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Spatial and Temporal Analyses of Citrus Sudden Death in Brazil

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ABSTRACT. Citrus Sudden Death (CSD) is a disease of unknown etiology that affects sweet orange grafted on Rangpur lime and Volkamer lemon rootstock in Brazil. The spatial and temporal patterns of CSD were monitored in 116 citrus plots from 37 citrus farms located in 10 counties of Minas Gerais (MG) and São Paulo (SP) states, Brazil, between January 2001 and September 2004. Maps of the spatial distribution of CSD infected trees were examined at three spatial scales. At the individual tree scale, ordinary runs analysis of CSD-symptomatic trees indicated clustering of symptomatic trees both within rows and across rows. At the middle scale of small groups of trees, the binomial index of dispersion for various quadrat sizes suggested aggregation of CSD-symptomatic trees for almost all plots within the quadrat sizes tested, and the index of aggregation increased with quadrat size. Estimated parameters of the binary form of Taylor's power law provided an overall measure of aggregation of CSD-symptomatic trees for all quadrat sizes tested and the intensity of aggregation was also a function of quadrat size and disease incidence. The largest scale tested was the entire plot level. Spatial autocorrelation analysis of proximity patterns suggested that aggregation often existed among quadrats of various sizes up to three lag distances. The results were interpreted as indicating the disease is caused by a biotic factor, and the disease was transmitted within a local area of influence approximately six trees in all directions. Annual rates of CSD progress based on the Gompertz model had the same range as *Citrus tristeza virus* (CTV) progress rates and many similarities were found between the spatial patterns of the CTV/*Toxoptera citricida* pathosystem and CSD. Based on the symptoms, spatial and temporal patterns of CSD support the hypothesis that CSD may be caused by a virus and vectored by aphids or insects with similar habits. The progress and spread of CSD in São Paulo state on a regional scale and its implications for the citrus industry were also discussed.

"Morte Súbita dos Citros" or Citrus Sudden Death (CSD) was first reported in an area south of Triângulo Mineiro in Minas Gerais State and north of São Paulo State, Brazil, in 2001 (7). CSD is now considered a potentially serious threat to the sustainable, commercial production of sweet oranges and some mandarins grafted on Rangpur lime and Volkamer lemon rootstocks, which represent more than 85% of 180 million trees of São Paulo State.

CSD decline symptoms are characterized by foliar discoloration, partial defoliation, reduction in new shoots, absence of internal shoots, rot and death of a large portion of the root system, and the characteristic development of a yellow stain in the phloem part of the rootstock (7). The rootstock bark near the bud union undergoes profound anatomical changes such as small phloem

cells, collapse and necrosis of sieve tubes, over production and degradation of phloem, accumulation of non-functioning phloem (NFP) and invasion of the cortex by old NFP (23). These symptoms intensify as the disease develops and culminate with the sudden collapse and death of the tree, normally without fruit abscission (7).

CSD is graft-transmissible (26) and shows many similarities to citrus tristeza (23). Based on symptoms and the similarities found between the spatial and temporal patterns of CSD in Brazil and the *Citrus tristeza virus* (CTV)/*Toxoptera citricida* pathosystem in Costa Rica and Dominican Republic it was proposed that CSD may be caused by a similar but undescribed pathogen (probably a virus) and probably vectored by an aphid (or insect with similar habits). The

search for graft-transmitted agents such as endogenous bacteria, viroids, and virus has produced negative results (1), although CTV and a new virus (possible *Tymoviridae*) have been found in diseased trees. The association of CSD affected trees with CTV variants (5, 19) and the new *Tymoviridae* (18, 20) has been studied, but the role that these viruses play in CSD is still not clear and Koch's postulate has not been completed.

The disease continues to increase and spread in and among groves in São Paulo State. The objective of this study was to examine and augment our current knowledge (1) of the spatial and temporal patterns of CSD at three different scales: rows, blocks and regional scales and to use this information to make inferences about the etiology and future progress of the disease.

MATERIALS AND METHODS

Citrus Block Scale

Data collection. The spatial pattern of CSD was monitored in 116 citrus plots (plot = block within a grove) from 37 citrus farms located in 10 counties of Minas Gerais (MG) and São Paulo (SP) states, Brazil, between January 2001 and September 2004. Each plot was composed of sweet orange (Hamlin, Natal, Valencia, Pera, Westin or Rubi) grafted on Rangpur lime rootstock. Incidence of CSD was assessed by visual inspection of the canopy of each tree in each plot. When necessary, diagnostic confirmation of CSD was performed by checking for the characteristic yellow stain in the phloem (7). Severity of CSD symptoms was assessed according to the following scale: 0 = healthy; 1 = initial, mild symptoms of decline (leaves turn pale green, become dull, some defoliation, and the rootstock bark below the bud union turns light yellow); 2 = severe defoliation, rootstock bark turns

bright yellow, with conspicuous root rot; 3 = death of tree due to CSD. The location of each symptomatic tree and the date when the symptoms appeared were recorded and spatial maps were prepared for each block studied.

