

SAMPLING OF *DIAPHORINA*
CITRI (HOMOPTERA: PSYLLIDAE) ON
 ORANGE JESSAMINE IN SOUTHERN FLORIDA

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ABSTRACT

Dispersion indices and related statistics of Asian citrus psyllid, *Diaphorina citri* Kuwayama, on orange jessamine [*Murraya paniculata* (L.) Jack] shoots in southern Florida from 1998 to 1999 were determined with 235 data sets and used to develop sampling plans. Three regression models, Taylor's power law, Iwao's patchiness regression, and $k = c + dm$ [$k = m^2 / (S^2 - m)$] (where k is the parameter for the negative binomial distribution) were used to analyze the data. Taylor's power law ($\alpha = 0.3407 \pm 0.03$, $b = 1.2971 \pm 0.03$, $r^2 = 0.88$) fit the data better than Iwao's model ($\alpha = -0.3217 \pm 0.12$, $\beta = 1.6979 \pm 0.06$, $r^2 = 0.76$). Taylor's b and Iwao's β were both significantly > 1 , indicating that *D. citri* populations were aggregated. Iwao's α was significantly < 0 , indicating that the basic distribution component of *D. citri* was the individual insect. The slope d (0.7489 ± 0.48) was indistinguishable from 0, indicating the existence of a common k (estimated as 1.2741). The incidence (P_i , proportion infested) and mean density (m) relationship was developed by negative binomial distribution (NBD) basis and Nachman's model [$\ln(m) = 0.2277 + 1.2444 \ln(-\ln(P_o))$] (where P_o = Proportion of uninfested sampling units in a sample). The NBD was appropriate for studying *D. citri* distribution based on comparison of NBD basis and Nachman's models. The relationship to determine sample sizes for fixed levels of precision and fixed-precision-level stop lines for sequential sampling was also developed.

Key Words: Asian citrus psyllid, Taylor's power Law, Iwao's patchiness regression, negative binomial distribution

RESUMEN

Índices de dispersión y estadísticas relacionadas con el psila de cítrico Asiático, *Diaphorina citri* Kuwayama, en brotes de *Muralla paniculata* (L.) Jack en el Sur de Florida entre 1998 y 1999 fueron determinados con 235 conjuntos de datos y usados para el desarrollo de planes de muestreo. Tres modelos de regresión, la ley de poder Taylor, la regresión Iwao, y $k = c + dm$ [$k = m^2 / (S^2 - m)$] (donde k es el parámetro para la distribución binomial negativa) fueron usados para analizar los datos. La ley de poder Taylor ($\alpha = 0,3407 \pm 0,12$, $b = 1,2971 \pm 0,03$, $r^2 = 0,88$) encaja los datos mejor que el modelo de Iwao ($\alpha = -0,3217 \pm 0,12$, $\beta = 1,6979 \pm 0,06$, $r^2 = 0,76$). La b de Taylor y el β de Iwao fueron ambos significativamente > 1 , indicando que poblaciones de *D. citri* fueron agregadas. El α fue significativamente < 0 , indicando que el componente básico de distribución de *D. citri* era el insecto individual. La pendiente d ($0,7489 \pm 0,48$) fue indistinguible de 0, indicando la existencia de una k en común (estimada a 1,2741). La relación entre incidencia (P_i , proporción infestada) y densidad promedio (m) fue desarrollada a base de la distribución negativa binomial (NBD) y el modelo de Nachman [$\ln(m) = 0,2277 + 1,2444 \ln(-\ln(P_o))$] (donde P_o = proporción de unidades de muestreo

sin infestar en una muestra). El NBD fue apropiado para estudiar la distribución de *D. citri* basado en la comparación entre la NBD y el modelo Nachman. La relación para determinar los tamaños de muestreo para niveles fijos de precisión y sus líneas que contienen este parámetro para muestreo secuencial también fueron desarrolladas.

