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Dispersion Pattern and Sampling of *Diaphorina citri* Kuwayama (Hemiptera:Psylidae) Populations on *Citrus suhuiensis* Hort. Ex Tanaka in Padang Ipoh Terengganu, Malaysia

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ABSTRACT

An understanding of the dispersion patterns of a pest is an important pre-requisite for developing an effective pest management program. In this study, fifty five (55) citrus trees were surveyed for adult *Diaphorina citri* once every four week for a period of ten months (March 2011 – December 2011). Analysis of spatial-distribution pattern using various indices of dispersion and regression models showed that *D. citri* exhibited an aggregated distribution on *Citrus suhuiensis*. Taylor's power law (a = 0.897, b = 1.267, R^2 = 0.74) fitted the data better than Iwoa's regression modal (α = 0.376, β = 0.196, R^2 = 0.409). The optimal sample sizes needed for fixed precision levels of 0.10, 0.15 and 0.25 were estimated using Taylor's regression coefficients, and the required sample sizes increased dramatically with increased levels of precision. Therefore, these sampling-plan presented should serve as a tool for an efficient estimation of *D. citri* population density in citrus orchard for pest management decision.

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INTRODUCTION

Diaphorina citri Kuwayama (Hemiptera: Psyllidae) is regarded as one of the most important pests of citrus worldwide (Boina

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et al., 2009; Sule *et al*., 2012a) because it is known to be the most efficient vector of citrus greening disease. It is believed to be of Far Eastern origin (Tsai *et al*., 2002), and has been reported in Malaysia since 1970 (see Abdullah *et al*., 2009; Sule *et al.,* 2012a). A recent report has also showed that most of the cultivated citrus varieties in Peninsular Malaysia, Sabah and Sarawak have been infected with citrus greening disease (Sijam *et al*., 2008).

Feeding by adult and immature *D. citri* can result in curled and notched leaves, defoliation, flower drop, irregular-shaped canopies and branch death (die back), especially when their populations are high (Yang *et al.,* 2006). In addition, honeydew excreted by nymphs leads to blemishing of foliage and fruit with subsequent reduction in photosynthesis (Wang *et al.,* 2001). Besides that, citrus tree infected with greening diseases produce bitter, inedible, misshapen fruits and eventually die within 5–10 years of infection (Mann *et al*., 2010; Sule *et al.*, 2012b).

Dispersion or distribution and abundance of organisms are important attributes of insect population and vital ecological properties of species (Siswanto *et al*., 2008). Knowledge about dispersion pattern of an organism is essential in understanding population biology, resource exploitation and dynamics of biological control agents (Fauvergue & Hopper, 1994). It provides a better understanding of the relationship that exists between organism and its environment which may be helpful in designing efficient sampling programs for population estimates, development of population models (Soemargono *et al*., 2008) and pest management strategy.

There are many methods used to describe the dispersion of arthropod populations, but most estimates are based on sample means and variances (Bisseleua *et al*., 2011), while the relationships between the variance and mean are used as indices of aggregation (Arnaldo & Torres, 2005). The models of Taylor's and Iwao's also depend on the relationship between the sample mean and the variance of insect numbers per sampling unit. The slope of the regression models are used as an index of aggregation. Designing sampling plans based on these indicators has been reported to reduce sampling effort and minimize variation of sampling precision (Kuno, 1991; Payandeh *et al*., 2010).

Despite the economic importance of *D. citri* to citrus growers, little is known about its dispersion pattern in Southeast Asia where *D. citri* still remains a major threat to citrus cultivation. Thus, there is an urgent need for such information as it will provide citrus pest managers, researchers, extension officers, and citrus farmers with a cost-effective sampling method for *D. citri.* Therefore, this study was undertaken to determine dispersion pattern of *D. citri* on *Citrus suhuiensis* (variety limau madu) in order to develop a suitable sampling plan for the pest.

