Preliminary Spatial Pattern Analysis of Huanglongbing in São Paulo, Brazil

R. B. Bassanezi[1], L. A. Busato[1], A. Bergamin Filho[2], L. Amorim[2], and T. R. Gottwald[3]

[1]Fundecitrus, Av. Dr. Adhemar P. de Barros 201, CEP 14807-040 Araraquara, SP, Brazil; [2]Departamento de Entomologia, Fitopatologia e Zoologia Agrícola, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Piracicaba, SP, Brazil; [3]USDA, ARS, USHRL, 2001 S. Rock Road, Fort Pierce, FL, 34945 USA

ABSTRACT. Huanglongbing (HLB) was reported in 2004 in São Paulo, Brazil. Assessments of diseased trees by visual symptoms were made in 36 plots from 8 farms in the central citrus region of São Paulo State. A total of 155 HLB spatial maps (varying from 0.14 to 25.99% disease incidence) were analyzed, considering quadrat sizes of 2 × 2, 4 × 4, 6 × 6 and 8 × 8 trees, by ordinary runs analysis, binomial index of dispersion and binary form of Taylor's power law. Aggregation among HLB-symptomatic trees was detected by ordinary runs analysis, with clustering in both within- and across-rows directions. However the percentage of aggregation within- and across-rows was low. The binomial index of dispersion for various quadrat sizes suggested aggregation of HLB-symptomatic trees for about 40% of the plots. The relationship between log (observed variance) and log (binomial variance) was highly significant for all four quadrat sizes. Estimated parameters of the binary form of Taylor's power law provided an overall measure of aggregation of HLB-symptomatic trees for all quadrat sizes tested. All power law estimates of b and A, were statistically different from 1, which indicated a general and significant pattern of aggregation of symptomatic plants for all quadrat sizes tested. The degree of aggregation was also positively related to disease incidence. Data from 20 plots ranging in disease incidence were also analyzed by spatial autocorrelation to examine the association among groups of infected trees using the 2 × 2 quadrat size. In 14 of 20 cases, clusters of HLB-infected trees were found to be associated with secondary clusters whose centers were at distances ranging from 4.2 to 22.1 tree spaces distant, indicating psyllid vector movement resulting in transmission to nearby trees causing clusters and to trees at considerable distance initiating new foci of infection.

Index words. Binomial analysis, disease incidence, infection foci, Taylor's power law.

Citrus Huanglongbing (HLB) is globally considered as one of the most important threats to commercial and sustainable citrus production. The disease is a limiting factor of citrus production in many areas of Southeast Asia, China, Japan, Taiwan, Indonesia, Philippines, Indian Ocean Islands, India, Africa, and the Arabian Peninsula (1, 2, 4, 5, 7, 11, 27, 29). In addition, HLB was discovered in Florida in late August 2005 and has since been detected in multiple commercial citrus plantings and numerous residential areas in multiple counties in south Florida. HLB-affected citrus tree show leaf yellowing and mottling, twig dieback, tree defoliation and general decline, small, lopsided and poorly colored fruit, fruit drop, and seed abortion (2, 24, 32). When the disease becomes endemic, affected trees are destroyed and the productive duration of fruit-bearing is shortened. The etiologic agent of HLB has been identified as a psyllid-borne, graft-transmissible and phloem-restricted bacterium (13, 15, 24, 27, 28) characterized as *Candidatus* Liberibacter in the α subdivision of the proteobacteria. *Candidatus* L. asiaticus has been associated with the Asian form of the disease and *Ca.* L. africanus with the African form (14, 23). Recently, leaf and fruit symptoms resembling those of HLB were observed in several sweet orange orchards in the Araraquara area of São Paulo State in Brazil, and *Ca.* L. asiaticus (12) and a third species, *Ca.* L. americanus, were found associated with the disease with the new species most prevalent

(30). *Ca.* L. americanus was detected in 214 symptomatic leaf samples from 47 citrus farms in 35 municipalities and *Ca.* L. asiaticus was found only four times within the 47 farms. The Asian psyllid vector of *Ca.* L. asiaticus, *Diaphorina citri* Kuwayama, reached Brazil 60 yr ago, is well established in São Paulo orchards, and is probably the candidate for the main HLB-agent vector in Brazil. However, due to its recent report in Brazilian orchards, the transmission of *Ca.* Liberibacter by *D. citri* in Brazil is not yet confirmed and no data is available with regard to HLB spatial distribution in Brazil. The distribution of psyllid vectors obviously plays a major role in HLB pathogen dissemination. HLB has been demonstrated to occur in aggregates or clumps of trees and direction or row effects have also been noted in China, Philippines and Reunion Island (16, 17, 18, 19). The purpose of this study was (i) to characterize the spatial pattern of HLB affected trees in groves of São Paulo State in Brazil and (ii) to compare Brazilian HLB spatial pattern with Asian HLB spatial pattern described in the literature.

MATERIALS AND METHODS

Data collection. Based on the survey of trees with visual symptoms of HLB the spatial pattern was determined in 36 citrus plots (i.e., a block within a grove) from 8 farms in the Central citrus region of São Paulo State, Brazil. The number of plants in each plot ranged from 320 to 10,944. Each plot was composed of sweet orange, lime and tangor grafted on Rangpur lime, Volkamer lemon, Swingle citrumelo, Cleopatra and Sunki mandarins (Table 1). Incidence of HLB was assessed by visual inspection of the canopies of all trees in each plot. The location of each symptomatic tree and the date

TABLE 1
DESCRIPTION OF THE CITRUS PLOTS IN SÃO PAULO STATE WHERE SYMPTOMATIC TREES WERE ASSESSED FOR HUANGLONGBING (HLB)

County	Number of plots	Scion/rootstock combination	Planting year	Tree spacing (m)	Number of trees	Analyzed maps
Araraquara	21	Westin/Rangpur, Hamlin/Rangpur, Valencia/Rangpur, Valencia/Swingle	1999-2002	7.0 × 3.0, 7.0 × 3.5, 7.0 × 4.0, 7.0 × 4.5, 7.5 × 5.0, 7.5 × 4.5, 8.0 × 3.5	320-2,080	47
Boa Esp. Sul	4	Natal/Rangpur, Valencia/Rangpur	2001	7.5 × 3.0, 7.5 × 3.5	780-3,280	8
Gavião Peixoto	3	Valencia/Volkamer.	2001	7.6 × 4.0	320	7
Itirapina	8	Pera/Rangpur, Pera/Sunki, Natal/Rangpur, Lime/Rangpur, Murcott/Rangpur, Murcott/Sunki	1998-2000	5.8 × 2.7, 6.0 × 3.0, 6.3 × 2.8, 6.3 × 2.9, 6.3 × 3.0, 6.5 × 3.0, 6.8 × 3.3	768-10,944	8
Luiz Antonio	24	Hamlin/Swingle, Pera/Rangpur, Pera/Sunki, Natal/Cleopatra, Valencia/Swingle, Lime/Cleopatra	1986-1999	7.0 × 3.0, 7.0 × 3.5, 7.0 × 4.0, 9.0 × 6.0	1,232-9,360	64
Rincão	8	Valencia/Rangpur, Valencia/Swingle	1996-2002	7.0 × 3.8, 7.0 × 4.0	1,408	16
São Simão	4	Valencia/Rangpur, Valencia/Swingle	2002-2003	7.5 × 4.0	704-1,408	5

when the symptoms appeared were recorded for each of the maps. A total of 155 binary (presence/absence) spatial maps of HLB, varying from 0.14 to 25.99% disease incidence were obtained.