Temporal analysis. The incidence of CSD (number of symptomatic plants divided by the total number of plants) for each evaluation date in each of 82 plots having multiple assessment dates (12 to 36) was plotted against time. An estimate of the annual rate of disease increase during the study period was obtained with the Gompertz model by the linear regression of transformed annual values of CSD incidence $\{-\ln[-\ln(y)]\}$ and time (3).

Spatial analysis. Binary (presence/absence) spatial maps of CSD were prepared for all assessment dates for each plot, resulting in a total of 927 maps. For the first level of spatial hierarchy, ordinary runs analyses were performed on each data set to determine if aggregation existed between adjacent symptomatic trees within rows and across rows (22) with the use of a Visual Basic EXCEL macro (T.R. Gottwald, unpublished). A nonrandom pattern (i.e., aggregation) of symptomatic trees was assumed if the observed number of runs was less than the expected number of runs at $P = 0.05$.

For the second level of spatial hierarchy, the data were examined for the presence of aggregation at various quadrat (group) sizes. The incidence data for each plot were partitioned into quadrats of four (2×2), sixteen (4×4), 36 (6×6), and 64 (8×8) trees with the use of a Visual Basic EXCEL macro (T. R. Gottwald, unpublished). When data are expressed as disease incidence, the binomial distribution provides the best assessment for random conditions (21) and the binomial index of dispersion (D) was used to test for the presence of randomness of CSD-symptomatic trees within each quad-

rat (group) size (21). For the binomial index, a large D (>1) combined with a small P (<0.05) suggests aggregation of symptomatic trees (21).

The binary form of Taylor's power law (16) relates the observed variance (V_{obs}) to the expected binomial variance (V_{bin}) for a random distribution of binary data. In this case, $\log(V_{\text{obs}}) = \log(A) + b \log(V_{\text{bin}})$, where A and b are parameters. Linear regression was performed for all plots using the least squares method, and the significance of the relationship between $\log(V_{\text{obs}})$ and $\log(V_{\text{bin}})$ was determined by the F-test, and the appropriateness of the model was evaluated using the coefficient of determination (R^2) and by the pattern of the residuals of regression. A random spatial distribution of symptomatic plants is inferred when $b = A = 1$; a constant level of aggregation is indicated when $b = 1$ and $A > 1$; and when $b > 1$, the degree of aggregation varies according to the incidence. A t -test was used to assess the equality of parameters b and A to unity, using the estimate of the parameter and its standard deviation (3).

In the third level of spatial hierarchy, the strength and directionality or orientation of aggregation 'among' quadrats of various sizes containing symptomatic citrus trees were examined with spatial autocorrelation analysis for nine plots (13, 14). For this analysis, data sets were parsed into quadrats of 2×2 , 4×4 , and 6×6 trees per quadrat. Due to the large number of assessment dates, only one assessment per year was examined. The x,y spatial location and disease incidence for each quadrat size on each assessment date in the individual citrus plots were used as input data. Autocorrelation proximity patterns were calculated consisting of positively (SL+), negatively (SL-), and non-correlated lag positions (13, 14). The size and shape of core and reflected clusters of SL+ were calculated, in which a core cluster is a group of

significant, positively correlated ($P = 0.05$), spatial lag distance classes that form a discrete and contiguous group with the origin (i.e., lag [0.0]) of the autocorrelation proximity pattern; a reflected cluster is a discrete group of two or more contiguous significant positive lag positions discontinuous with the origin and the core cluster. The 'core cluster saturation' is a measure of the saturation of the core clusters with significantly positive lags (i.e., the proportion of lag positions within the extents of the cluster that were significantly positive). Effects were evaluated as the number of significant lag positions within the first row (within-row) or within the first column (across-row) of the autocorrelation proximity pattern that are contiguous with the origin, and for 'edge' effects, at the distal edges of the proximity pattern (3, 11, 24).

Regional Scale

Data collection and analysis.

Two annual surveys were conducted by Fundecitrus in all citrus blocks of sweet orange grafted on Rangpur lime and Volkamer lemon older than 2 years in the north and northeast municipalities of São Paulo state to determine the location and incidence of CSD symptomatic trees. In the first survey 8,705,526 trees in 3,338 blocks on 524 farms in seven municipalities were inspected between June and September 2002. In the second survey, over 65 million trees in 39,945 blocks on 9,588 farms in 62 municipalities were inspected between September and December 2003. All blocks with CSD-symptomatic trees were geo-referenced with a hand held GPS unit and the incidence of the disease was assessed. The temporal and spatial progress of CSD in São Paulo state were analyzed by comparing the two surveys, at municipality and state scales, for number of CSD symptomatic trees, affected blocks, affected farms, affected municipalities, and extent of affected area.

