SPATIAL ANALYSIS OF PLUM LEAF SCALD IN SÃO PAULO STATE, BRAZIL

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SUMMARY

This study characterizes the spatial dynamics of Plum Leaf Scald on three commercial orchards located in São Paulo State, Brazil. All trees from each orchard were evaluated during four consecutive years (one orchard), and for three consecutive years (two orchards). The incidence and characteristics of spatial patterns were assessed by the binomial dispersion index, Ripley's K-function and average minimum distance. Autologistic models were fitted for different neighboring structures with model selection guided by the Akaike Information Criteria. The autologistic model relates the probability of an infection with the presence of the disease on neighboring trees within and between the rows and also on the diagonals, on the same and/or previous time of assessment. Aggregated patterns were detected by different methods, except for the evaluations with incidence below 10%. The selected autologistic model consistently showed an increased risk of disease for trees with infected neighboring in the same row on the same time of assessment, whereas effects between rows were only detected for the higher incidences. Overall, the Plum Leaf Scald epidemic shows random patterns at the beginning and aggregation on later stages.

Keywords: *Prunus salicina*, *Xylella fastidiosa*, epidemiology.

INTRODUCTION

The Plum Leaf Scald (PLS) is a disease caused by the bacterium *Xylella fastidiosa* (Wells *et al*., 1987) that inhabits exclusively the xylem vessels of various plant species (Hartung *et al*., 1994). Symptoms associated with PLS are initially chlorotic spots at the tip of the leaves, which develop into necrotic, and then extend towards leaf blades. Necrotic areas assume gray or dark brown color, giving to the affected branches a burned aspect, hence the name "scald" (Ducroquet *et al*., 2001).

From 2008 to 2013, the amount of plums imported by the Brazilian market increased by 75%, amounting 32,219 tons of imported fruit costing US$ 46 million in 2013 (Aliceweb, 2014). Demand for imports is mainly a result of disease problems occurrence in Brazil's main producing regions. Minas Gerais State, on Southeastern Brazil, was once considered the largest Brazilian state producer of plums in the 70s, with a significant decrease in planting due to the occurrence of PLS. Also in the 70s and 80s, the disease was responsible for the eradication of orchards on Brazilian Southern States, Rio Grande do Sul to Paraná (Alvarenga *et al*., 2007; Oliveira *et al*., 2011; Eidam *et al*., 2012).

The main agents of bacteria transmission are insects belonging to the suborder Homoptera, family *Cicadellidae*. Popularly known as leafhoppers, these insects suck plant sap directly from the xylem vessels, transmitting bacteria propagules from plant to plant (Redak *et al*., 2004). Another important way of disease introduction is the use of infected seedlings, especially in newly deployed orchards (Roberto *et al*., 2002). Seedlings or other propagative infected material, often asymptomatic, can carry the disease to long distances, crossing borders of states and countries. As examples, reports of seedling introductions infected with *X. fastidiosa* in various species in the American states of California, Arizona and even in island territories such as French Polynesia and Hawaii (Janse and Obradovic, 2010). Saponari *et al*. (2013) reported the occurrence of *X. fastidiosa* in oleander, almond and olive trees exhibiting leaf scorch symptoms in southeastern Italy. *X. fastidiosa* presents a major risk to the European Union territory due to its potential to cause disease in the risk assessment area once it establishes, as hosts are present and the environmental conditions are favorable. *X. fastidiosa* may affect several crops in Europe, such as citrus, grapevine and stone fruits as almond, peach, plum (EFSA, 2015; Saponari *et al*., 2016).

In Brazil, since the 90s it has been reported the emergence of cultivars that allow the time extension of the orchards exploration, allowing farmers to produce besides the disease occurrence in some regions. However,

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Table 1. Description of commercial plum orchards assessed about their spatial distribution of Plum Leaf Scald, in the municipality of Paranapanema, São Paulo State, Brazil.

Orchard	Cultivars[1]	Orchard orientation	Number of trees	Year of planting	Evaluation period	Spacing (m)
1	Gulf Blaze	North-Northeast (NNE)	1338	1998	2000 to 2003	6,0×4,0
2	Reubennel	Northeast (NE)	375	1996	2005 to 2007	6,0×4,0
3	Reubennel	East (E)	298	1998	2005 to 2007	6,0×4,0

[1] *Prunus salicina* Lindl.

