DISPERSION PATTERN AND SAMPLING PLAN FOR ASIAN CITRUS PSYLLID, *Diaphorina citri* Kuwayama (HEMIPTERA: PSYLLIDAE) IN A CITRUS ORCHARD

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ABSTRACT

The ecology of the psyllid and its spatial distribution as the basis for the development of a reliable sampling plan are very important. The abundance and spatial distribution of *Diaphorina citri* (Hemiptera: Psyllidae) were studied in a commercial citrus orchard in southwestern Sarawak, Malaysia from April 2013 to December 2014. The spatial distribution of *D. citri* eggs, nymphs and adults were analysed using Taylor's power law and Iwao's patchiness regression. Taylor's power law fitted the data better and produced higher values of R^2 than Iwao's regression model but did not work well with the egg populations. Based on both regression models, the field dispersion patterns of *D. citri* eggs, nymphs and adults were aggregated among flush shoots in individual trees as indicated by the regression slopes that were significantly >1. By homogeneity tests on both regression methods, the slopes of Taylor's power law and Iwao's regression model did not differ significantly for the *D. citri* population on honey tangerine for both years. The minimum number of flush shoots per tree required for estimates of *D. citri* densities varied from 2, 4 and 6 flush shoots for adults, nymphs and eggs, respectively, for the average density of each developmental stage obtained during our studies. Prediction suggested that a sampling plan consisting of 10 trees with the optimum number of six flush shoots per tree was required for a reasonably accurate density estimation of the three life stages of *D. citri* acceptable enough for population studies and pest management program in citrus orchards.

Keywords: *Diaphorina citri*, dispersion, sampling units, sample size, citrus

ABSTRAK

Ekologi psilid dan taburan penyebaran adalah sangat penting sebagai asas untuk pembangunan rancangan persampelan yang boleh dipercayai. Kelimpahan dan taburan spasial *Diaphorina citri* (Hemiptera: Psyllidae) telah dikaji di kebun limau komersil di bahagian barat selatan Sarawak, Malaysia bermula dari April 2013 hingga Disember 2014. Taburan reruang bagi telur, nimfa dan dewasa *D. citri*, telah dianalisis menggunakan undang-undang kuasa Taylor dan regresi model Iwao. Undang-undang kuasa Taylor menganalisis data dengan lebih baik dan menghasilkan nilai R^2 lebih tinggi berbanding model regresi Iwao tetapi tidak berfungsi dengan baik terhadap populasi telur. Berdasarkan kedua-dua model regresi tersebut, corak penyebaran lapangan telur, nimfa dan dewasa *D. citri* telah diagregatkan di antara tunas pucuk pada pokok individu seperti yang ditunjukkan oleh lereng regresi yang ketara >1. Ujian keseragaman pada kedua-dua kaedah regresi iaitu, undang-undang kuasa Taylor dan model regresi Iwao menunjukkan tidak berbeza dengan ketara terhadap populasi *D. citri* pada pokok limau tangerin madu untuk tempoh kedua-dua tahun. Jumlah minimum tunas pucuk bagi setiap pokok yang diperlukan untuk menganga kepadatan *D. citri* adalah dari dua, empat dan enam untuk dewasa, nimfa dan telur, masing-masing untuk purata kepadatan bagi setiap peringkat kitar hidup yang diperoleh semasa kajian kami. Ramalan mencadangkan bahawa satu pelan persampelan yang terdiri daripada 10 pokok dengan jumlah optimum enam tunas pucuk setiap pokok diperlukan untuk penganggaran kepadatan yang tepat dari tiga tahap kitar hidup *D. citri* yang boleh diterima untuk kajian populasi dan program pengurusan serangga ini di kebun limau.