RESULTS

Citrus Block Scale

CSD temporal progress. The estimated rates per year of disease increase were variable among blocks, independent of variety, age, farm and municipality, with an average of 0.42 (maximum rate = 2.55 and minimum rate = 0.04) (Table 1). By fitting Gompertz model to the disease progress curves, we found that disease increased rapidly in some plots (requiring two to ten

yr for CSD incidence to go from five to 95%), whereas in other plots disease progress was slower and CSD incidence was < 0.01 (< 4 affected trees) 2 yr after the appearance of the first symptomatic tree. The increase of disease incidence was higher at the end of the dry season and the beginning of rainy season which generally starts at the end of August and beginning of September in the north of São Paulo and south of Triângulo Mineiro, Brazil (Fig. 1). The majority of affected trees display initial symp-

TABLE 1
THE ANNUAL RATE OF INCREASE IN SYMPTOMS OF CITRUS SUDDEN DEATH
ESTIMATED USING THE GOMPERTZ MODEL IN PLOTS IN BRAZIL

Municipality/State ^a	Variety ^b	Number of plots	Year planted	Number of monthly assessments	Range of Gompertz annual rate of CSD increase ^c
Altair/SP	Natal	5	1988-1995	21	0.06-0.29 (0.15)
Barretos/SP	Hamlin	1	1993	22	0.25
Barretos/SP	Natal	2	1991-1993	22-38	0.30 (0.30)
Barretos/SP	Pera	1	1995	21	0.33
Barretos/SP	Valencia	1	1996	22	0.32
Campo Florido/MG	Natal	1	1992	23	0.16
Campo Florido/MG	Pera	5	1994	23-24	0.15-0.33 (0.23)
Colômbia/SP	Hamlin	3	1995-1996	25-38	0.11-0.51 (0.28)
Colômbia/SP	Natal	5	1989-1994	15-37	0.15-0.78 (0.36)
Colômbia/SP	Pera	5	1991-1995	11-23	0.06-0.19 (0.13)
Colômbia/SP	Valencia	3	1995-1996	11-25	0.06-0.41 (0.19)
Colômbia/SP	Westin	1	1998	25	0.26
Comendador Gomes/MG	Natal	2	1990	25	0.24-0.26 (0.25)
Comendador Gomes/MG	Pera	5	1990-1991	29-35	1.36-2.20 (1.86)
Comendador Gomes/MG	Valencia	4	1990-1991	21-29	0.20-2.55 (1.87)
Comendador Gomes/MG	Westin	1	1991	23	2.27
Frutal/MG	Hamlin	4	1991-1996	19-23	0.22-0.37 (0.29)
Frutal/MG	Natal	5	1990-1991	12-25	0.27-0.41 (0.33)
Frutal/MG	Pera	4	1995-1996	24-25	0.08-0.48 (0.23)
Frutal/MG	Valencia	8	1995-1997	12-25	0.10-0.34 (0.21)
Guaraci/SP	Natal	2	1985-1992	21	0.06-0.15 (0.11)
Guaraci/SP	Valencia	1	1994	16	0.06
Monte A Minas/MG	Natal	1	1994	22	0.37
Nova Granada/SP	Natal	1	1995	24	0.09
Nova Granada/SP	Rubi	1	1997	23	0.04
Nova Granada/SP	Valencia	3	1995	23	0.06-0.18 (0.13)
Planura/MG	Natal	1	1995	24	0.06
Planura/MG	Valencia	2	1995	25-26	0.05-0.15 (0.10)
Prata/MG	Natal	4	1995	23	0.12
Riolândia/SP	Natal	3	1989	20	0.07-0.21 (0.12)

^aSP = State of São Paulo; MG = State of Minas Gerais.

^bAll varieties grafted on Rangpur lime.

^cMinimum, maximum and average values of estimated annual rate of disease increase predicted by the Gompertz model.