Table 2. Different parametrizations tested of the autologistic model considering temporal and spatial covariates assessing incidence of Plum Leaf Scald, caused by *Xylella fastidiosa*.

Models	Covariates
M1	Current Time: row and row spacing.
M2	Current Time: row, row spacing and diagonal.
M3	Former time: row and row spacing
M4	Former time: row, row spacing and diagonal.

more knowledge is needed to guide more efficient management in the orchard, including balanced fertilization, water availability, and removal of diseased material and especially, control of insect vectors (Dalbó and Feldberg, 2009). Difficulty in using these techniques has limited the permanence of small farmers or those with low levels of technology.

For similar pathosystems, such as Huanglongbing (HLB), Citrus Variegated Chlorosis (CVC) and Coffee Leaf Scorch (CLS), the management is based on the epidemiology of diseases in the field, including temporal and spatial dynamics of the disease (Gottwald *et al*., 1993; Nunes *et al*., 2001; Tubajika *et al*., 2004; Rocha *et al*., 2010), vector behavior (Redak *et al*., 2004; Ott *et al*., 2006; Muller, 2008, 2013) and regional management (Bassanezi *et al*., 2005). Therefore, the study of the spatial distribution of a particular disease depends on the pathogen dispersion characteristics, plants architecture and plants spatial arrangement (Gilligan, 1982; Redak *et al*., 2004; Spósito *et al*., 2007). Knowledge of this spatial distribution can be an important tool to assist the development of disease control strategies such as to PLS in Brazil and worldwide.

There are no epidemiological studies on PLS in Brazil, and the works that have been made with this pathogen focus mainly on the pathogen's interaction with the vector and alternative hosts (Muller, 2008, 2013). This highlights the importance of those studies to develop strategies of disease management in the field, considering especially the dispersion model of the disease. Other relevant information from the literature is that there are different strains of the bacteria in different regions and specializations between cultures (Lopes *et al*., 2003; Janse and Obradovic, 2010). Therefore, regionalized studies to better understanding the pathosystem and for the recommendation of disease management are relevant.

This study aims to: i) characterize the spatial dynamics of the disease in commercial orchards of plum cultivated in subtropical climate in São Paulo state, Brazil, ii) evaluate results provided by different spatial statistical methods to the understanding of PLS development over the time.

MATERIALS AND METHODS

Study area description. The PLS incidence data were collected in commercial orchards in the municipality of Paranapanema, São Paulo State, Southeastern Brazil, located at the geographical coordinates 23°23' 19" S and 48°43' 22" W, altitude of 610 meters. The climate belongs to the type Cwa, warm temperate climate, with dry winter and hot summer, according to the Köppen climate classification. The city's average temperature of the coldest month is 17.3°C and average temperature of the warmest month is 24.3°C. The average annual temperature is 21.1°C. Average annual rainfall in Paranapanema is 1407 mm, being 40.1 mm the average in the driest month and 203.8 mm in the rainiest month.

Three orchards were evaluated (Table 1) in two selected properties in São Paulo State. Orchard 1 was evaluated for four years (2000-2003) and the other two orchards (orchards 2 and 3) for three years (2005-2007). All trees of each orchard were inspected, being recorded the presence or absence of the typical symptoms of the disease (incidence) in summer time, between January and March. The assessment in the study region was carried out 3-4 months after harvest, when the symptoms are more evident and easily identified in the field.

Data analysis. Exploratory analyzes were performed observing the ratio between diseased and healthy plants over time and the amount of missing data (NA's). Kernel smoothing was used to filter the main features of the spatial variability of the data whilst still retaining the essential characteristics of the fields (Bailey and Gatrell, 1995).

Spatial patterns of aggregation or randomness were firstly inspected by the analysis of quadrat counts with quadrats sizes of 2×4, 3×6, 4×8 e 5×10 trees. Binomial dispersion index ($Id = Vobs/Vteor$) was computed. The Id is the ratio between the observed variance (Vobs) and variance (Vteor) expected under randomness. The null hypothesis that Id = *1* indicates random pattern and rejection is considered as an indication of aggregation (Madden *et al*., 1995). A t-test was used and conclusions are reported under 0.01 and 0.05 significance levels.