Kata kunci: *Diaphorina citri*, penyebaran, unit persampelan, saiz sampel, sitrus

INTRODUCTION

The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama, (Hemiptera: Psyllidae), one of the two known effective vectors of huanglongbing (HLB) disease , formerly known as citrus greening (Hall & McCollum 2011); is a debilitating and one of the world's most serious citrus diseases which is caused by a phloem-limited Gram-negative bacterium, non-culturable causal agent '*Candidatus liberibacter asiaticus*' (α -Proteobacteria) (Bové 2006). HLB is a highly destructive and fastidious severe disease of citrus, particularly on sweet orange and mandarin assortments (De Graca & Korsten 2004; Etebu & Nwauzoma 2014). It is the primary cause of losses in citrus production in Asia (Mao 2016), South Africa, and USA (Tiwari et al. 2011), and yet there is no successful treatment for infected trees. According to Beattie and Holford (2008), HLB detected in the year after 1970 posed a destructive threat to the Malaysian citrus industry. HLB is best controlled through integrated disease management using healthy and PCR certified HLB-free planting material, removal of diseased branches or trees and integrated vector control. HLB and its vectors pose a major threat to the profitability and sustainability of the Malaysian citrus and nursery industries, especially in citrus rehabilitation. It has spread rapidly and gained national importance owing to the dramatic spread and transmission of HLB between 1989 and 1992, which devastated citrus orchards in Peninsular and East Malaysia, including Sarawak (Teo et al. 2000). The once flourishing citrus industry in

Sarawak was destroyed completely by HLB in 1992 (Teo et al. 2000). By 1991, the disease had destroyed a total area of 1,143 ha with approximately 310,000 trees resulting in an estimated yield loss of more than 6,500 metric tons of fruit and an economic loss of RM 6.5 million or USD 1.6 million (Teo et al. 2000). Currently the presence of the vector *D. citri* in citrus orchards which previously were regarded as HLB- free in Malaysia are still under the potential threat of one of the most serious diseases of citrus which may pose a destructive threat to the profitability and sustainability of the Malaysian citrus industry.

Information on the spatial distribution of *D. citri* within Malaysian citrus orchards is critical for understanding the biology and ecology of the psyllid, furthermore, spatial distribution of an insect pest is the basis for the development of a reliable sampling plan (Sule et al. 2012) which is required for the development and implementation of the integrated pest management (IPM) program. Investigations of this kind were undertaken for various citrus species in China (Zhao 2017), Taiwan (Wang 1981), India (Dharajothi et al. 1989), USA (Kunta et al. 2012), and Brazil (Teixeira et al. 2008), but with very little or no published information on dispersion pattern of life stage and sampling plan for *D. citri* on honey tangerine (*Citrus aurantium*) orchard in Asia. Thus, results of the present research on the dispersion pattern as well as sampling plan for *D. citri* in a Malaysian citrus are quite novel, and while the study is linked to other investigations elsewhere in Asia, our methodological approach is radically different and the results are influential.

Control of *D. citri* with pesticides and bio-control agents relies on studies of relationships between population density and spatial distribution pattern of the vector in major citrus growing countries, and dispersion indices calculated for eggs, nymphs and adults in *D. citri* populations underlie reliable sampling plan for the pest management. The use of selective insecticides and bio-control agents such as *Tamarixia radiata* and *Diaphorencyrtus aligarhensis* are recommended for integrated pest management (IPM) program of *D. citri*. However, the efficiency of these parasites is limited by the presence of hyperparasitic wasps. These specialist parasitoids are currently being evaluated for potential use in suppressing psyllid population in citrus (Hoy & Nguyen 2000; Leong et al. 2011). Khan et al. (2016) reported that several ladybeetle species are found to prey on *D. citri*.

