RBF Magazine
version ISSN 0100-2945

Rev. Bras. Frutic. vol.32 no.3 Jaboticabal September Epub 2010 20-Aug-2010

doi: 10.1590/S0100-29452010005000077

ARTICLES
GENETICS AND BREEDING

Spatial distribution of *HLB* (Greening) on citrus using geostatistics [1]

Renata Moreira Leal [I] , José Carlos Barbosa [II] ; Gregolin Marilia Costa[III] ; Belasque José Junior [IV] ; Pedro Takao Yamamoto [V] ; Joseph Dragone [VI]

[I] Dr in Plant Production, Depto. Exact Sciences. FCAV / UNESP, Access Route
[II] Prof. Paulo Donato Castellane, s / n º, 14884-900, Jaboticabal-SP. renatamleal@yahoo.com.br
[III] Prof. Dr. Depto. Exact Sciences FCAV / UNESP. jcbarbosa@fcav.unesp.br
[IV] Dr. in Agricultural Entomology, Depto. Agricultural Entomology. FCAV / UNESP. mgcosta@hotmail.com
[V] Researcher's Defense Fund Citrus - Fundecitrus, CP 391, 18801-970, Araraquara-SP.belasque@fundecitrus.com.br ; ptyamamoto@fundecitrus.com.br
[VI] Agronomist responsible for Rey Rancho farm

ABSTRACT

The objectives were to assess the spatial distribution and spread of *HLB* (greening) of citrus in plots of agricultural land in the municipality of Araraquara-SP, using geostatistics. To determine the number of trees with greening, periodic inspections were performed at intervals of three months, from March 2005 to July 2007, counting on each block, the number of plants with symptoms of the disease. We conducted a descriptive analysis of data and to verify the spatial distribution of greening, we used geostatistics by adjusting the semivariogram and kriging interpolation of the data. The spatial dependence of greening plants showed aggregation radius from 300 to 560 m, indicating an aggregated distribution of the disease. Through the kriging maps, it was observed that the initial focus of diseased plants occurred in the limits of the farm, with expansion of the greening of the area. The inspection interval of three months was not adequate to reduce the greening on the farm.

Index terms : semivariogram, kriging, *Diaphorina citri.*

ABSTRACT

The aim of this study was to use Geostatistics to verify the spatial distribution of *HLB* (Greening) in oranges orchards on agricultural property located in the city of Araraquara, São Paulo. To determine the number of plants with greening, periodic inspections constitute the three months from March 2005 Were Made Until July 2007, counting the number of plants in each stand with the characteristic Symptoms of the disease. A descriptive analysis of the data was undertaken, and used to verify Geostatistics Were the spatial distribution of greening through the adjustment of semivariograms and kriging interpolation of data by. The spatial dependence of greening plants formed with a beam of aggregation of 300 to 560 m, Indicated an aggregated distribution of disease. Diagrams of kriging Showed That initial focus of greening started with plants at the border in the farm and there was an expansion of disease through The entire area. The interval of inspections constitute the three months was not Appropriate to Reduce the greening in the farm.

Index terms: Semivariogram, kriging, *Diaphorina citri.*

INTRODUCTION

Brazil, led by rising exports, is currently the world's largest producer of oranges, with some 18 million tons. In 2007, the State of São Paulo accounted for 80% of national production, obtained in the cultivated area of 565 hectares, which corresponds to an area of 71% of all citrus production in Brazil (AGRIANUAL, 2008).

The orange crop is a crop with the greatest number of pests and, among these, currently, the psyllid has great merit *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). It is a small insect that sucks the sap from plants and has caused in recent years, major damage to the citrus groves, it is the insect vector of prokaryotic cause greening.

