SPATIAL DISTRIBUTION OF THE ASIAN CITRUS PSYLLID, *DIAPHORINA CITRI* KUWAYAMA (HOMOPTERA: PSYLLIDAE) ON CITRUS AND ORANGE JASMINE

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Abstrak: Taburan ruang kutu *psyllid Diaphorina citri* (Homoptera: Psyllidae) pada pokok limau dan bunga melur yang telah dianalisis menggunakan pendekatan indeks matematik model taburan dan regresi menunjukkan taburan berkelompok. Hukum kuasa Taylor dan model regresi Iwao sangat sesuai dengan kesemua set data, manakala model regresi $k = c + dm$ [$k = m^2/(s^2 - m)$] didapati tidak memadai bagi perkelompokan *psyllid* limau kerana k tidak stabil dan bergantung kepada kepadatan kutu *psyllid*. Pada amnya, hukum kuasa Taylor lebih sesuai dengan data, memberi nilai R^2 yang lebih tinggi berbanding model Iwao bagi kesemua kes, sama ada bagi limau sahaja, bunga melur sahaja atau gabungan kedua-duanya. Populasi *D. citri* pada kuadran pokok, iaitu utara, barat, selatan dan timur, didapati tidak berbeza secara signifikan. Sungguhpun begitu, taburan di antara sudur setengah atas dan bawah didapati berbeza secara signifikan di mana sudur setengah atas dinaungi lebih banyak *psyllid* daripada setengah bawah.

Kata kunci: Taburan Ruang, Tumbuhan Perumahan, *Diaphorina citri,* Taburan

Abstract: The analysis of spatial distribution of the psyllid *Diaphorina citri* (Homoptera: Psyllidae) on citrus and orange jasmine analyzed using various mathematical indices of dispersion and regression models showed an aggregated distribution. Taylor's power law and Iwao's regression model fitted very well to all data sets, while the regression of $k = c + dm$ [$k = m^2/(s^2 - m)$] was an inadequate model for the aggregation of the citrus psyllid since k was unstable and dependent upon the density of the insect. In general, Taylor's power law fitted the data better, yielding higher values of R^2 than the Iwao's model for all cases, whether citrus only, orange jasmine only or pooled. The population of *D. citri* on the tree quadrants, i.e. north, west, south and east, did not differ significantly. However, distribution between the upper and lower half of the canopy was significantly different where the upper canopy harbored more psyllids than the lower half.

Keywords: Spatial Distribution, Host Plants, *Diaphorina citri*, Dispersion

INTRODUCTION

One of the main benefits accrued from studies in population ecology is the understanding on the distribution and abundance of organisms (Andrewartha &

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Birch 1954). An important aspect of distribution and abundance is the dispersion pattern. Knowledge of the dispersion pattern in an insect is very important because its population biology is a result of the interaction between individuals of the species and their habitat (Sevacherian & Stern 1972). It allows a better understanding of the relationship between an insect and its environment, and provides basic information for interpreting spatial dynamics, designing efficient sampling programs for population estimation and pest management (Sevacherian & Stern 1972; Taylor 1984; Binns 1986; Kuno 1991), and the development of population models (Croft & Hoyt 1983).

Most insect populations are spatially aggregated (Southwood 1978; Taylor *et al*. 1978, 1980; Turchin & Kareiva 1989; Faeth 1990; Morris *et al*. 1992), but the degree of aggregation often varies among populations and among species (Root & Cappuccino 1992). There are varieties of approaches to characterize the spatial distribution of insects (Kuno 1991; Binns & Nyrop 1992), which generally based on the sample mean ($\bar{x}$) and variance (s^2) (Feng & Nowierski 1992).

