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RESEARCH ARTICLE / *ARTIGO*

Comparative epidemiology of citrus tristeza in Cuba and citrus sudden death in Brazil

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ABSTRACT

Citrus Sudden Death (CSD) is a disease of unknown etiology, reported only in Brazil. Due to similarities between the epidemiology of CSD in Brazil and Citrus Tristeza (CT) in other countries, this work aimed to compare CSD and CT in Cuba. Besides testing the hypothesis of shared epidemiological patterns, the data generated can be used to carry out control strategies if CSD breaks out in Cuba. Seven epidemic data sets were statistically compared for each disease by analyzing their progress in time and the dependence among symptomatic or infected plants at two levels of spatial scale. CSD and CT epidemics could not be differentiated based on mean progress rate, area under disease progress curves, proportion of aggregated sequences (Ordinary runs test), index of Dispersion (*D*) or both parameters, log (*A*) and *b*, of the binary power law model. The progress rate of both diseases varied from low (0.0003/month) to very high (0.045/month). The proportion of aggregated sequences was low (<0.16) and *D*, log(*A*) and *b* values indicated aggregation in groups of 4 to 16 plants for both diseases. These results suggest that measures currently used for CT eradication and management in Cuba may be applied in case of an eventual introduction of CSD in that country.

Keywords: Citrus tristeza virus, CTV, Closterovirus, Citrus sudden death associated virus, CSDaV, temporal pattern, spatial pattern.

RESUMO

Epidemiologia comparativa da tristeza dos citros em Cuba e morte súbita dos citros no Brasil

A morte súbita dos citros (MSC) é uma doença de causa desconhecida relatada apenas no Brasil. Em função de semelhanças com a tristeza em outros países, objetivou-se comparar a MSC com a tristeza em Cuba. Além de testar a hipótese de similaridade dos padrões epidemiológicos, os dados gerados podem ser utilizados no delineamento de estratégias de controle no caso de uma eventual introdução da MSC em Cuba. Sete epidemias de cada doença foram comparadas estatisticamente por meio de análise do progresso no tempo e da dependência entre plantas sintomáticas ou infectadas em dois níveis de escala espacial. As epidemias de MSC e tristeza não puderam ser distinguidas com base na taxa média de progresso, na área abaixo da curva de progresso da doença, na proporção de seqüências agregadas (*Ordinary Runs* test), no índice de dispersão (*D*) ou nos parâmetros log(*A*) e *b* após ajuste da lei de Taylor binária. A taxa de progresso das epidemias de ambas as doenças foi variável, de baixa (0,0003/mês) a muito alta (0,045/mês). A proporção de seqüências agregadas foi baixa (<0,16) e os valores de *D*, log(*A*) e *b* indicaram agregação em grupos de 4 a 16 plantas tanto para a MSC quanto para a tristeza. Esses resultados indicam que as atuais medidas usadas no programa de erradicação e manejo da tristeza em Cuba poderiam ser usadas na eventualidade da introdução da MSC nesse país.

Palavras-chave: Citrus tristeza virus, CTV, Closterovirus, Citrus sudden death associated virus, CSDaV, padrão temporal, padrão espacial.

INTRODUCTION

Tristeza is the most important viral disease of citrus. Losses close to 100 million plants in citrus-producing countries have been estimated since 1930 (Rocha Peña *et al*., 1995, Cambra *et al*., 2000). The causal agent is *Citrus tristeza virus* (CTV, genus *Closterovirus*, family *Closteroviridae*) (Karasev, 2000) transmitted by grafting and by aphids. The most efficient vector is *Toxoptera citricida* Kirkaldy (Rocha Peña *et al*., 1995). CTV produces two main effects: decline of trees grafted on sour orange (*Citrus aurantium* L.) and stem pitting, which weakens the plants and reduces yield (Cambra *et al*., 2000).

The introduction of *T. citricida* in the Caribbean Basin has caused an increase in the number of CTV-infected plants, and the death of millions of trees grafted on sour orange rootstock. Studies on citrus tristeza in Cuba, from 1992-1995, show that CTV incidence was very low in all areas, except Isla de la Juventud (Peña *et al*., 2002). A later survey (2000-2004) indicated that the proportion of infected plants had increased in most areas (Batista *et al*., 2005). On the other hand, the characterization of Cuban CTV isolates indicated that weak ones predominate, although more severe isolates have been sporadically detected (Batista *et al*., 1996; Herrera *et al*., 2000). Tristeza is considered a menace for

Cuban citriculture and this pathosystem differs from what is found in other countries where tristeza epidemics occur. Thirteen years after the presence of *T. citricida* in Cuba, CTV incidence has increased, but symptoms of decline are observed only in isolated trees even though the main rootstock used is sour orange (Batista *et al*., 2005).