Quadrat based methods rely on arbitrary choices of quadrat sizes. Further evidence on aggregation was then gathered from two continuous distances based methods, the average minimum distance test (AMD) and Ripley's K-function. Such methods were used, for instance, in

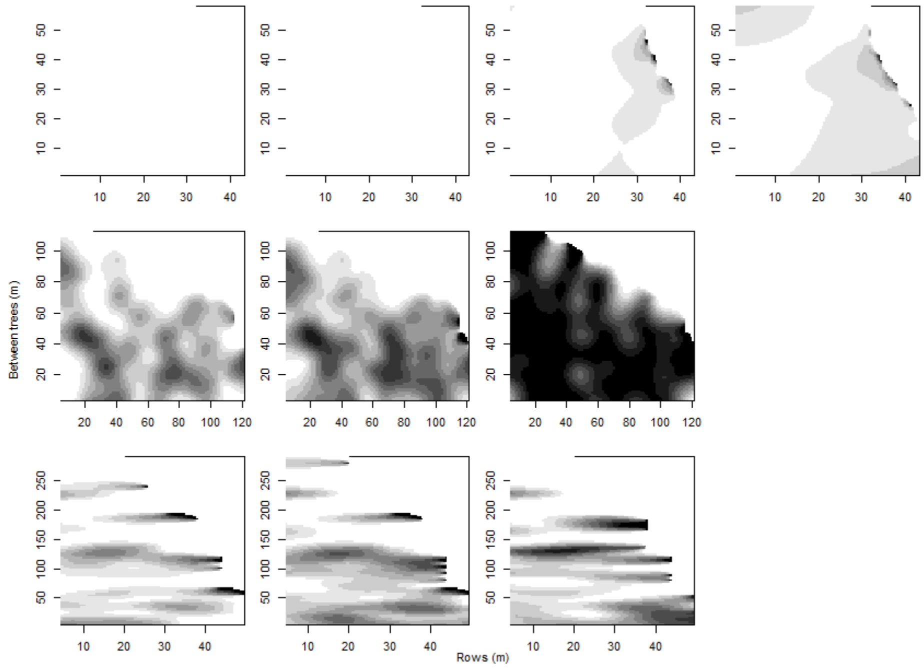

Fig. 1. Maps of the intensity of Plum Leaf Scald incidence in four years of evaluations, by Kernel smoothing method. From the left side, Line 1 data in Orchard 1 in different years of evaluations from 2000 to 2003, Line 2 the different years of evaluations in Orchard 2, from 2005 to 2007 and Line 3 the different years of evaluations in Orchard 3, from 2005 to 2007. Municipality of Paranapanema, São Paulo State, Brazil.

detecting spatial patterns of citrus black spots by Spósito *et al.* (2007). For the AMD the distances between each tree and the closest infected tree are averaged and the value is compared with a reference distribution obtained by Monte Carlo simulations under randomness. Observed averages below the 0.01 or 0.05 quartiles of the distribution obtained by randomization indicate aggregated patterns.

The Ripley's K function (Ripley, 1981) is widely used in the analysis of point patterns to detect aggregated or random patterns. The function computes the average density of points on a sequence of circles with increasing radius in the vicinity of each the events (here a diseased tree). The empirical function is compared with bands (envelopes) obtained by the functions computed from simulation under randomness. The result is typically given as a plot of scaled densities against the radius. Empirical curves showing parts outside the bands indicates deviation from the random pattern. The method only considers the diseased plants ignoring the spacing and therefore is only meaningful when assessing a large number of plants and the empirical curves should be interpreted only for distance values above the spacing between plants.

Additional insight can be gained by fitting models which relates the status of the disease with the status on neighborhood of the trees. Such models not only allow for detecting spatial patterns, identifying plausible neighborhood structure but also quantify the odds of disease in relation of its presence in such neighborhoods. Inference must be carried out accounting for the fact that data are used as response and also defining the neighboring status (Gumpertz *et al.*, 1997). Different neighborhood structures can be defined and tested, such as within and between rows and diagonal effects. The neighboring structures can be fitted separately or jointly, depending on the interest and the spacing between the trees. Disease status on such neighborhoods can be considered at the same or previous times of assessment. Such models were used for instance in Franciscon *et al.* (2008) in citrus sudden death disease and Kaiser *et al.* (2014) in bean pod mottle virus. The neighborhoods considered here are given in Table 2, accounting separately for influences of diseased tree within the rows, between the rows, on diagonals and on current and previous times. The Akaike Information Criteria (AIC) was used to select between the fitted models and coefficients for neighboring structures assessed for $p < 0.01$, 0.05 and 0.10 significance levels.