METHODOLOGY

Study Site

The study was carried out from March 2011 to December 2011 at a pesticide-free

citrus orchard at Pusat Pertanian Padang Ipoh, Kuala Berang, Terengganu, Malaysia (N 05 02' 55.6" E 103 00' 54.6"). The orchard has an area of 20.4 hectares, which is divided into sixteen blocks. Crops planted in the plantation were citrus (seven blocks), vegetables (five blocks), rambutans (three blocks) and bananas (one block). Two plots measuring 1.2 hectare and containing 596 citrus trees were selected for the study. The citrus trees were planted at 5m x 4m spacing with agronomic practices such as weeding and application of manure are given to the citrus plants at regular intervals.

Population Sampling

Fifty five citrus trees of similar size (1.6m – 1.7m high) making 1/12 of the total trees planted in the two blocks were selected randomly as the sample trees. One sampling visit was made every four weeks for a total of ten sampling visits to survey the population of *D. citri* in the selected trees. The canopy of each tree was partitioned into upper and lower strata, and thereafter, each stratum was divided into four quadrants, namely, north, south, west and east. From each quadrant, three young shoots with newly expanded leaves (mostly light yellow in colour) were randomly selected during each sampling visit for observation, counting and recording the number of *D. citri* (adults).

Analysis

Distribution Pattern

Based on the *D. citri* counts from the two canopy strata and the four cardinal points, a mean number of *D. citri* per tree over time was calculated to be used in calculating the various dispersion indices. Furthermore, the spatial distribution of *D. citri* was determined by using different methods. The simplest method is the variance to mean ratio $\frac{S^2}{m}$, where the value of $\frac{S^2}{m} < 1$ indicates a uniform dispersion, while $\frac{S^2}{m} = 1$ indicates random dispersion and $\frac{S^2}{m} > 1$ indicates an aggregated dispersion.

Lloyd's index of patchness is described as ratio of the mean of mean crowding (m^*) to mean density (m). The mean crowding was calculated as described by Southwood (1978) using the following formula:

$$m^* = x + \left[\left(\frac{S^2}{x}\right) - 1\right]$$

where x is the mean density and S^2 is the variance, where Lloyd's index =1 indicates a random dispersion, Lloyd's index > 1 indicates aggregated dispersion, and Lloyd's index < 1 indicates regular dispersion.

The degree of aggregation was determined by using the three most commonly used dispersion indices, i.e., the Green coefficient (Cx), Taylor's power law and Iwao's patchiness regression. The Green coefficient was calculated as described by Green (1979) using the following formula:

$$Cx = \frac{\left(S^2/m\right) - 1}{\Sigma x - 1}$$

where S^2 = variance of mean, m = mean number of *D. citri* per shoot and Σx = total number of *D. citri,* where $Cx = 1$ the coefficient indicate a random dispersion; where $Cx > 1$, it indicates aggregated dispersion; and where $Cx < 1$ indicating a regular dispersion.

Taylor's power law describes the regression between logarithm of the population variance and logarithm of population mean according to the following equation:

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

where S^2 is the population varience, m is the population mean, a is the Y intercept and b is the slope of the regression, which is an index of aggregation. When b =1, it indicates a random dispersion; when it is > 1, it indicates aggregated dispersion; and when it is < 1, it indicates regular dispersion.

Iwao's Method: The Iwao's patchiness regression method quantifies the relationship between the mean crowding index (m^*) and the mean (m) by the following formula: $m^* = \alpha + \beta m$, where m^* was determined as [$m(S^2/m-1)$]. The intercept (α) is the index of the basic component of a population or basic contagion (where α <, =, and > 1 represent regularity, randomness, and aggregation of populations in spatial patterns, respectively), and the slope (β) is the density contagiousness coefficient interpreted in the same manner as b of Taylor's regression.

Sampling Plan

Based on the sample counts, the optimal sample sizes (n) was calculated with a and b from Taylor's Power Law to develop the enumerative sampling plan of Green (1970), with precision levels of 0.10, 0.15, and 0.25 for ecological and pest management purposes, as recommended by Southwood (1978), using the following formula:

$$n = am^b / D^2$$

where a and b are Taylor's power law coefficients, m is the *D. citri* density and D is the desired precision. The sampling stop line was calculated as suggested by Pedigo and Buntin (1994) and Namvar *et al*. (2012) using the following formula:

$$T_n = \left[\frac{an^{1-b}}{D^2} \right]^{\frac{1}{(2-b)}}$$

where, T_n, n and D are the cumulative total for sample n, the maximum number of sampling units, (sample size) and the fixed level of desired precision. The parameters a and b were determined from Taylor's power law (Southwood & Henderson, 2000).