Spatial analysis. Binary spatial maps of HLB were prepared for all assessment dates for each plot. 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 with the use of a Visual Basic EXCEL macro (26, T. R. Gottwald, unpublished). The analysis was applied only for rows with more than one symptomatic tree. 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 sizes. The incidence data for each plot were partitioned into quadrats of four (2 by 2), 16 (4 by 4), 36 (6 by 6), and 64 (8 by 8) trees with the use of the aforementioned Visual Basic EXCEL macro. When data are expressed as disease incidence, the binomial distribution provides the best fit for random conditions (25). Randomness within quadrats was thus assessed via binomial analysis. The binomial index of dispersion D was used to test for the presence of randomness of HLB-symptomatic trees at each quadrat size (25). For the binomial index, a large D (>1) combined with a small P (<0.05) suggests aggregation of symptomatic trees (25).

The binary form of Taylor's power law (22) relates the observed variance (V_{obs}) and the expected binomial variance (V_{bin}) for a random distribution of binary data. In this case, $\log(V_{obs}) = \log(A) + b \log(V_{bin})$, where A and b are parameters. Linear regression was performed for all plots using the least squares method. The significance of the relationship between $\log(V_{obs})$ and $\log(V_{bin})$ was determined by F-test, and the appropriateness of the model was evaluated by the coefficient of determination (R^2) and by the pattern of the residuals of regression. A random condition in the spatial distribution of symptomatic plants is inferred when $b = A = 1$. There is a constant level of aggregation for all incidence values when $b = 1$ and $A > 1$. When $b > 1$ the degree of aggregation varies according to the incidence. The equality of parameters b and A to unity was tested by the *t*-test, using the estimate of the parameter and its standard deviation (6).

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 20 plots (21). Data were parsed into three quadrat sizes, i.e., 2 by 2, 4 by 4 and 6 by 6 trees. The *x,y* spatial location and disease incidence of trees within 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 from which an evaluation of spatial patterns of disease incidence was performed. 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 discontiguous with the origin and the core cluster. The strength of aggregation 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). Row effects were evaluated as the number of significant lag positions within the first row (within) or within the first column (across) of the autocorrelation proximity pattern that are contiguous with the origin (6, 20, 31).

RESULTS

Spatial arrangement of HLB-symptomatic trees. The first level of spatial hierarchy examined was the association of symptom status between adjacent trees. Overall, for the 155 maps examined, aggregation within rows was detected in 57.4% of the maps and aggregation across rows was detected in 46.5% of the maps (Table 2). From the 1,588 rows tested, 211 (13.3%) were aggregated. From the 2,649 across rows tested, only 189 (7.1%) were aggregated (Table 2).

The next level of spatial hierarchy to be examined was the association of symptomatic plants within quadrats of various sizes. The interpretation of the values of the binomial index of dispersion *(D)* suggests a spatial structure of symptomatic plants significantly random for the majority of the plots and quadrat sizes of 2 by 2 (67.1% of plots), 4 by 4 (59.4% of plots) and 6 by 6 (59.6% of plots) (Table 2). For the majority of the plots and quadrat size 8 by 8, D values were higher than 1, i.e., aggregated (50.4% of plots), especially for incidence values higher than 0.01 (56.8% of plots). D values were usually higher for the largest quadrat sizes, with averages of 1.10, 1.29, 1.51, and 1.82 for 2 by 2, 4 by 4, 6 by 6, and 8 by 8 quadrat sizes, respectively.

The relationship between $\log(V_{obs})$ and $\log(V_{bin})$ was highly significant ($P < 0.001$) for the four sizes of quadrat (Fig. 1). Estimates of b and $\log(A)$ were, respectively, 1.02 (SE = 0.01) and 0.09 (SE = 0.01) for the 2 by 2 quadrat ($R^2 = 0.99$); 1.07 (0.01) and 0.28 (0.04) for the 4 by 4 quadrat ($R^2 = 0.98$); 1.11 (0.03) and 0.49 (0.09) for the 6 by 6 quadrat ($R^2 = 0.92$); and 1.23 (0.04) and 0.99 (0.14) for the 8 by 8 quadrat ($R^2 = 0.90$). All estimates of b and A were statistically different from 1 ($P < 0.05$), which indicated a general and significant pattern of aggregation of symptomatic plants within all quadrat sizes tested. Values of b higher than 1 also indicated that the degree of aggregation was a function of the incidence.

The final level of spatial hierarchy examined was the association among groups (quadrats) of trees as estimated by spatial autocorrelation. For the largest quadrat size tested (6 by 6 tree groups) 14 of the 20 data sets were large enough to be quadratized at this dimension. Spatial autocorrelation analyses results indicated that core clusters existed for 17/20, 9/20, and 6/14 plots for quadrat sizes 2 by 2, 4 by 4, and 6 by 6, respectively (Table 3). Core clusters with the greatest number of significant spatial lags tended to occur most frequently for plots in the mid-range of HLB disease incidence for all three quadrat sizes. The number of plots with core clusters decreased with quadrat size and as expected, quadrat size 2 by 2 demonstrated the largest core cluster sizes. In the majority of cases, core clusters were complete (i.e., saturated with significant positive lags) for all quadrat sizes (Table 3). However, in those cases in which core clusters were not completely saturated, strength of aggregation varied from 0.36 to 0.90 and was often associated with an asymmetry of the core cluster. No trends in strength of aggregation of the core clusters were noted over time for any quadrat size by plot combination. Row effects were detected by spatial lag autocorrelation for many plots and there did not seem to be a prevalence for within- versus across-row effects (Table 3). The occurrence of within- and across-row effects diminished with increasing quadrat size. Significant

TABLE 2
DISEASE INCIDENCE, ORDINARY RUNS, BINOMIAL DISPERSION INDEX (*D*) ANALYSES OF CITRUS HUANGLONGBING (HLB) IN BRAZIL BASED ON SYMPTOMATIC TREES OF SEVERAL SCION-ROOTSTOCK COMBINATIONS