In Brazil, CTV was first detected in 1937 and destroyed millions of trees grafted on sour orange rootstock in the following years. The problem was solved by replacing sour orange by tolerant rootstocks like Rangpur lime (*C. limonia* Osbeck) (Pompeu Júnior, 2005). Today, more than 85% of the 180 million citrus trees in Brazil are grafted on Rangpur lime rootstock, and susceptible varieties such as Pêra sweet orange (*C. sinensis* Osbeck) have been cross-protected with mild CTV isolates (Müller *et al*., 2005; Pompeu Júnior, 2005). Since the 1930´s, *T. citricida* has been the main CTV vector in Brazil, but other CTV vectors such as *Aphis gossypii* Glover and *A. spiraecola* Patch exist as well (Müller *et al*., 2005).

“Morte Súbita dos Citros” or Citrus Sudden Death (CSD) was first reported in an adjacent area south of the Triângulo Mineiro in Minas Gerais State and north of São Paulo State, Brazil, in 2001 (Gimenes-Fernandes & Bassanezi, 2001). CSD is considered a potential threat to the sustainable commercial production of sweet oranges and some mandarins (*C. reticulata* Blanco) grafted on Rangpur lime or Volkamer lemon (*C. volkameriana* Pasq.) rootstocks, which represent the majority of citrus trees of São Paulo State (Bassanezi *et al.*, 2007). Despite its severity, CSD is present only in São Paulo and Minas Gerais states and therefore is considered a quarantined disease for Cuba and other countries as well as for other Brazilian States.

CSD is graft-transmissible (Yamamoto *et al.*, 2004) but the search for graft-transmitted agents such as endogenous bacteria, viroids and viruses has produced negative results (Bassanezi *et al.*, 2003). However, CTV and a new marafivirus (Tymoviridae) provisionally called Citrus sudden death associated virus (CSDaV) have been found in diseased trees. The association of CSD affected trees with CTV variants (Coletta-Filho *et al.*, 2005; Maccheroni *et al.*, 2005) and the new marafivirus (Jesus Junior *et al.*, 2005; Maccheroni *et al.*, 2005) has been studied, but the role these viruses play in CSD is still unclear and Koch’s postulates have not yet been fulfilled.

The study of the spatial-temporal dynamics of tristeza in Cuba has shown differences in apparent infection rates between citrus varieties and regions. Aggregation of infected plants was found in the second and third level of spatial hierarchy (Batista *et al.*, 2008). For CSD, a preliminary epidemiological study was carried out and the results were compared to previously described tristeza epidemics in the Caribbean Basin (Bassanezi *et al.*, 2003). Symptomatic plants were aggregated in most spatial scales. Based on symptoms and the similarities found between the temporal and spatial patterns of CSD in Brazil and the CTV/*T. citricida* pathosystem in Costa Rica and the Dominican Republic, it was proposed that CSD may be caused by a similar pathogen (likely a virus) and is probably vectored by aphids (or an insect with similar behavior) (Bassanezi *et al*., 2003). More detailed analysis of CSD epidemiology led to similar conclusions (Jesus Junior & Bassanezi, 2004; Bassanezi *et al.*, 2005a). Nevertheless, these comparisons were not based on statistical tests. The results were compared taking into account only their similarities. Moreover, since CSD is a novel disease, enough temporal data were not available and this important epidemic feature could not be thoroughly analyzed.