The greening affects all citrus varieties and is considered currently the most important disease of citrus in the world, therefore, difficult to control, is highly destructive to the orchards (Bove, 2006). In Brazil, the disease was identified in July 2004 in the region of Araraquara-SP (COLLECTION-Filho et al., 2004) and currently has a presence in several cities of the states of Sao Paulo, Minas Gerais and Paraná.

tween the causative agents of disease, there is a Gram-negative bacteria, only in the phloem of the host plant, named *Candidatus* Liberibacter sp. There are three species: *Candidatus* Liberibacter africanus, *Candidatus*Liberibacter asiaticus

and *Candidatus* Liberibacter americanus, however, only *C.* asiaticus Liberibacter and *C* .Liberibacter americanus were found in Brazil (COLLECTION-Filho et al., 2004).

Still, as the causative agent of the disease for the phytoplasmas. Teixeira et al. (2008) evaluated the characteristic symptoms of greening in citrus orchards located in the northern, central and southern state of Sao Paulo. The authors found, through laboratory analysis, that a group 16Sr IX fioplasma was associated with typical symptoms of greening in citrus orchards of the regions evaluated.

The characteristic symptom of the disease occurs initially in an industry that stands out for having yellow leaves, in contrast with the green branches not affected, causing death and dry the hands and reduction in plant growth, in addition, the fruits become small, deformed and asymmetric (Ayres et al., 2005).

Because of the importance of this disease, some studies have been developed in recent years, mainly addressing measures to control the insect vector, but research to evaluate the spatial dependence of greening in citrus groves are scarce.

To study the spatial dependence, geostatistics is the most suitable tool because it considers the position in space of the variable under study (LIEBHOLD et al., 1993). With the use of geostatistics, it is possible to determine the spatial dependence of disease through the development of semivariograms that are fitted to a model that provides the radius of aggregation of diseased plants and from this semivariogram, is to produce maps that show as is the spread of disease in the area.

Given the above, the objectives were to assess the spatial distribution and expansion of *HLB* (greening) of citrus in plots of agricultural land in the municipality of Araraquara-SP, using geostatistics.

MATERIAL AND METHODS

The research was conducted on the farm Rancho Rey (Araraquara-SP), with the objective of studying the spatial dependence of the greening and expansion in stands of sweet orange [*Citrus sinensis* (L.) Osbeck]. The experimental area lies between the coordinates 21 º 44 '83 "south latitude and 48 º 15' 90" longitude west of Greenwich and altitude of 647 m (central part of the farm). The experiment consisted of 46 stands of orange trees (Figure 1) with different ages, spacing, rootstocks (Rangpur lime and Cleopatra) and varieties (Hamlin, Murcott, Natal, Pera and Valencia). The total number of plants on the farm at the beginning of the experiment, was 162,534, and each block had a number of plants that ranged from 756 to 9226. Plots received the treatments (fertilization, weeding, applying herbicides) and plant (application of acaricides, insecticides and fungicides) recommended to the culture. To determine the number of trees with greening, periodic inspections were performed at intervals of about three months, from March 2005 to July 2007, counting the number of plants with symptoms of the disease in each stand. When they met with greening plants, these were eliminated from the plot, according to Instruction No. 10 (Ministry of Agriculture, Livestock and Supply). The sampling units were composed by the study plots, and to geocode them, used a GPS Trimble 4600 LS model, determining the geographic coordinates of

the center of each plot. We conducted a descriptive analysis of data and to verify the spatial dependence and the spread of disease, we used geostatistics, by adjusting the semivariogram and kriging interpolation of data (Robert et al., 2002). The first step in geostatistical semivariogram was the setting that provided the type and form of spatial dependence, which is nothing more than a plot of semivariance versus distance. The semivariance is described as:

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

where, γ (*h*) is the semivariance estimated by a distance h, *N* (*h*) is the number of paired observations ([*Z* (*xi*) - *Z* (*x i* + *h*]), separated by distance h. Thereafter, data were fitted to the model semivariogram spherical, exponential, Gaussian or power. In this study, the semivariogram model that best fitted the data was the Gaussian, which is described as:

$$\gamma(h) = C_0 + C_1 \left[1 - \exp\left(-\frac{3h^2}{a^2} \right) \right]$$

where, C_0 is the nugget effect or minimum semivariance, $C_0 + C_1$ is the threshold or maximum semivariance and*the* radius is the range or aggregation. Next step was to construct maps using kriging the spatial dependence of the semivariograms modeled and estimated values in any field position without trend and with minimum variance, allowing us to visualize the behavior of the variable under study by means of maps and surface. For data analysis, statistical programs were used: MINITAB 14 (descriptive statistics) and SURFER 7.0 (geostatistics).