All the analyses were performed using the add-on package Rcitrus (Krainski and Ribeiro Jr, 2006) for the R software (R Development Core Team, 2015).

RESULTS

The incidence of PLS varied from 0.48 to 9.2% in Orchard 1; from 27.3 to 80.3% in Orchard 2 with increasing incidence over time; in orchard 3, the incidence varied from 14.1 to 23.3% and it was observed a decrease in the number of failures (absence of plants) throughout the study period, from 287 in 2006 to 282 in 2007, due to substitution of dead plants.

Kernel smoothing (Fig. 1) shows borders of the evaluated areas with higher concentration of infected plants,

Table 3. Binomial dispersion index of plants with Plum Leaf Scald symptoms caused by *Xylella fastidiosa* in different quadrats sizes. Municipality of Paranapanema, São Paulo State, Brazil.

Quadrats size	Year[a]	Orchard 1				Orchard 2				Orchard 3			
		N[1]	P[2]	ID[3]		N	P	ID		N	P	ID	
2×4	1	166	0.4	1.0	*ns*	38	30.0	2.1	*	30	15.0	1.4	*ns*
	2	166	2.6	1.1	*ns*	38	41.6	2.1	*	30	24.1	1.3	*ns*
	3	166	4.5	1.1	*ns*	38	88.1	1.9	*	30	23.3	1.7	*
	4	166	8,5	1,3	*	–	–	–	–	–	–	–	–
3×6	1	68	0.4	0.9	*ns*	13	31.4	2.3	*	11	15.6	1.9	*
	2	68	2.6	1.0	*ns*	13	42.5	2.4	*	11	24.2	2.6	*
	3	68	4.4	1.0	*ns*	13	88.4	1.4	*ns*	11	20.7	2.6	*
	4	68	8.4	1.8	*	–	–	–	–	–	–	–	
4×8	1	36	0.3	0.9	*ns*	6	31.6	2.9	*	5	16.8	1.5	*ns*
	2	36	2.7	1.6	*	6	45.0	2.4	*	5	26.2	2.3	*ns*
	3	36	4.1	1.8	*	6	89.3	1.4	*ns*	5	22.5	1.2	*ns*
	4	36	8.4	1.9	*	–	–	–	–	–	–	–	
5×10	1	19	0.4	0.8	*ns*	4	26.5	1.1	*ns*	3	16	2.8	*ns*
	2	19	2.5	2.6	*	4	45.0	2.1	*ns*	3	16.6	4.1	*
	3	19	4.0	2.4	*	4	92.0	0	*r*	3	18	1.7	*ns*
	4	19	7.0	2.1	*	–	–	–	–	–	–	–	–

[a] Annual evaluations, Evaluation 1 of Orchard 1 = year 2000 and Orchards 2 and 3 = 2005.
[1] Number of complete estimated quadrats.
[2] Quadrats percentage with at least one diseased plant.
[3] Binomial dispersion index: If ID > 1 and significant means aggregate; If ID < 1 or not significant means random.
* significant or $p < 0.05$ and ns = not significant.

especially in orchards 1 and 3. In Orchard 2 (second line Fig. 1) there was a large concentration of diseased plants spreaded over the area, with no obvious pattern.

The dispersion index (Table 3) showed random distribution pattern of the disease in the first assessment, and aggregated in the last evaluation for all quadrats sizes tested for Orchard 1. In the second and third evaluations, only the larger quadrats (4 × 8 and 5 × 10) had pointed to aggregated spatial pattern. In Orchard 3, there was also wide prevalence of the random pattern, except for the 3 × 6 arrangement, which presented aggregated pattern across all assessments dates, and the 2 × 4 arrangement, which showed aggregation in the last assessment. Orchard 2 showed prevalence of aggregate patterns, except in the latest assessments of the intermediate sized quadrats (3 × 6 and 4 × 8) and also the largest quadrats arrangement (5 × 10), the latter presenting random pattern in for the first two reviews, and regular pattern, characteristic of high incidences, for the last evaluation.