Very little is known about the dispersion of the Asian citrus psyllid, *Diaphorina citri* Kuwayama, the most efficient vector of citrus greening disease, on citrus plants, *Citrus reticulata* var. Madu and orange jasmine, *Murraya paniculata* L., causing reduced production and death of trees. As such, the objective of the study was to determine the spatial distribution pattern of *D. citri* on citrus and orange jasmine ecosystem. As recommended by Mollet *et al*. (1984), several indices of dispersion, frequency distribution and regression models were used to analyze spatial pattern before conclusions about dispersion were drawn.

MATERIALS AND METHODS

Population Sampling
Fifteen trees of similar size, each of citrus and orange jasmine in Serdang, Malaysia, were randomly selected as sample trees. The canopy of each tree was partitioned into two strata; upper and lower halves, with each stratum split into four compass quadrants or directions, namely north, west, south and east. Hence, there were a total of eight sectors in one tree for observations. From each sector, ten shoots were randomly chosen, making a total of 80 shoots per tree for estimation of the population density. Counting and recording the presence of insects were conducted *in situ* as the number of insects per shoot was tabulated for each tree.

The population densities on these shoots were used to calculate dispersion indices. Since the sample data consisted of the number of individuals per shoot, termed as a sampling unit (SU), and the total number of shoots was 80 per tree, the data was arranged on the original counts in the form of frequency distribution. This conformed with the recommendation by Ludwig and Reynolds (1988) that, when the number of SUs is more than 30, the data should be arranged as a frequency distribution, i.e. the number of SUs begins with 0, 1, 2

individuals and so on. Initially, the data was analyzed to determine the trends of population distribution based on the variance-to-mean ratios ($s^2/\bar{x}$).

Computation

The $s^2/\bar{x}$, which is the simplest index of dispersion and the most fundamental (Taylor 1984), indicates a random dispersion when it is equaled to 1.0; a uniform dispersion when it is less than 1.0; and an aggregated dispersion when it is more than 1.0 (Myers 1978). A chi-square (χ^2) test was performed for the $s^2/\bar{x}$ to determine its significant departure from 1.0 by the following expressions:

$$\chi^2 = (s^2/\bar{x})(N-1)$$

and
$$d = \sqrt{2x^2} - \sqrt{2(N-1)-1}$$

where d is statistical test and N is the total number of SUs. If $|d| < 1.96$, agreement with a random dispersion is accepted; if $|d| < -1.96$, a regular dispersion is suspected; and if $|d| > 1.96$, a clumped dispersion is likely (Elliott 1973).

Lloyd's index patchiness, an index that does not depend on either sample size or mean density (Hurlburt 1990), was calculated as ratio of mean crowding (m^*) to mean density (m). The m^* was computed with the formula as described by Southwood (1978):

$$m^* = \bar{x} + [(s^2/\bar{x}) - 1]$$

where $\bar{x}$ is the mean density and s^2 is the variance. When Lloyd's index = 1, it indicates a random dispersion; when it is > 1, it indicates an aggregated dispersion; and when it is < 1, it indicates regular dispersion. The dispersion parameter k for the negative binomial was estimated as $m^2/(s^2 - m)$ and then fitted to a negative binomial distribution. The fitness of the negative binomial model to the data of frequency distribution was measured by a χ^2 goodness-of-fit test statistic. The value of k was also used to test whether a common k (k_c) for a negative binomial exists or not by linearly regressing on m (Southwood 1978; Feng & Nowierski 1992), such that $k = c + dm$. A value of d significantly > 0 indicates the dependence of k on m. In addition, on the basis of variance and mean values, the other dispersion indices were determined and the statistical tests were applied to confirm the distribution pattern of the insects.

The degree of aggregation was measured by three commonly used dispersion indices, namely the Green coefficient (C_x) (Green 1966), Taylor's power law (Taylor *et al*. 1978), and Iwao's patchiness regression (Iwao 1968 *in* Southwood 1978). These indices were chosen in an attempt to get a consensus on dispersion because use of a single index can be misleading (Myers 1978). Mollet *et al*. (1984) and Davis (1994) recommended that in evaluating dispersion of an arthropod, one should use several different techniques before drawing conclusions about dispersion.