Citrus is one of the most important economic crops in the U.S. with about 500,000 ha in citrus orchards mostly in California, Florida, Texas, and Arizona. In Florida alone, citrus encompasses 389,857 planted hectares with a total of 107 million trees in the 33 citrus producing counties. The annual earning on citrus is estimated at \$1.1 billion (Tsai 1998). Citrus greening disease or Huanglungbin is the most serious disease of citrus in the world (Aubert et al. 1996, Tsai et al. 1988). The Asian citrus psyllid, *Diaphorina citri* Kuwayama, is the most efficient vector of citrus greening bacterium, *Liberobacter asiaticum* Jagoueix, Bove & Garnier, throughout Asia and the Far East (Catling 1970, Pande 1971, Tsai et al. 1988). The combined presence of a psyllid vector and a greening agent has been the limiting factor in citrus production in these areas (Ke et al. 1988, Tsai et al. 1988). On June 3, 1998 the Asian citrus psyllid was first found in southern Florida, with the subsequent discovery of *D. citri* in Broward, Palm Beach, Martin, Dade, St. Lucie, Hendry, and Collier Counties in a 3-month period (Halbert et al. 1998). Given high reproductive potential of this vector during favorable conditions of weather and food availability (J. H. T., unpublished data), this pest is expected to spread throughout citrus producing area in Florida in 2-3 years. It poses a serious threat to other citrus producing states in the future. Based on our observations, this pest is most abundant on orange jessamine, *Murraya paniculata* (L.) Jack (J. H. T., unpublished data), which is widely planted as hedges in the urban landscape in southern Florida. It could serve as an alternate host for maintaining psyllid populations when young citrus shoots are not available.

Data on dispersion of pest populations is an important aspect of population biology because it is a result of the interaction between individuals of the species and their habitat (Sevacherian & Stern 1972). Knowledge of this dispersion 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 (Harcourt 1961, Iwao 1970, Sevacherian & Stern 1972, Taylor 1984), and the development of population models (Croft & Hoyt 1983). Methods that are commonly used to describe dispersion of arthropod populations have been summarized by Southwood (1978). Several estimates based on sample mean (m) and variance (S^2) are used as indices for aggregation (Lloyd 1967) and the dispersion parameter k for the negative binomial distribution (Southwood 1978). Moreover, these indices are often convertible from one to another. Sampling plans based on these descriptions of dispersion (Kuno 1969, Green 1970) reduce sampling effort and minimize variation of sampling precision (Hutchison et al. 1988, Kuno 1991, Trumble et al. 1989). Little is known about the dispersion of *D. citri* because of its new pest status in USA. To fill this void, we gathered data on the dispersion of *D. citri* adults on orange jessamine in southern Florida from 1998 to 1999. From this information, two incidence-density relationships, the optimal sample sizes for estimating density, and the sequential sampling plans suitable for intensive population research and pest surveys were developed.

MATERIALS AND METHODS

Sampling of Citrus Psyllid Population

A field survey for sampling populations of citrus psyllids was conducted from October 1998 to May 1999 in ten orange jessamine fields in Broward County, Florida. The plants were not sprayed with insecticides during the course of the study.

For the purpose of sampling, the field in each location was divided into five areas (10×2 m). At weekly intervals, one shoots (about 6-10 cm long) was selected at random from each square meter area by throwing a pointed object. Thus a total of 20 shoots were selected from each of the 5 areas on each sampling date. Numbers of citrus psyllid adults per shoot were counted and recorded.

Variance-Mean Relationships

The mean density (m) per shoot and variance (S^2) were calculated for shoots in each field per sampling date and related to each other using Taylor's power law (Taylor 1961, 1971, Taylor et al. 1978) and Iwao's patchiness regression (Lloyd 1967, Iwao 1968, Iwao & Kuno 1971).

