Spatial Pattern Analysis of Citrus Greening In Shantou, China

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ABSTRACT. Assessments of individual trees for citrus greening disease were made in 1986 and again in 1988 in a citrus grove near Poutai Shantou Prefecture, China, consisting of ca. 20,000 Tankan/Fuzhou mandarin trees. In 1986, 16 trees displayed greening symptoms. The diseased trees were near the southern edge of the grove immediately adjacent to a small garden that contained ca. 24 trees with greening symptoms. By 1988, 14% of the trees in the Poutai grove were assessed as greening-diseased. The spatial pattern of disease was analyzed by 1) indices of dispersion, 2) ordinary runs analysis, 3) spatial autocorrelation, and 4) isopath mapping. Disease was considered to be aggregated based on Lloyd's-index-of-patchiness. With ordinary runs analysis, greater aggregation was found in 78% of north-south rows compared to 44% for east-west groups of trees. The greater north-south aggregation corresponded to the general grove traffic and closer spacing between trees on beds running north-south. Spatial autocorrelation indicated that clusters of diseased trees oriented north-south from one another were related. Clustering on the southern, eastern, and northern borders of the grove was verified by mapping of isopaths. Such clustering was probably indicative of ingress of psyllid vectors from the heavily infested small planting to the south, from an adjacent town to the east, and aggregation of the psyllids along a northern bordering canal, respectively.

Index words. indices of dispersion, spatial lag autocorrelation analysis, *Diaphorina citri.*

Citrus greening disease (CGD) is a serious problem in the People's Republic of China (PRC) where it is referred to as 'Huanglungbin' or 'yellow shoot.' Mandarins and oranges are highly sensitive to CGD. CGD is one of the major limiting factors to citrus production in the PRC and other Asian countries (1,2,9,14).

Citrus greening is presumably caused by a phloem-limited, fastidious bacterium. The bacteria are disseminated over long distances primarily by contaminated budwood and nursery stock occasionally carrying the pathogen and eggs or nymphs of the vector. The pathogen is subsequently disseminated by endemic populations of the vector from nearby villages and neglected groves (3,4). Researchers in the PRC have attempted to establish pathogen-free citrus clones via thermotherapy and shoot tip grafting to decrease pathogen dissemination via infected budwood and nursery plants. Trees resulting from these efforts are kept in remote foundation groves and are indexed regularly to insure that they remain free of CGD as well as several other diseases. An effort is also being made to estasblish disease-free nurseries in isolated regions devoid of the vector, where diligent application of insecticides is used to ensure a supply of clean planting material.

Of the two potential psyllid vector species known worldwide, only the Asian psyllid, *Diaphorina citri* Kuwayama, is presently known to exist in the PRC (4). Control of psyllid populations is still best accomplished by the use of chemical insecticides, although some attempts are being made to introduce a hymenopterous parasite of *D. citri* for biocontrol. Unfortunately, insecticides are expensive and often unavailable in some areas of the PRC and often these chemicals are not economically feasible, especially for the small farmer.

The distribution of psyllid vectors obviously plays a major role in CGD pathogen dissemination. Greening has been demonstrated to occur in aggregates or clumps of trees and direction or row effects have also been noted (5,6,7). The size and shape of these clusters of trees in orange groves in China and Reunion have been examined previously (8). Observed clusters of diseased trees in larger mandarin plantings in southeast PRC appeared to be larger than those reported in orange groves in south central PRC. This study was conducted to examine the clustering of CGD in a large tangor grove near Shantou, China, to determine relative cluster size and shape (7).