Spatial distribution patterns and sampling plans for *D. citri* have been studied in the United States in orange jasmine, *Murraya paniculata* (L.) Jack (Tsai et al. 2000) and grapefruit (*Citrus paradisi*) Macfad (Sétamou et al. 2008). Dharajothi et al. (1989) used a sample unit of one new flush shoot (4-5 cm in length) in sampling for *D. citri* on lime (*Citrus aurantifolia* (Christm.) Swingle) in India. To the best of the author's knowledge, no research information on sampling citrus flush in Malaysia was available. In view of possible differences in citrus culture, climate, and other factors among citrus industries around the world, the appropriateness of data presented by Dharajothi et al. (1989) and Sétamou et al. (2008) relative to *D. citri* population densities on citrus honey tangerine in Malaysia is not known. Taylor's power law and Iwao's patchiness regression were used to evaluate and outline the dispersion trend of *D. citri* eggs, nymphs and adults (Taylor

1961, 1984; Iwao 1977*a*; Southwood & Henderson 2000). The objectives of our research therefore were to study the spatial distribution pattern of *D. citri* population on honey tangerine and to determine the optimum number of flush and tree samples required for reliable estimates of its population densities on citrus in the field. This will provide a base line information and will be essential for the formulation of any integrated pest management program of *D. citri* in citrus orchards.

MATERIALS AND METHODS

The studies were conducted in a 1 ha commercial citrus orchard that housed 200 grafted non-bearing, PCR-certified disease-free honey tangerine (*Citrus aurantium*) of similar size in south western Sarawak, Malaysia. The trees received sprays containing 0.35% *n*C24 D-C-Tron Plus and mancozeb M80 @ 0.15% a.i. when necessary during the study period to control mite infestation and fungal disease on the foliage. All other practices were in accordance with the farmer's normal schedule. A monthly fertilizer application and weed control was carrying out in the citrus orchard when necessary. The fungicide mancozeb (Dithane M45 80 WP) at 3 g product/L of water was added to all spray tank mixed and applied to all tress when necessary for controlling mite/scale infestation and greasy spot.

Data Collection

The selected citrus orchard was divided into 10 blocks with 20 trees each. Stratified random sampling was adopted in this study for all developmental stages of *D. citri* between April 2013 and December 2014. Young flush (about 10–15 mm in length) with five selected young leaves were selected as the sample unit and 100 random samples of young flushes were collected weekly from the 200 trees for 79 consecutive weeks. On each sampling occasion, 10 young flushes were sampled at random from 10 trees of each block. Each flush was carefully examined and the number of *D. citri* adults per flush was counted and recorded. Eggs and nymphs were counted *in situ* and recorded per flush with the aid of a 10× hand held lens.

Statistical Analysis

Dispersion indices were calculated using Taylor's power law dispersion indices (Taylor 1961) and Iwao's patchiness regression (Iwao 1968). The dispersion index of aggregation (b) and the density contagiousness coefficient (β) were tested for significance of departure from 1 (randomness) by using a two-tailed t-test (with df=n-1 and P=0.05). In addition, slope coefficients of the respective regression models were subjected to test for homogeneity using ANOVA SPSS version 25 computer package for the analysis of the spatial dispersal of *D. citri* populations. The fit of a common to different data sets was tested using *F*. The mean number (m) of *D. citri* eggs, nymphs or adults and the related variances (S^2) per flush per tree were calculated. Data analysed was the average number of citrus psyllid *D. citri* per flush per tree. The optimum number of trees and flushes per tree required for each developmental stage of *D. citri* was determined for three precision levels (D=5%, 10% and 25%), respectively.

Dispersion Analysis of *D. citri* Eggs, Nymphs and Adults in A Citrus Orchard

Subsequently finding the distribution trend from the dispersion indices, spatial distribution patterns of the developmental stages of *D. citri* among flushes per tree were compared by applying the Taylor's power law (Taylor 1961) and Iwao's patchiness regression (Iwao 1968) for the citrus. For each data set (one fitted to a Poisson distribution and the other fitted to a negative binomial distribution), separate regressions were calculated for year 2013 and 2014.