The C_x was determined using the following formula (Green 1966):

$$C_x = \frac{\left(s^2/m\right) - 1}{\sum x - 1}$$

where s^2 = variance of mean, m = mean number of *D. citri* per shoot and $\sum x$ = total number of *D. citri*. When C_x = 0, the coefficient indicates a random dispersion; and when C_x = >0 – 1, it indicates an aggregated dispersion.

The Taylor's power law states that the variance (s^2) of a population is proportional to a fractional power of the arithmetic mean: $s^2 = am^b$. The coefficient *a* and *b* were estimated from the regression model:

$$\log s^2 = \log a + b \log m$$

where the slope *b* is an index of aggregation. When $b < 1$, it indicates a regular dispersion; when $b = 1$, it indicates a random dispersion; and when $b > 1$, it indicates an aggregated dispersion.

The Iwao's patchiness regression is the regression of m^* on m in the linear model $m^* = \alpha + \beta m$, where the value of m^* is derived from $[m + (s^2/m - 1)]$. The intercept α is the index of basic contagion and β is the density contagiousness coefficient, a measure of aggregation as *b* of Taylor's regression above. A student *t*-test was used to determine significance of departure from randomness for both regression methods. In addition, slope coefficients of the respective regression models were subjected to test for homogeneity using methods as described by Gomez and Gomez (1984). The fit of a common to different data sets was tested using *F*.

To examine if the mean population differed significantly among sectors in the canopies, data for the differences in insect number from all the eight sectors was subjected to analysis of variance (SAS 2001). Data analyzed was the average number of insects per shoot. The experimental design used was split-plot, with the tree as a block or replication (n = 15), the stratum as the main plot (n = 2), and the quadrant as the sub-plot (n = 4).

RESULTS AND DISCUSSION

During the study, it was evident that the adult psyllids were observable while immature stages were quite rare on most of the sampled trees, both citrus and orange jasmine, despite the presence of abundant new flushes on these trees. Therefore, the spatial distribution pattern observed herein was that of the adult citrus psyllid on citrus and orange jasmine trees.

Distribution Pattern
The spatial distribution patterns of the citrus psyllid both on citrus and orange jasmine were found in accordance with various indices of dispersion, to be highly aggregated (Tables 1 and 2). In all cases the $s^2/\bar{x}$ were > 1 (ranging from 2.40 to 4.92); the index of m^* and Lloyd's index of patchiness were greater than unity;

Table 1: Statistical distribution and indices of dispersion of *D. citri* on citrus.

Tree no.	Mean $\bar{x}$	Variance s^2	$s^2/\bar{x}$ ratio	Lloyd's mean crowding m^*	Index patch.	Exponent k	Goodness-of-fit test χ^2	Critical Value χ^2	Index disp.	Green coeff. (C_x)
1	0.39	0.98	2.51	1.90	4.87	0.2558	0.124	3.841	7.38	0.050
2	0.43	1.03	2.40	1.83	4.26	0.2974	0.760	3.841	6.94	0.042
3	0.60	1.48	2.47	2.07	3.45	0.4074	2.816	5.991	7.23	0.031
4	0.81	2.41	2.98	2.79	3.44	0.4139	0.881	5.991	9.17	0.031
5	0.35	0.76	2.17	1.52	4.34	0.2973	1.252	3.841	5.99	0.043
6	0.58	1.39	2.40	1.98	3.41	0.4073	0.103	5.991	6.94	0.031
7	0.28	0.61	2.18	1.46	5.21	0.2278	0.031	3.841	6.03	0.056
8	0.38	0.92	2.42	1.80	4.74	0.2576	0.212	3.841	7.02	0.049
9	0.69	1.86	2.70	2.39	3.46	0.4020	1.020	5.991	8.12	0.032
10	0.41	1.11	2.71	2.12	5.17	0.2453	0.180	3.841	8.16	0.053
11	0.55	1.80	3.27	2.82	5.13	0.2430	0.365	3.841	10.20	0.053
12	0.85	2.59	3.05	2.90	3.41	0.4165	1.362	5.991	9.42	0.031
13	0.64	1.93	3.02	2.66	4.16	0.3144	0.838	5.991	9.31	0.040
14	0.71	2.26	3.18	2.89	4.07	0.3285	0.505	3.841	9.89	0.039
15	0.46	1.06	2.30	1.76	3.83	0.3569	0.086	5.991	6.53	0.036