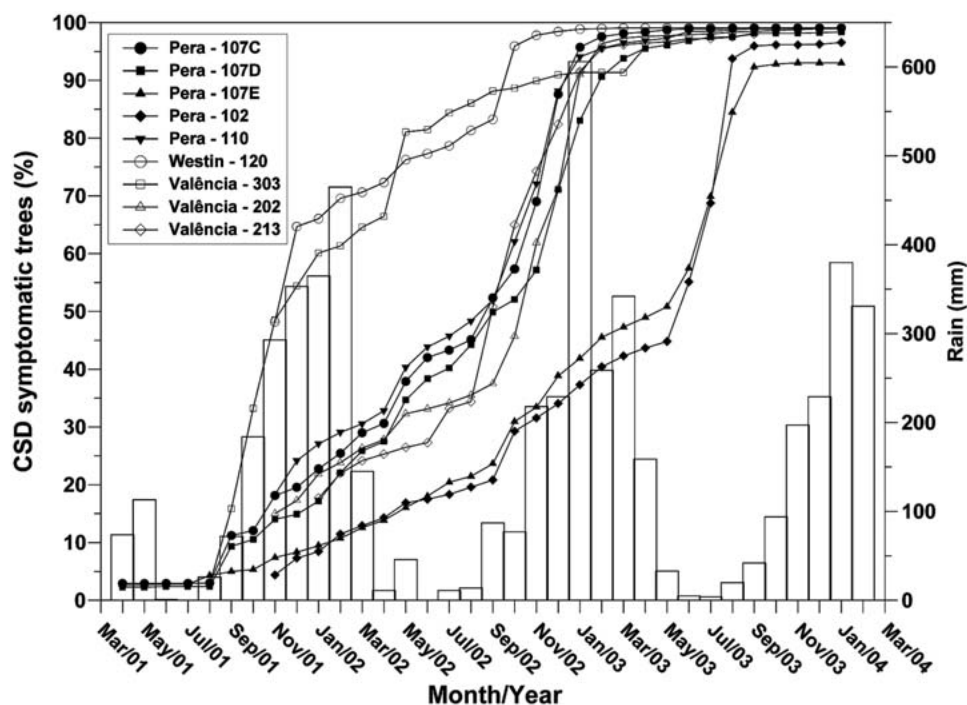


Fig. 1. Temporal increase of citrus sudden death for various plots and varieties, assessed as incidence of symptomatic trees of sweet orange grafted on Rangpur lime on a farm in Comendador Gomes, Minas Gerais state, Brazil. Monthly rainfall is also indicated.

toms (level 1) within the first 2 yr after a symptomatic tree appears in the block, after which the incidence of trees with symptoms severity of 2 and 3 increases (Fig. 2).

Spatial arrangement of CSD-symptomatic trees. The smallest scale of spatial hierarchy examined was the association of symptom status between adjacent trees. Of the 927 maps examined, aggregation was detected in 75.0% and 63.6% of cases within and between rows respectively (data not shown). Of the 12,726 within-row tests, 15.3% were aggregated and of the 17,888 across-rows tests, only 9.2% were aggregated. Aggregation increased with the increase in CSD incidence, especially in older plots.

At the medium level of spatial hierarchy the association of symptomatic plants within quadrats or groups of trees of various sizes was investigated. The binomial index of

dispersion (D) suggested a spatial structure of symptomatic plants that was non-random (i.e., aggregated) for the majority of the plots and quadrat sizes tested, especially between incidence values of >0.01 and <0.9 (data not shown). Values of D were usually higher for the largest quadrat sizes, with averages of 1.15, 1.50, 1.87, and 2.29 for 2×2 , 4×4 , 6×6 , and 8×8 quadrat sizes, respectively.

The relationship between $\log(V_{obs})$ and $\log(V_{bin})$ was highly significant ($P < 0.001$) for the four quadrat sizes tested. All estimates of b and A were statistically different from 1 ($P < 0.05$), which indicated a pattern of aggregation of symptomatic plants in all quadrat sizes tested (Table 2). Values of $b > 1$ also indicated that the degree of aggregation was a function of symptom incidence.

The final level of spatial hierarchy examined was the association

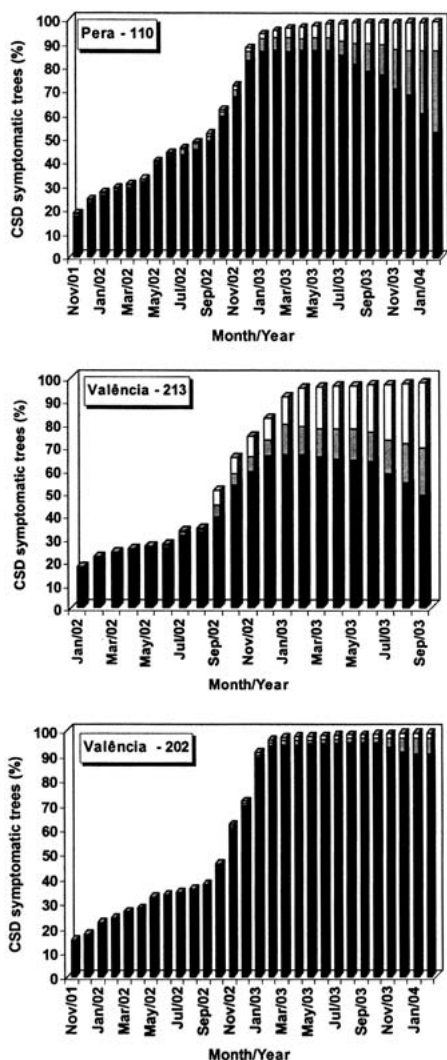


Fig. 2. Temporal increase of incidence and severity of symptoms of citrus sudden death on two varieties (Pera and Valencia) and three plots (110, 213 and 202) from a farm in Comendador Gomes, Minas Gerais state, Brazil. Symptom severity (black = 1-initial symptoms; gray = 2-severe symptoms; white = 3-tree death).