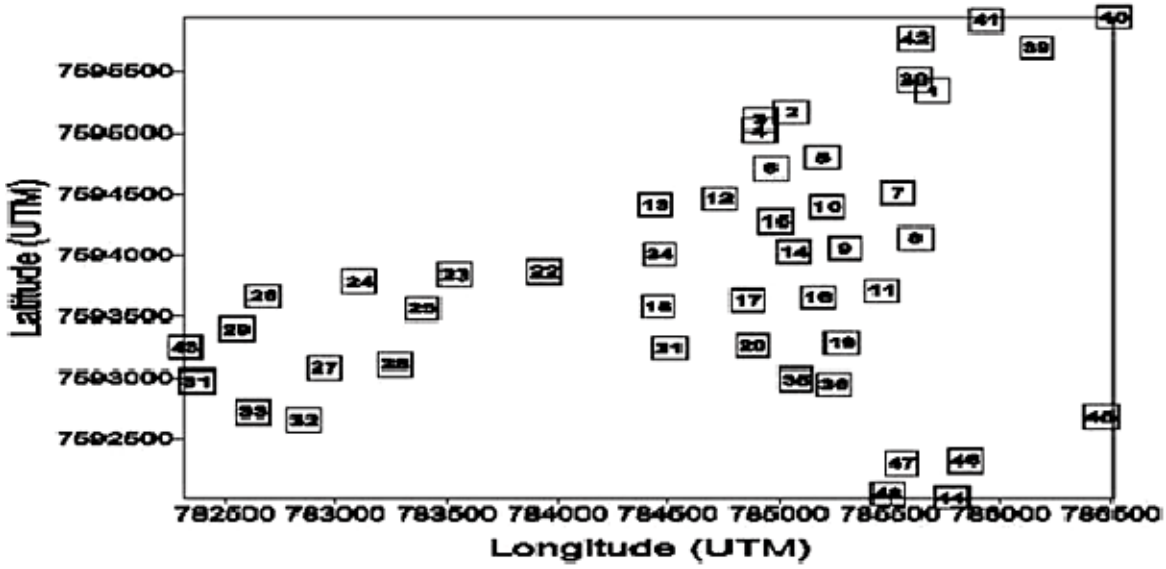


FIGURA 1 - Mapa de localização dos talhões de citros na Fazenda Rancho Rey (Araraquara-SP).

RESULTS AND DISCUSSION

There were, at first inspection, 426 plants with greening, ie 0.3% of all plants grown on the farm. On second inspection, there was an increased number of diseased plants corresponded to 6,533 (4%), and, on the farm during the inspection period, there were always plants with disease symptoms (Figure 2). It can be inferred that the interval of three months between an inspection and another in the same block, may not have been appropriate because greening plants, which have not yet had symptoms of the disease at the time of inspection, remained on the field for more than three months, increasing the sources of inoculum in plots that were responsible for the infection of those plants still healthy.