			Ordinary runs[b]		Dispersion index (*D*)[c]			
Plots	Dates[a]	Disease incidence (proportion)	Within row	Across rows	Quadrat size 2 × 2	Quadrat size 4 × 4	Quadrat size 6 × 6	Quadrat size 8 × 8
10	26/07/04	0.0933	0.00	0.12	1.15	1.28	0.60	—[d]
	25/08/04	0.0993	0.00	0.11	1.13	1.20	0.56	—
	29/09/04	0.1172	0.00	0.10	1.02	1.06	0.60	—
101	01/10/04	0.0844	0.00	0.13	1.06	1.05	—	—
15	26/08/04	0.1308	0.00	0.18	1.12	1.64*	2.28*	—
	30/09/04	0.2002	0.04	0.12	1.14	1.51*	2.58*	—
17	26/07/04	0.1669	0.13	0.07	0.96	1.23	1.54*	1.41
	25/08/04	0.2116	0.15	0.05	0.96	1.17	1.63*	1.77*
	29/09/04	0.2230	0.13	0.05	0.96	1.17	1.62*	1.90*
1A	12/08/04	0.2352	0.00	0.06	1.20*	1.44*	—	—
	03/09/04	0.2566	0.00	0.05	1.11	1.36	—	—
	01/10/04	0.2599	0.00	0.05	1.08	1.25	—	—
1B	12/08/04	0.2414	0.18	0.06	1.17*	1.41*	0.98	2.14*
	03/09/04	0.2427	0.19	0.06	1.15*	1.36*	0.95	2.06*
	01/10/04	0.2447	0.18	0.06	1.15*	1.35*	0.95	1.97*
1C	03/09/04	0.2429	0.06	0.05	1.17	1.47*	—	—
	04/10/04	0.2543	0.06	0.07	1.21*	1.42*	—	—
2	28/07/04	0.0023	0.00	0.00	1.00	0.99	0.97	—
	13/08/04	0.0038	0.00	0.00	0.99	0.97	0.92	—
	04/10/04	0.0045	0.00	0.00	0.99	0.95	0.89	—
25	27/07/04	0.1184	0.11	0.00	1.11	1.11	1.15	—
	26/08/04	0.1268	0.06	0.00	1.12	1.05	1.28	—
	30/09/04	0.1437	0.06	0.00	1.05	1.12	1.27	—
2A	12/08/04	0.2024	0.03	0.05	1.06	1.16	1.42	2.14*
	06/09/04	0.2045	0.06	0.05	1.07	1.15	1.40	2.07*
	04/10/04	0.2145	0.06	0.05	1.09	1.19	1.45*	2.48*
2B	18/06/04	0.1355	0.21	0.05	1.03	1.32*	1.90*	—
2BC	18/06/04	0.1306	0.21	0.06	1.09	1.37*	1.94*	1.95*
	15/07/04	0.2037	0.21	0.04	1.09	1.22	1.53*	1.58*
2C	18/06/04	0.1476	0.21	0.05	1.15	1.17	0.99	—
2D	16/07/04	0.1475	0.14	0.01	1.12	1.46*	1.25	—
3A	06/09/04	0.0043	0.00	0.00	1.39*	1.36*	1.28	1.34
3AB	28/07/04	0.0040	0.00	0.00	0.99	1.24*	1.47*	1.38
3B	13/08/04	0.0043	0.00	0.00	1.39*	1.36*	1.28	1.34
	06/09/04	0.0085	0.00	0.00	1.20*	1.34*	2.11*	2.12*
	05/10/04	0.0123	0.33	0.00	1.12	1.30	1.86*	2.32*
3C	15/07/04	0.1961	0.13	0.04	1.19*	1.46*	1.45	—
6	27/07/04	0.0221	0.22	0.00	1.07	1.43*	1.58*	—
	24/08/04	0.0257	0.22	0.00	1.04	1.28	1.42	—
	28/09/04	0.0264	0.22	0.00	1.03	1.25	1.41	—
7	26/07/04	0.0683	0.10	0.03	1.10	1.29*	1.01	1.74*
	24/08/04	0.0750	0.10	0.02	1.11*	1.33*	1.07	1.84*

[a] Day/month/year.

[b] Values shown for each plot in each assessment date are the proportion of the number of test rows with significant aggregation ($P = 0.05$) considering the total number of rows tested (row with more than 1 diseased tree).

[c] Binomial index of dispersion (*D*) values for indicated quadrat size by plot and assessment date for citrus plots in Brazil with HLB symptomatic trees. Values presented for each assessment date are *D* (=observed variance/binomial variance). Significances (*) were calculated by comparison of df×*D* with the chi-square distribution. Values of *D* not significantly different from 1 ($0.95 > P > 0.05$) indicate that the pattern of symptomatic trees is indistinguishable from random. A large (>1) *D* and a small *P* (≤ 0.05) suggest rejection of H_0 (random pattern) in favor of H_1 (aggregated pattern of symtomatic trees).

[d] (—) Too few numbers of quadrats (<15) were available to allow calculation.

TABLE 2 (CONTINUED)
DISEASE INCIDENCE, ORDINARY RUNS, BINOMIAL DISPERSION INDEX (D) ANALYSES OF CITRUS HUANGLONGBING (HLB) IN BRAZIL BASED ON SYMPTOMATIC TREES OF SEVERAL SCION-ROOTSTOCK COMBINATIONS

			Ordinary runs[b]		Dispersion index (D)[c]			
Plots	Dates[a]	Disease incidence (proportion)	Within row	Across rows	Quadrat size 2 × 2	Quadrat size 4 × 4	Quadrat size 6 × 6	Quadrat size 8 × 8
	28/09/04	0.0827	0.10	0.02	1.12*	1.46*	1.11	1.81*
9	26/07/04	0.1829	0.00	0.00	1.05	1.12	—	—
	24/08/04	0.1944	0.00	0.00	1.14	1.22	—	—
	28/09/04	0.2361	0.00	0.03	1.24*	1.81*	—	—
105	13/07/04	0.0015	0.00	0.00	1.00	0.98	0.96	0.92
	07/10/04	0.0019	0.00	0.00	1.00	0.98	0.94	0.90
107	14/06/04	0.0038	0.00	0.00	0.99	1.00	—	—
	06/10/04	0.0051	0.00	0.00	0.99	0.98	—	—
108	14/07/04	0.0052	0.00	0.00	0.99	0.92	0.81	—
	07/10/04	0.0060	0.00	0.00	0.98	0.91	0.78	—
201	09/08/04	0.0037	0.50	0.00	1.16*	1.14	1.29*	1.17
	06/10/04	0.0043	0.50	0.50	1.13*	1.25*	1.54*	1.39*
3A	05/10/04	0.0113	0.50	0.00	1.13	1.16	1.67*	1.71*
12A	10/09/04	0.0125	0.00	0.00	0.97	1.39	—	—
	08/10/04	0.0156	0.00	0.00	0.96	1.23	—	—
12B	10/09/04	0.0438	0.20	0.00	1.18	1.27	—	—
	08/10/04	0.0469	0.20	0.00	1.15	1.16	—	—
13	11/08/04	0.0063	0.00	0.00	0.99	0.95	—	—
	10/09/04	0.0250	0.00	0.00	0.93	1.19	—	—
	08/10/04	0.0281	0.00	0.00	0.92	1.32	—	—
A06	16/06/04	0.0486	0.15	0.02	1.14*	1.58*	2.23*	3.29*
A10	16/06/04	0.0090	0.29	0.00	1.12*	1.37*	1.57*	2.23*
B0204	16/06/04	0.0182	0.09	0.11	1.08	1.33*	2.01*	1.91*
B06	16/06/04	0.0134	0.36	0.20	1.19*	1.75*	2.15*	2.20*
B13	16/06/04	0.0457	0.39	0.20	1.45*	2.90*	3.31*	6.50*
N18	16/06/04	0.0318	0.62	0.18	1.54*	3.40*	6.12*	8.37*
Q04	10/06/04	0.0404	0.00	0.00	1.01	1.13	1.14	—
S03	16/06/04	0.0156	0.13	0.08	1.12*	0.99	0.90	1.11
144	08/06/04	0.0713	0.28	0.23	1.29*	1.91*	4.10*	2.16*
	19/08/04	0.0738	0.07	0.10	1.10*	1.33*	2.13*	1.34
	20/09/04	0.0775	0.07	0.10	1.08	1.29*	2.08*	1.43
205A	19/07/04	0.0149	0.25	0.33	1.05	1.37*	1.80*	2.36*
	18/08/04	0.0241	0.11	0.25	1.17*	1.31*	1.82*	2.33*
	08/10/04	0.0277	0.10	0.22	1.13*	1.27*	1.56*	1.72*
205B	19/07/04	0.0291	0.11	0.11	1.11	1.42*	1.49*	1.77*
	08/10/04	0.0334	0.00	0.00	1.08	1.29*	1.24	1.43
205C	17/08/04	0.0135	0.00	0.50	1.07	1.13	0.90	0.61
	08/10/04	0.0149	0.00	0.50	1.05	1.37*	1.14	0.79
205D	19/07/04	0.0142	0.33	0.00	1.16*	1.20	1.03	1.16
	17/08/04	0.0213	0.22	0.00	1.07	1.09	1.08	1.08
206A	20/07/04	0.0234	0.29	0.00	1.18*	1.59*	2.47*	3.03*
	17/08/04	0.0313	0.10	0.00	1.14*	1.47*	2.07*	2.28*

[a] Day/month/year.