RESULTS AND DISCUSSION

Distribution Pattern

The distribution patterns of *D. citri* on *C. suhuiensis* were established in accordance with the various indices of dispersion. The result of the current study reveals the dispersion patterned of *D. citri* to be highly aggregated within *C. suhuiensis*.

In all the trees sampled, the variance to mean ratio ($\frac{S^2}{x}$) was greater than the one with the values ranging from 4.67 to 1.24 (see Table 1). The Lloyd's Patchiness mean crowding ($\frac{m^*}{m}$) was also greater than one, indicating an aggregated distribution of the psyllid within *C. suhuiensis.* Similarly, the Green coefficient (Cx) values were greater than one, confirming the distribution of *D. citri* on *C. suhuiensis* to be aggregative in nature. However, the Lloyd's mean crowding (m^*) reveals a variable distribution pattern (Table 1), with 63.63% of the sampled trees showing aggregated distribution, while 20.0 and 16.36% showing regular and random distribution, respectively. These results corroborate with the previous finding by Soemargono *et al.* (2008), who showed the distribution of *D. citri* to be spatially aggregated on both the *Citrus reticulate* variety madu and *Murraya paniculata*. In addition, other results similar to ours were reported by Van den Berg *et al.* (1991) on other psylla species (*Trioza erytreae* Del Guercio and *Cacopsylla mali* Schmidtb).

Many authors have reported that an aggregated distribution pattern is a characteristic of arthropods and regular distributions are rarer, which are mainly found in the population where there is strong competition between individuals (Agrov *et al.*, 1999). The aggregated distribution pattern display by *D. citri* in the present study might be attributed to food source and mate, since *D. citri* was reported to be more attracted to flush leaves for feeding and

TABLE 1

Distribution statistics and dispersion indices of *Diaphorina citri* on *Citrus suhuiensis*

Tree No.	x	S^2	S^2/x	m^*	m^*/m	Cx
1	0.78	1.73	2.22	2.00	2.35	6.93
2	0.67	0.89	1.33	1.00	1.17	4.56
3	0.78	1.51	1.94	1.71	1.98	6.16
4	0.33	0.89	2.67	2.00	5.33	8.11
5	0.67	1.56	2.33	2.00	2.67	7.22
6	0.22	0.40	1.78	1.00	3.72	5.74
7	0.89	1.21	1.36	1.25	1.30	4.63
8	0.89	2.32	2.61	2.50	2.70	7.96
9	0.22	0.40	1.78	1.00	3.72	5.74
10	0.44	0.91	2.06	1.50	2.82	6.48
11	0.22	0.40	1.78	1.00	3.72	5.74
12	0.33	0.44	1.33	0.67	1.33	4.56
13	0.33	0.44	1.33	0.67	1.33	4.56
14	0.33	0.44	1.33	0.67	1.33	4.56
15	0.89	3.65	4.11	4.00	4.39	11.96
16	0.33	0.44	1.33	0.67	1.33	4.56
17	0.33	0.89	2.67	2.00	5.33	8.11
18	0.78	3.51	4.51	4.29	5.29	13.02
19	0.22	0.40	1.78	1.00	3.72	5.74
20	0.33	0.89	2.67	2.00	5.33	8.11
21	0.33	0.44	1.33	0.67	1.33	4.56
22	0.33	0.44	1.33	0.67	1.33	4.56
23	0.44	0.91	2.06	1.50	2.82	6.48
24	0.33	0.89	2.67	2.00	5.33	8.11
25	0.33	0.44	1.33	0.67	1.33	4.56
26	0.44	0.91	2.06	1.50	2.82	6.48
27	0.44	0.91	2.06	1.50	2.82	6.48
28	0.33	0.89	2.67	2.00	5.33	8.11
29	0.56	0.91	1.64	1.20	1.72	5.39
30	0.56	0.91	1.64	1.20	1.72	5.39
31	0.44	0.91	2.06	1.50	2.82	6.48
32	0.56	0.69	1.24	0.80	1.00	4.32
33	0.22	0.40	1.78	1.00	3.72	5.74
34	0.33	0.44	1.33	0.67	1.33	4.56
35	0.44	0.69	1.56	1.00	1.69	5.13
36	0.56	1.14	2.04	1.60	2.44	6.45
37	0.56	2.47	4.44	4.00	6.76	12.85
38	0.67	2.44	3.67	3.33	4.67	10.78
39	0.33	0.44	1.33	0.67	1.33	4.56
40	0.78	1.51	1.94	1.71	1.98	6.16
41	0.33	0.89	2.67	2.00	5.33	8.11
42	0.78	2.17	2.80	2.57	3.08	8.45
43	1.11	1.88	1.69	1.80	1.73	5.50
44	0.56	0.91	1.64	1.20	1.72	5.39
45	0.44	0.91	2.06	1.50	2.82	6.48
46	0.44	1.58	3.56	3.00	6.19	10.48
47	0.67	0.89	1.33	1.00	1.17	4.56
48	1.00	4.67	4.67	4.67	4.67	13.44