among groups of trees estimated by spatial autocorrelation and indicated that core clusters existed for less than half of the plot/quadrat size/assessment date combinations examined (Fig. 3A). The number of significant spatial lags in the core clusters tended to be small (≤ 3) in all but 4 cases (Fig. 3A). The number of core

clusters detected tended to decrease as CSD incidence increased, which is expected as aggregation tends to decrease as disease incidence approaches an asymptote.

In the majority of cases, core clusters were complete (i.e., saturated with significant positive lags). In those cases in which core clusters were not completely saturated, strength of aggregation varied from 0.40 to 0.92 and was often associated with an asymmetry of the core cluster (Fig. 3B). No trends in saturation of the core clusters were noted over time for any quadrat size by plot combination. Where reflected clusters existed, they existed predominately for the smallest quadrat size tested (2×2 trees) and for all assessment dates during the monitoring period (Fig. 3C-D). The maximum distance between the centroids of the core and reflected clusters was 6.0 to 53.3 meters with an average of 26.8 meters (Fig. 3E).

Row effects were detected by spatial lag autocorrelation for most plots. Although both within- and across-row effects were detected, within-row effects were often stronger and were more frequently detected (Fig. 3F-G). When row effects were detected in both 2×2 and 4×4 quadrat sizes for a given assessment, row effects were frequently stronger for the 2×2 quadrat size. Interestingly, edge effects were detected and were sometimes significant (Fig. 3H).

Regional Scale

CSD temporal and spatial progress. In the first survey, the number of municipalities with the disease was five and in the second survey it was 18. An increase of CSD incidence was observed in the seven municipalities inspected in both surveys (Colômbia, Barretos, Altair, Guaraci, Olímpia, Onda Verde, and Nova Granada). In the 15 mo between the two surveys in those seven municipalities, the number of

TABLE 2
THE EFFECT OF QUADRAT SIZE ON THE LINEAR REGRESSION BETWEEN LOG
OF OBSERVED VARIANCE (V_{OBS}) VERSUS LOG OF BINOMIAL VARIANCE (V_{BIN}) USING
TAYLOR'S POWER LAW MODEL* (ALL CITRUS BLOCK WITH PLANTS SHOWING
CSD SYMPTOMS WERE USED IN THE ANALYSIS)

Quadrat size	log(A)	SE	B	SE	R ²
2 × 2	0.179*	0.008	1.064*	0.004	0.987
4 × 4	0.540*	0.024	1.156*	0.009	0.943
6 × 6	0.774*	0.041	1.198*	0.014	0.888
8 × 8	1.107*	0.072	1.271*	0.022	0.844

*Taylor's Power Law model variables: Intercept = (log(A)), angular coefficient = (b), respective standard errors = (SE) and determination coefficient (R²).

*Value of log(A) significantly higher than 0 or B value significantly higher than 1 ($P < 0.05$) by *t*-test.

citrus farms with the disease increased from 88 to 97 and the number of affected citrus blocks from 407 to 678. The number of symptomatic trees in the field increased from 22,100 to 44,300. When including eradicated trees and newly symptomatic trees from the second survey, there were 436,477 trees with symptoms of CSD (Table 3).

The CSD epidemic in São Paulo State appeared to originate from the Minas Gerais municipalities of Comendador Gomes and Frutal, where the disease was first reported and where the highest incidence of CSD-symptomatic trees are found, with approx. 1.5 million affected trees. The municipality Colômbia is close to Frutal, and was the first affected municipality in the state of São Paulo, and has the highest levels of symptomatic trees, followed by Barretos, Altair, and Guaraci (Table 3). Other municipalities had an incipient disease incidence such that the regional distribution resulted in a disease gradient from the extreme northeast (Colômbia) to the south and west of São Paulo state.

In 2002, the disease was restricted to the north region of the State (64 km east to west and 44 km north to south). Fifteen months later, CSD was detected 58 km further west (total 122 km) and 61 km further south (total 105 km), thus occupying four times more area and affecting important citrus areas in

the northwest and center regions of the State (Fig. 4).

DISCUSSION

The objective of this study was to augment our knowledge of the spatial and temporal patterns of CSD at three different scales (1), and to use this information to make inferences about the etiology and future progress of the disease. Temporal analysis of the development of CSD epidemics based on symptoms at the citrus block scale does not provide an insight into the infection process as the interpretation is based solely on symptom expression. Indeed, symptom expression follows a latent or incubation period. For nursery trees the latent period is at least 14 mo (26), and is likely longer for established field trees. Thus, CSD disease and epidemic progress may have started many months prior to the expression of symptoms.