Taylor's power law model states that the variance (s^2) is proportional to a fractional power of the arithmetic mean (x): $s^2=ax^b$ which expresses variance-mean relationships and provides a quantitative analysis of the index of aggregation in terms of some ecologically meaningful parameters (Kuno 1972). Taylor's power law relates the variance to the mean density through: $s^2=ax^b$ (where x=mean and s^2=variance, the constant a represents a sampling factor, and the constant b represents the true index of aggregation characteristic of a species. The coefficient a and b were obtained from the regression model:

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

The index b is specific and only a is affected by sampling procedure; where the slope b is an index of aggregation, with a constant graduation in dispersion from regular ($b<1$), through random ($b=1$), to aggregated ($b>1$) (Taylor 1961).

Iwao's patchiness regression model regression quantifies the linear relationship between the mean crowding index (m^*) and the sample mean (m) while parameters α and β were obtained by linear m^*/m regression ($m^*=\beta m+\alpha$; Iwao (1968)). The intercept α is the index of basic contagion and β is the density contagiousness coefficient in *D. citri* population, a measure of aggregation as b of Taylor's regression above (Iwao 1977; Taylor 1984); with $0<\beta<1$, $\beta=1$, and $\beta>1$ indicating a uniform, random and contagious dispersion, respectively. Taylor's power law regression coefficients (a and b) and the index of basic contagion (α) and the density contagiousness coefficient (β) of Iwao's regression were determined for each developmental stages of *D. citri* on honey tangerine.

Optimum Sample Size Determination

A two-level sampling method was employed to include sampling of trees and of flush shoots within trees. The precision of the overall mean will depend on the variance between flush shoots within tree samples (s^2_F), and the variance between-tree samples. It is also on the costs of sampling a flush within the same tree (C_F) or of moving to another tree and sampling within it in the same citrus orchard. The two levels of our sampling scheme were characterized as follows: T is the number of trees sampled per orchard (T=10) and F is the number of flushes sampled per tree (F=10). For our study, it took between 3 and 5 min to randomly select a tree, with a mean time of 3.5 min in the orchard. The time to collect and evaluate a flush from each canopy was between 5 s to 60 s with a mean time of 30 s within each tree. On this basis, the optimum number of trees or number of flushes per tree to minimize sampling variance for any given development stage of insect given by Harcourt (1961) is as follows:

$$N_s = \sqrt{\frac{S_F^2}{S_T^2} X \frac{C_T}{C_F}}$$

where C_T is the basic cost (i.e., 3.5 min) per tree (s2T), and CF is the additional cost for each sampled flush and 30 s is the time spent to sample and evaluate each flush (s2F). s2T and s2F are the variance components between-tree and flushes within-tree respectively associated to the mean number of eggs, nymphs and adults of *D. citri*. These variance components were analysed using ANOVA of SAS.

The sample variance of the mean for any developmental stage in the samples of trees and of flushes within trees can be computed by the equation provided by White (1978) as follows:

$$\text{V (mean)} = \frac{S_F^2}{f\ t} + \frac{S_T^2}{t}$$

where f and t are the number of flushes and number of trees respectively per sample, and s2T and s2F are the variance components as previously defined. To select the optimum sample size (t), which need to be sampled for each developmental stage as to obtain any desired approximate standard error of the sample mean can be computed using the following equation (Southwood and Henderson 2000).

$$N_t = \frac{\left(\frac{S_S^2}{N_F}\right) + S_T^2}{(\bar{x}\ X\ D)^2}$$

where N_t is the optimum number of trees for each developmental stage of *D. citri* was determined for three precision levels (D = 5%, 10% and 25%) and $(x)\bar{}$ is the sample mean. A precision level of 25% standard error of the mean is usually applied for general estimates of insect populations and generally accepted for pest sampling in IPM programs, while a precision of 10% standard error of the mean allows detection of smaller changes in population dynamic and is generally desired for research purposes (Southwood and Henderson 2000).