[b] Values shown for each plot in each assessment date are the proportion of the number of test rows with significant aggregation ($P = 0.05$) considering the total number of rows tested (row with more than 1 diseased tree).

[c] Binomial index of dispersion (D) values for indicated quadrat size by plot and assessment date for citrus plots in Brazil with HLB symptomatic trees. Values presented for each assessment date are D (=observed variance/binomial variance). Significances (*) were calculated by comparison of df$\times D$ with the chi-square distribution. Values of D not significantly different from 1 ($0.95 > P > 0.05$) indicate that the pattern of symptomatic trees is indistinguishable from random. A large (>1) D and a small P (≤ 0.05) suggest rejection of H_0 (random pattern) in favor of H_1 (aggregated pattern of symtomatic trees).

[d] (—) Too few numbers of quadrats (<15) were available to allow calculation.

TABLE 2 (CONTINUED)
DISEASE INCIDENCE, ORDINARY RUNS, BINOMIAL DISPERSION INDEX (D) ANALYSES OF CITRUS HUANGLONGBING (HLB) IN BRAZIL BASED ON SYMPTOMATIC TREES OF SEVERAL SCION-ROOTSTOCK COMBINATIONS

Plots	Dates[a]	Disease incidence (proportion)	Ordinary runs[b]		Dispersion index (D)[c]			
			Within row	Across rows	Quadrat size 2 × 2	Quadrat size 4 × 4	Quadrat size 6 × 6	Quadrat size 8 × 8
	16/09/04	0.0447	0.12	0.05	1.16*	1.44*	1.93*	2.02*
206B	20/07/04	0.0270	0.00	0.00	1.03	1.14	1.09	1.23
	17/08/04	0.0305	0.00	0.09	1.05	1.07	1.37	1.25
	17/09/04	0.0334	0.00	0.09	1.08	1.02	1.22	1.18
206C	20/07/04	0.0149	0.20	0.25	0.96	0.98	1.29	1.40
	17/08/04	0.0199	0.14	0.14	1.01	1.29*	2.05*	1.88*
	16/09/04	0.0256	0.22	0.11	0.98	1.19	1.94*	1.69*
206D	20/07/04	0.0206	0.11	0.00	1.08	1.05	1.02	0.88
	17/08/04	0.0270	0.17	0.00	1.19*	1.08	0.91	0.60
	16/09/04	0.0334	0.14	0.07	1.21*	1.16	1.03	0.67
207A	20/07/04	0.0327	0.00	0.31	1.31*	2.14*	2.97*	4.49*
	17/08/04	0.0369	0.08	0.31	1.29*	2.17*	3.01*	4.36*
	16/09/04	0.0426	0.07	0.25	1.25*	2.03*	2.90*	4.52*
207B	20/07/04	0.0149	0.00	0.67	1.05	1.17	1.18	0.70
	17/08/04	0.0206	0.20	0.17	1.01	1.05	1.11	0.83
	16/09/04	0.0227	0.10	0.17	1.00	1.11	1.14	1.00
207C	20/07/04	0.0135	0.00	0.00	1.07	1.02	0.86	0.70
	16/08/04	0.0220	0.11	0.00	1.07	1.14	1.21	1.14
	13/10/04	0.0263	0.11	0.00	1.03	1.16	1.27	1.34
207D	20/07/04	0.0249	0.00	0.00	1.04	1.22	0.99	1.44
	16/08/04	0.0298	0.17	0.00	1.11	1.54*	1.57*	2.03*
	13/10/04	0.0362	0.14	0.00	1.09	1.47*	1.54*	2.24*
207T	08/06/04	0.0185	0.20	0.08	1.09*	1.17*	1.36*	1.47*
208A	20/07/04	0.0412	0.11	0.25	1.38*	2.07*	2.80*	4.61*
	16/08/04	0.0447	0.09	0.20	1.40*	2.05*	2.89*	4.26*
	13/09/04	0.0455	0.08	0.20	1.38*	2.01*	2.89*	4.10*
	13/10/04	0.0469	0.08	0.20	1.37*	1.97*	2.78*	3.98*
208B	20/07/04	0.0185	0.29	0.00	1.10	1.12	1.38	1.80*
	13/10/04	0.0185	0.14	0.00	1.02	1.04	1.15	1.06
208C	20/07/04	0.0156	0.00	0.00	1.05	1.24	1.02	1.07
	13/10/04	0.0192	0.00	0.00	1.02	1.25	1.20	1.06
208D	20/07/04	0.0078	0.00	0.00	0.98	1.08	1.16	1.64*
	16/08/04	0.0107	0.00	0.00	0.97	0.98	1.07	1.69*
	13/10/04	0.0114	0.00	0.00	0.97	0.96	1.01	1.55
209A	21/07/04	0.0497	0.11	0.00	1.18*	1.56*	2.62*	3.39*
	16/08/04	0.0504	0.11	0.00	1.17*	1.56*	2.62*	3.39*
	13/09/04	0.0518	0.05	0.00	1.16*	1.56*	2.66*	3.39*
	13/10/04	0.0526	0.09	0.00	1.15*	1.53*	2.68*	3.49*
209B	16/08/04	0.0130	0.00	0.33	0.96	1.20	1.13	0.81
	13/10/04	0.0187	0.00	0.17	1.03	1.35*	1.16	1.02
214A	18/08/04	0.0114	0.00	0.50	1.10	0.96	1.01	1.08
	17/09/04	0.0149	0.50	0.50	1.35*	1.37*	1.29	1.97*

[a] Day/month/year.

[b] Values shown for each plot in each assessment date are the proportion of the number of test rows with significant aggregation ($P = 0.05$) considering the total number of rows tested (row with more than 1 diseased tree).

[c] Binomial index of dispersion (D) values for indicated quadrat size by plot and assessment date for citrus plots in Brazil with HLB symptomatic trees. Values presented for each assessment date are D (=observed variance/binomial variance). Significances (*) were calculated by comparison of dfx$D$ with the chi-square distribution. Values of D not significantly different from 1 ($0.95 > P > 0.05$) indicate that the pattern of symptomatic trees is indistinguishable from random. A large (>1) D and a small P (≤ 0.05) suggest rejection of H_0 (random pattern) in favor of H_1 (aggregated pattern of symtomatic trees).

[d] (—) Too few numbers of quadrats (<15) were available to allow calculation.