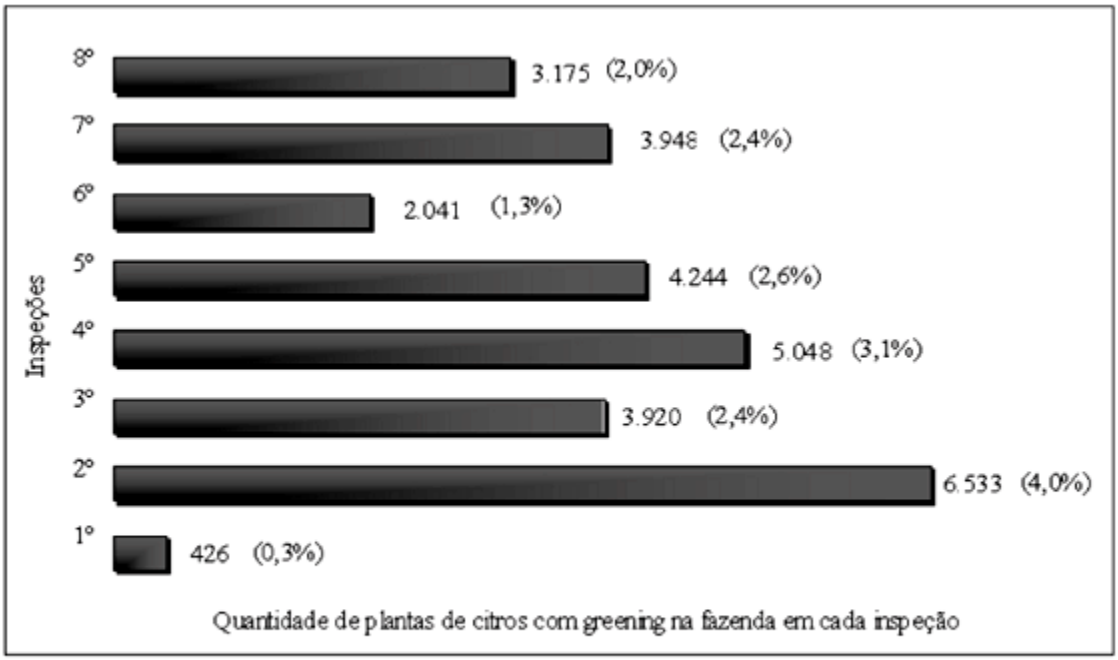


FIGURA 2 - Número total de plantas com greening dos 46 talhões de citros, em cada inspeção, da Fazenda Rancho Rey (Araraquara-SP).

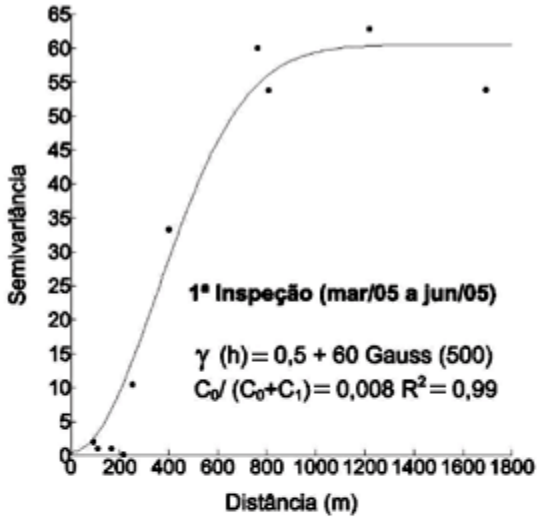
We found great variability of the study area, because when comparing the mean and variance, it appears that the variance values were always higher than the average (Table 1), indicating that the diseased plants are found in area in aggregate. In the same table, there are high values of the coefficient of variation, which in this type of study can be considered normal, since comparisons were made with greening plants between plots that had many diseased plants and other stands that had few or no plants with the disease.