Taylor's power law states that the variance (S^2) of a population is proportional to a fractional power of the arithmetic mean (m): $S^2 = am^b$. To estimate a and b , the values of $\ln(S^2)$ were regressed against those of $\ln(m)$ using the model

$$\ln(S^2) = \ln(a) + b \ln(m) \quad (1)$$

where the parameter a is a scaling factor related to sample size (Southwood 1978), the slope b is an index of aggregation which indicates a uniform, random and aggregated dispersion when $b < 1$, $b = 1$, $b > 1$, respectively.

Iwao's patchiness regression method quantifies the relationship between Lloyd's (1967) mean crowding index (m^*) and mean (m) by:

$$m^* = \alpha + \beta m \quad (2)$$

where m^* was determined as $[m + (S^2 / m - 1)]$ (Lloyd 1967). The intercept (α) is the index of basic contagion and the slope (β) is the density contagiousness coefficient interpreted in the same manner as b of Taylor's regression.

Estimation of Incidence

The relationship between the proportion of samples with one or more animals (the incidence, P_s) and the density of animals (m) per sample unit was developed using the following two methods.

One was developed by assuming that a negative binomial distribution (NBD) with variable k would describe the distribution of psyllids on the shoots. This assumption was later tested. The NBD-based relationship was chosen because of the close relationship between NBD and Taylor's power law (Binns 1986). Estimated S^2 was described as a function of m (Taylor 1961). With this relationship, k of the NBD can be

calculated as $[m^2 / (am^b - m)]$. The incidence is then one minus the zero term of the NBD (Wilson & Room 1983, Nyrop et al. 1989):

$$P_1 = 1 - 1 / [(1 + m/k)^k] \quad (3)$$

Another P_i and m relationship was developed using the model proposed by Nachman (1984). Because this model does not use a theoretical probability distribution as a basis, it was fit to the data to check the assumed applicability of the NBD (Nyrop et al. 1989). In Nachman's model, the proportion of sample units with no animal (P_0) is related to the mean density as:

$$P_0 = \exp(-\delta m^\gamma) \quad (4)$$

where δ and γ are parameters of the model. To fit the model to the data, the data were calculated based on the sampling dates and locations. The model is linearized with the mean density regressed on P_0 (Nyrop et al. 1989) as:

$$\ln(m) = A + B \ln[-\ln(P_0)] \quad (5)$$

To test the applicability of the negative binomial distribution, the proportion of sample units with no psyllids (P_0) was calculated for different means using the two incidence and mean relationships and compared by chi square test.

Estimation of Common k for the NBD

The estimates of the dispersion parameter k for the NBD, computed as $m^2 / (S^2 - m)$, were linearly regressed on m ,

$$k = c + dm \quad (6)$$

to test for the existence of a common k (k_c) for each of the data sets (Southwood 1978, Feng & Nowierski 1992). A d value significantly > 0 indicates the dependence of k on mean density. The variance and mean within each area where the variance exceeded the mean were used to estimate k_c for a negative binomial distribution (Fleischer et al. 1991). Estimates of k_c were made using Elliot's (1977) techniques, which estimates k_c by regressing $y' = (S^2 - m)$ on $x' = (m^2 - S^2 / n)$, and k_c was defined by $k_c = 1/\text{slope}$. An index for spatial aggregation of arthropod populations, $1/k$, which is equal to $m^* / m - 1$ (Southwood 1978) and is the same as Cassie's index C (Cassie 1962), was also employed to evaluate the dispersion patterns.

The general linear model procedure (GLM) of SAS (SAS Institute 1988) was used to estimate the linear regression. Student's t tests were used to determine if the slopes (b) of the regression lines were significantly > 1.0 (equations 1, 2) or 0 (equation 6) (Sokal & Rohlf 1981).

Sampling Plans

We determined the sample sizes for fixed levels of precision by substituting Taylor's variance-mean relationship into the usual expression for the standard error of the mean and rearranging:

$$n = am^{b-2} / D^2 \quad (7)$$

where n is the sample size and D is the required level of precision expressed as a proportion of the mean, and a and b are the coefficients from Taylor's power law (Pena & Duncan 1992, Walker & Allsopp 1993). We used two values of D , 0.10 and 0.25; the latter allows detection of doubling or halving of sample means (Southwood 1978), whereas the former would be useful in detecting smaller changes in ecological studies (Walker & Allsopp 1993).