MATERIALS AND METHODS

Disease assessments of all individual trees were made in 1986 and 1988 in the Poutai Citrus Cooperative Grove near Shantou, Guangdong Province, PRC (7). The cooperative plot is managed by ca. 76 different farmers who are each responsible for their portion of the grove. However, the grove receives a local standard range of care with primitive hand-powered sprayers for insecticide treatment. The grove consists of ca. 20,000 trees of Tankan/Fuzhou mandarin on land converted to citrus from swamp rice. The trees were planted on beds running north-south. The planting was established from disease-free planting materials in 1983. Individual trees were visually scored plus or minus for CGD. Because of the large number of trees, the data were divided into quadrats of 2 X 2, 3 X 3, 4 X 4, 6 X 6, 8 X 8, 16 X 16, and 32 X 32 trees per quadrat. Quadrat scores were the total number of greening-affected trees per quadrat. The spatial distribution of trees with CGD was analyzed by 1) ordinary runs analysis (10), 2) several indices of dispersion (12,13), 3) spatial lag autocorrelation (11), and 4) computer-assisted isopath mapping and response surface analysis (8).

RESULTS

In 1986 only 16 trees (0.08%) displayed symptoms of CGD. These were primarily near the southern edge of the grove. During a visit to the grove later in 1988, a small planting of 24 trees associated with a small vegetable garden was noted immediately adjacent to a farmhouse on the southern edge of the grove. These trees were all severely infected with greening and did not originate from disease-free nursery stock as did the rest of the grove. By 1988, 2880 trees (14.4%) were assessed as having CGD.

Results from ordinary runs analysis indicated a higher degree of aggregation (155/199 = 77.9% of rows tested) in the north-south direction compared with (91/205 = 44.4% of rows tested) in the east-west direction (Table 1). This greater-north-south aggregation was in the same orientation as the raised planting beds.

Results of analysis of aggregation by variance-to-mean ratio, Lloyd's-Index-of-Patchiness, and Morisita's-Index-of-Dispersion all indicated aggregation was present for all quadrat sizes except the smallest (2 X 2) quadrat size (Table 2). Morisita's index values remained relatively stable for all but the largest quadrat sizes. Morisita's-Index-of-Mean-Crowding values increased sharply from 2 X 2 to the 3 X 3 quadrat size then continued to rise gradually for the larger quadrat size (Fig. 1).

Isopath contour maps of disease incidence demonstrated numerous aggregates which were mostly coalesced into larger clumps of complex shape (Fig. 2B). Isopath contour lines were closest together on the southern and eastern borders of the

TABLE 1
ORDINARY RUNS ANALYSIS OF CITRUS GREENING IN SHANTOU, POUTAI GROVE (PEOPLE'S REPUBLIC OF CHINA

Plot	Row direction	Proportion of aggregated rows[z]
West	EW	25/98 = 0.25
	NS	104/124 = 0.84
East	EW	66/107 = 0.61
	NS	51/75 = 0.68
Total	EW	91/205 = 0.44
	NS	155/199 = 0.78

[z]Diseased trees were considered to be aggregated within rows if the observed number of runs was significantly different from the expected at $P = 0.05$ on a one-sided test for $Z < -1.64$.

TABLE 2
INDICES OF DISPERSION OF CITRUS GREENING IN THE SHANTOU, POUTAI GROVE, PEOPLE'S REPUBLIC OF CHINA

Quadrat size[z]	Variance-to-mean-ratio[y]	Lloyd's-Index-of-patchiness[y]	Lloyd's-index-of-mean-crowding[x]	Morisita's index[y]
2 x 2	0.456	0.808	2.287	0.808
3 x 3	2.999	2.624	3.230	2.625
4 x 4	4.347	2.427	5.692	2.426
5 x 5	6.232	2.417	8.923	2.533
6 x 6	8.442	2.429	12.647	2.426
8 x 8	12.609	2.244	20.939	2.237
10 x 10	18.662	2.248	31.812	2.238
16 x 16	32.180	1.839	68.360	1.839
32 x 32	51.629	1.340	199.351	1.341

[z]Quadrats are groups of trees oriented q X w where q = the number of trees in rows north-south and w = the number of trees in rows east-west.
[y]Variance-to-mean ratio, Lloyd's-Index-of-Patchiness, and Morisita's-Index Values <1, = 1, and <1 indicate regular, random, and aggregated distributions of trees with disease symptoms.
[x]Lloyd's-Index-of-Mean-Crowding values indicate the number of times more crowded the test population is than it would be if the population were randomly distributed.

plot, indicating that a steep disease gradient and large clusters of disease occurred in these areas. The greatest disease incidence was at the southern and eastern grove periphery and disease tapered toward the center of the plot from the south edge to the north and from the east edge to the west (Fig. 1A).