RESULTS AND DISCUSSION

Dispersion indices of *D. citri* eggs, nymphs and adults in a citrus orchard

Dispersion indices calculated using Iwao's patchiness regression and Taylor's power law are presented (Table 1). Taylor's power law regression indicated a highly significant and positive relationship ($P < 0.001$) between the ln(variance) and ln(mean) of *D. citri* adults, nymphs, or eggs per flush per tree, while for Iowa's regression a highly significant positive ($P < 0.001$) relationship was found between the mean crowding index and the actual mean density of *D. citri* eggs, nymphs, or adults per flush shoot per tree. However, Taylor' power law was not well fitted for the egg distribution based on the linear relationship between variance and mean density as the index of aggregation was negative for eggs (b = -29.3; Table 1) for pooled data on honey tangerine during 2013-2014 may probably due to low number of data points available.

To understand the distribution further, Iwao's patchiness regression ($m^* = \beta m + \alpha$) was fitted, based on the linear relationship between mean crowding index and mean density, over a range of different densities; $m^* = 3.38 + 14.9m$ in 2013, and $m^* = 3.29 + 30.5m$ in 2014. The intercept value of the Iwao's patchiness regression for the egg populations where index of basic contagion (α) = 3.38 and 3.29 (Table 1) for both years in 2013 and 2014, respectively in the field were fairly close to each other but greater than one. This confirmed the aggregation among egg populations with stronger aggregation tendency.

The values of Iwao's patchiness regression, $m^*=8.3m+2.04$ in 2013 and $m^*=2.88m+7.72$ in 2014, further confirmed the aggregated distribution for *D. citri* nymphs. For Taylor's power law, the value of b was 2.15 and 2.6 in 2013 and 2014, respectively (Table 1). This outcome also confirmed aggregated distributions for nymphs, as the values for b were > 1. The spatial distribution of eggs and nymphs is an outcome of the movement trends of gravid females to oviposition sites as D. citri only disperses in the adult stage of its life cycle (Boina et al. 2010). *D. citri* adults are not strong fliers and will leap short distances when disturbed (Aurambout et al. 2009). They typically fly short distances (3-5m) from one tree to the other (Hall and Hentz 2011).

Our studies on the spatial distribution of *D. citri* population in a Malaysian honey tangerine orchard showed that the eggs and nymphs followed a contagious distribution on flushes within trees and that the distribution could be expressed as a negative binomial distribution (Bliss and Owen 1958). The contagious distribution of eggs on flushes indicated that female adults tend to lay eggs on trees on which they land before searching for a new host plant. These data also suggested a preference of females for 4 to 7 day-old (3 mm to 10 mm long) new young flush leaves on which to lay eggs rather than older, longer flushes. Female adults preferred flush growth < 6mm in length to longer flush lengths and numbers of eggs laid on a flush decline rapidly as the flush length increases and when individual leaves attain lengths >10mm. (Leong et al. 2010). Taylor's power law usually indicated that degrees of aggregation increased as population density increased. Adults were observed to have aggregated distribution (b = 7.5) in 2013 but almost random distribution (b = 0.81) in 2014 (Table 1) due to migration or dispersal. Dispersion of adults is density-related. They dispersed when population density increased and aggregated when population density declined. The dispersion behaviour minimises intraspecific competition for resources. The aggregated behaviour of *D. citri* developmental stages on citrus flush can probably be attributed to their preference for new young flushes for oviposition, feeding, and development. *D. citri* eggs are laid in groups or clusters on new flush leaves where nymphs feed during their developmental stage. An increase in mobility and inter-habitat movement of individuals as they mature and increase mortality with developmental stages resulting in the decreased aggregation of *D. citri* from immature to adult stage. The values of the index of basic contagion (α) were 3.38 and 3.29, respectively for the egg populations in 2013 and 2014 season (Table 1), indicating positive associations between individuals in the colonies. Because the coefficient of density-contagiousness (β) was > 1, the aggregation patterns among the colonies were confirmed. Although adult females always tended to lay eggs contagiously, as indicated by the aggregated distribution of eggs they may avoid infested flushes for oviposition. However, Martini et al. (2014) reported that female psyllids avoided conspecific female cues in a density-dependent manner and the conspecific females were attractive to odours from citrus plants actively damaged by psyllid feeding. They initiated dispersion when population density increased, resulting in a change from the initially contagious to almost random dispersion on infested citrus.