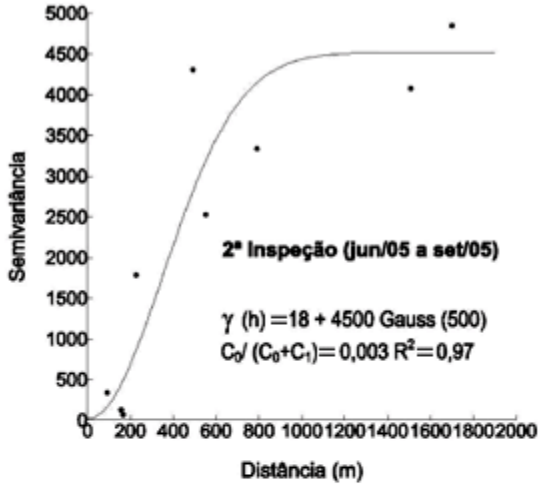
TABELA 1 - Estatística descritiva das plantas com greening dos 46 talhões de citros, em cada inspeção, da Fazenda Rancho Rey (Araraquara-SP).

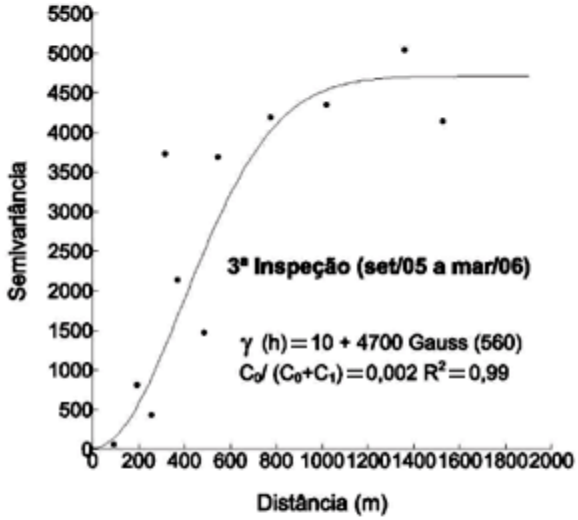
Inspeções	Média*	Mediana	Variância	Coeficiente de Variação	Assimetria	Curtose
1ª	9,26	1	840,51	313,05	5,83	36,91
2ª	142,0	30	57435,3	168,75	2,37	5,51
3ª	104,7	47	57279,2	228,56	5,19	29,99
4ª	124,0	81	22234,9	120,25	2,34	5,16
5ª	106,1	54	25801,6	151,39	3,54	15,19
6ª	53,7	14	18053,3	250,16	4,81	25,67
7ª	106,7	74	8955,6	88,69	1,05	0,41
8ª	85,8	59	5440,3	85,95	1,41	1,24

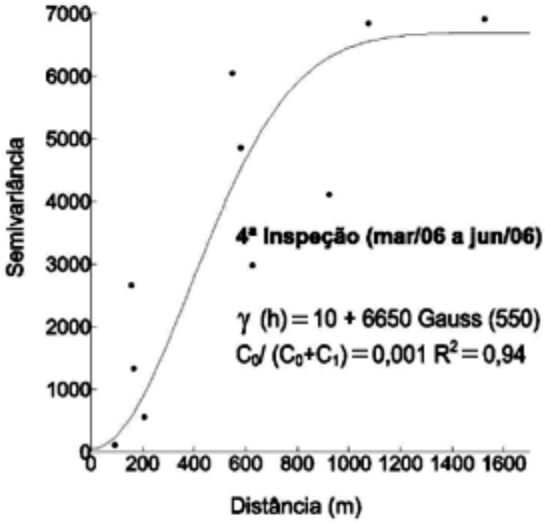
* Média do número de plantas com greening dos 46 talhões de citros.

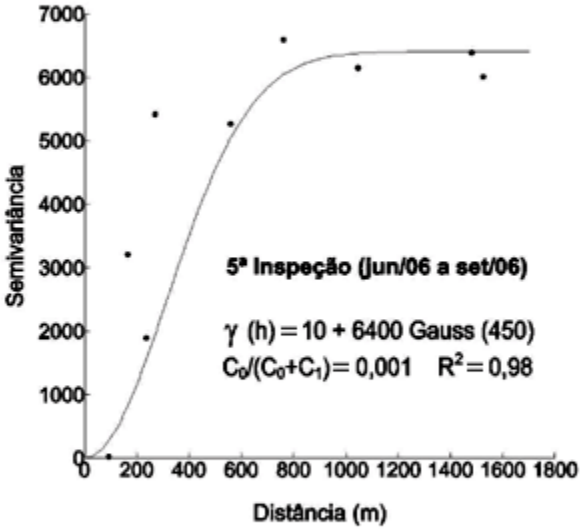
In geostatistical analysis, the variables were the number of plants greening present in each of the 46 plots of citrus. The spatial dependence of the number of plants with citrus greening was studied by means of semivariograms and the model that best fitted the data was a Gaussian, with the highest coefficient of determination (Figure 3).