The AMD method (Fig. 2) patterns of randomness of PLS incidence ($p > 0.05$) in the first 3 evaluations in Orchard 1 (Fig. 2, A-C), with aggregation in the last assessment (Fig. 2D). The AMD observed between diseased plants in Orchard 1 ranged from 48.8 to 8.2 meters in the first and last assessment respectively. Orchards in 2 and 3 (Fig. 2, from E to J), there was prevalence of aggregate pattern ($p < 0.05$), except on the second evaluation in Orchard 3. Average distances observed ranged from 4.8 to 4.0 meters between the assessments made in Orchard 2, and from 6.5 to 5.2 meters in Orchard 3.

The plots of Ripley's K functions (Fig. 3) show that aggregated patterns prevails for different evaluations at the different orchards, with exception to the first evaluation of the Orchard 1 (Fig. 3A).

The best fitted autologistic models (Table 4) for Orchards 1 and 3 include effects of diseased plants on rows, between rows and the diagonals on the same assessment (Model 2). However, estimated parameters for the first orchard were not significant ($p > 0.05$) in any of the dates, not significantly differing, at this probability level, from simulated populations with random pattern. The parameter considering plants on the same row was significant only at the $p < 0.10$ significance level at the fourth year of evaluation, repeating the aggregated pattern observed in the methods described above.

In Orchard 2, the best adjustment was also observed in Model 2 for the first and third assessments, evidencing the influence of diseased plants presence in the same assessment date and considering the influence of the diagonals. For the second assessment, Model 4 was better adjusted, thus also indicating influence of the previous stage of the disease on the neighboring plants within the rows, between rows and diagonals. Diseased plants on same row neighboring plants increase the odds of disease for all assessment dates and assessed orchards (Table 4).

DISCUSSION

In this work, the aggregated pattern in the spatial distribution of PLS incidence was prevalent for different fields and methods used, beside a random start. The methods that divided the fields in different quadrats sizes brought divergent conclusions. It was found the prevalence of disease onset at the borders of the orchards. The disease increase was more likely to occur among plants within the same row planting, regardless of geographic orientation of orchards.

The highest concentration of diseased plants on the borders of orchards 1 and 3 is shown according to the description of Redak *et al.* (2004) for Pearce's disease (*X. fastidiosa*) in vines, which the author attributed to the entrance pattern in the field of vectors that inhabit the vegetation in the edge of orchards. Laranjeira *et al.* (2004) also observed pattern of higher concentration of the disease from the borders of the three citrus orchards evaluated for CVC incidence, using an isopath area determination method, visually similar to Kernel smoothing, used in this study.

As well as on data from PLS, the pattern of higher concentration on the orchards borders in papaya sticky disease was attributed to the presence of isolated outbreaks within the lots. In this case, the internal outbreaks could be explained by the formation of vectors colonies into the orchard, and the subsequent transmission from secondary inoculum (Vidal *et al.*, 2004).