However, Iwao's patchiness regression can test the dual nature of aggregation, basic contagion (α) concerned with the aggregation (β) or clustering habit characteristics, and aggregation tendency or distribution pattern of clusters. Contagious distribution of infested flushes can therefore be indicated by b and β being > 1. The parameter β is an index of the aggregation in the *D. citri* psyllids population, when $\beta > 1$, the conveyance is contagious, and when $\beta = 1$, the essential parts of the dispersion are randomly circulated. The intercept α and β of the regression therefore appears to be valuable as an index to depict the diverse aspects of the scattering patterns of *D. citri* populations. Although α values (Table 1) were relatively lower for adults than for eggs and nymphs, positive intercepts ($\alpha > 0$) indicated that generally more than 1 egg, nymph or adult could be found on a flush. This may probably due to the behaviour of females, which tend to lay eggs successfully on newly emerged young flushes, especially during flushing cycle, while avoiding oviposition, thereby leading to distributions of eggs being dependent on the densities of flushes within the tree.

The positive value of α of Iwao's patchiness regression suggests a preference for particularly favourable spots in the habitat, but more generally it seems to be related to the mode of life characteristics of the species. This implied a positive interaction (mutual attraction) between individuals (Southwood & Henderson 2000) of the citrus psyllid *D. citri*, therefore the individuals would tend to aggregate even at a very low density in the same habitat. It was found to be true in nature, where they lived together in colonies on the host plant, especially during their nymphal stages provided biotic and abiotic factors are favourable for *D. citri* population. A high mean colony size of D. *citri* adults between 20 and 51 per shoot per tree on citrus and orange jasmine, respectively was reported by Xu et al. (1989) in Fujian, China Soemargono et al. (2009) in Malaysia. Distribution of nymphs per flush were clearly related to the original clumped distribution of eggs distribution, as α and β were 3.38 and 14.9, and 3.29 and 30.5, respectively in 2013 and 2014 (Table 1). This indicated a strong aggregation tendency.

The intercept of the Iwao's patchiness regression was negative for adults on honey tangerine ($\alpha = -0.75$) for 2013 season. The value of the index of basic contagion (α) of -0.75 for 2013 was smaller than zero whereas the α value of 2.52 for 2014 was greater than zero. The observed negative value of α (-0.75) for adults (Table 1) suggests that *D. citri* adults do not form colonies on honey tangerine and Tsai et al. (2000) reported that a single individual is the basic component of *D. citri* population on orange jasmine. Similar observations were made by Dharajothi et al. (1989) for *D. citri* adults on acid lime. The lack of colonies of *D. citri* adults may be a means by which competition is limited, specifically for the progeny of this highly prolific or reproductive species. This may be related to behaviour that allows females either to avoid oviposition on flushes on which they encounter eggs laid earlier by other females during abundant flush growth or by forcing them to lay eggs on flushes with eggs during non-flushing periods. These circumstances would result in egg distributions becoming less uniform. The intercept α values or index of basic contagion was lower in the adult populations than in the egg and nymph populations for 2013 and 2014, respectively. This suggest that the basic component of the adult population was smaller than that of the egg and nymph populations. The changeable and complicated natural environment in the orchard led to marked fluctuations of egg, nymph and adult populations. This could have been related to larger migration space and higher mortality of *D. citri* in a natural environment. However, Water et al. (2013) claimed that negative intercept values are not biologically meaningful and suggested passing the regression through the origin for obtaining biologically intuitive results.