Iwao's patchiness regression: $x^* = 0.85 + 2.49^{**} x$ ($R^2 = 0.76$); Taylor's power law: log $0.51 + 1.30^{**}$ log x ($R^2 = 0.96$)

Table 2: Statistical distribution and indices of dispersion of *D. citri* on orange jasmine.

Tree no.	Mean $\bar{x}$	Variance s^2	$s^2/\bar{x}$ ratio	Lloyd's mean crowding $m*$	Index patch.	Exponent k	Goodness-of-fit test χ^2	Critical Value χ^2	Index disp.	Green coeff. (C_x)
1	1.23	3.97	3.23	3.46	2.81	0.5459	4.213	7.815	10.06	0.023
2	1.39	4.70	3.38	3.77	2.71	0.5819	0.209	7.815	10.58	0.022
3	1.54	5.82	3.78	4.32	2.81	0.5518	1.137	9.488	11.91	0.025
4	1.55	7.62	4.92	5.47	3.53	0.3959	2.493	9.488	15.35	0.032
5	1.06	3.86	3.64	3.70	3.49	0.4040	4.341	7.815	11.45	0.029
6	1.11	4.13	3.72	3.83	3.45	0.4106	1.318	7.815	11.71	0.029
7	1.49	5.70	3.83	4.32	2.90	0.5258	3.139	9.488	12.07	0.028
8	1.41	5.64	4.00	4.41	3.13	0.4722	1.800	7.815	12.61	0.028
9	0.88	2.54	2.89	2.77	3.15	0.4595	3.308	7.815	8.84	0.025
10	1.05	3.67	3.50	3.55	3.38	0.4211	3.703	7.815	10.99	0.030
11	1.63	7.28	4.47	5.10	3.13	0.4673	1.336	9.488	14.05	0.027
12	1.45	5.90	4.07	4.52	3.12	0.4729	0.998	7.815	12.83	0.027
13	1.21	4.47	3.69	3.90	3.22	0.4509	1.011	7.815	11.62	0.028
14	1.59	6.83	4.30	4.89	3.08	0.4809	2.469	7.815	13.54	0.026
15	1.46	5.99	4.10	4.56	3.12	0.4715	1.334	7.815	12.92	0.027

Iwao's patchiness regression: $x* = 0.55 + 2.71^{**} x$ $(R^2 = 0.80)$; Taylor's power law: $\log 0.51 + 1.56^{**} \log x$ $(R^2 = 0.93)$

values of test statistic, d exceeded 1.96 and the C_x values were more than zero, indicating that the spatial distribution of the citrus psyllids was aggregative in nature. The analysis of frequency distribution showed that the values of the calculated χ^2 goodness-of-fit tests in all cases (0.031–5.354) were less than those of the tabulated χ^2 (3.841–9.488) at the 5% level of probability, which again was in agreement with an aggregated distribution.

Taylor's power law and Iwao's patchiness regression appeared to describe the distribution of *D. citri* well and consistently regardless of host plant types. The slope values of Taylor's power law for the citrus psyllid on citrus and orange jasmine (b = 1.30 and 1.56, respectively) were significantly greater than 1 (t = 4.286 and 4.667; df = 14; P < 0.05), indicating clumped or aggregated distributions on these trees. Similarly, the slopes (β) of Iwao's model on citrus and orange jasmine (2.49 and 2.71, respectively) were also significantly greater than 1 (t = 3.921 and 4.526; df = 14; P < 0.05), suggesting that the distribution pattern of the citrus psyllids to be aggregated on both host plants. Hence, these regression models supported the conclusion that the population of *D. citri* was aggregated. By homogeneity tests on the regression slopes, it was determined that there was no significant difference between the slopes of Taylor's power law (t = 1.79; df = 26; P > 0.05) for the two host plants. In the same way, the slopes of Iwao's regression model did not significantly differ for the population on the two host plants (t = 0.41; df = 26; P > 0.05). Therefore, all data were pooled and an overall regression was calculated and graphically presented in Figures 1A and 1B for Taylor's power law and Iwao's patchiness regression, respectively.