Comparative epidemiology is considered an important research approach that allows the analysis and synopsis of plant disease epidemics (Kranz, 1988) and seeks to define the differences and similarities among diseases (Madden *et al*., 2007). With a diversity of tools, it enables validation of hypotheses and theories. According to Madden *et al*. (2007), disease progress curves used in comparative epidemiology studies may originate from planned experiments or from observations of natural epidemics in commercial fields. However, special care should be taken to perform appropriate statistical comparisons to avoid conclusions based on superficial resemblance (Kranz, 1988; Madden *et al*., 2007). Due to similarities between tristeza and CSD, and considering that the latter is not present in Cuba, a comparison between them should bring important information for quarantine authorities of that country. For example, based on spatial pattern results, it is possible to take the decision to use a different sampling procedure for CSD detection than the one used for CT detection. In addition, taking into account the reported differences between tristeza in Cuba and in other countries of the Caribbean Basin (Batista *et al*., 2008), the comparative epidemiology of CSD and tristeza epidemics should reinforce and/or clarify concepts about the spread of these diseases. The objectives of this study were to test the hypothesis that epidemiological patterns of CSD in Brazil and citrus tristeza in Cuba are indistinguishable and to improve our knowledge regarding the possible causal agents of CSD.

MATERIALS AND METHODS

Areas. To study tristeza in Cuba, seven commercial areas of sweet orange were selected in five provinces. For CSD in Brazil, seven sweet orange plots – comparable to Cuban areas regarding variety and tree spacing - were selected in the States of São Paulo and Minas Gerais. The characteristics of each field are listed in Table 1.

Evaluations. CTV in Cuba. A total of eight evaluations (twice a year) were performed between 1999 and 2006. In each field, all trees included in a 400-plant section were sampled: 20 plants within rows x 20 plants across rows for all fields except HVN02 which had 16x25 and SC01 which had 17x24. Virus presence was determined by double antibody sandwich indirect (DASI) enzyme linked immunosorbent assay (ELISA) (Batista *et al*., 2008).

TABLE 1 - Province or State, spacing, age, variety and rootstock in fourteen fields evaluated for CTV infection in Cuba or CSD symptoms in Brazil

Disease	Plot	Province or State	Spacing (meters)	Age at the beginning of evaluations (years)	Scion	Rootstock
CTV	HVN01	La Habana	8X4	29	Washington Navel sweet orange	Sour orange
	HVN02	La Habana	8X4	21	Valencia sweet orange	Sour orange
	MTZ-O	Matanzas	7x4	1	Valencia sweet orange	Swingle citrumelo
	CF-O	Cienfuegos	8x4	30	Valencia sweet orange	Sour orange
	CA-O	Ciego de Ávila	8X4	35	Valencia sweet orange	Sour orange
	SC01	Santiago de Cuba	8X4	28	Washington Navel sweet orange	Sour orange
	SC02	Santiago de Cuba	8X4	15	Valencia sweet orange	Sour orange
CSD	NM9	Minas Gerais	8x4	3	Valencia sweet orange	Rangpur lime
	AST8	Minas Gerais	7.5x4	11	Valencia sweet orange	Rangpur lime
	VV303	Minas Gerais	7.5x4	10	Valencia sweet orange	Rangpur lime
	SF508	Minas Gerais	7.5x4.5	7	Valencia sweet orange	Rangpur lime
	SF513	Minas Gerais	7.5x4.5	6	Valencia sweet orange	Rangpur lime
	SA10A	São Paulo	8x4	6	Valencia sweet orange	Rangpur lime
	VV213	Minas Gerais	7.5x4	12	Valencia sweet orange	Rangpur lime

CSD in Brazil. Plot size was 12x48 plants for SF508, 16x48 for SA10A, 20x48 for VV303 and VV213, 24x40 for SF513 and AST8, and 42x90 for NM9. Since there is no reliable serological or molecular diagnostic test for CSD, incidence was assessed only by visual inspection of the canopy of each tree. When necessary, diagnostic confirmation of CSD was performed by checking for the characteristic yellow stain in rootstock phloem in the graft union region (Gimenes-Fernandes & Bassanezi, 2001).

Each plant was assigned to a category: not diseased (0) or diseased (1), their location and the detection date were recorded. The results were used to make cumulative maps of CTV and CSD incidence, represented by the proportion of infected or symptomatic plants in each evaluation. All areas were evaluated twice a year, but the date of the first evaluation differed among plots. Evaluations started in 2001 for VV213 and VV303, in 2002 for SA10A and AST8, and 2003 for NM9, SF508 and SF513. Total number of assessment dates ranged from seven (NM9 and VV303) to nine (VV213).

Temporal analysis. Three models were selected (logistic, Gompertz and monomolecular) to fit them to CSD data by non-linear regression. The best model was selected based on the determination coefficient (R^2) between observed and expected values, and the presence or absence of patterns in the graphic of residuals *versus* expected values (Campbell & Madden, 1990). A model with a good R^2 was accepted as appropriate only if its residuals showed no detectable patterns. Results for CT have been described elsewhere (Batista *et al.*, 2008).