The variability of CSD disease progress rates, susceptibility of orange cultivars, management practice and distribution of farm locations make disease behavior difficult to predict. The Gompertz annual disease progress rates for CSD were compatible with calculated rates previously reported (1) and similar to those published for CTV (2, 11, 4, 6, 9, 10), although it should be noted that CSD was assessed visually, while CTV incidence was determined serologically.

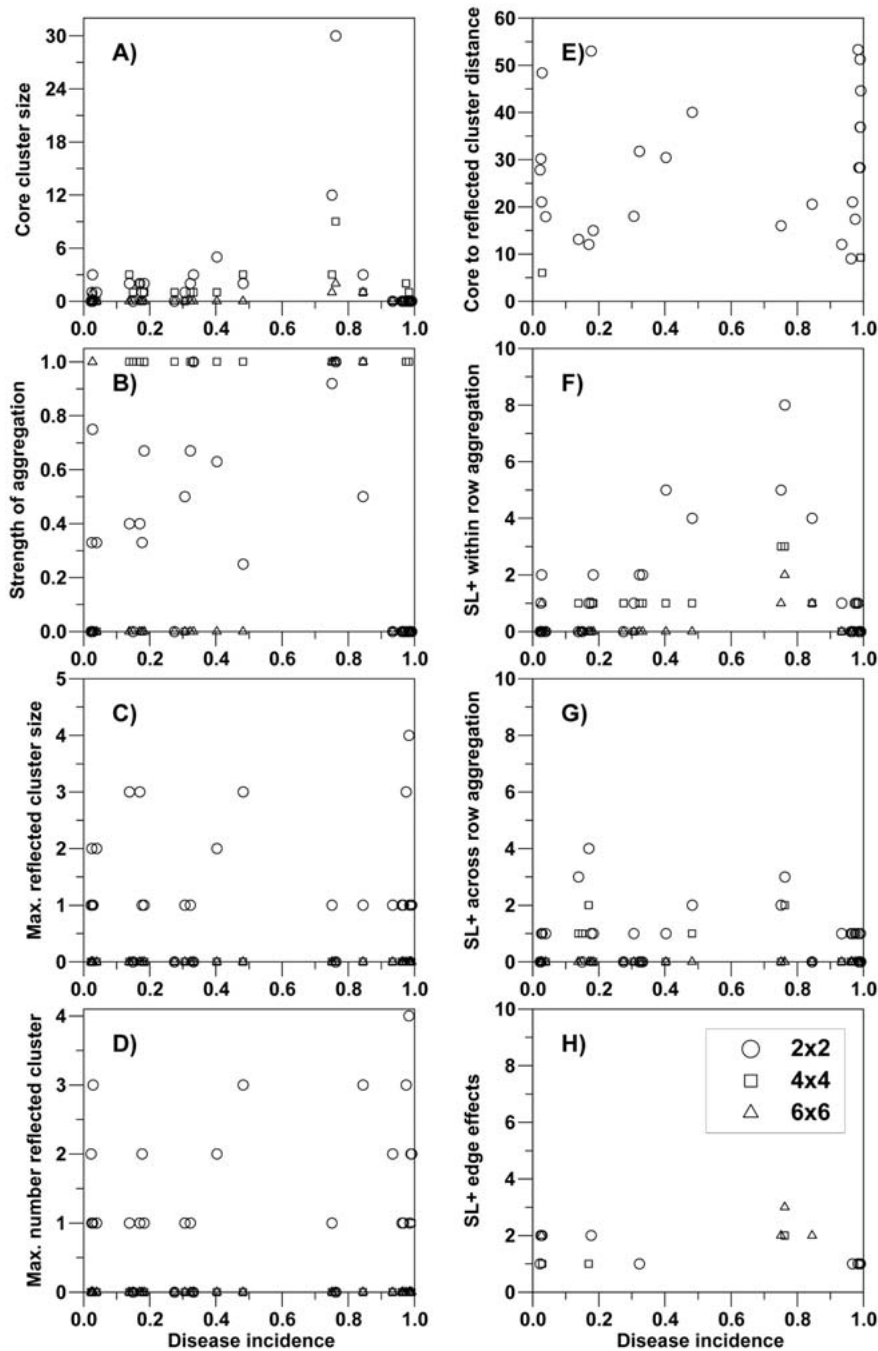


Fig. 3. Spatial autocorrelation analysis for citrus sudden death symptomatic trees in citrus orchards in Brazil (quadrat sizes 2×2 , 4×4 , and 6×6). A) Core cluster size = the number of lags significantly greater than expected by chance at $\alpha = 0.05$ level (SL+) contiguous with the [0,0]lag position that form a discrete group; B) Strength of aggregation = number of SL+ in core cluster/total number of SL+; C) Maximum reflected cluster size = the maximum number of contiguous SL+ in various clusters not contiguous with the core cluster; D) Maximum number of reflected clusters; E) Core to reflected cluster distance in meters; F) The number of SL+ within-row of the row defined by the [0,0] lag; G) The number of SL+ across-row of the column defined by the [0,0] lag; H) Significant edge effects = the number of SL+ at the distal edges of the proximity pattern / total number of SL+ $\geq 5\%$.