In general, Taylor's power law fitted the data better than the Iwao's model. This is based on the higher values of R^2 for each of the two situations considered. In addition, Taylor's power law provided a slightly more even distribution of points along the line and showed no evidence of curvilinearity when compared to the plot of Iwao's regression (Fig. 1). Iwao's patchiness regression, nonetheless, allows the ecological implications of the parameter to be interpreted, since this equation was originally derived with close reference to theoretical distribution models (Kuno 1991). The positive value of α of Iwao's patchiness regression in all situations (citrus, orange jasmine, or pooled) implied a positive interaction (mutual attraction) between individuals of the citrus psyllid, so that even at a very low density, the individuals would tend to aggregate in the same habitat. This was observed to be true in nature, when biotic and abiotic factors are favorable for the psyllids, where they lived together in colonies, especially during the nymphal stages. In Fujian, China, a mean colony size reaching as high as 20 and 51 adults of *D. citri* per shoot on citrus and orange jasmine, respectively, had been reported by Xu *et al*. (1989 cited by Aubert 1990).

Unlike in Taylor's power law and Iwao's patchiness regression, the homogeneity test on the slopes (d) of the regression of k on m was not significantly different at the 5% level of significance, hence all data were not pooled. The regression of k on m generated the equations of k = 0.16 + 0.31 m (R^2 = 0.60) and k = 0.36 + 0.08 m (R^2 = 0.12) for *D. citri* population on citrus and orange jasmine, respectively. The slope for the population on citrus was significantly > 0 (t = 4.39; P < 0.01) while that on orange jasmine was

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$log\,(s^2) = log\,0.54 + 1.39\,log\,(m)\ \ (R^2 = 0.98)$

(a)
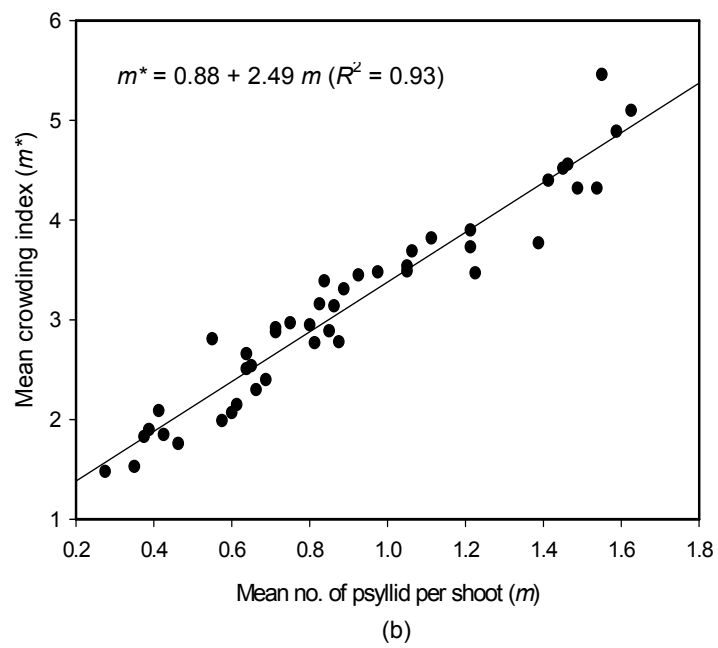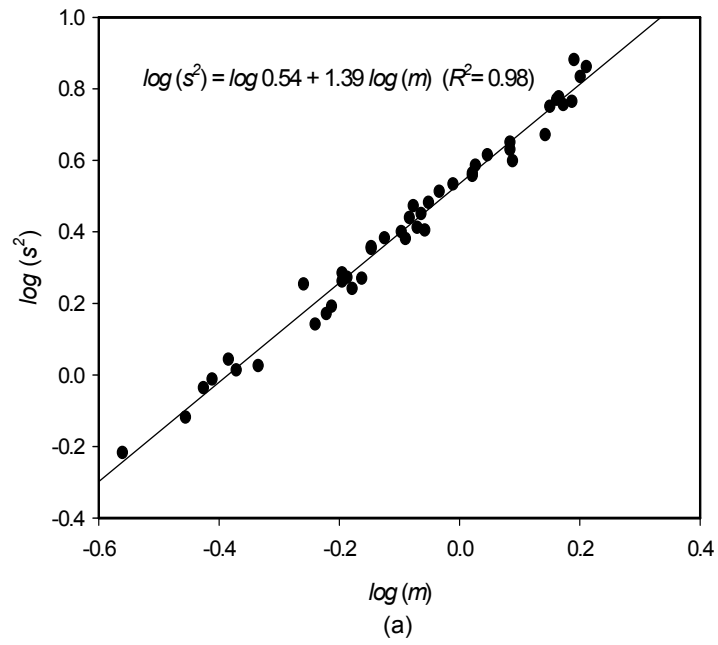
$m^* = 0.88 + 2.49\,m\ (R^2 = 0.93)$