Spatial analysis. Binary (presence/absence) spatial maps were prepared for all assessment dates for each plot, resulting in a total of 55 maps for CSD and 49 maps for CTV. For the first level of spatial hierarchy, ordinary runs analyses were performed on each data set to determine if aggregation existed between adjacent symptomatic/infected trees within rows and across rows (Madden *et al.*, 1982). A nonrandom pattern (i.e., aggregation) of symptomatic/infected trees was assumed if the observed number of runs was less than the expected number at P=0.05. Sequences with less than one diseased or one healthy plant were excluded from the analysis. Based on these results, the proportion of aggregated and random sequences for every field, evaluation and direction were calculated. The proportion of aggregated sequences between directions and diseases was compared using the t-test (P≤0.05).

For the second level of spatial hierarchy, the data were examined for the presence of aggregation at two quadrat sizes. The incidence data for each plot were partitioned into quadrats of four (2x2) and sixteen (4x4) plants. When data are expressed as disease incidence, the binomial distribution provides the best assessment for random conditions and the binomial index of dispersion (D) was used to test for the presence of randomness of CSD-symptomatic or CTV-infected trees within each quadrat size. D values statistically greater than 1 (P≤0.05) indicate aggregation (Madden & Hughes, 1995). For the comparative analysis, the mean of $D>1$ values was calculated for both diseases including a range of incidence from 0.15 to 0.77, which includes most of the data. Comparison was performed using the t test (P≤0.05), taking into account the assumptions and procedures described by Zar (1996) and the precedent of Bassanezi & Laranjeira (2007).

The binary form of Taylor's power law relates the observed variance (V_{obs}) to the expected binomial variance (V_{bin}) for a random distribution of binary data (Madden & Hughes, 1995). In this case, $\log(V_{obs}) = \log(A) + b \log(V_{bin})$, where A and b are parameters. For both diseases and all quadrat sizes linear regression of Vobs on Vbin was performed using the least squares method, and the significance of the relationship between $\log(V_{obs})$ and $\log(V_{bin})$ was determined using the F-test. The appropriateness of the model was evaluated using the determination coefficient (R^2) and by the pattern of the residuals of regression. A random spatial distribution of symptomatic/infected plants is inferred when b=1 and $\log(A)$=0; a constant level of aggregation is indicated when b=1 and $\log(A)>0$; when $b>1$, the degree of aggregation varies according to the incidence. The *t*-test was used to assess the equality of parameters b and $\log(A)$ to 1 and 0, respectively, using the estimate of the parameter and its standard error (Madden *et al.*, 1995). Also, parameters were compared between diseases and quadrat sizes using the t-test ($P \leq 0.05$).

RESULTS

Temporal analysis. It was not possible to fit any progress model to the CSD incidence data (low R^2 and existence of residual patterns, data not shown). Conversely, tristeza progress in Cuba was best described by a logistic model for most areas (Batista *et al.*, 2008). Nevertheless, CT progress in two Cuban fields could not be represented by any model (Figure 1D). For this reason general monthly rates were compared, i.e. the computed rate considering the proportion of symptomatic/infected plants at the first and last evaluations and the time (months) between them as well as areas under disease progress curves (AUDPC).

The progress of both diseases was variable and the epidemics could be sorted into three groups according to their incidence at the last evaluation (Figure 1). Five out of seven CT and CSD progress curves had medium to high incidences at the last evaluation. However, for both diseases there were also epidemics whose incidence hardly increased over time.

Some CSD epidemics had a higher progress rate compared to those of CT in Cuba (Figure 1C and 1F), with a range between 0.001 and 0.045 compared to 0.0003 to 0.022 for tristeza. However, when mean progress rates were compared there was no statistical difference between diseases (0.011±0.008 [CT] and 0.014±0.019 [CSD] P=0.73, t-test). AUDPCs of both diseases were also statistically indistinguishable using t-test (16.1±12.3 [CT] and 6.2±5.9 [CSD] P=0.08).