The number of samples after which sampling can be terminated (T_n) for a constant precision, D , of the mean [$D = (S^2 / n)^{-2} / m$], was determined using the equation derived by Green (1970):

$$\log T_n = \frac{\log(D^2/a) + (b-1)}{b-2} \log n \quad (8)$$

where T_n is the stop line for sample size n , a and b are from Taylor's power law, and D is defined as above.

RESULTS AND DISCUSSION

The complete data set consisted of 235 psyllid samples from ten locations covering the period of October 1998 through March 1999. The mean density of *D. citri* in samples ranged from 0.1 to 8.5 adults per shoot. The highest number of psyllid adults on a shoot was 43. These 235 psyllid data sets were used for dispersion analysis.

Variance-Mean Relationships

The results of Iwao's regression of m^* on m and Taylor's power law analysis are listed in Table 1. Iwao's patchiness regression described well the relationship between mean crowding and density for *D. citri* (Table 1, Fig. 1). The constant α indicates the tendency to crowding (+ ve) or repulsion (- ve) defined as the 'Index of Basic Contagion' by Iwao (1970). For *D. citri*, the value of α was < 0 ($t = -2.546$, $df = 233$, $P < 0.05$), indicating that for adults the basic component of the population is a single individual. Estimate of β , the density contagiousness coefficient, was significantly > 1 ($t = 11.27$, $df = 233$, $P < 0.001$).

Taylor's power law provided a highly significant relationship between variance (S^2) and mean density (Table 1, Fig. 2). Taylor's intercept, $\ln(a)$, was significantly > 0 ($t = 11.57$, $df = 233$, $P < 0.001$; Table 1). Estimate of b was significantly > 1 ($t = 9.26$, $df = 233$, $P < 0.001$).

TABLE 1. PARAMETER ESTIMATIONS OF FOUR LINEAR MODELS FOR *D. CITRI* ON ORANGE JESSAMINE.

Model	Slope \pm SEM ¹	Intercept \pm SEM ²	N	r ²
Taylor's	1.2971 \pm 0.03***	0.3407 \pm 0.03***	235	0.8753
Iwao's	1.6979 \pm 0.06***	-0.3217 \pm 0.12*	235	0.7637
Nachman's	1.2444 \pm 0.06***	0.2277 \pm 0.04***	47	0.9189
Equation 6	0.7489 \pm 0.48	1.4216 \pm 0.99	235	0.0102

¹Table entries are significant at level of $P < 0.05$ (*) or 0.0001 (***) for $H_0: \pi = 1$ for Taylor's and Iwao's model ($t = [\text{slope} - 1] / \text{SE}_{\text{slope}}$, $df = N - 1$), and $H_0: \pi = 0$ for Nachman's model and equation 6 ($t = \text{Slope} / \text{SE}_{\text{slope}}$, $df = N - 1$).

²Table entries are significant at level of $P < 0.05$ (*) or 0.0001 (***) for $H_0: \pi = 0$.