TABLE 2 (CONTINUED)
DISEASE INCIDENCE, ORDINARY RUNS, BINOMIAL DISPERSION INDEX (*D*) ANALYSES OF CITRUS HUANGLONGBING (HLB) IN BRAZIL BASED ON SYMPTOMATIC TREES OF SEVERAL SCION-ROOTSTOCK COMBINATIONS

			Ordinary runs[b]		Dispersion index (*D*)[c]			
Plots	Dates[a]	Disease incidence (proportion)	Within row	Across rows	Quadrat size 2 × 2	Quadrat size 4 × 4	Quadrat size 6 × 6	Quadrat size 8 × 8
214B	22/07/04	0.0078	1.00	0.00	1.16*	1.08	0.80	1.06
	18/08/04	0.0085	1.00	0.00	1.15*	1.05	0.74	0.78
	17/09/04	0.0092	0.00	0.00	1.13*	1.03	0.74	0.70
214C	18/08/04	0.0036	0.00	0.00	0.99	1.36*	0.94	0.90
	17/09/04	0.0057	0.00	0.00	0.99	1.18	0.89	1.09
214D	22/07/04	0.0163	0.00	0.40	1.13*	1.12	2.12*	1.85*
	18/08/04	0.0234	0.18	0.13	1.12	1.15	1.88*	1.50
	17/09/04	0.0256	0.33	0.11	1.15*	1.31*	2.40*	1.82*
422A	23/07/04	0.0057	0.00	0.00	0.99	0.92	0.83	0.99
	20/08/04	0.0092	0.00	0.00	0.97	0.87	0.86	1.64*
	24/09/04	0.0163	0.00	0.00	1.04	1.03	0.87	2.29*
422B	23/07/04	0.0107	0.00	0.00	0.97	0.98	1.03	0.86
	20/08/04	0.0128	0.20	0.00	1.08	1.16	1.43	0.88
	24/09/04	0.0142	0.20	0.00	1.06	1.10	1.35	0.79
422C	23/07/04	0.0107	0.00	0.00	0.97	1.12	1.03	1.08
	20/08/04	0.0114	0.00	0.00	0.97	1.09	0.97	1.12
	24/09/04	0.0135	0.50	0.00	1.18*	1.56*	1.48*	1.41
422D	23/07/04	0.0078	1.00	0.00	0.98	1.26*	1.09	0.95
	24/09/04	0.0199	0.14	0.00	1.01	1.15	1.18	1.39
427A	24/09/04	0.0021	0.00	0.00	1.00	1.65*	1.63*	1.60*
427C	24/09/04	0.0021	0.00	0.00	1.00	0.98	0.94	0.90
427D	24/09/04	0.0036	0.00	0.00	0.99	0.96	0.89	0.79
443B	23/08/04	0.0014	0.00	0.00	1.00	0.99	0.97	0.95
	21/09/04	0.0021	0.00	0.00	1.00	0.98	1.63*	0.90
36A	02/08/04	0.0028	0.00	0.00	0.99	0.97	0.91	1.37
	27/09/04	0.0085	0.00	1.00	1.31*	1.56*	2.03*	1.83*
36B	27/09/04	0.0114	1.00	0.00	0.97	1.11	—	—
36D	27/09/04	0.0057	1.00	0.00	1.24*	1.18	1.12	1.69*
37D	30/08/04	0.0021	0.00	0.00	1.00	0.98	0.94	0.90

[a] Day/month/year.

[b] Values shown for each plot in each assessment date are the proportion of the number of test rows with significant aggregation ($P = 0.05$) considering the total number of rows tested (row with more than 1 diseased tree).

[c] Binomial index of dispersion (*D*) values for indicated quadrat size by plot and assessment date for citrus plots in Brazil with HLB symptomatic trees. Values presented for each assessment date are *D* (=observed variance/binomial variance). Significances (*) were calculated by comparison of df×*D* with the chi-square distribution. Values of *D* not significantly different from 1 ($0.95 > P > 0.05$) indicate that the pattern of symptomatic trees is indistinguishable from random. A large (>1) *D* and a small *P* (≤0.05) suggest rejection of H_0 (random pattern) in favor of H_1 (aggregated pattern of symtomatic trees).

[d] (—) Too few numbers of quadrats (<15) were available to allow calculation.

edge effects were detected in 5/20, 5/20, 1/14 for 2 by 2, 4 by 4, and 6 by 6 quadrat sizes, respectively (Table 3).

Data from 20 plots ranging in disease incidence were also analyzed by spatial autocorrelation to examine the association among groups of infected trees using the 2 by 2 quadrat size. In 14 of 20 cases, clusters of HLB-infected trees were found to be associated with secondary clusters whose centers were at distances ranging from 4.2 to 22.1 tree spaces distant, indicating psyllid vector movement resulting in transmission both to nearby trees causing clusters and to trees at considerable distance initiating new foci of infection.

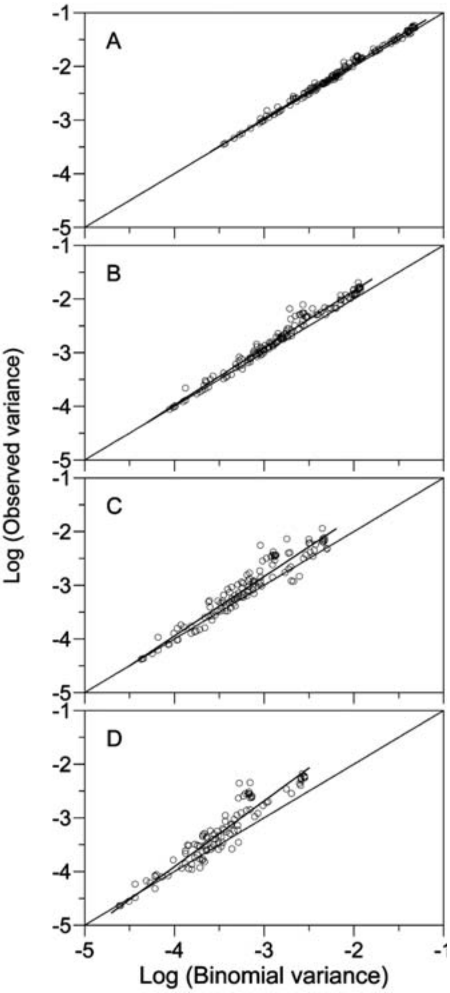

Fig. 1. The relationship between the observed and the theoretical binomial (random) variance of the incidence of huanglongbing (HLB). Each data point represents a HLB assessment (symptomatic trees) in a plot in Brazil. The solid lines represent the relationship $\log(V_{obs}) = \log(A) + b \log(V_{bin})$ fitted to the data by ordinary least squares regression. The dashed lines represent the binomial line (i.e., observed variance = binomial variance). (A) quadrat size 2 by 2: $b = 1.02$ (SE = 0.01), $\log(A) = 0.09$ (SE = 0.01), $R^2 = 0.99$; (B) quadrat size 4 by 4: $b = 1.07$ (0.01), $\log(A) = 0.28$ (0.04), $R^2 = 0.98$; (C) quadrat size 6 by 6: $b = 1.11$ (0.03), $\log(A) = 0.49$ (0.09), $R^2 = 0.92$; (D) quadrat size 8 by 8: $b = 1.23$ (0.04), $\log(A) = 0.99$ (0.14), $R^2 = 0.90$.

