GREENING, STUBBORN, AND OTHER DISEASES

Analysis of the Distribution of Citrus Greening in Groves in the Philippines

T. R. Gottwald, C. I. Gonzales, and B. G. Mercado

ABSTRACT. Disease assessments were made in two citrus groves on Luzon and Mindinao Islands of the Philippines. Both groves were composed of Szinkom mandarin interplanted with Calamansi and occasional pummelo trees. The Luzon grove (VP) consisted of an older, heavily diseased block of trees and three younger blocks immediately adjacent to the east and northeast. All blocks were established with nursery stock, some of which was infected with greening. The Mindinao grove (BJ), consisting of 920 trees, was established from nursery stock a portion of which was probably infected. Based on analysis of isopath maps of each block in the VP grove, aggregation of diseased trees existed in the three newer blocks but was not often associated with rows. Aggregates of diseased trees appeared to be coalescing over time and small individual clusters were generally indeterminate in shape. The oldest block of trees was > 90% diseased and no longer showed aggregation due to the nearly uniform distribution of trees with severe greening symptoms. Isopath maps of the BJ grove were inconclusive. Ordinary runs analysis confirmed the general lack of within-row aggregation in both groves. Morisita's-index-of-dispersion was interpreted to indicate that aggregates of 2 X 2, 3 X 3, 4 X 4, and 6 X 6 trees existed and that aggregates of diseased trees larger than 6 X 6 also existed during the last disease assessments. This diffuse aggregation may have been the result of the random distribution of diseased planting materials followed by subsequent confluent and nondirectional spread of greening by psyllid vectors.

Index words. Spatial patterns of disease, indices-of-dispersion, *Diaphorina citri*, psyllid vectors.

In many places throughout Southeast Asia, citrus groves are established from plants grown in own-use nurseries or from small local nurseries. These nurseries do not produce citrus exclusively, but also provide numerous species of fruit trees for local fruit growers. Budwood registration programs are infrequent, and in most cases, budwood from indexed and registered disease-free mother trees is rare or not available (1,11). On the local level, there is often minimal awareness or knowledge of greening and citrus virus diseases. Thus, many citrus plantings in Southeast Asia are established from nursery stock infected with greening, tristeza, and other pathogens. The extent of infection in such nursery stock is unknown, however, groves established from such stock do not appear to be uniformly infected. Thus, the introduction of disease contagions, especially that of citrus greening disease (CGD), from nurseries into groves significantly shortens the productive life of these plantings and contributes to their ultimate demise. Following random introduction, the CGD pathogen is spread by the Asian psyllid vector *Diaphorina citri*. Clusters of diseased plants frequently occur in the grove (2,3,4). The percentage of diseased nursery plants outplanted combined with high populations of psyllids and their patterns of migration determines the number of such clusters, their rate of coalescence, and contributes to the rate of disease progress.

The effect of populations of psyllid vectors on greening has been studied previously (4,5). Aggregations of greening, as a result of natural spread of the pathogen by psyllid vectors, has also been demonstrated in China and in the Philippines (6,7,8).

The purpose of this study was to: 1) determine the presence and significance of aggregations of diseased trees in two groves in the Philippines established from infected nursery stock; and 2) estimate the change in the spatial size of such aggregates over time. The study is in contrast to a similar study in China in which the dynamics of aggregation in groves established from disease-free nursery materials are being investigated (8).

MATERIALS AND METHODS

Disease assessments were made in two groves in the Philippines. Both groves consisted of mixed plantings of Szinkom mandarin interplanted with Calamansi and occasional pummelo trees, and both were established from contaminated nursery materials obtained locally. The Venacio Pecho grove (VP) in Tanauan, Batangas Province, Luzon, consisted of a 9- to 10-yr-old heavily diseased block of trees to the west and three younger blocks of 1- to 3-yr-old trees to the east and northeast. All groves were planted at a relatively high density of 2-4 m between trees (7). The Baio Joaquin grove, (BJ) near Devao, Mindinao, consisted of a single irregularly elongate planting of trees with varied but greater planting distance of ca. 7-10 meters between trees. Control of vectors was minimal in both groves. All trees were assessed on a 0 to 40 rating scale on each assessment date as previously described (9). The VP grove was assessed four times, twice in 1988 and 1989, whereas the BJ grove was assessed only once in 1989. Data from the VP grove were divided into quadrats of 2 X 2, 3 X 3, 4 X 4, and 6 X 6 trees. Data from the BJ