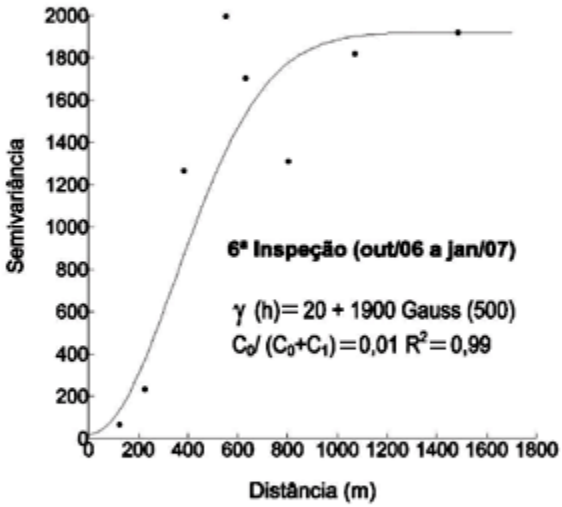

1ª Inspeção (mar/05 a jun/05)
γ (h) = 0,5 + 60 Gauss (500)
C0/ (C0+C1) = 0,008 R2 = 0,99
Semivariância
Distância (m)


2ª Inspeção (jun/05 a set/05)
γ (h) = 18 + 4500 Gauss (500)
C0/ (C0+C1) = 0,003 R2 = 0,97
Semivariância
Distância (m)


3ª Inspeção (set/05 a mar/06)
γ (h) = 10 + 4700 Gauss (560)
C0/ (C0+C1) = 0,002 R2 = 0,99
Semivariância
Distância (m)


4ª Inspeção (mar/06 a jun/06)
γ (h) = 10 + 6650 Gauss (550)
C0/ (C0+C1) = 0,001 R2 = 0,94
Semivariância
Distância (m)


5ª Inspeção (jun/06 a set/06)
γ (h) = 10 + 6400 Gauss (450)
C0/(C0+C1) = 0,001 R2 = 0,98
Semivariância
Distância (m)


6ª Inspeção (out/06 a jan/07)
γ (h) = 20 + 1900 Gauss (500)
C0/ (C0+C1) = 0,01 R2 = 0,99
Semivariância
Distância (m)

Semivariograms were prepared from plants with citrus greening of eight inspections (Figure 3), which presented a range of spatial dependence from 450 to 560 m, with the exception of the last inspection, which was 300 m (Figure 3). The range of spatial dependence of the last inspection was lower than the previous because over the course of inspections, as they were diseased plants in plots, they were eliminated and, in addition, some sites were completely eradicated from the farm because of the large number of diseased plants. The number of plots in the first inspection was 46, leaving the last inspection, only 37 plots.

The range of spatial dependence is of great importance, as well as indicate the radius of aggregation of plants with citrus greening on the farm, allows us to analyze the time interval in which the inspections in the same block, will be performed. By Ray aggregation of plants with citrus greening, provided by the semivariograms during eight inspections, we can conclude that the interval of three months between an inspection and the other in the same plot, it was not appropriate because in the course of inspections not decreased markedly or relevance of the radius of aggregation (Figure 3).