Spatial analysis. According to ordinary runs test, the proportion of aggregated sequences was generally below 0.2 for both diseases, ranging from 0.02 to 0.22 (CTV) and 0.016 to 0.18 (CSD). Differences were found between the proportions of aggregated sequences within and across row for both diseases, with a higher proportion within rows [CSD:

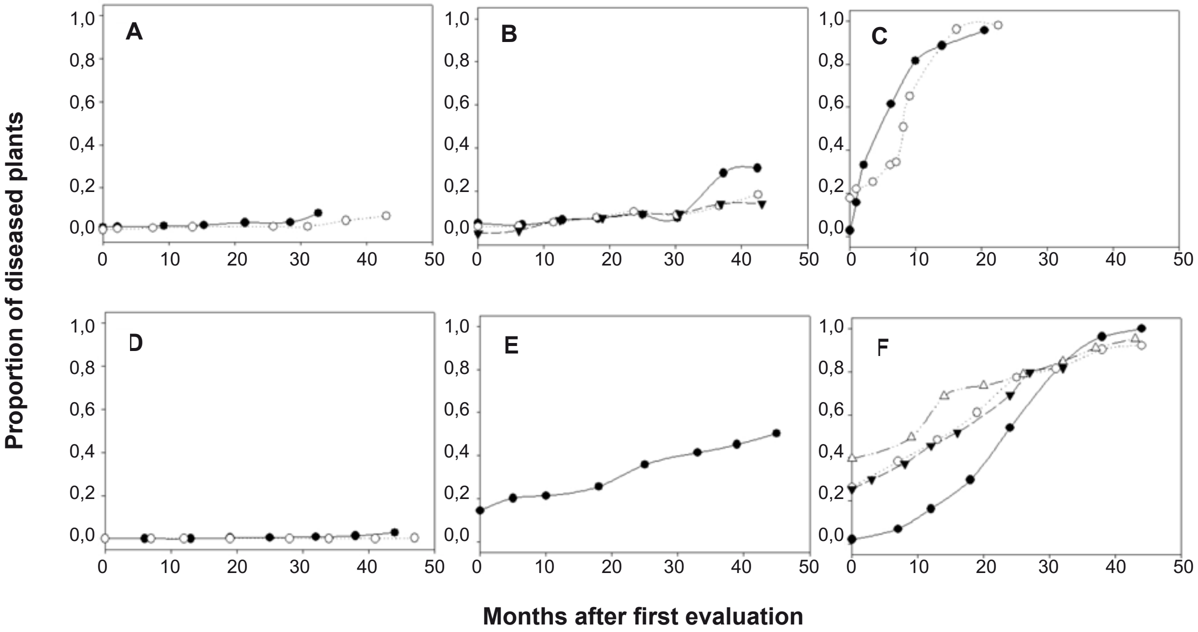

FIG. 1 - Progress curves of Citrus Sudden Death (CSD) symptomatic plants in Brazil (A, B, C) and *Citrus tristeza virus* (CTV) infection in Cuba (D, E, F) considering three final incidence ranges, below 0.10 (A and D), between 0.15 and 0.40 (B and E), and above 0.80 (C and F).

0.16 (within rows) x 0.04 (across rows); CT: 0.10 (within rows) x 0.06 (across rows)]. The proportion of within-row aggregated sequences was higher for CSD (0.16±0.02) than for CT epidemics (0.10±0.001), while across-rows proportion did not differ between diseases.

A high frequency of *D* values, significantly higher than 1, was recorded for both diseases, for both quadrat sizes. Those proportions were not significantly different between diseases, regardless of quadrat size (P≥0.24). Due to large differences of disease incidence values between CSD and CT, the mean *D* values between diseases were compared for a selected common incidence range (0.15 to 0.77). Means were different for 2x2 quadrat size [1.24±0.12 (CSD); 1.52±0.31 (CT), P=0.0002)], but similar for 4x4 size [1.87±0.41 (CSD); 1.90±0.52 (CT), P=0.83)]. Despite this difference, all mean values were statistically higher than 1 (P≤0.05).

The relationship between log (observed variance) and log (binomial variance) was significant for both diseases and quadrat sizes (P≤0.05) (Figure 2). Regression parameters log(*A*) and *b* were always higher than 0 and 1, respectively, regardless of disease or quadrat size. When log(*A*) and *b* were compared between diseases, CT parameters were significantly higher for 2x2 (P≤0.01) quadrat size but similar to CSD for 4x4 size (P≥0.18).

