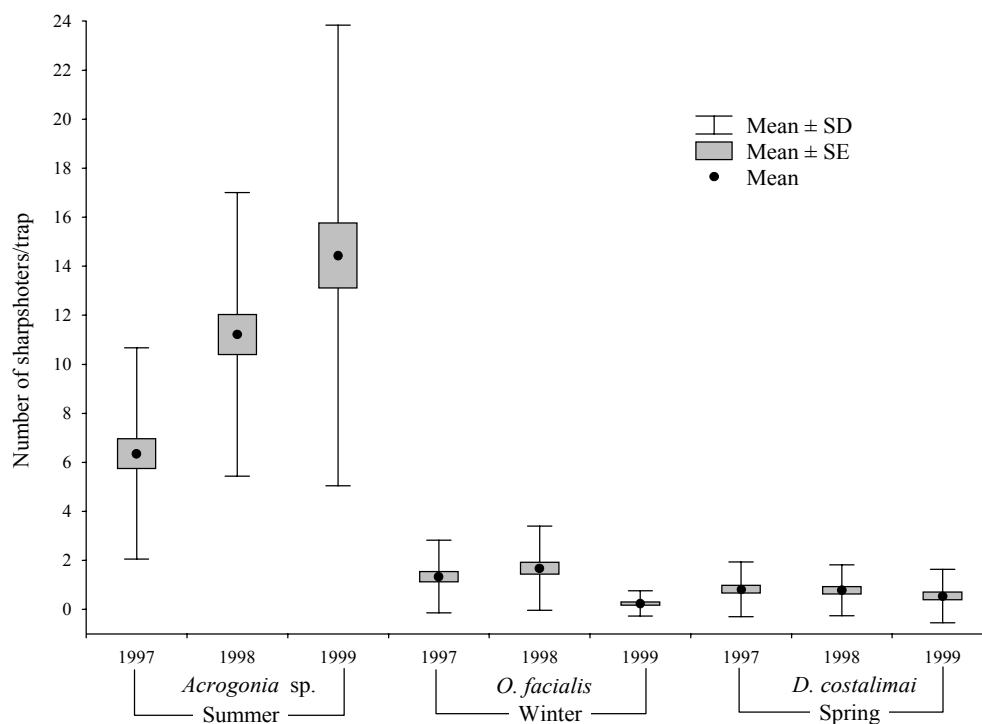


Figure 2. Number of *Acrogonia* sp., *D. costalimai* and *O. facialis* collected per trap during the different seasons in a sweet orange grove in Bebedouro, SP. SD = Standard Deviation; SE = Standard Error of Mean

Table 1. Semivariogram models for the spatial distribution of *Acrogonia* sp., *D. costalimai* and *O. facialis* in a sweet orange grove. Bebedouro, SP. 2002.

Year	Semivariogram parameters			Model	R ²	area (m ²) ^a	k ^b
	C ₀	C ₁	a (m)				
<i>Acrogonia</i> sp. (summer)							
1997	0.90	17.50	65.00	Spherical	0.75	13,273	0.05
1998	12.00	24.00	85.00	Spherical	0.83	22,698	0.33
1999	2.00	85.00	60.00	Spherical	0.65	11,309	0.02
<i>O. facialis</i> (winter)							
1997	0.06	0.85	69.00	Gaussian	0.77	14,957	0.07
1998	0.20	1.75	55.00	Gaussian	0.90	9,503	0.10
1999	0.10	3.30	57.00	Gaussian	0.80	10,207	0.06
<i>D. costalimai</i> (spring)							
1997	0.24	1.00	54.00	Spherical	0.72	9,160	0.19
1998	0.34	0.86	53.00	Spherical	0.55	8,824	0.28
1999	0.06	0.85	69.00	Spherical	0.90	14,957	0.07

^aArea calculated by πr^2 , where $p = 3.14$ and $r = a$

^b $C_0 / C_0 + C_1$ ratio

population levels varying from 0 to 42 sharpshooters per trap (Fig. 4A-C).

Different from the other two species, the data for *O. facialis* adjusted to a Gaussian (Table 1, Fig. 3D-F) during the winter of all three years, with a dependency band of from 55 m to 69 m. The kriging maps of *O. facialis* showed an aggregated spatial distribution of this sharpshooter (Fig. 4D-F).

The species *D. costalimai* fitted to the spherical model during the spring of the three years (Fig. 3G-I). The variation in dependence was 53 m to 69 m (Table 1). In Fig. 4G-I it is possible to observe the aggregation areas and the variation in population size for this species, from 0-5 sharpshooters/trap, depending on the year.