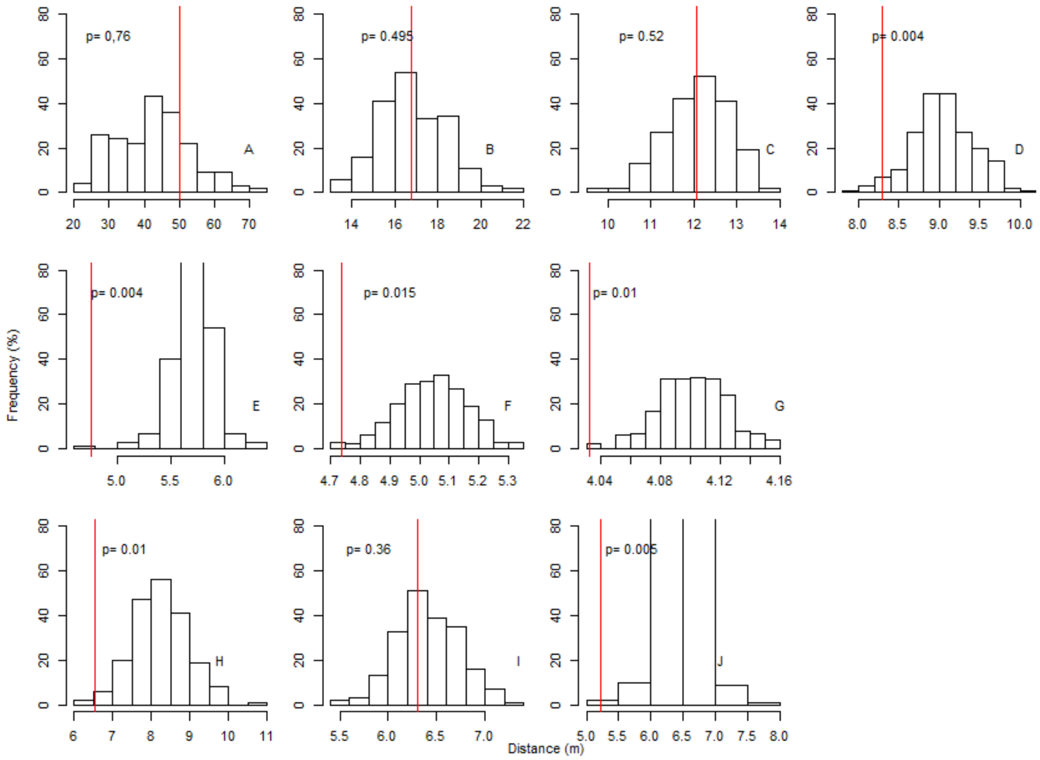

Fig. 2. Frequency of Average Minimum Distances between healthy plants and its neighbors with Plum Leaf Scald symptoms in Orchard 1. A = 2000; B = 2001; C = 2002, D = 2003, Orchard 2. E = 2005; F = 2006; G = 2007, Orchard 3. H = 2005; I = 2006; J = 2007. The probability (p) indicates whether there is a statistical difference between the studied population and a simulated population with random pattern. Municipality of Paranapanema, São Paulo State, Brazil.

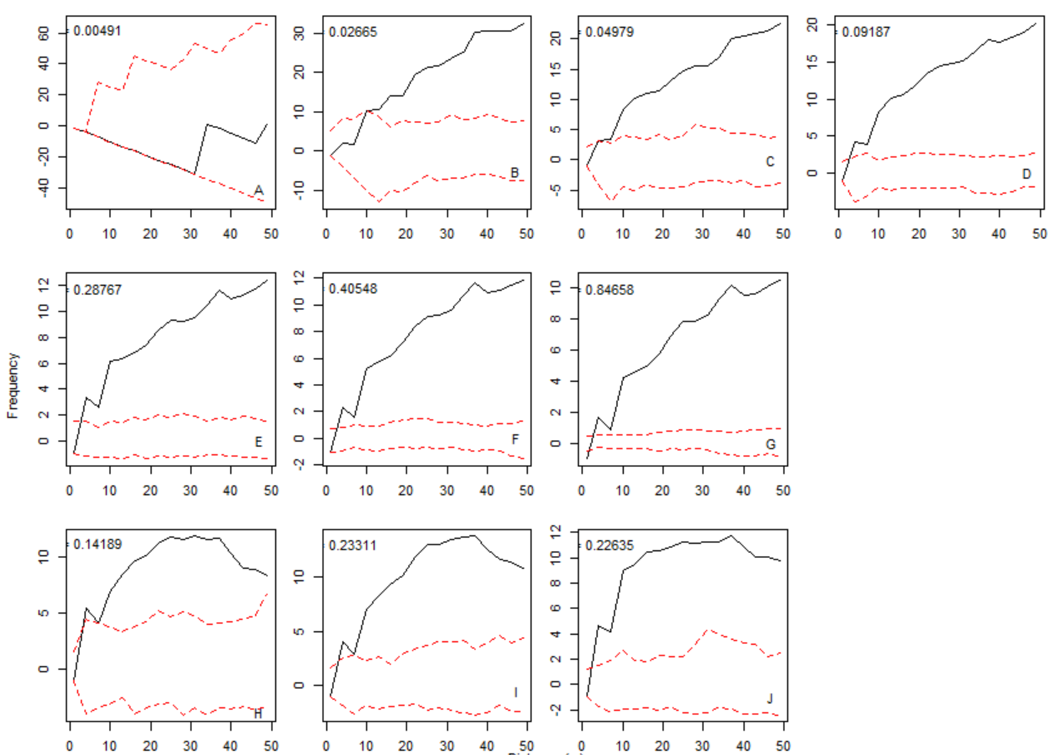

Fig. 3. Ripley's K Function fitted to Plum Leaf Scald incidence data in Orchard 1. The dashed line indicates the function's confidence envelope, and values within this range indicate randomness. Orchard 1. A=2000; B=2001; C=2002; D=2003, Orchard 2. E=2005; F=2006; G=2007, Orchard 3. H=2005; I=2006; J=2007. Decimal numbers on the left of the graphics indicate disease incidence at each evaluation. Axis y unit is percentage (%). Municipality of Paranapanema, São Paulo State, Brazil.