DISCUSSION

Comparisons carried out in the present study are valuable to improve quarantine measures and to help devise adequate eradication strategies in case of introduction of CSD in Cuba. CT epidemics in Cuba displayed distinct patterns when compared to those in other Caribbean countries (Batista *et al.*, 2008). The most important difference is that sweet orange trees in Cuba are normally grafted onto sour orange, and this combination is one of the most sensitive to CTV. Nevertheless, citrus trees in Cuba rarely show decline symptoms, even after the introduction of the most efficient vector (*T. citricida*) in 1993.

In order to achieve comparable epidemiological patterns, CSD areas in Brazil were selected based on similar sweet orange variety (Valencia) and tree spacing (~8mx4m) than CT areas in Cuba. Despite previous reports on CSD epidemiology (Bassanezi *et al.*, 2003, 2005a), the data sets used here had not yet been analyzed. Patterns of CT epidemics in Cuba have been discussed elsewhere (Batista *et al.*, 2008), but for this work they had to be rearranged to meet the criteria used for CSD analysis.

Variation on disease progress rates and on final proportion of symptomatic/infected trees occurred for CT as well as for CSD epidemics. Moreover, mean monthly rates and AUDPC could not be statistically differentiated. It is important to note that this is the first time that temporal patterns of these diseases have been compared by statistical tests as advised by Kranz (1988) and Madden *et al.* (2007). When CSD and CT epidemics were compared for the first time, the amount of data was limited and most CSD progress curves (five out of nine) did not reach 5% of incidence (Bassanezi *et al.*, 2003). For this reason, it was assumed that CSD progress could be represented by a Gompertz model as reported for CT in Caribbean countries and the comparison of disease progress rates was done directly, without a formal statistical test (Bassanezi *et al.*, 2003)

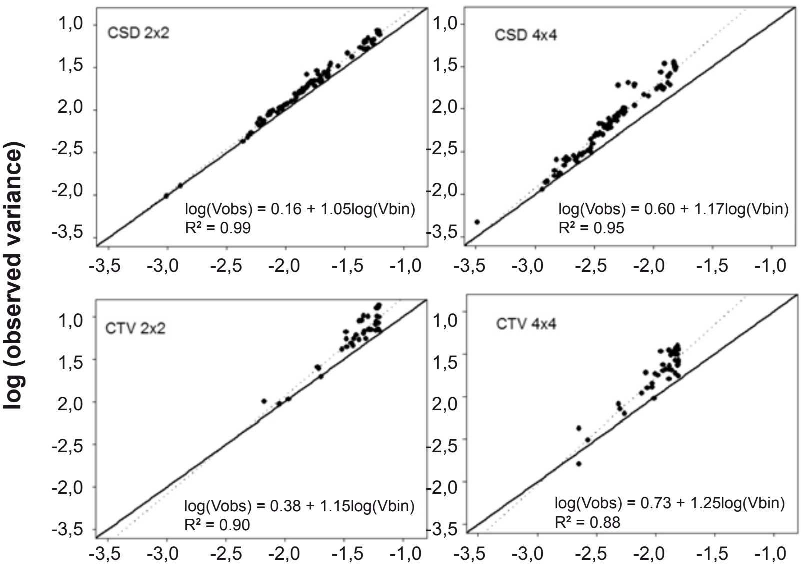

FIG. 2 - Relationship between logarithm of observed variance (log *Vobs*) and logarithm of binomial variance (log *Vbin*) of spatial data of citrus sudden death (CSD) symptomatic plants in Brazil and *Citrus tristeza virus* (CTV) infected plants in Cuba for quadrat sizes 2x2 and 4x4. log(*A*) and *b* values were significantly higher than 0 and 1, respectively (P≤0.05) for both diseases and quadrat sizes.

Unfortunately, since it was not possible to fit any model to CSD progress curves, we could not compare curve shapes between the two diseases. It must be noted that disease assessments for CSD were made visually, whereas CTV incidence was determined serologically. Favorable conditions for CSD-symptom expression could be different for CTV infection. Despite the similarities concerning absolute progress rates, and indistinguishable AUDPCs, a stronger evidence of comparable temporal patterns would be possible if the same model could be made to fit CSD and CT epidemics. In the absence of a diagnostic test for CSD, a better approach to compare the temporal pattern of both diseases would be to assess CT symptom progress whenever it is possible.