TABLE 1 (continue)

49	0.67	1.11	1.67	1.33	1.67	5.44
50	0.22	0.40	1.78	1.00	3.72	5.74
51	0.67	1.56	2.33	2.00	2.67	7.22
52	0.44	0.91	2.06	1.50	2.82	6.48
53	0.67	1.56	2.33	2.00	2.67	7.22
54	0.22	0.40	1.78	1.00	3.72	5.74
55	0.33	0.44	1.33	0.67	1.33	4.56

x = mean, S^2 = variance,
m^* =Lloyd's mean crowding,
m^*/m =Lloyd's Patchiness mean crowding and
Cx = Green index

oviposition (Sule *et al.*, 2012b), or to either active aggregation on the part of this psyllid or to some variations of the environment such as microclimate, preferred part of plant, and natural enemies (Tsai *et al.,* 2000).

Taylor's power law analysis appeared to illustrate the distribution of *D. citri* well by showing highly significant relationships between the variance and mean of *D. citri* population (Fig.1), and the model was found to be significantly different from 0 (t = 12.35, p < .0001). The slope values of Taylor's power law for the psyllid on *C. suhuiensis* (b = 1.267) was significantly greater than 1 (t = 9.71, df = 54, p < .0001), indicating an aggregated or clumped distribution pattern for *D. citri* on *C. suhuiensis*. However, Iwao's patchiness regression based on the same sampled trees did not show any significant relationship between the mean crowding index (m^*) and the mean (m) of *D. citri* (Fig.2) (t = 1.69, p = 0.896). It also produced a slope value (β = 0.196) below 1 (t = 0.13, df = 55, p = 0.13), indicating a regular distribution. Nevertheless, the constant α in the Iowa's model indicates the tendency to crowding when it is positive (+) or repulsion when it is negative (-) as it is the 'Index of Basic Contagion' defined by Iwao (1970).