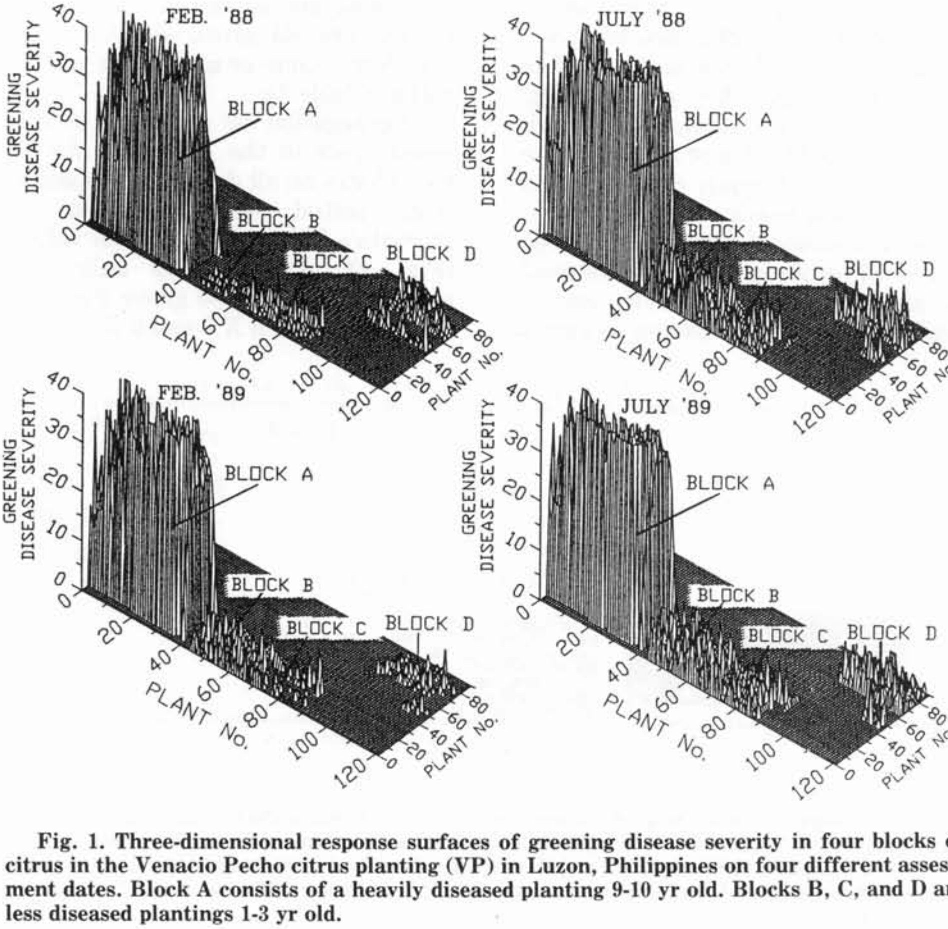

Fig. 1. Three-dimensional response surfaces of greening disease severity in four blocks of citrus in the Venacio Pecho citrus planting (VP) in Luzon, Philippines on four different assessment dates. Block A consists of a heavily diseased planting 9-10 yr old. Blocks B, C, and D are less diseased plantings 1-3 yr old.

grove were divided into quadrats of 3 X 3 and 6 X 6, only, due to the limitations of grove configuration. Larger quadrat sizes were not possible due to the limited number of trees in the plots. Individual quadrat scores were the sums of the individual scores of the trees within the quadrat. The data were examined by 1) ordinary runs analysis (10) to determine the presence or absence of directionality of aggregation, 2) isopathic contour mapping (9) and three-dimensional response-surface plots were utilized to examine the extent and position of aggregates of diseased plants (7,9), and 3) various indices of dispersion to examine the relative significance and size of aggregates (9,12,13).