The slope of the regressions or coefficient of density-contagiousness (β) were positive and significantly >1 for all three developmental stages on honey tangerine for both 2013 and 2014 suggesting a contagious distribution of all *D. citri* populations on honey tangerine. Both α and β were complementary and useful as indices for describing different aspects of the dispersion pattern of *D. citri* population.

By homogeneity tests on both regression methods, the slopes of Taylor's power law and Iwao's regression model did not differ significantly (F=3.51, 5.82; P>0.05) for the *D. citri* population on honey tangerine for both years. However, the distribution pattern of *D. citri* population was aggregated for both years. Therefore, both regression models appeared to describe the spatial distribution of *D. citri* population well and consistently regardless of year.

In general, Taylor's power law and Iwao's patchiness regression provided good indications of the relationship between variance and mean density of adults, nymphs or eggs per flush on honey tangerine respectively during 2013 and 2014 season. The lower fit of Taylor's power law regression for eggs may be due to few data points available. However, the Taylor's power law model was appropriate and provided an adequate description of variance-mean relationships of all *D. citri* developmental stages and fitted the data better than the Iwao's regression model based on the relatively higher values of R^2 for each of the two models considered. Furthermore, Taylor's power law provided a slightly more even distribution of points along the line when compared to Iwao's regression model. The intercept ln(a) of Taylor's power law was significantly >0 and the dispersion coefficient b was significantly >1, indicating an aggregated spatial pattern of each *D. citri* stage on honey tangerine. However, the slopes for the three developmental stages of *D. citri* were unequal as shown by the significant ln(mean) developmental stage interaction (F=5.62; df=2; P<0.0001).

The present study on the spatial distribution of *D. citri* revealed that eggs and nymphs followed a similar contagious distribution which is stronger than that of adults. This corroborates the aggregative nature of dispersion of *D. citri* population in the field as reported by other researchers for various citrus species in the major citrus growing region in USA (Tsai et al. 2000; Sétamou et al. 2008), Brazil (Teixeira et al. 2008), India (Dharajothi et al. 1989), Taiwan (Wang 1981), China (Xu et al. 1989) and Malaysia

(Soemargono et al. 2008). The distribution of adults did not show any definite pattern. The psyllid's distribution pattern would have been affected by dispersal of adults, initial distribution of eggs, and nymphal mortality. However, from pest management point of view, the egg and nymphal stages matter most, because immediately after hatching, the nymphs start sucking the sap from the plant and thus any control strategy needs to be directed at early stages. Therefore, egg sampling is crucial to fix a time of spray. The trend of the pest to change from an early aggregated distribution to random distribution in adults indicates the role of key mortality factors operating. This, therefore, opens new vistas in the study of *D. citri* for which the sampling plan outlined here will be applicable (Dharajothi et al. 1989).

Table 1. Dispersion patterns of *D. citri* egg, adult, and nymph counts on honey tangerine flush shoots for individual trees as determined by Taylor power law and Iwao's patchiness regression in a Malaysian citrus orchard.

Year	***D.citri* stage**	**intercept**	**slope**	**SE_b**	**R^2**	**p**
Taylor's power law						
2013 + 2014	Egg	42.6	-29.3	0.18	0.07	ns
2013	Nymph	0.18	2.15	0.23	0.74	<0.0001
	Adult	0.05	7.5	3.35	0.66	<0.0001
2014	Nymph	0.22	2.6	0.32	0.85	<0.0001
	Adult	0.20	0.81	2.18	0.61	<0.0001
Iwao's patchiness regression						
2013	Egg	3.38	14.9	7.92	0.56	<0.0001
	Nymph	2.04	8.31	1.37	0.53	<0.0001
	Adult	-0.74	23.0	1.79	0.58	<0.0001
2014	Egg	3.29	30.5	7.80	0.84	<0.0001
	Nymph	7.72	2.88	2.72	0.46	<0.0001
	Adult	2.52	11.7	3.65	0.45	<0.0001

Intercepts are β and α for Taylor's power law and Iwao's patchiness regression, respectively

Ns = nonsignificant ($P > 0.05$), * = significant ($P< 0.05$), ** = highly significant ($P < 0.01$), SE_b = is the standard error of the slope, R^2 = regression coefficient, P = the significance level of the t-value.