Another important index obtained from the semivariogram is the ratio $C_0 / (C_0 + C_1)$, that indicates the distribution of the disease. According to Journel and Huijbregts (1978), the values of the $C_0 / (C_0 + C_1)$ below 0.8 indicate an aggregated distribution of the variable. In this study, the values obtained in eight inspections ranged from 0.01 to 0.008 (Figure 3), indicating that plants with greening were distributed in an aggregated form, ie in the form of clumped on the farm.

Some research using geostatistics to evaluate spatial dependency and expanding other citrus diseases have been developed. Gottwald et al. (1996) examined the temporal and spatial distribution of citrus tristeza and found patchy distribution of disease and spread of diseased plants throughout the area. Roberto et al. (2002) studied the spatial dependence of citrus variegated chlorosis (CVC) and also observed aggregated distribution of disease in the field, with a range of spatial dependence of diseased plants from 10 to 14 m. The authors found through the kriging maps, expansion of the CVC in the area and concluded that the use of geostatistics was extremely important to verify the behavior of the disease on the farm.

Geostatistical analysis was also used to study the spatial dependence of various pathogens that cause diseases in other crops such as *Fusarium oxysporum* on tomato (REKAH et al., 1999), *Phytophthora infestans* on tomato and potato (JAIME-GRACIA et al., 2001) , *Venturia inaequalis* in apple (Charest et al., 2002) and *Colletotrichum*spp. bean and cotton (Alves et al., 2006). In all studies, we determined the spatial dependence of diseased plants with subsequent spread of the disease occurred almost throughout the area.

To better visualize the evolution of greening on the farm, were prepared kriging maps. There was, at first inspection, the initial focus of diseased plants originated in the blocks closest to the boundaries of the farm, later forming clusters from a greening plants with aggregate radius of 500 m. Probably, this outbreak initially

came psyllids infected by coming from the neighboring farm to the study, which had already greening plants (Figure 4).

Gottwald et al. (1989), through periodic inspections and found that the greatest amount of greening plants found within the limits of the plot, especially when the plot next door also had a greater incidence of diseased plants.

On second inspection, the radius of aggregation of citrus trees was maintained patients (500 m), however, the number of diseased plants was much higher than this inspection (0 to 1,500 plants) found in the first inspection (0 to 240 plants). Moreover, there was the emergence of another outbreak of greening plants from the plots above the first Reboleira initial inspection (Figure 4).

In the third, fourth, fifth and sixth inspection, it was observed besides the appearance of new foci of greening plants, the expansion of the disease that almost took over half the farm area, forming new foci of aggregation with a radius of 500 to 560 m (Figure 4). So by these results, we conclude once again that the interval of three months between an inspection and the other in the same block, was not adequate because it always existed in the area with greening plants, whose range of aggregation of diseased plants hardly was decreased.

In the seventh and eighth inspections, there was the expansion of the greening of the whole farm, ie the emergence of new foci and greening plants with subsequent formation of foci on the opposite side from the one that emerged first in Reboleira farm (Figure 4).

The rapid spread of disease throughout the farm due to the presence of the psyllid in orchards throughout the year, and, according to Yamamoto et al. (2001), its population peak occurs in spring / summer to fall in autumn / winter, so even during periods in which the insect population was low, it transmitted the bacteria from infected plants to healthy ones yet.

The interval between an inspection and the other (three months) in the same block, is also related to the spread of disease, since plants with greening stay long in the field, serving as a source of inoculum for the contamination of plants still healthy .

CONCLUSIONS

1-Spatial dependence of greening plants showed aggregation radius of 300 to 560 m, indicating an aggregated distribution of the disease.

2-Through the kriging maps, notes that the initial focus of diseased plants occurred in the limits of the farm with the expansion of greening throughout the area.

3-The inspection interval of three months was not adequate to reduce the greening on the farm.