(b)

Figure 1: Regression analysis of (a) Taylor's power law, and (b) Iwao's model for *D. citri* on total pooled samples.

indistinguishable from 0 ($t = 1.31$; $P > 0.05$). This reflected the density-dependent relationships of k to m, suggesting there is no common k for the population on citrus. In contrast, a common k seemed to exist for the population on orange jasmine and was estimated as 0.461 using the formula given by Southwood (1978). As k is found to be unstable and dependent upon density, in this study k was not an adequate model for the determination of aggregation of the citrus psyllid.

Within Plant Distribution

Distribution of *D. citri* population within host plants, based on the analysis of variance, was not affected by directions and interactions between direction and stratum (height). This situation was very similar for both citrus and orange jasmine canopy. No significant difference of the population density of *D. citri* was observed among cardinal directions (north, west, south and east), both on citrus and orange jasmine trees. However, the psyllid population was significantly higher at the upper halves than the lower halves of the canopies (Table 3).

Table 3: Population density of *D. citri* on citrus and orange jasmine with respect to quadrant and stratum.

| Quadrant | No. of *D. citri* per shoot | | | | | |
| | Citrus | | | Orange jasmine | | |
	Upper	Lower	Avg.[a]	Upper	Lower	Avg.[a]
North	0.65	0.41	0.53a	1.61	0.95	1.28a
West	0.73	0.40	0.56a	1.85	0.94	1.39a
South	0.67	0.38	0.52a	1.70	0.95	1.32a
East	0.68	0.41	0.54a	1.69	1.00	1.34a
Avg.[a]	0.68a	0.40b		1.71a	0.96b	

[a] Means within the respective columns and rows for each plant followed by the same letter are not significantly different at $P = 0.05$ according to LSD.

The causes of aggregation in these psyllids might be due to their inherent active aggregative behavioral response such as in a situation where the presence of one individual attracts the others, perhaps for the purpose of feeding and reproduction, and also due to some heterogeneity of the environment such as microclimate and preferred part of the plant (Poole 1974; Southwood 1978; Wratten & Fry 1980). It was known that the ovipositing and feeding sites of *D. citri* are always on young flushes. The difference in mean counts of *D. citri* between upper and lower halves of the plant canopies (Table 3) seemed to be most influenced by physical factors such as exposure to the sun.

ACKNOWLEDGEMENT

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