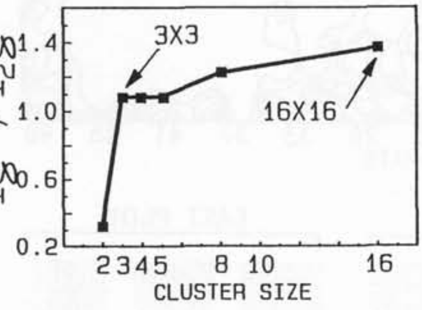

Fig. 1. Analysis of clump or aggregate size of citrus greening diseased trees in the Poutai grove in Shantou, PRC. The abscissa is the clump in quadrat size and the ordinate is the ratio of Morisita's Index value for each quadrat size and the ordinate is the ratio of Morisita's Index value for each quadrat size to the index for the quadrat size twice that large. The plateau at the 3 X 3 quadrat size indicates the smallest significant clump size. The continued rise in the ratio indicates that larger clump sizes predominate and that quadrat size is approaching the ultimate clump size.

Spatial lag autocorrelation analysis was used to examine the size and shape of clusters by dividing the grove into seven subplots and examining the predicted cluster shape in each (Table 3) (Fig. 2C). Clusters ranged from ca. 20 to 200 plants. Edge effects influenced the analysis in the easternmost subplot. Correlations of quadrats in the north-south direction indicated the existence of secondary clusters in four of the seven analyses. These were located ca. 13-14 quadrats, or ca. 25 to 30 m apart.

DISCUSSION

The Poutai grove is bordered on the western and northern edges by canals. The town of Poutai borders the grove on the southern and eastern edges. There are numerous ornamental backyard citrus trees with occasional plantings of *Murraya paniculata* which presumably could act as a reservoir for *D. citri*. The 16 CGD-diseased plants discovered during the 1986 assessment were located primarily on the southern edge of the grove immediately adjacent to a small planting of 24 trees with severe CGD symptoms, near a farmhouse ca. 10 m from the southern edge of the grove. This was an obvious source of CGD