Using geostatistics to demonstrate the distribution

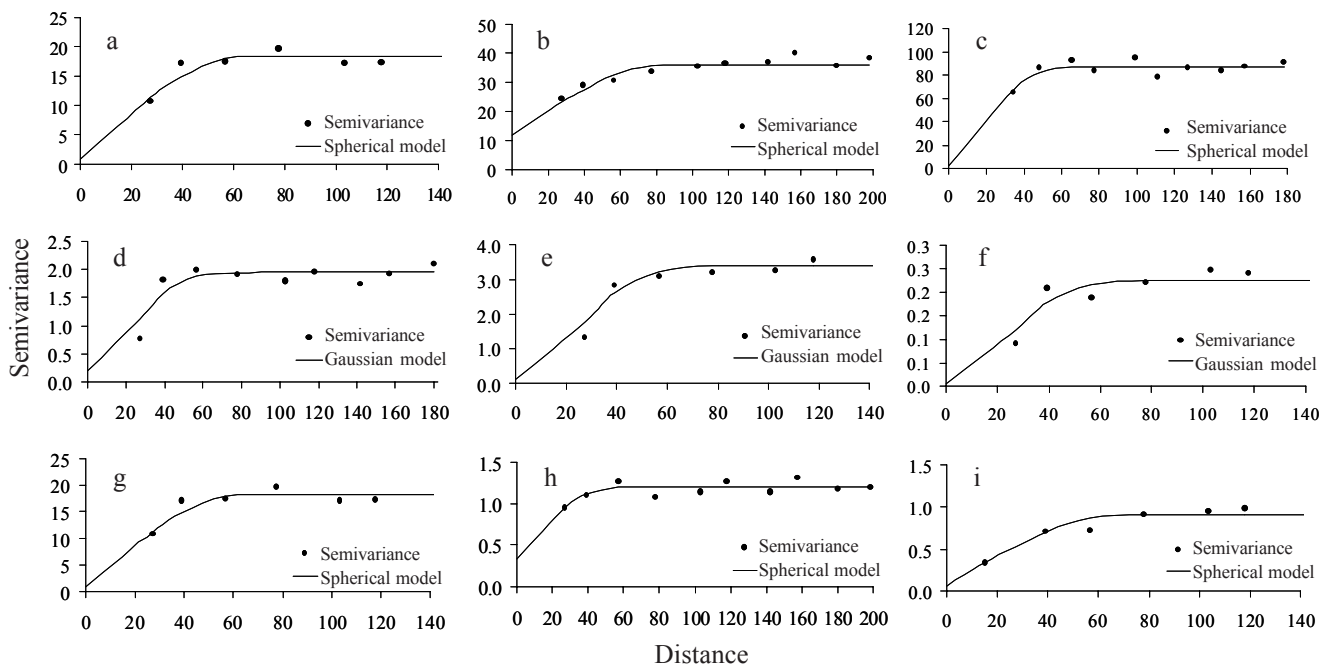


Figure 3. Semivariograms of sharpshooters collected per trap during different seasons. *Acrogonia* sp. collected in summer (a = 1997; b = 1998; c = 1999), *O. facialis* collected in winter (d = 1997; e = 1998; f = 1999) and *D. costalimai* collected in spring (g = 1997; h = 1998; i = 1999)