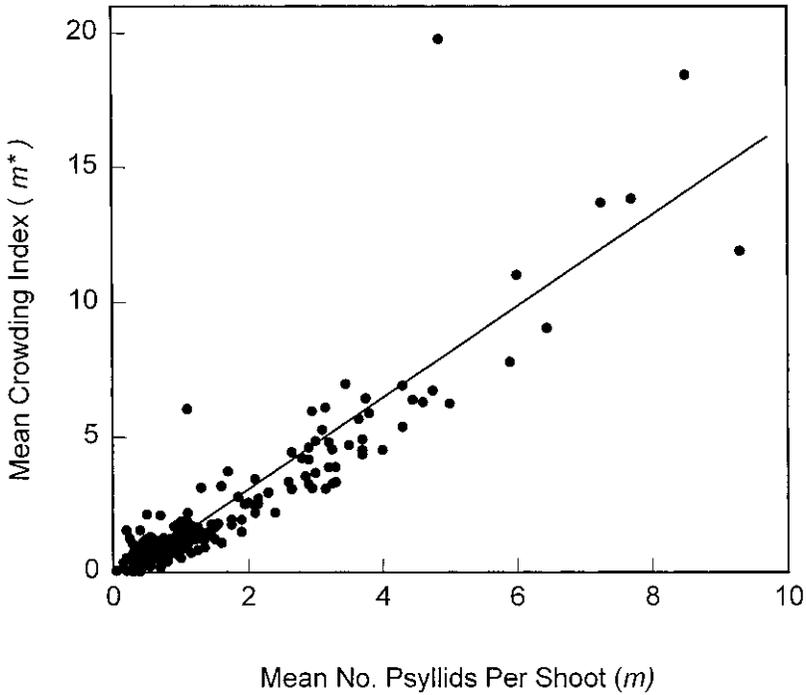


Fig. 1. Regression analysis of Iwao's mean crowding index (m^*) on mean density (m) for *D. citri* populations on orange jessamine ($m^* = -0.3217 + 1.6979m$, dots are estimated values based on observations).

Taylor's power law generally fit the data better, yielding higher value of r^2 (0.8753) than Iwao's model (0.7637). The aggregation indices (slopes, b and β) of Taylor's power law and Iwao's patchiness regression were all significantly > 1 ($P < 0.05$), indicating an aggregated dispersion distribution of *D. citri* on orange jessamine (Table 1). The causes of aggregation could be attributed to either active aggregation on the part of this psyllid (such as behavior and reproductive biology), or to some heterogeneity of the environment (such as microclimate, preferred part of plant, and natural enemies) (Southwood 1978). The similar observations were also reported by Van den Berg et al. (1991) for the citrus psylla (*Trioza erythrae* Del Guercio), and Tret'yakov (1984) for the apple psylla (*Cacopsylla mali* Schmidt). Van den Berg et al. (1991) reported that higher numbers of citrus psylla adults were apparently related to the prevailing wind direction. Catling (1970) stated the population fluctuations of psyllids were closely correlated with flushing rhythm and flush quality because eggs are laid exclusively on young flush points and nymphs develop on immature leaves. Although no data on *D. citri* is currently available for direct comparison, however, the observed values for β and b are similar to those for many moderately aggregated insects (Taylor 1961, 1971). Comparing to other aggregated Homopterans, the values of β and b for *D. citri* were lower than those for the citricola scale *Coccus pseudomag-*