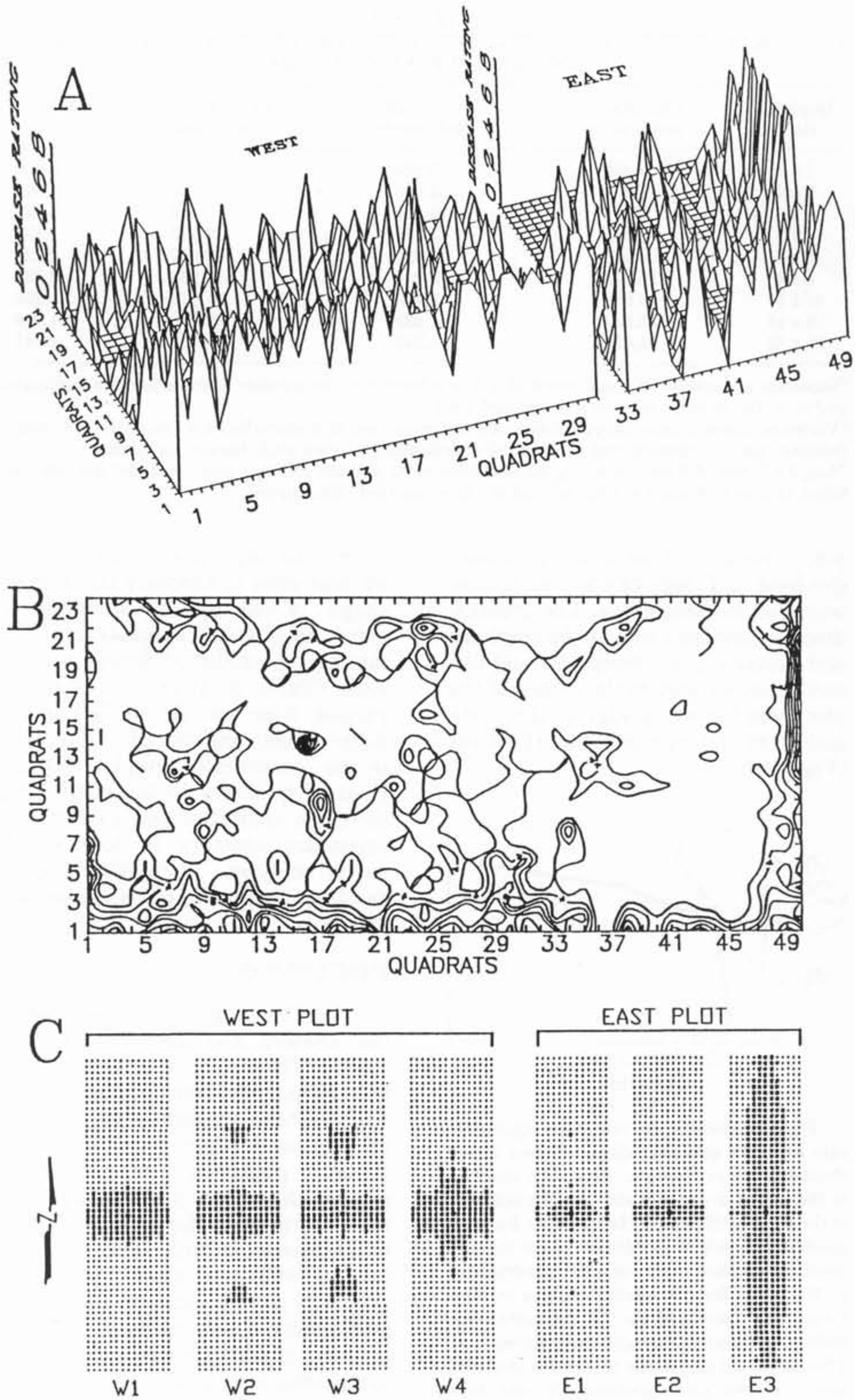
A
WEST
EAST
DISEASE RATING
0 2 4 6 8
QUADRATS
1 3 5 7 9 11 13 15 17 19 21 23
1 5 9 13 17 21 25 29 33 37 41 45 49
B
QUADRATS
1 3 5 7 9 11 13 15 17 19 21 23
1 5 9 13 17 21 25 29 33 37 41 45 49
QUADRATS
C
WEST PLOT
EAST PLOT
N
W1
W2
W3
W4
E1
E2
E3

TABLE 3
EXAMPLE OF SPATIAL LAG AUTOCORRELATIONS OF INCIDENCE OF CITRUS GREENING DISEASE IN SUBPLOT W3 IN A MANDARIN ORANGE GROVE IN SHANTOU, PEOPLE'S REPUBLIC OF CHINA

Lag quadrats N-S	Lag quadrats E-W							
	0	1	2	3	4	5	6	7
0	1.00*[z]	.687*	.698*	.649*	.727*	.724*	.656*	.632*
1	.650*	.567*	.598*	.589*	.610*	.564*	.591*	.562*
2	.456*	.393*	.475*	.470*	.423*	.391*	.447*	.496*
3	.222*	.197*	.193*	.226*	.256*	.151	.239*	.206
4	.118*	.590*	.064	.087	.129	.067	0.57	.082
5	0.73	.097	0.43	.094	.118	.027	.102	.057
6	–.043	–.033	–.047	.001	–.039	–.046	–0.14	–.022
7	–.024	.001	–.023	.035	–.004	–.003	.099	.050
8	–.030	–.041	–.090	–.013	–.084	–.014	.021	.003
9	–.071	.070	.008	.072	.030	.102	.069	.031
10	.041	.041	.020	.078	0.37	.095	.021	.054
11	.056	.060	.138	.116	.031	.085	.083	.134
12	.202*	.168*	–.142	.163	.122	.165	.165	.217
13	.207*	.161*	.149	.137	.209	.135	.144	.168
14	.220*	.221*	.222*	.184	.215	.218	.289	.329
15	.079	–.009	.045	.029	.025	.003	.039	.419
16	–.052	–.117	–.034	–.047	–.026	–.124	–.037	–.060
17	–.033	–.029	–.062	–.045	–.011	–.111	–.072	–.125