DISCUSSION

Monitoring the occurrence of HLB symptoms can be somewhat problematic. The typical smaller fruit, leaf mottling and veinal chlorosis, and interveinal chlorosis are similar to zinc pattern deficiency and are usually followed by retarded growth, but these symptoms are often non distinct and/or restricted to one branch or side of the tree (15, 27, 32). These symptoms can be sometimes attributed to other diseases present in Brazil, especially blight, citrus variegated chlorosis, citrus sudden death, and *Phytophthora*-diseases. Eventually, twig dieback and a general decline ensue due to the severe effect of pathogens on the phloem of the host. Infections by HLB pathogens are often expressed in sectors on infected trees, indicating incomplete systemic infection or perhaps variable pathogen titer levels throughout infected trees. The lag in time between transmission of the pathogen by psyllid vectors or by propagation and the onset of visual symptoms for Asian and African HLB can be quite variable depending on the time of the year when infection took place, environmental conditions, tree age, or tree species/cultivar (1, 10, 16, 28, 32). It appears that the same may be true for the American HLB isolate. Thus quantifying the severity or expression of disease symptoms in individual trees may not be a precise indication of pathogen content. Additionally, due to the temporal variation in symptom expression, trees infected at the same time may express the onset of symptoms with great variability over one or more years. This inherently broad and variable lag period compromises the accuracy of spatial and temporal studies to some extent. HLB epidemics can be established by introduction of infected plant materials and by transmission due to insect vectors. The unintentional introduction of infected plant materials establishes the disease in new areas or countries and subsequent unregulated movement can spread the disease over large areas. Natural trans-mission appears to be

TABLE 3
SPATIAL AUTOCORRELATION ANALYSIS FOR HUANGLONGBING (HLB) IN BRAZIL IN SEVERAL CITRUS SCION-ROOTSTOCK COMBINATIONS

Plot	Quadrat size	Disease incidence[a]	Significant lags[b]		Strength of aggregation[c]	Core cluster size[d]	Core cluster saturation[e]	Reflected cluster size[f]	Total no. of reflected clusters[g]	SL+ Reflected Clusters		Effects[h]		
			SL+	SL-						Max. X Dist.	Max. Y Dist.	Within row	Across row	Edge
207D	2 × 2	0.1307	4	0	0.25	1	1.00	1	3	10	3	2	1	0 ns
	4 × 4	0.3864	3	0	0.33	1	1.00	1	2	5	2	1	1	1S
	6 × 6	0.6286	1	0	1.00	1	1.00	0	0	NA	NA	0	1	0 ns
209A	2 × 2	0.1818	3	2	0.33	1	1.00	1	2	0	13	2	1	0 ns
	4 × 4	0.4545	1	0	1.00	1	1.00	0	0	NA	NA	0	1	0 ns
	6 × 6	0.7143	0	0	0.00	0	0.00	0	0	NA	NA	0	0	0 ns
108	2 × 2	0.0238	0	0	0.00	0	0.00	0	0	NA	NA	0	0	0 ns
	4 × 4	0.1111	0	0	0.00	0	0.00	0	0	NA	NA	0	0	1 ns
	6 × 6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
2A	2 × 2	0.6051	1	0	0.00	0	NA	1	1	4	0	0	1	0 ns
	4 × 4	0.9773	3	0	1.00	3	1.00	0	0	NA	NA	1	1	0 ns
	6 × 6	1.0000	0	0	0.00	0	0.00	0	0	NA	NA	0	0	0 ns
1B	2 × 2	0.6410	0	0	0.00	0	0.00	0	0	NA	NA	0	0	0 ns
	4 × 4	0.9783	0	0	0.00	0	0.00	0	0	NA	NA	0	0	0 ns
	6 × 6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	2 × 2	0.3923	2	0	0.00	0	NA	1	2	6	2	0	0	1S
	4 × 4	0.8667	0	0	0.00	0	0.00	0	0	NA	NA	0	0	0 ns
	6 × 6	1.0000	0	0	0.00	0	0.00	0	0	NA	NA	0	0	1 ns
7	2 × 2	0.2808	1	1	0.00	0	NA	1	1	7	0	0	1	1S
	4 × 4	0.6538	1	0	0.00	0	NA	1	1	2	1	0	0	1S

[a] Disease incidence = Quadrat disease incidence.

[b] Number of [X,Y] lags significantly greater (SL+), or less (SL-) than expected by chance at a = 0.05 level.

[c] Strength of aggregation = number of SL+ in core cluster/total number of SL+.

[d] Core cluster size = the number of significant SL+ lags contiguous with the [0,0]lag position that form a discrete group.

[e] Core cluster saturation = core cluster size/ total number of possible lags determined by the extents of the core cluster, not including the [0,0] lag.

[f] Reflected cluster size = the number of contiguous SL+ in various clusters not contiguous with the core cluster.

[g] Total number of clusters = the number of contiguous clusters of SL+ in the proximity pattern.

[h] Effects = the number of SL+ within-row and within-column of the row and column defined by the [0,0] lag. Edge effects are significant (S) or non significant (ns) if the number of SL+ at the distal edges of the proximity pattern/total number of SL+ is ≥5%, and <5%, respectively.

TABLE 3 (CONTINUED)
SPATIAL AUTOCORRELATION ANALYSIS FOR HUANGLONGBING (HLB) IN BRAZIL IN SEVERAL CITRUS SCION-ROOTSTOCK COMBINATIONS

			Significant lags[b]							SL+ Reflected Clusters		Effects[h]		
Plot	Quadrat size	Disease incidence[a]	SL+	SL-	Strength of aggregation[c]	Core cluster size[d]	Core cluster saturation[e]	Reflected cluster size[f]	Total no. of reflected clusters[g]	Max. X Dist.	Max. Y Dist.	Within row	Across row	Edge
	6 × 6	0.9412	0	0	0.00	0	0.00	0	0	NA	NA	0	0	1 ns
36A	2 × 2	0.0284	5	0	0.20	1	1.00	1,2	3	13	4	0	1	1S
	4 × 4	0.1023	1	0	0.00	0	NA	1	1	5	1	0	0	1S
	6 × 6	0.1714	0	0	0.00	0	0.00	0	0	NA	NA	0	0	0 ns
427A	2 × 2	0.0085	1	0	1.00	1	1.00	0	0	NA	NA	1	0	0 ns
	4 × 4	0.0227	0	0	0.00	0	0.00	0	0	NA	NA	0	0	0 ns
	6 × 6	0.0571	0	0	0.00	0	0.00	0	0	NA	NA	0	0	0 ns
144	2 × 2	0.2650	20	1	0.90	18	0.41	1	2	5	7	7	1	0 ns
	4 × 4	0.6500	6	0	0.83	5	0.45	1	1	0	5	4	1	0 ns
	6 × 6	0.9231	5	0	1.00	5	0.45	0	0	NA	NA	3	1	1S
207A	2 × 2	0.1420	11	0	0.36	4	0.57	1,2	4	12.5	0.5	4	4	1S
	4 × 4	0.3977	4	1	0.75	3	0.60	1	1	5	0	2	2	1S
	6 × 6	0.6571	1	0	1.00	1	1.00	0	0	NA	NA	1	0	0 ns
6	2 × 2	0.0971	0	0	0.00	0	0.00	0	0	NA	NA	0	0	0 ns
	4 × 4	0.2933	0	0	0.00	0	0.00	0	0	NA	NA	0	0	1 ns
	6 × 6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
201	2 × 2	0.0134	11	0	0.09	1	1.00	1,2	9	10	13	1	2	0 ns
	4 × 4	0.0500	0	0	0.00	0	0.00	0	0	NA	NA	0	0	0 ns
	6 × 6	0.1026	1	0	0.00	0	NA	1	1	2	3	0	0	0 ns
206C	2 × 2	0.0597	10	0	0.00	0	NA	2,1	7	9	4	0	4	0 ns
	4 × 4	0.2159	4	0	0.00	0	NA	2,1	3	1	6	0	1	1S

[a] Disease incidence = Quadrat disease incidence.