TABLE 3
RESULTS OF TWO SURVEYS IN 2002 AND 2003 TO ASSESS DISTRIBUTION AND INCIDENCE OF SYMPTOMS OF CITRUS SUDDEN DEATH
IN SÃO PAULO STATE, BRAZIL

Municipality	Year														
	2002 (June-September)							2003 (September-December)							
	NAF	NAB	NAT	%AF	%AB	%AT/AB	NAFF	NABF	NATF	NAFE	NABE	NATE	%AF	%AB	%AT/AB
Colômbia	37	317	21,310	86.05	70.92	1.44	42	364	37,079	2	86	385,731	95.65	85.88	20.82
Barretos	28	57	515	12.73	4.54	0.19	36	146	6,796	0	20	183	16.59	13.23	1.30
Altair	14	22	165	17.07	3.72	0.08	5	7	145	0	22	6,201	5.68	3.82	33.78
Guaraci	5	6	80	4.59	0.83	0.29	8	13	251	0	4	35	7.14	2.45	1.01
Olimpia	4	5	30	0.64	0.19	0.30	2	2	8	0	3	22	0.32	0.19	0.57
Onda Verde	0	0	0	0.00	0.00	0.00	1	10	24	0	0	0	7.69	2.53	0.04
Nova Granada	0	0	0	0.00	0.00	0.00	1	1	2	0	0	0	1.56	0.30	0.08
Paulo de Faria*							3	3	58	0	0	0	21.43	1.88	0.56
Cajobi*							1	3	52	0	0	0	0.84	0.49	0.46
Riolândia*							1	3	24	0	0	0	16.67	9.38	0.49
Ibirá*							1	1	10	0	0	0	1.30	0.43	0.39
Bálsamo*							1	1	2	0	0	0	1.59	0.50	0.08
Ipigua*							1	2	2	0	0	0	2.38	1.17	0.04
Tanabi*							1	1	2	0	0	0	0.53	0.14	0.07
Bebedouro*							1	1	1	0	0	0	0.21	0.04	0.03
Cosmorama*							1	1	1	0	0	0	0.97	0.26	0.04
Embaúba*							1	1	1	0	0	0	0.67	0.19	0.06
Monte Azul Paulista*							1	1	1	0	0	0	0.33	0.06	0.04
Total	88	407	22,100				108	561	44,459	2	135	392,172			

NAF = Number of affected citrus farms; NAB = Number of affected citrus blocks; NAT = Number of affected citrus trees; %AF = Percentage of affected citrus farms in the municipality (NAF/Number of citrus farms in the municipality); %AB = Percentage of affected citrus blocks in the municipality (NAB/Number of citrus blocks in the municipality); %AT/AB = Percentage of affected citrus trees per affected citrus block in the municipality (NAT/Number of citrus trees in affected blocks in the municipality); NAFF = Number of affected citrus farms with trees in the field; NABF = Number of affected citrus blocks with trees in the field; NATF = Number of affected citrus trees in the field; NAFF = Number of affected citrus farms eradicated due to CSD; NABE = Number of affected citrus blocks eradicated due to CSD; NATE = Number of affected citrus trees eradicated due to CSD.

*Municipalities not inspected in the CSD survey 2002.

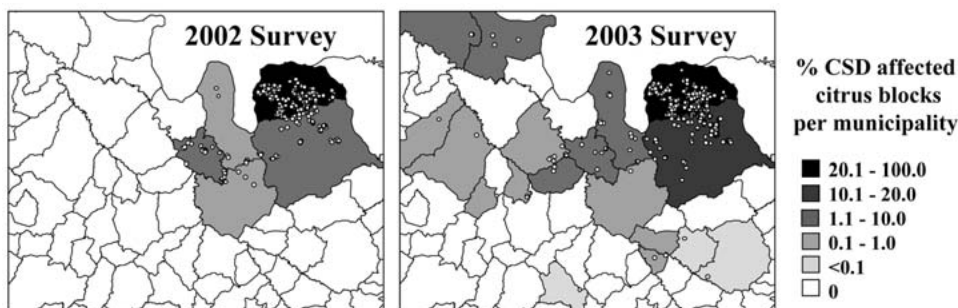


Fig. 4. Distribution of citrus sudden death in the State of São Paulo, Brazil.