Similarities between CSD and CT were found for all spatial scales and associated statistical tests. Considering the first hierarchical level – spatial dependence among adjacent plants – it was shown that for both diseases the proportion of aggregated sequences was low, no matter the direction. These results are comparable to those found for other citrus diseases transmitted by aerial vectors, such as huanglongbing (Bassanezi *et al.*, 2005b) and leprosis (Bassanezi & Laranjeira, 2007). However, they are very different from what has been reported for Bahia bark scaling, which was shown to have a very high proportion of aggregated sequences (0.47) in the downwind direction (Laranjeira *et al.*, 2006).

Another characteristic shared by CSD and CT is the higher proportion of aggregated sequences within rows compared to across rows. This was also observed for huanglongbing (Bassanezi *et al.*, 2005b) and leprosis (Bassanezi & Laranjeira, 2007), indicating that even when spatial dependence is low, plant spacing plays an important role in the spread of citrus vector borne diseases, considering that in citrus groves the within-row spacing is always less than between rows.

Analysis at the second level of spatial hierarchy revealed that symptomatic/infected plants of both diseases were aggregated in a group (quadrat), for all quadrat sizes and tests (index of dispersion or binary power law). The strong aggregation of CTV-infected plants in the 2x2 quadrat size may reflect the different evaluation procedures carried out for each disease. In general, at this spatial scale, parameters of one disease could not be differentiated from those of the other. Similar results were reported by Bassanezi *et al*. (2003), but they were only based on data resemblance. At this spatial scale, the aggregation level of symptomatic/infected plants of both diseases can be considered slight, but even so, there is an indication of local transmission among plants.

CSD epidemics in Brazil and CT epidemics in Cuba are not statistically different for most epidemiological criteria and thus can be considered to share temporal and spatial patterns. Comparable results have been reported when analyzing CSD in Brazil and CT in Costa Rica and the Dominican Republic (Bassanezi *et al.*, 2003) but these authors focused on the nature of CSD´s putative etiological agent. Although our results reinforce their observations, this was not our main purpose. We aimed at testing whether CSD and CT in Cuba shared patterns as hypothesized by Bassanezi *et al*. (2003), and if so, to propose quarantine and control strategies for that country.

Considering all similarities found between CSD and CT epidemics, it is advisable that quarantine authorities in Cuba establish a surveillance program to detect CSD as soon as the first affected plants appear. Taking into account that CSD can reach high progress rates, it would be very difficult to eradicate it if it were not detected at the early stages of dissemination.

It would also be important to use results of spatial patterns to devise joint strategies for sampling and detection of CT and CSD symptomatic plants. For example, the information about aggregation in sub-areas and a trend to be more aggregated within rows could be used to refine sampling. In practice, instead of waiting for the first report of CSD, Cuban authorities could incorporate the search for symptomatic plants in every citrus region, in the same way as is done for detection of trees with symptoms of CTV-induced decline and stem pitting on tolerant rootstocks. Of course, despite similar sampling procedures, while the CT search is mainly focused on sour orange rootstock groves, the CSD search would be directed towards groves established on Volkameriana and Rangpur lime rootstocks.

On the other hand, epidemiological similarities indicate that CT management measures currently adopted in Cuba (Batista *et al.*, 2005) could be used for CSD as well. Those measures include national surveys and early detection of infected plants, eradication of symptomatic plants, use of certified budwood and tolerant rootstocks, and control of vectors.

In Cuba, most inspections are based on immunological tests since citrus trees grafted onto sour orange only occasionally show CTV decline symptoms. This is a research challenge when considering CSD assessments since there is no diagnostic test for CSD yet. CSD vectors, if any, are also still unknown, but considering the spatial pattern similarities with tristeza, insect management programs could be directed to aphids or to insects with a similar behavior. In Cuba, the most frequent aphids in citrus groves are *T. citricida*, *A. spiraecola* and *T. aurantii* Boyer de Fonscolombe (Batista *et al.*, 2008). The probability of CSD introduction and/or detection in Cuba is increasing because of the rootstock diversification program due to CTV. In this scenario, *C. volkameriana* is one of the most suitable and popular rootstocks in Cuba, but it has been demonstrated to be susceptible to CSD in Brazil. In addition, it is not advisable to disregard the possibility of an eventual CSD epidemic in Cuba following the same pattern observed for CTV, viz. symptomless trees of the most susceptible scion-rootstock combinations.

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