Optimum Sample Size

The variance components were used for the flush shoots and within trees sampling units. The optimum number of citrus flush shoots required per tree for estimating *D. citri* densities were 6 ,4 and 2 for eggs, nymphs, and adults, respectively. These sample sizes were lower than the 10 flushes actually sampled per tree during our studies. Therefore, estimates of mean *D. citri* densities per tree in the present studies were adequate. These optimum numbers of flushes required per tree for each developmental stage are consistent with the relative contribution of the between flushes within trees variance to the total variance component (Table 2). The number of citrus trees to be sampled at a precision level of 5%, 10% and 25% for the calculated optimum number of flushes per tree (t_{opt})

and for the actual number of flushes sampled (f=10) were determined. In this study, the optimum sample size to adequately estimate populations for different developmental stage of *D. citri* in a citrus orchard is 6 flushes per 1 and 5 trees, 4 flushes per 1 and 3 trees and 2 flushes per 1 and 8 trees to achieve a precision level of 10% standard error of the mean. The optimum sample size of 6 flushes per 1 and 4 trees, 3 flushes per 1 and 4 trees, and 7 flushes per 1 and 2 trees to achieve a precision level of 25% standard error of the mean for eggs, nymphs and adults, respectively. It appeared that a decrease in *D. citri* mean densities will lead to an increase in sample sizes and vice versa. However, to achieve a relative precision level of 25% required for field studies, a sampling scheme that needs a total of 60 (six flushes 1 and 10 trees) flush shoots per orchard may provide adequate density estimates of *D. citri* for all developmental stages. These numbers of flush shoots are considerably lower than the actual 10 trees sampled during our study. For management purposes of *D. citri* on citrus, Dharajothi et al. (1989) recommended sample sizes of 40, 38, and 19 flush shoots per tree for eggs, nymphs and adults, respectively for a sampling plan that is based on 10 trees per orchard. Based on 40 trees per orchard, Sétamou et al. (2008) recommended optimum sample sizes of eight flush shoots per 1 and 10 trees would provide adequate density estimates of the three developmental stages of *D. citri* with a percentage relative precision of 25% required for field studies.

Table 2. Optimum number of citrus trees (t) and flush shoots (f) per tree required to achieve a given precision level for field estimation of *D. citri* population densities in a Malaysian citrus orchard.

Developmental Stage	**Sampling Unit**	**Standard Error of Mean, SE (mean)**		
		0.05	**0.10**	**0.25**
	Optimum no. of flushes per tress (f $_{opt}$)[a]			
Eggs	Trees	6	5	4
	Flushes	6	6	6
Nymphs	Trees	4	3	3
	Flushes	4	4	4
Adults	Trees	10	8	7
	Flushes	2	2	2

[a] f $_{opt}$ is calculated after Harcourt (1961)

CONCLUSION

The present research study illustrated that the spatial distribution of eggs and nymphs is a result of the movement patterns of gravid females in response to the oviposition sites. The dispersion indices were used to confirm the aggregated or contagious distribution pattern of *D. citri* population on the flushes within the tree and could be expressed by the negative binomial distribution. Increased population density in the field resulted in greater dispersion of population was the consequence of females' dispersal and their selection of oviposition sites. Because the exponential increase in dispersion can be predicted by means of the population density of immature stages, a sampling plan was developed from the relationship between the dispersion behaviour and population density rather than the relationship between economic damage and population density. This sampling method provides a viable alternative to the tedious enumerative sampling plan for growers to obtain a rapid estimate of citrus psyllid population level with minimal effort and allows control action to be based on infestation level rather than on a calendar or prophylactic schedule.

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