RESULTS

Three-dimensional response surfaces of the VP grove were used to demonstrate the difference in severity between the A plot and the other three plots in the grove (Fig. 1A,B,C,D). The convoluted network of isopaths in the A plot as seen in the corresponding isopath contour maps is typical of a heavily diseased grove. Three-dimensional response surfaces of the VP grove over time were used to show minor fluctuations and a gradual increase in disease severity from the four disease assessments over the 17 months of the study. Small aggregates of diseased plants which were beginning to coalesce within rows were seen in isopath maps of the B and C plots. A more general coalescence of aggregates irrespective of row effects was seen in isopath maps of the D plot (Fig. 2). Similarly, a more diffuse aggregation was seen in isopath maps of the BJ grove with only a few coalescing clusters, mostly in the southern half of the grove where greater disease severity was seen by 3-D response surface graphs (Fig. 3).

In the VP grove no aggregation in the A block was indicated by ordinary runs analysis because of the uniform infection of all plants there forming one large cluster. Only little aggregation within rows both north-south and east-west was indicated by ordinary runs analysis in the B, C and D blocks. The BJ grove also indicated very low within- or across-row aggregation (Table 1).

Aggregation or clumping of diseased trees in the VP grove for all four blocks on all dates at all quadrat sizes tested was confirmed by Morisita's-Index-of-Dispersion values (Fig. 4). Analysis via indices-of-aggregation of the BJ grove was only possible for the 3 X 3 and 6 X 6 quad-

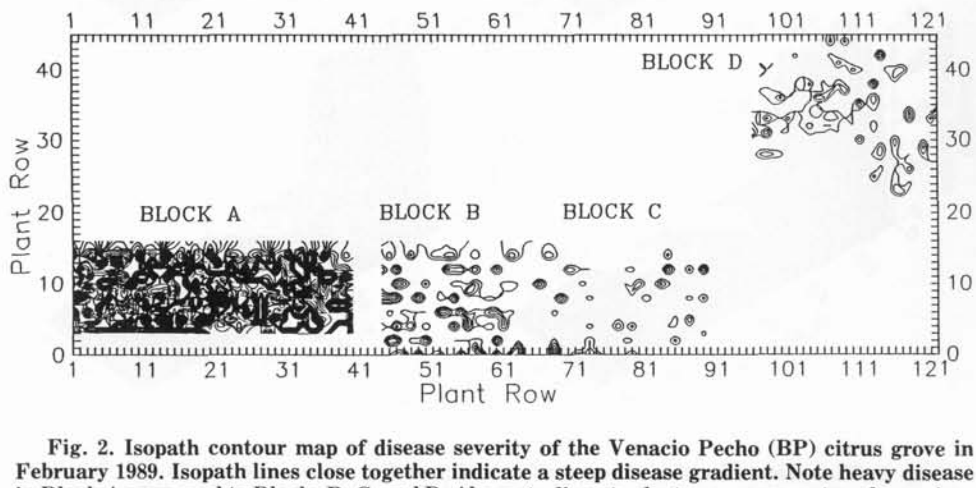

Fig. 2. Isopath contour map of disease severity of the Venacio Pecho (BP) citrus grove in February 1989. Isopath lines close together indicate a steep disease gradient. Note heavy disease in Block A compared to Blocks B, C, and D. Also note discrete clusters or aggregates of greening-diseased trees in Block C and to some extent in Block B and more diffuse or coalescing clusters in Block D.