Based on the higher value of R^2 produced

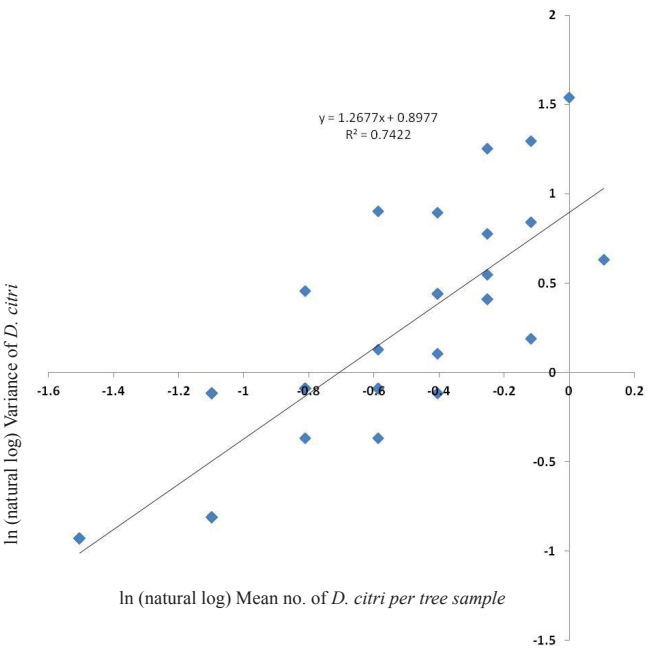

Fig.1: Regression analysis of Taylor's power law for *Diaphorina citri* populations on *Citrus suhuiensis*

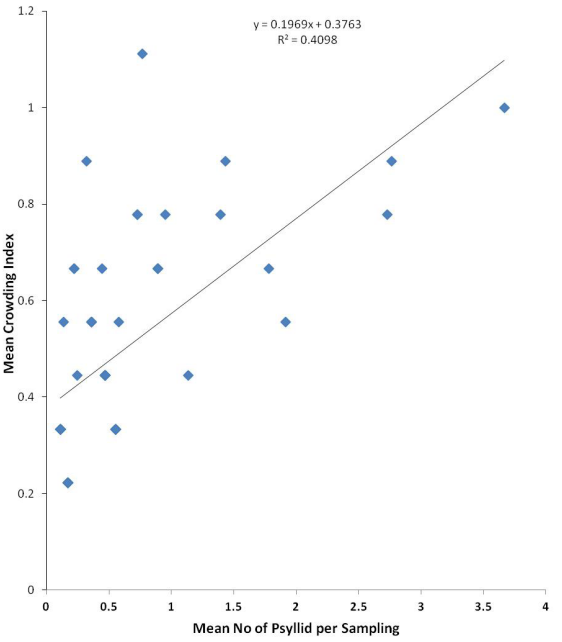

Fig.2: Regression analysis of Iwao's mean crowding index (m^*) on mean density (m) for *Diaphorina citri* populations on *Citrus suhuiensis*

by Taylor's power law compared to Iwao's patchiness regression, it could be stated that Taylor's model fitted the data better than Iwao's modal. Furthermore, Taylor's power law provides a more even distribution of the points along the line than Iwao's model. In spite of Iwao's model inability to fit the data very well, it could still give an insight into the interpretation of implication of ecological parameters (Kuno, 1991). For instance, the positive value of α of Iwao's patchiness regression in the present study is indicative of a mutual attraction (positive interaction) between the individuals even at a very low density.

Sampling Plans

The relationship between the mean psyllid density and the required sample size for the fixed precision levels of 10, 15 and 25% is shown in Fig.3. The stop line of the fixed precision level of 25% of the mean for sequential sampling is presented in Fig.4. Since the variance mean regression in Taylor's model provided a good description of the data (Fig.1), the regression variability would only have a minor effect at very low mean density.

In order to achieve high fixed precision levels of 10 and 15% for precise density estimate, quite a large number of samples are required (Fig.3). Thus, the level of the precision needed is a choice made based on the purpose of a sampling plan. From the results of the present study, the optimal sample size for a precision of 25% ranged from 14 to 443 trees, depending on the mean. However, the sample sizes increased