Table 4. Parameters estimated by fitting the data of Plum Leaf Scald incidence to the autologistic model under four parameterizations. Three commercial orchards located in the city of Paranapanema, São Paulo State, Brazil.

Orchard	Year[1]	Model[2]	Estimated parameters								AIC[5]
			Rows		Between trees		Diag. A[3]		Diag. B[4]		
1	1	M1	0	ns[6]	0	ns	–		–		71.12
	1	M2	0	ns	0	ns	0	ns	0	ns	63.62
	2	M1	–1.14	ns	0.43	ns	–		–		301.87
	2	M2	–1.12	ns	0.47	ns	0.33	ns	0.69	ns	296.80
	2	M3	0	ns	0	**	–		–		303.02
	2	M4	0	ns	0	**	0	**	0	**	297.45
	3	M1	0.74	ns	0.47	ns	–		–		440.27
	3	M2	0.79	ns	0.25	ns	–0.71	ns	0.19	ns	427.88
	3	M3	0.80	ns	0.19	ns	–	ns	–	ns	448.82
	3	M4	0.80	ns	0.19	ns	0.15	ns	0.20	ns	444.75
	4	M1	0.84	.	0.12	ns	–		–		717.64
	4	M2	0.83	.	0.07	ns	0.04	ns	0.17	ns	703.49
	4	M3	0.68	.	0.01	ns	–		–		721.21
	4	M4	0.63	ns	0.17	ns	0.18	ns	0.36	ns	705.33
2	1	M1	1.20	**	0.36	ns	–		–		314.14
	1	M2	1.35	**	0.25	ns	0.25	ns	0.01	ns	295.02
	2	M1	0.83	**	0.23	ns	–		–		365.22
	2	M2	0.83	**	0.22	ns	–0.17	ns	0.083	ns	347.67
	2	M3	1.26	**	0.16	ns	–		–		348.68
	2	M4	1.27	**	0.01	ns	0.20	ns	0.21	ns	330.24
	3	M1	1.89	**	0.98	*	–		–		162.87
	3	M2	2.09	**	1.54	**	–0.73	.	0.02	ns	144.50
	3	M3	0.89	**	0.38	*	–		–		195.85
	3	M4	0.80	**	0.23	.	0.16	ns	0.11	ns	189.24
3	1	M1	1.42	ns	0.17	ns	–		–		133.72
	1	M2	1.48	.	0.38	ns	0.08	ns	–0.92	ns	127.64
	2	M1	1.23	*	0.27	ns	–		–		161.39
	2	M2	1.49	**	0.59	ns	0.16	ns	–0.86	ns	146.81
	2	M3	1.42	*	0.69	ns	–		–		163.88
	2	M4	1.44	*	0.96	*	0.17	ns	0.59	ns	158.04
	3	M1	1.47	*	0.69	ns	–		–		146.74
	3	M2	1.56	*	0.80	ns	0.44	ns	–0.41	ns	137.27
	3	M3	1.12	**	0.83	*	–		–		150.58
	3	M4	1.36	**	1.13	*	0.03	ns	–0.76	ns	138.96

[1] Annual evaluations, being evaluation 1 of Orchard 1 = year 2000 and Orchards 2 and 3 = year 2005.

[2] Parametrizations of autologistic model were: M1 = Current Time: row and row spacing; M2 = Current Time: row, row spacing and diagonal; M3 = Former time: row and row spacing; M4 = Former time: row, row spacing and diagonal.

[3] Diagonal towards (1,1).

[4] Diagonal towards (–1,1).

[5] Akaike's Information Criterion, the lower the value the better fit.

[6] Significance of the coefficients estimated by the Monte Carlo method: "**" $p < 1\%$; "*" $p > 1$ and $< 5\%$, "." $p > 5$ and $< 10\%$; "ns" = not significant.

The presence of isolated foci can also be explained by the action of more than one spread agent, for example, the use of infected seedlings in the implementation of the orchard (Cati, 2009; Janse and Obradovic, 2010), being this aggravated by the endemic nature of the disease in the study region, in different cultures and alternative hosts (Muller, 2013).