[b] Number of [X,Y] lags significantly greater (SL+), or less (SL-) than expected by chance at a = 0.05 level.

[c] Strength of aggregation = number of SL+ in core cluster/total number of SL+.

[d] Core cluster size = the number of significant SL+ lags contiguous with the [0,0]lag position that form a discrete group.

[e] Core cluster saturation = core cluster size/ total number of possible lags determined by the extents of the core cluster, not including the [0,0] lag.

[f] Reflected cluster size = the number of contiguous SL+ in various clusters not contiguous with the core cluster.

[g] Total number of clusters = the number of contiguous clusters of SL+ in the proximity pattern.

[h] Effects = the number of SL+ within-row and within-column of the row and column defined by the [0,0] lag. Edge effects are significant (S) or non significant (ns) if the number of SL+ at the distal edges of the proximity pattern/total number of SL+ is ≥5%, and <5%, respectively.

TABLE 3 (CONTINUED)
SPATIAL AUTOCORRELATION ANALYSIS FOR HUANGLONGBING (HLB) IN BRAZIL IN SEVERAL CITRUS SCION-ROOTSTOCK COMBINATIONS

Plot	Quadrat size	Disease incidence[a]	Significant lags[b]		Strength of aggregation[c]	Core cluster size[d]	Core cluster saturation[e]	Reflected cluster size[f]	Total no. of reflected clusters[g]	SL+ Reflected Clusters		Effects[h]		
			SL+	SL-						Max. X Dist.	Max. Y Dist.	Within row	Across row	Edge
	6 × 6	0.3714	1	0	1.00	1	1.00	0	0	NA	NA	1	0	0 ns
17	2 × 2	0.5341	4	2	0.50	2	1.00	1	2	12	10	0	3	1S
	4 × 4	0.9205	2	1	1.00	2	1.00	0	0	NA	NA	0	2	0 ns
	6 × 6	0.9714	1	1	0.00	0	NA	1	1	0	2	1	0	0 ns
105	2 × 2	0.0059	2	0	0.00	0	NA	1	2	6	11	0	0	0 ns
	4 × 4	0.0256	0	0	0.00	0	0.00	0	0	NA	NA	0	0	0 ns
	6 × 6	0.0588	0	0	0.00	0	0.00	0	0	NA	NA	0	0	0 ns
3C	2 × 2	0.5603	1	1	1.00	1	1.00	0	0	NA	NA	1	0	0 ns
	4 × 4	0.9464	0	0	0.00	0	0.00	0	0	NA	NA	0	0	0 ns
	6 × 6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
2BC	2 × 2	0.5770	3	1	0.33	1	1.00	2	1	4.5	0	1	2	0 ns
	4 × 4	0.9732	0	0	0.00	0	0.00	0	0	NA	NA	0	0	0 ns
	6 × 6	1.0000	0	0	0.00	0	0.00	0	0	NA	NA	0	0	1 ns
2B	2 × 2	0.4409	4	0	1.00	4	0.50	0	0	NA	NA	2	1	0 ns
	4 × 4	0.8736	0	0	0.00	0	0.00	0	0	NA	NA	0	0	1 ns
	6 × 6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
2D	2 × 2	0.4471	3	0	0.33	1	1.00	1	2	0	3	2	1	0 ns
	4 × 4	0.8765	0	0	0.00	0	0.00	0	0	NA	NA	0	0	1 ns
	6 × 6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

[a] Disease incidence = Quadrat disease incidence.

[b] Number of [X,Y] lags significantly greater (SL+), or less (SL-) than expected by chance at a = 0.05 level.

[c] Strength of aggregation = number of SL+ in core cluster/total number of SL+.

[d] Core cluster size = the number of significant SL+ lags contiguous with the [0,0]lag position that form a discrete group.

[e] Core cluster saturation = core cluster size/ total number of possible lags determined by the extents of the core cluster, not including the [0,0] lag.

[f] Reflected cluster size = the number of contiguous SL+ in various clusters not contiguous with the core cluster.

[g] Total number of clusters = the number of contiguous clusters of SL+ in the proximity pattern.

[h] Effects = the number of SL+ within-row and within-column of the row and column defined by the [0,0] lag. Edge effects are significant (S) or non significant (ns) if the number of SL+ at the distal edges of the proximity pattern/total number of SL+ is ≥5%, and <5%, respectively.

related to high vector populations and to the extensiveness of the inoculum reservoir (1, 11). Psyllid migrations appear to be highest when host plants are flushing and psyllid sedentary populations are frequent when foliage is mature (1, 8). In Africa, natural spread is probably greatest in late spring when new flush is available and psyllid populations are highest (1, 8, 9). Psyllid vectors are also attracted to yellow wavelengths of light, and thus preferentially to foliage expressing HLB symptoms.

Specific studies have not been done to relate vector populations and transmission rates for the new American isolate of HLB. In addition, insufficient time has elapsed since the discovery of the new isolate in Brazil to collect multiyear incidence data necessary to study the temporal increase of the isolate under Brazilian conditions. However, we can compare the spatial patterns of the American isolate and those from previous studies of the Asian isolate. From a spatial perspective, results of the study of the Asian isolate of HLB at various spatial analyses conducted to date can be interpreted in combination to obtain a more comprehensive picture of spatial patterns that existed in Asian HLB-infected plantings. The combined analyses indicate two mechanisms of vector spread of HLB, within local areas and over longer distances (3, 16, 17, 18, 19).

The study performed in Brazil was restricted to the spread of HLB within local areas. According to the ordinary runs analysis, the spread of disease between neighboring plants occurs but is not very frequent, unlike the situation described in Reunion Island (3). Aggregation within quadrats was not observed in the majority of plots when disease incidence was low. This indicates that infective vectors land at random in a field at the beginning of the epidemic or that they fly at random, spreading the pathogen, before they become established in a tree.

For the Asian isolate, within local areas, aggregations of infected trees occur that at times can be quite large, encompassing as many as 1672 trees. This does not mean that every tree in these local areas will become infected, but that a high proportion of them will, as demonstrated by the 'strength of aggregation' calculation associated with spatial autocorrelation results and defining a focus of infection (Gottwald, unpublished data). In this case vectors are apparently spreading the disease to either adjacent or nearby trees only a few spaces away. The same kind of aggregation of groups of trees was only hinted at for the American HLB spatial analyses. Certainly aggregation of large numbers of adjacent trees was seen but not to this same extent. This is likely due to the maturity of the Asian HLB epidemics examined versus the relatively lower incidence and presumed younger American HLB infections in Brazilian plantations.

Spatial autocorrelation also identifies a prevalence of reflected clusters or areas of aggregation that are discontinuous with the main cluster. These are interpreted as indicative of the presence of secondary foci. For the Asian HLB epidemics, reflected clusters are quite prevalent and are at a distance of about 25-50 m from the main cluster of disease and each other (17). For the American HLB epidemics examined, there was also a prevalence of reflected clusters in many plots. Such a pattern of widely spaced foci perhaps indicates a spatial mechanism associated with longer distance vector movement. That is, when vectors move, either naturally in search of new feeding opportunities or when disturbed, they occasionally do so to other than nearby trees and when this occurs they move at least 25 to 50 m (17).