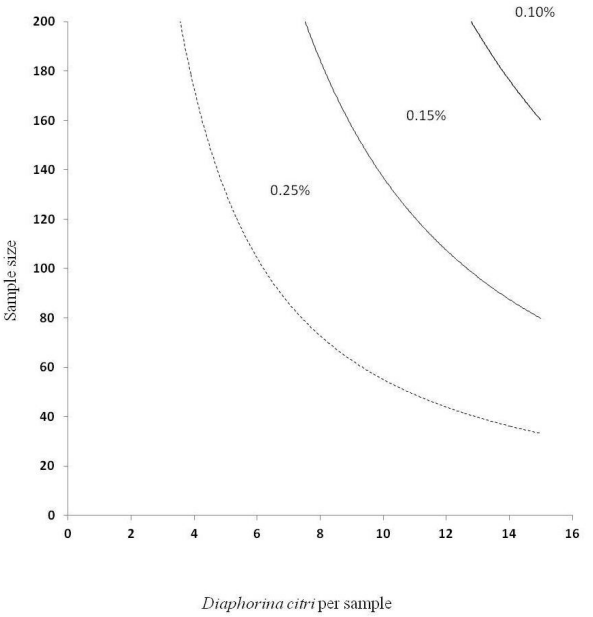

Fig.3: The relationship between required sample size and mean density for achieving fixed precision levels of 0.10, 0.15 and 0.25% for *Diaphorina citri* populations on *Citrus suhuiensis*

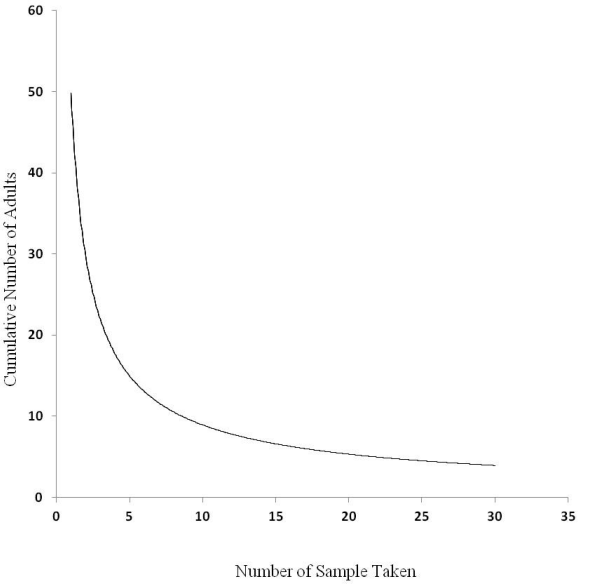

Fig.4: Sampling stop line at a fixed precision level of 25% for *Diaphorina citri* populations on *Citrus suhuiensis*

considerably when the precision level required also increased. For instance, for the precision level of 10%, the sample size ranged from 90 to 2772 trees.

Furthermore, considering a desired accuracy of 25% in the present study, the number of sample trees needed for mean densities of around fourteen *D. citri* per tree (assuming this number to be the action threshold for *D. citri* in the present condition) was approximately 35. This number of trees is considerably lower than the actual 55 trees sampled during our survey. However, if the level of precision was raised to 10%, the number of the samples required became 215 trees, for the same density. In their work, Setamou *et al*. (2008) used forty citrus orchards with the sampling of ten trees per orchard and twenty new flush per tree, and recommended using eight flush per tree and ten trees per orchard to provide a density estimate of *D. citri* with a percentage relative precision of 25%. Meanwhile, Dharajothi *et al*. (1986) recommended a sample size of nineteen flush per tree for a sampling plan that is based on one tree per orchard to achieve a 25% precision level. Although Setamou *et al*. (2008) and Dharajothi *et al*. (1986) recommended small number of trees per orchard and shoots per tree than the present study, these differences might be attributed to the total number of trees sampled, the number of sampling unit and abundance of psyllid. For instance, it has been reported that a decrease in the mean number of insects sampled normally leads to an increase in the sample size and vice versa (Naranjo & Flint, 1994). Furthermore, sampling a higher number of trees with a low number of sampling unit per tree will yield smaller sampling error than sampling a small number of trees with a higher number of sampling unit per tree. Nevertheless, the findings of this study will go a long way in alleviating the problem faced by growers on decision making with respect to pests.

CONCLUSION

The distribution analyses using various indices of dispersion and regression models have shown that *D. citri* was spatially aggregated on *C. suhuiensis* variety limau madu. The fixed precision sampling plan developed in this study will provide useful insights into efficient estimation of *D. citri* population density in citrus orchard. Furthermore, information on the density level of *D. citri* provides a sound base for selecting appropriate decision making in designing IPM programmes for this particular pest.

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