An important observation made in this study was that CSD symptoms generally do not appear widespread in plantings for at least 12 mo following the appearance of the first symptomatic tree. Therefore, constant surveying for CSD symptomatic trees in blocks near affected regions provides an opportunity for early disease detection and eradication of symptomatic and exposed plants. The optimal time for CSD surveys is spring, when new flush on healthy trees allows easy differentiation from diseased trees with little or no flush. During drought both healthy and diseased trees are water stressed with little or no flush, which makes them difficult to differentiate. Towards the end of the rainy season diseased trees can also recover somewhat and partially regenerate the root system and foliar canopy.

The aggregation among symptomatic trees detected by ordinary runs analysis confirmed previous findings (1) and indicates that CSD-symptomatic trees influence the status of adjacent trees. This spatial distribution is consistent with a biotic agent, for example, a vector-borne pathogen similar to CTV (12). The association of symptomatic trees within quadrats of various sizes using the index of dispersion D and the estimated parameters of the binary form of Taylor's power law corroborated previous results (1). Even though the values of D and the parameters b and $\log(A)$ in this study were slightly smaller, they

were still in the range of values for CTV-infected trees in the presence of *T. citricida* or *A. gossypii* as the predominant vector (11, 12, 15). Taylor's power law analysis (D , b and A significantly >1 , and increasing with quadrat size) suggested a significant nonrandom spatial structure of symptomatic trees for most plots and quadrat sizes over time for disease incidences >0.01 to <0.9 with the degree of aggregation having a positive relationship with CSD incidence (17, 25). Sources of infection external to the plot can result in low incidences (<0.01) and a random pattern of infection (12). A trend toward aggregation at higher incidences is a result of movement of vectors within plots, and mostly among neighboring plants within or across rows (8, 11, 12, 22).

CSD aggregation was also detected among groups of trees by spatial autocorrelation methods, but it was not as great or as extensive as aggregation within groups. The aggregation was greater within- compared to across-row and could be indicative of branches of adjacent trees touching within rows providing a means for vectors to move from tree to tree along rows. With CTV a greater within-row aggregation was found when trees became large enough for their branches to intertwine within-rows (11). Reflected clusters were most common at the smallest quadrat size and indicates an association among groups of CSD affected trees over considerable dis-

tances. The location (maximum distance) of reflected clusters relative to the core cluster was 6.0 to 53.3 meters with an average of 26.8 meters between the centroids of the core and reflected clusters. This supports the contention that transmission of CSD is not to the nearest neighbor unless the canopies within row are closed. Edge effects suggest that many transmissions may originate from outside the plot, indicating even longer distance transmissions.

Considering all three levels of spatial hierarchy CSD appears to exhibit a weak aggregation within a local area of influence, with groups of infected trees related over distance, which is good evidence that CSD is a contagion and transmission occurs both to trees in the immediate vicinity and to trees over some distance.

The spatial dynamics of CSD could be a result of a shift of the predominant vector from a migratory to a resident species (as happened with the CTV/*Aphis gossypii* pathosystem when *T. citricida* was introduced). A recent study on a farm in the north of São Paulo showed that young orchards (3-yr-old trees) contained a prevalence of *A. gossypii* and *A. spiraecola*, while older orchards (9-yr-old trees) were dominated by *T. citricida*, although all species were present in both orchards (E. L. V. Primiano et al., personal communication). The similarities of spatial patterns of CSD and CTV are striking and the spatial analyses and inferences give clues to the underlying processes and possible etiology of CSD, although the similarities are not conclusive in the absence of an identified pathogen.

Unfortunately, CSD continues to spread through São Paulo state. The

future rate of CSD progress will likely be affected by soil and weather conditions, inarching and use of tolerant rootstocks, eradication of diseased trees, relative proximity of citrus orchards, and movement of contaminated propagation material. The role of water deficit on CSD development has not been determined and until now the disease has been restricted to an area with an annual water deficit >80 mm. In advance of the epidemic front, inarching with tolerant but drought susceptible rootstocks will necessitate the use of irrigation in the north and northwest citrus regions. It is estimated that >4 million trees on Rangpur lime were inarched to date. According to Fundecitrus reports of São Paulo citrus nursery inspection, the production of new nursery citrus scions grafted onto Rangpur lime has decreased from 84.6% in March 2001 to 38.4% in November 2004. Effective management which mitigates disease will result in greater cost, and could decrease the competitiveness of the Brazilian citrus industry. Annual surveys of CSD are required to monitor the increase in disease and spread to new regions and to define areas of short and long-term risk, providing information which is fundamental for strategic planning of new citrus projects. Knowledge of the disease distribution and spread is critical for making decisions regarding feasibility and adoption of management strategies for CSD.

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