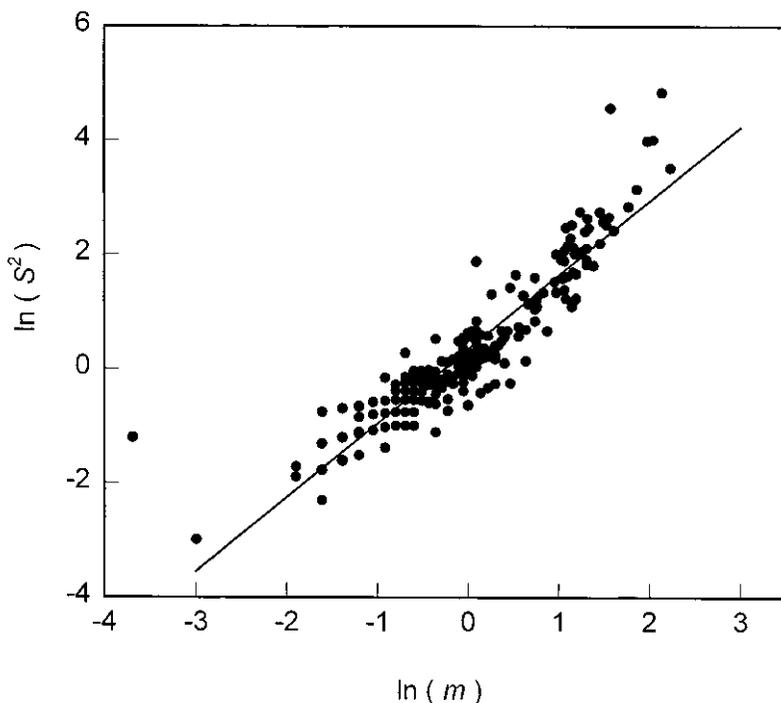


Fig. 2. Regression analysis of Taylor's power law ($\ln(S^2) = \ln(0.3407) + 1.2971 \ln(m)$) for *D. citri* populations on orange jessamine (dots are estimated values based on observations).

noliarum (Kuwana) on citrus (Trumble et al. 1995), the wooly apple aphid *Erisoma lanigerum* (Hausmann) on apple trees (Asante et al. 1993), and the Russian wheat aphid *Diuraphis noxia* (Mordvilko) in wheat field (Feng & Nowierski 1992), but was higher than those for the pea aphid *Acyrtosiphon pisum* (Harris) in alfalfa field (Hutchison et al. 1988).

Estimation of Incidence

Nachman's model gave an excellent fit to the relationship between the proportion of shoots without psyllids (P_0) and mean density (m) of *D. citri* (Table 1, Fig. 3). Using the parameter estimates (Table 1), the proportion of shoots with or without psyllids can be estimated from mean density with equation 5. For example, samples with mean densities of 0.5 and 2 psyllids per shoot correspond to ≈ 38 and $\approx 77\%$ infested shoots, respectively. This model could be used for grove managers who wish to develop the decision-making plans when economic threshold of *D. citri* becomes available in the future.

The values of P_0 for various means calculated according to Nachman's model and the NBD model are presented in Figure 4. Values of P_0 calculated with the NBD model

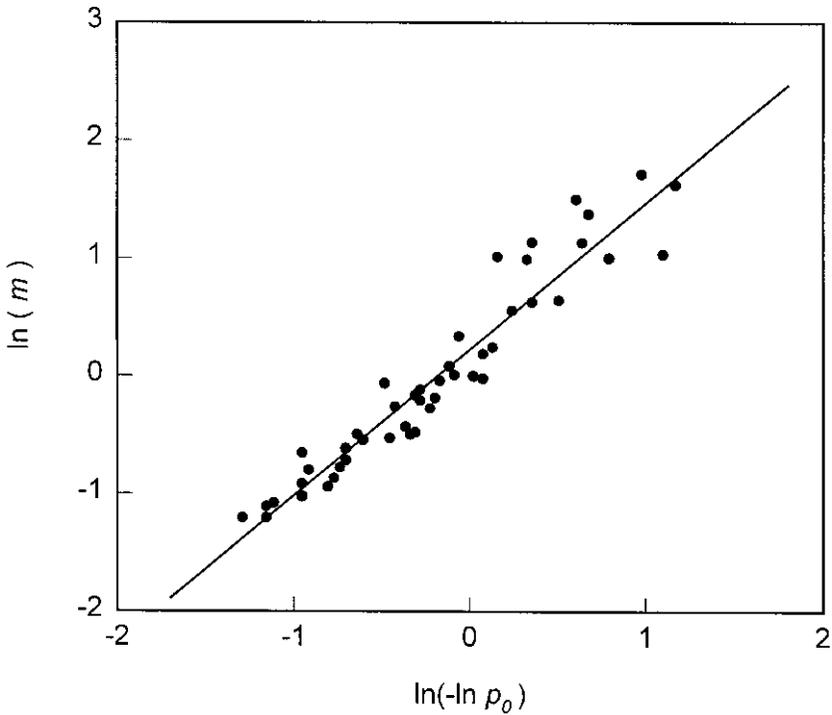


Fig. 3. Regression analysis of Nachman's model ($\ln(m) = 0.2277 + 1.2444 \ln(-\ln(P_0))$) for *D. citri* populations on orange jessamine (dots are estimated values based on observations).

were greater than those calculated by using Nachman's model at lower population density. On the contrary, at higher population density level, the values of P_0 become smaller. Generally, this deviation was small (<0.01) and the chi square test indicated that the two models were similar and interchangeable ($P > 0.05$).