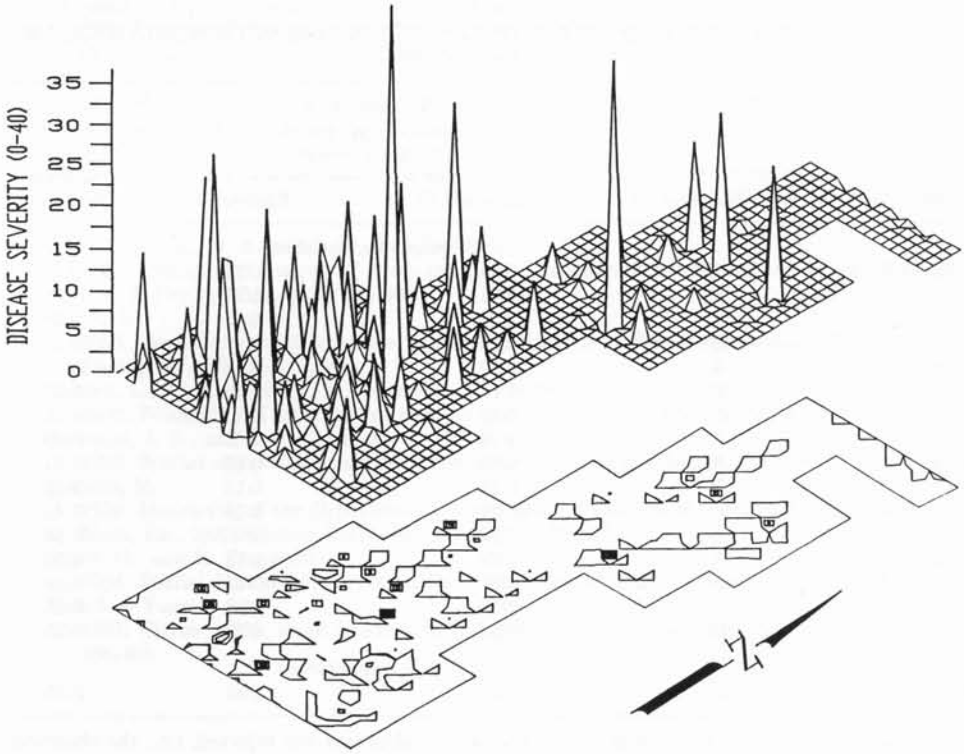

Fig. 3. Three-dimensional response surface and isopath map of the Biao Joaquin (BJ) citrus grove in April 1989. Note higher disease severity in southern end of planting. Isopath contour lines are inconclusive for aggregation.

rat sizes due to the plot size and configuration. Significant aggregation was determined by variance-to-mean ratio as well as Lloyd's-Index-of-Patchiness and Morisita's-Index-of-Dispersion for both quadrat sizes tested (table 2). The larger values associated with each of these tests for the 6 X 6 quadrat size compared to the 3 X 3 quadrat size may indicate that aggregates greater than 6 X 6 trees also exist at this point in the epidemic.

DISCUSSION

The aggregation of citrus greening disease found in the two groves examined in the Philippines was similar to that found in China (6,8,9). Apparently only a low percentage (ca. 10-20%) of trees originating from the local nurseries were infected at the time of planting. Spread of citrus greening bacteria by the psyllid vector during the next 2-3 yr gave rise to the clumps of diseased plants which were detected during subsequent assessments. Thus, the amount of aggregation found in the Philippine groves that resulted from spread from nursery introductions was similar to that detected in Shantou, China, from natural spread by psyllid vectors from exogenous inoculum sources (8). However, unlike the Shantou PRC plot, only a relatively small amount of within-row aggregation was detected. This was likely due to a more random distribution of diseased trees when outplanted in the Philippine plots. Spread from these foci of introduction and coalescence of the aggregates of diseased trees was nearly equivalent in the north-south and east-west directions. Thus, the movement of psyllid vectors was likely to be more random in this case with no apparent directionality. Values of Morisita's-Index-of-Dispersion decreased with larger quadrat sizes.

TABLE 1
ORDINARY RUNS ANALYSIS OF CITRUS GREENING DISEASE IN TWO CITRUS GROVES IN THE PHILIPPINES

Date	Block	No. rows with disease aggregated/ total no. rows[z] North-south	East-west	Disease incidence[y]
		Venacio Pecho Grove		
3/88	A	0/39	0/12	0.95
	B	0/21	1/12	0.10
	C	2/29	3/12	0.38
	D	0/24	3/27	0.46
10/88	A	0/39	0/12	0.91
	B	1/24	0/12	0.42
	C	0/24	0/12	0.34
	D	4/24	4/27	0.39
2/89	A	0/39	0/12	0.91
	B	0/21	0/15	0.51
	C	0/24	1/15	0.43
	D	3/24	10/27	0.48
7/89	A	1/39	1/12	0.92
	B	0/21	0/12	0.44
	C	0/27	0/12	0.25
	D	2/24	4/27	0.25
		Biao Joaguin Grove		
4/89	—	1/52	1/50	0.35

[z]Rows were considered to be aggregated for disease if randomness was rejected, i.e., the observed number of runs was significantly different from the expected at $P = 0.05$ on a one-sided test for $Z < -1.64$.
[y]Disease incidence = the porportion of diseased trees.