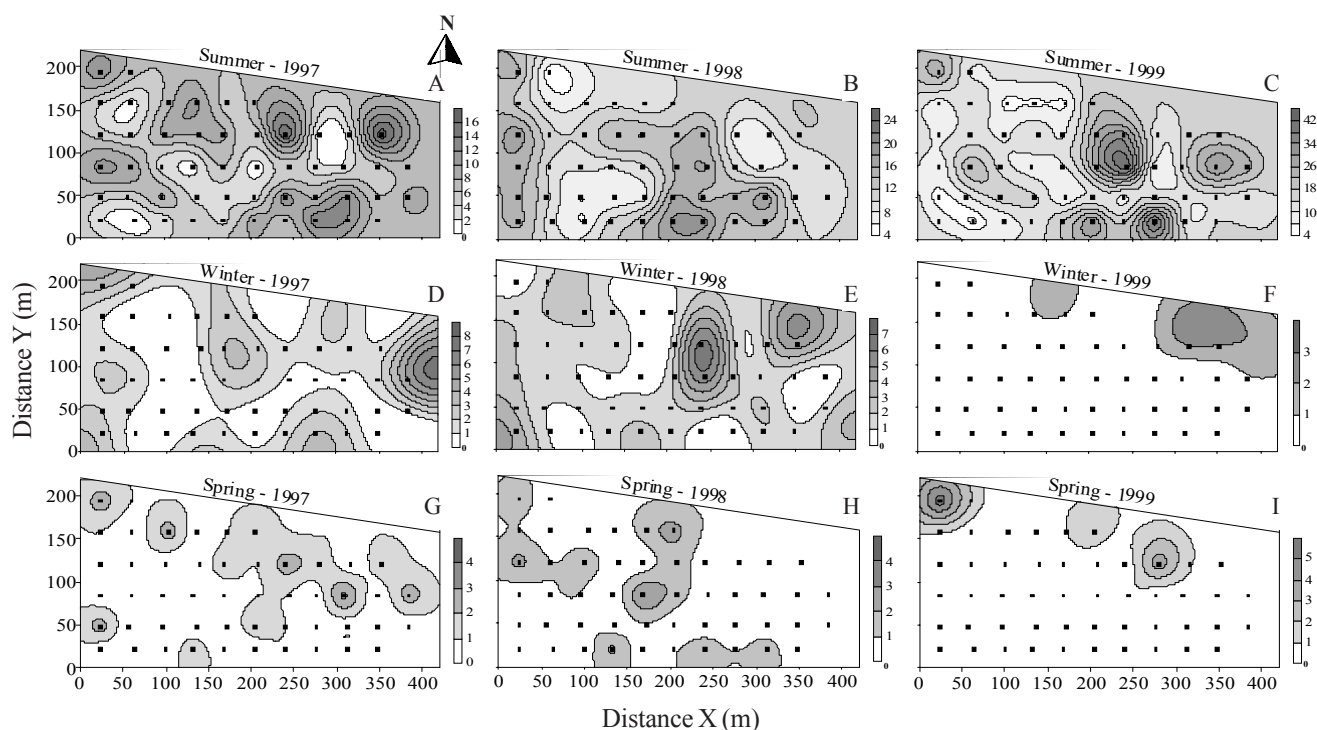


Figure 4. Kriging maps showing the spatial distribution of *Acrogonia* sp. (A, B and C), *O. fascialis* (D, E, F) and *D. costalimai* (G, H, I). Dots represent the sampling locations (yellow sticky traps).

dynamics of CVC, Roberto *et al.* (2002) reported that the disease is aggregated in the field. According to the authors, the disease distribution is initially at random in the grove and then aggregated within foci at less than 10-14 m apart. The spherical model is the best fit for disease distribution and kriging maps also reveals that the incidence of CVC increases in periods during which the trees undergo vegetative growth, coinciding with greater expected occurrence of insect vectors of the bacterium in the field. Besides, the author concluded that the rate of disease spread is predicted to be proportional to the number of initial foci and occurrence and efficiency of vectors. Thus, the results observed in this research confirm the role of sharpshooter vectors in causing a limited bacterial dispersal range, as previously predicted by Gottwald *et al.* (1993).

The seasonal variability found in the spatial distribution models for each of the three species of sharpshooters is probably due to a combination of various factors, including the feeding and egg laying preferences of the adults, as well as the climate, or more precisely the relationship between rainfall and temperature, which has a direct effect on vegetative growth in citrus trees.

During months of more intensive vegetative growth and flush, the sharpshooters might concentrate their activity on patches of citrus trees that offer the best nutritional conditions for feeding, breeding or oviposition. Adults of the glassy-winged sharpshooter, *Homalodisca coagulata* (Say) for example, tend to select host plants with higher concentrations of certain nutrients in the xylem sap (Brodbeck *et al.* 1990). The nutritional quality of xylem sap can vary considerably in plants depending on several environmental factors, such as

soil fertility and humidity, shade, plant age and location, etc (Andersen *et al.* 1993, Andersen & Brodbeck 1991). This behavior could explain the aggregated pattern observed for *Acrogonia* sp. and *D. costalimai* in the summer and spring, respectively. In the case of *O. fascialis*, a similar behavior could occur in the winter, since this species can feed on woody stems of citrus trees (Gravena *et al.* 1998). We certainly need more background information on behavior and nutritional ecology of these insects in order to understand the ultimate reasons for their seasonal aggregation.

At this time, the information gained from the analysis of spatial distribution of sharpshooters can be valuable to improve the management of CVC by establishing an appropriate number and density of sticky traps in citrus groves for monitoring the populations of vectors.

To summarize: it was possible to fit models to the semivariograms of the spatial distribution of *Acrogonia* sp., *D. costalimai* and *O. fascialis* in the sweet orange grove. During the three years the sharpshooter populations were aggregated only in the summer, winter and spring for *Acrogonia* sp., *O. fascialis* e *D. costalimai*, respectively. The control of sharpshooters should be initiated on the beginning of citrus grow season to reduce vector populations. According to the range of models, at least one trap must be placed per hectare in order to obtain a reliable estimate of sharpshooter populations. As the increase in CVC incidence is very rapid in the field (Roberto *et al.* 2002), the need for intensive insect vectors and disease symptoms monitoring are essential to reduce initial inoculum. Failure to follow this practice means growers will face increasing risks of CVC losses in exposed groves.

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