Longer or regional scale vector transmission has not been investigated for any HLB isolate, i.e., Asian, African or American. It is obvious that this likely happens as well, but is beyond the scope of the present study and other studies conducted to date.

LITERATURE CITED

1. Aubert, B.
   1987. *Trioza erytrea* Del Guercio and *Diaphorina citri* Kuwayama (Homoptera: Psylloidae), the two vectors of citrus greening disease: Biological aspects and possible control strategies. Fruits 42: 149-162.
2. Aubert, B., M. Garnier, D. Guillaumin, B. Herbagyandono, L. Setiobudi, and F. Nurhadi
   1985. Greening, a serious threat for the citrus production of the Indonesian archipelago. Future prospects of integrated control. Fruits 40: 549-563.
3. Aubert, B., A. Sabine, P. Geslin, and L. Picardi
   1984. Epidemiology of the greening disease in Reunion Island before and after the biological control of the African and Asian citrus psyllas. Proc. Int. Soc. Citricult. 1: 440-442.
4. Bové, J. M. and M. Garnier
   1984. Greening and psylla vectors of the disease in the Arabian Peninsula. In: *Proc. 9th Conf. IOCV*, 109-114. IOCV Riverside, CA.
5. Broadbent-Barkley, P.
   1983. Citrus greening and virus diseases in China and Southeast Asia. Report to the Plant Pathology section at the Int. Plant Pathology Congress of Melbourne. 21 pp.
6. Campbell, C. L. and L. V. Madden
   1990. *Introduction to Plant Disease Epidemiology.* John Wiley & Sons, New York.
7. Catling, H. D.
   1968. Distribution and biology of *Diaphorina citri*, the insect vector of leaf mottling (greening) disease of citrus. Report to the Government of Philippines, UNDP FAO 2589, 16 pp.
8. Catling, H. D.
   1969. The bionomics of the South African citrus psylla, *Trioza erytreae* D.G. (Homoptera: Psyllidae). 3. The influence of extremes of weather on survival. J. Entomol. Soc. Sthn. Afr. 32: 273-290.
9. Catling, H. D.
   1970. The bionomics of the South African citrus psylla *Trioza erytreae* D.G. (Homoptera: Psyllidae). 4. Influence of predators. J. Entomol. Soc. Sthn. Afr. 33: 341-348.
10. Catling, H. D. and P. R. Atkinson
    1974. Spread of greening by *Trioza erytreae* (Del Guercio) in Swaziland. In: *Proc. 6th Conf. IOCV*, 33-39. IOCV Riverside, CA.
11. Chao, B. Y., Y. H. Chiang, S. L. Lee, C. S. Chiu, and W. F. Su
    1979. Preliminary study on the relation between the prevalence of the citrus yellow shoot (Huanglongbing) and the citrus psyllid *Diaphorina citri* Kuwayama. Acta Phytopathol. Sinica 2: 121-126.
12. Coletta-Filho, H. D., M. L. P. N. Targon, M. A. Takita, J. D. De Negri, J. Pompeu Jr., M. A. Machado, A. M. do Amaral, and G. W. Muller
    2004. First report of the causal agent of Huanglongbing ("*Candidatus* Liberibacter asiaticus") in Brazil. Plant Dis 88: 1382.
13. Garnier, M. and J. M. Bové
    1983. Transmission of the organism associated with citrus greening disease from sweet orange to periwinkle by dodder. Phytopathology 73: 1358-1363.
14. Garnier, M. and J. M. Bové
    1996. Distribution of the huanglongbing (greening) Liberibacter species in fifteen African and Asian countries. In: *Proc. 13th Conf. IOCV*, 388-391. IOCV Riverside, CA.
15. Garnier, M., J. Latsille, and J. M. Bové
    1984. The greening organism is a Gram negative bacterium. In: *Proc. 9th Conf. IOCV*, 115-124. IOCV Riverside, CA.
16. Gottwald, T. R. and B. Aubert
    1988. Modeling of natural transmission of greening by vectors. Proc. 1st FAO/UNDP Citrus Greening Workshop, 16-23.
17. Gottwald, T. R., B. Aubert, and H. K. Long
    1990. Spatial pattern analysis of citrus greening in Shantou, China. In: *Proc. 11th Conf. IOCV*, 421-427. IOCV Riverside, CA.
18. Gottwald, T. R., B. Aubert, and X. Z. Zhao
    1989. Preliminary analysis of citrus greening (Huanglongbing) of China and French Reunion Island. Phytopathology 79: 687-693.
19. Gottwald, T. R., C. I. Gonzales, and B. G. Mercado
    1990. Analysis of the distribution of citrus greening in groves in the Philippines. In: *Proc. 11th Conf. IOCV*, 414-420. IOCV Riverside, CA.

20. Gottwald, T. R., S. M. Garnsey, and J. Borbón
    1998. Increase and patterns of spread of citrus tristeza virus infections in Costa Rica and the Dominican Republic in the presence of the brown citrus aphid, *Toxoptera citricida*. Phytopathology 88: 621-636.
21. Gottwald, T. R., S. M. Richie, and C. L. Campbell
    1992. LCOR2-spatial correlation analysis software for the personal computer. Plant Dis. 76: 213-215.
22. Hughes, G. and L. V. Madden
    1992. Aggregation and incidence of disease. Plant Pathol. 41: 657-660.
23. Jagoueix, S., J. M. Bové, and M. Garnier
    1994. The phloem-limited bacterium of greening disease of citrus is a member of the α subdivision of the proteobacteria. Int. J. Syst. Bacteriol. 44: 379-386.
24. Lin, K. H.
    1956. Observation on yellow shoot of citrus. Etiological study of yellow shoot of citrus. Acta Phytopathol. Sinica 2: 1-42.
25. Madden, L. V. and G. Hughes
    1995. Plant disease incidence: distributions, heterogeneity, and temporal analysis. Annu. Rev. Phytopathol. 33: 529-564.
26. Madden, L. V., L. R. Nault, D. J. Murral, and M. R. Apelt
    1995. Spatial pattern analysis of the incidence of aster yellows disease in lettuce. Res. Popul. Ecol. 37: 279-289.
27. Martinez, A. L. and J. M. Wallace
    1967. Citrus leaf mottle-yellows disease in the Philippines and transmission of the causal virus by a psyllid, *Diaphorina citri*. Plant Dis. Rept. 51: 692-695.
28. McLean, A. P. D. and P. C. J. Oberholzer
    1965. Citrus psylla, a vector of the greening disease of sweet orange. S. Afr. J. Agr. Sci. 8: 297-298.
29. Moll, J. N. and S. P. van Vuuren
    1977. Greening disease in Africa. Proc. Int. Soc. Citricult. 3: 903-912.
30. Texeira, D.C., A. J. Ayres, E. W. Kitajima, F. A. O. Tanaka, J. L. Danet, S. Jagoueix-Eveillard, C. Saillard, and J. M. Bové
    2005. First report of a Huanglongbing-like disease of citrus in Sao Paulo State, Brazil and association of a new liberibacter species, "*Candidatus* Liberibacter americanus", with the disease. Plant Dis. 89: 107.
31. Upton, G. and B. Fingleton
    1985. *Spatial Data Analysis by Example, Vol. I: Point Pattern and Quantitative Data.* John Wiley & Sons, Chichester.
32. Zhao, X. Y.
    1981. Citrus yellow shoot (Huanglungbin) in China: a review. Proc. Int. Soc. Citricult. 1: 466-469.