Such a decrease is interpreted to indicate that quadrat size is approaching actual diseased tree cluster size. Thus, the 6 X 6 quadrat size, or 36-tree clump, may be approximately the size of the average coalesced aggregate of diseased trees at this point in the epidemic. Apparently the initial aggregates resulting from introduction of diseased nursery trees were small during the initial stages of the epidemic, but these coalesced and formed larger clumps of diseased trees rapidly as the epidemic progressed. The shape of these coalesced clumps was highly amorphous.

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TABLE 2
INDICES OF DISPERSION AND ANALYSIS OF AGGREGATION OF CITRUS GREENING DISEASE IN THE BIAO JOAQUIN GROVE.

Quadrat size[z]	Variance to mean ratio[y]	Lloyd's-index-of-patchiness[y]	Lloyd's index-of-mean-crowding[x]	Morisita's-index-of-dispersion[y]
3 X 3	1.480	1.627	1.246	1.630
6 X 6	3.679	2.076	5.156	2.056

[z]Quadrats are expressed as 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 infected trees.
[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.

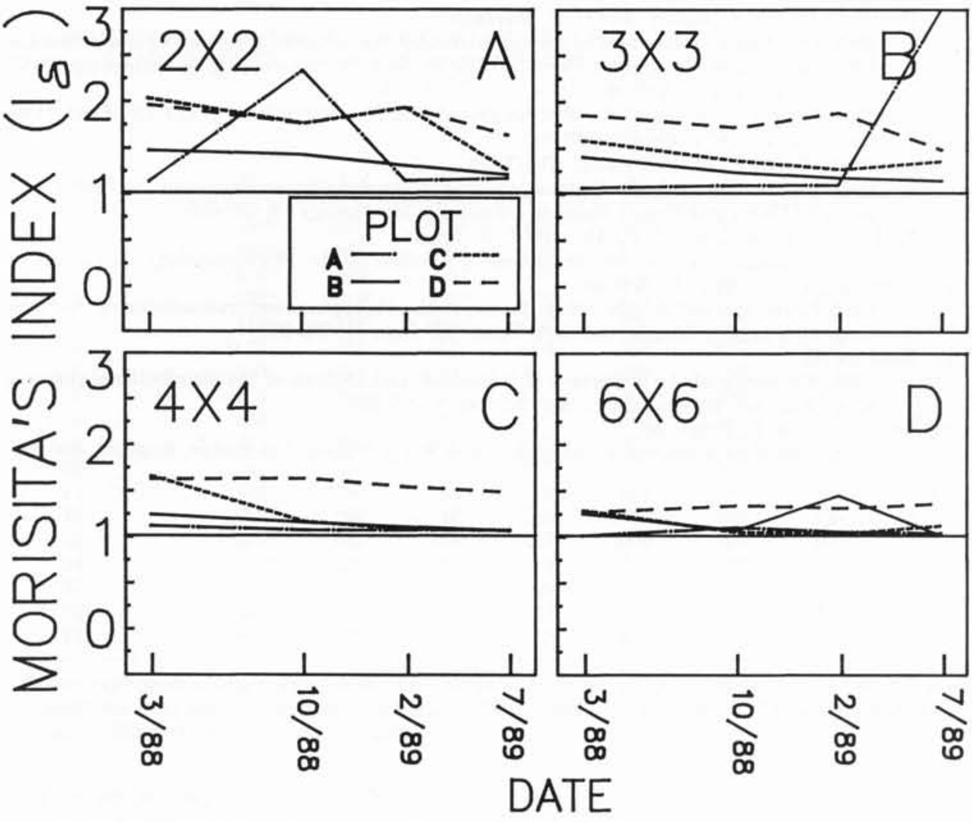

Fig. 4. Analysis of aggregation by Morisita's-Index-of-Dispersion of four blocks of the Venacio Pecho (VP) citrus grove, on four assessment dates and for four different quadrat sizes. Index values <1, = 1, and >1 indicate regular, random, and aggregated populations of disease plants, respectively. The more the index increases above 1, the more aggregation is indicated. Thus, note less aggregation for the largest quadrat size tested (6 X 6), indicating diseased clusters are similar to this size.

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