In Orchard 2 it was not observed this pattern of concentration on the border due to the high incidence of disease since the beginning of the assessments, with a maximum of 80% and dispersion index equal to the regular distribution pattern of incidence in the last evaluation date.

The aggregation patterns in higher incidences and randomness in the lower ones were confirmed by the different methods used in this study (dispersion index, AMD and Ripleys's K function). The dispersion index indicates whether in a given evaluation the diseased plants were aggregated or not, however, the quadrats sizes may interfere with the biological interpretation of the test results (Laranjeira *et al*., 2004).

Therefore, as for HLB in citrus, the random beginning of the epidemic can be assigned to the initial contribution by vectors. Thus, in low incidences, the distribution pattern of the disease would be random, due to dependence on the erratic arrival of the vector to the study area. Later, with the increased incidence, the pattern would become aggregated because diseased plants serve as the source of inoculum (secondary) to other plants in the study area and the presence of the vector within the orchard (Gottwald *et al*., 2007).

The graphics of the Ripley's K function didn't detect aggregation pattern of the disease in distances less than 10 meters in all evaluations. This weakness in the detection of the aggregation by this method in smaller rays agrees with Spósito *et al.* (2007), who commented that this method does not consider spacing between plants, but a fixed radius, and so has better applicability to large areas of study or at greater distances within the cultivated areas. The same authors remarked that the method of AMD is very useful for determining the aggregation patterns, considering the area as it is and not dividing the area arbitrarily.

The autologistic parameterization model that uses the same evaluation date as a covariate (Models 1 and 2) are those that have better descriptive capacity of epidemics. The models containing parameterization using the previous evaluation (Models 3 and 4), however, give the model a predictive power (Franciscon *et al*., 2008). The incidence detection by serological method was more efficient than the visual observation of symptoms according to Nunes *et al.* (2001). The best fit of the models with parameterization considering the same evaluation date (Models 1 and 2) in this work, showed that the comparison between different years bumped into the possibility of any plants being infected but asymptomatic.

The results of the autologistic model adjustments are in accordance with the descriptive comments, with the orchards 1 and 2 distribution of diseased plants increasing in both directions over time (within the rows and between rows), but with prevalence of developments in the y-axis, i.e., between plants of the same row planting. The increase in the number of symptomatic plants with CVC in two orchards in São Paulo in the row direction, by the Foci

Form Index method, was observed by Nunes *et al.* (2001) in agreement which the PLS data of this work.

The pattern of increased likelihood of disease in the same row disagrees with Gottwald *et al.* (1993) conclusion, which highlighted the largest increase in the Citrus Variegated Chlorosis towards northeast to southeast, so diagonally in the studied Orchard. Because of this diagonal effect, the authors cited above completely ruled out any mechanical transmission effect by cultural practices in that pathosystem.

By ordinary runs method, Laranjeira *et al.* (2004) found the spatial pattern of disease increase in citrus plants in the Northeast, Central and South of the São Paulo State. In the Northeast and State Center, prevalence of aggregated pattern was observed in the planting row up to 40% and 70% of incidence in the regions, respectively. In the southern region of São Paulo, there was no difference between increases of disease within the row or between rows. In spite of the above results, Laranjeira *et al.* (2004) concluded that considering the observed in the three regions, it cannot be said that *X. fastidiosa* has a predominant spread within or between rows, discarding the influence of man and mechanical means for the bacteria's dissemination. Furthermore, the authors reasoned that for other pathosystems involving this pathogen, there was no information on the use of this type of analysis or even influence of cultural practices, such as pruning, in the spread of the bacteria. Using pruning shears, Krell *et al.* (2007) had a low efficiency (4.7%) in the *X. fastidiosa* inoculation test in vine plants (*Vitis vinifera*), but showing that this modality is possible.

The mechanical transmission ability should be better studied, particularly in the case of PLS, due to the higher intensity of pruning on this culture when compared to citrus or grapevine, and also because either on different orientations of the orchards, trees of the same rows were the preferential direction of increase of the disease in this study.

The findings of this study may help the producers to develop strategies of the disease management, focusing on the most efficient methods to control vectors on the edges of the orchards, as soil applied insecticides and regular aerial sprays of insecticides on all plants before the onset of symptoms. After that, with the pathogen entrance, measures could be made on plants on the same rows of diseased ones, as pruning, eradication and replant, and local control of vectors with application of insecticides.

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