REFERENCES

AGRIANUAL 2007: Yearbook of Brazilian agriculture. São Paulo: FNP Survey, 2008. 520p. [Links]

ALVES, MC; POZZA, EA, MACHADO, JC; ARAÚJO, DV; TALAMINI, V., Oliveira, MS Geostatistics as a methodology to study the spatiotemporal dynamics of diseases associated with C *olletotrichum* spp. transmitted by seed.**Fitopatologia Brasileira** , Brasília, v.31, n.6, p. 557-563, 2006. [Links]

AYRES, AJ; BELASQUE, JJ; Sposito, MB; Bassanezi, RB; LOPES, SA **Handbook of greening** . Araraquara: Fundecitrus, 2005. 12 p. [Links]

Bove, JM Huanglongbing: a destructive, newly-emerging, century-old disease of citrus. **Journal Plant Pathology**, Bari, v.88, n.1, p. 7-37, 2006. [Links]

Charest, J., Dewdney, M.; PAULITZ, T.; PHILION, V.; CARISSE, O. Spatial distribution of *Venturia inaequalis*airborne ascospores in orchards. **Phytopathology** , Davis, v.92, n.7, p. 769-779, 2002. [Links]

COLLECTION-Filho HD, Takita, MA, Targon, MLPN; CARLOS, FE, AX, MA The bacterium *Candidatus* Liberibacter plants with HLB (ex grenning) in São Paulo. **Orange** , Cordeirópolis, v.25, n. 2, p.367-374, 2004. [Links]

GOTTWALD, TR; AUBERT, B., ZHAO, XZ Preliminary analysis of citrus greening (HLB) of China and French Reunion Island. **Phytopathology** , Davis, v.79, n. 6, p. 687-693, 1989. [Links]

GOTTWALD, TR; CAMBRA, M., MORENO, P., Camarasa, E., Piquer, J. Spatial and temporal analyzes of citrus tristeza virus in Eastern Spain. **Phytopathology** , Davis, v.86, n. 1, p. 45-55, 1996. [Links]

JAIME-GARCIA, R., Orum, TV, Felix-Gastelum, R.; TRINIDAD-CORREA, R.; VANETTEN, HD, Nelson, MR Spatial analysis of *Phytophthora infestans* genotypes and late blight severity on tomato and potato in the Del Fuerte Valley using Geostatistics and geographic information systems. **Phytopathology** , Davis, v.91, n. 12, p. 1156-1165, 2001. [Links]

Journel, AG; HUIJBREBTS, CJ **Mining Geostatistics** . London: Academic Press, 1978. [Links]

LIEBHOLD, AM, ROSSI, RE, Kemp, WP Geostatistic and geographic information system in Applied insect ecology.**Annual Review of Entomology,** Palo Alto, v.38, n.1, p.303-327, 1993. [Links]

REKAH, Y.; SHTIENBERG, D., Katan, J. Spatial distribution and temporal development of Fusarium crown and root rot of tomato and Dissemination pathogen in field soil. **Phytopathology** , Davis, v.89, n. 9, p. 831-839, 1999.[Links]

ROBERTO, SR; FARIAS, PRS; Bergamim SON, A. Geoestatistical analysis of spatial dynamics of citrus variegated chlorolis. **Fitopatologia Brasileira** , Brasília, v. 27, n.6, n. 6, p. 599-604, 2002. [Links]

TEIXEIRA, DC; WULFF, NA; MARTINS, CE; Kitajima, EW; Bassanezi, R.; AYRES, AJ; Eveillard, S., Saillard, C., Bove, JM phytoplasma Closely related to the pigeon pea witches'-broom phytoplasma (16Sr IX) Is Associated with citrus huanglongbing SYMPTOMS in the State of São Paulo, Brazil. **Phytopathology** , Davis, v.98, n. 9, p. 977-984, 2008. [Links]

YAMAMOTO, PT; PAIVA, PEB; Graven, S. Population fluctuation of *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) in citrus groves in northern São Paulo state. **Neotropical Entomology** , Londrina, v. 30, n. 1, p. 165-170, 2001. [Links]

Received: 03/06/2009.
Accepted for publication: 08/04/2010.