[z]Values represent average correlation coefficients of tests for all quadrats located at the indicated lag positions from each other. Negative values indicate negative correlations. *Autocorrelations significantly different from zero at $P = 0.05$.

and infective psyllid vectors. Numerous other groves are also in the vicinity. An influx of psyllids on the eastern edge of the Poutai grove likely originated from these nearby groves to the east and south. This could account for much of the disease intensity determined on the southern and eastern edges of the Poutai plot during the 1988 assessment.

In previous work, the authors estimated that greening epidemics approached disease asymptotes in 7 to 14 yr in orange groves in the PRC and in Reunion (8). These observations were made in groves established with disease-free planting material, but not receiving sufficient insecticide protection. Disease progress in the Poutai grove during the 5 yr since planting was similar to that which occurred in other mandarin plantings in southwest PRC. The Poutai plot reached a CGD incidence level of 14% during the first 5 yr from planting. If the present rate of disease increase continues, an asymptote of disease would be expected to occur in the Poutai grove within the next 2 to 4 yr. Thus, the range of epidemic dura-

Fig. 2. All analyses were conducted on the data set reduced to 2 X 2 quadrats for the Poutai grove near Shantou, PRC: A) 3-D response surface of the grove showing higher disease ratings on southern and eastern grove boundaries. B) Isopath contour map of disease incidence. Note the general predominance of irregularly shaped clumps of diseased trees that probably resulted from coalescence of smaller clusters. The closeness of isopath lanes on eastern and southern grove borders are indicative of steep disease gradients. C) Graphic presentation of correlogram obtained by spatial-lag-autocorrelation-analysis. Grove was divided into seven north-south subplots to examine cluster size. Clusters of 20-200 trees predominated. Note noncontagious clusters of diseased plants ca. 13-14 lags (quadrats) distant from main clusters, indicating an association between clusters located north-south from each other. See Table 3 for an example of a correlogram.

tion found in preliminary work seems applicable here.

Aggregation was indicated by all tests of indices of dispersion of quadrat sizes of greater than 2 X 2 trees. The use of Morisita's-Index-of-Clumping was especially useful, indicating clumping at the 3 X 3 and 4 X 4 quadrat size but not for the 2 X 2 quadrat size. Thus, aggregates of 9 (3 X 3 quadrat) and 16 trees (4 X 4 quadrats) were found to be significant whereas aggregates of 4 trees (2 X 2 quadrats) were not. In addition, the significance of larger quadrats was also seen. However, decreasing Morisita's-Index-of-Dispersion values for larger quadrat sizes of 16 X 16, and 32 X 32, (256 and 1024 tree groups) were indicative of quadrat sizes which are approaching the size of the largest significant clumps of CGD-diseased trees. Apparently early in the epidemic, small clumps of 9-16 diseased trees occurred. As the epidemic progressed these small clusters became numerous and being to coalesce. At the time of the second disease assessment, clump sizes of ca. 256-1024 diseased trees were common.

The north-south directionality was indicated by ordinary runs analysis and corresponded to the permanent traffice in the grove. Because of the mounds on which the grove was planted and the irrigation ditches between rows running north-south, traffic was almost entirely restricted to this direction. Psyllids, when disturbed, often move to the next tree or even several trees away in the opposite direction from the disturbance. Infective vectors entering the grove from the south would have been disturbed and moved along the rows primarily to the north. This would account for the predominance in clustering within north-south oriented rows. The secondary clumps or clusters of diseased trees determined by the spatial autocorrelation analysis were also oriented in this direction from one another. Vector movement appears to be generally from one tree to the next; however, there is some indication that longer distances are traversed at times and new foci of disease initiated. This could possibly be stimulated by population crowding, or major disturbances due to horticultural practices, combined with insufficient insecticide control. This distance was estimated to be 25-30m.

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