Estimation of Common k for the NBD

Figure 5 gives an overall picture on the relationship between k and the mean number of psyllids from the 177 samples where the variance exceeded the mean. Regression of k on the mean density per shoot using all data was not significant ($F = 2.388$, $r^2 = 0.0102$, $P = 0.1236$). Moreover, the slope of regression (d) was not significantly greater than 0 ($t = 1.545$, $df = 233$, $P = 0.1236$). Independence of k with the mean density suggests the existence of common k for the NBD of the psyllid populations. The estimates of a common k was 1.2741 using Elliot's (1977) method.

Southwood (1978) states that changes in the density of an insect often lead to changes in the distribution. However, we did not detect apparent density-dependent distribution changes in the psyllid population (Table 1, Fig. 5). Similar results were reported by Feng & Nowierski (1992) for the summer populations of Russian wheat

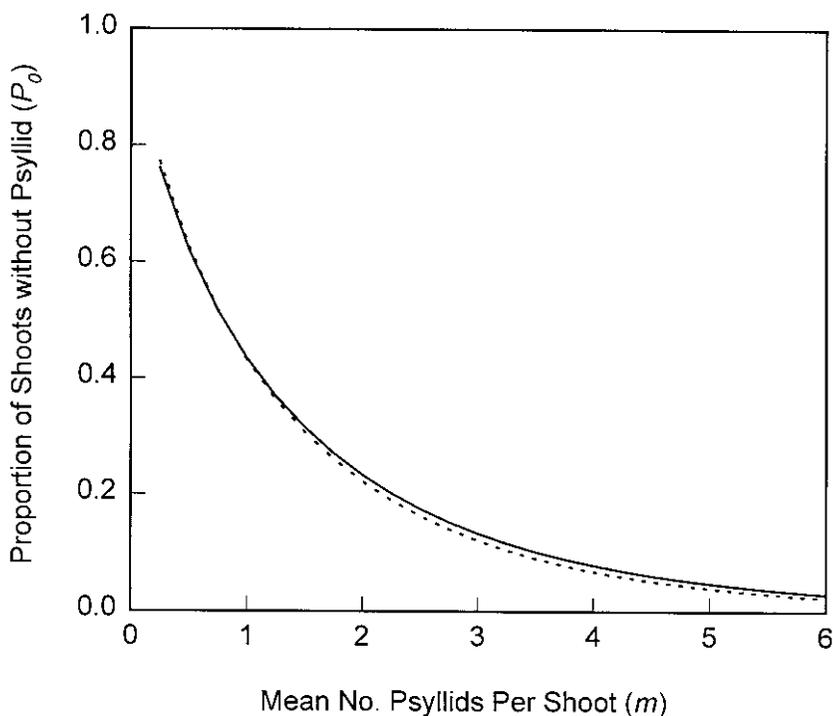


Fig. 4. Proportion of shoots without *D. citri* predicted from the density per shoot using NDB (dotted line) and Nachman's models (solid line).

aphid, *Diuraphis noxia* (Mordvilko), on winter wheat. The values of aggregation index ($1/k$) $<$, $=$, and $>$ 0 represent regularity, randomness, and aggregation of populations in spatial patterns, respectively (Cassie 1962, Southwood 1978). In our study, 177 sample observations were $>$ 0. This indicates that the populations of *D. citri* generally were highly aggregated on orange jessamine. This further supports the results obtained using Taylor's power law and Iwao's models. It should be noted that the values of $1/k$ of the remaining 58 observations ($m < 0.5$) were $<$ 0 which suggested a spatial distribution of regularity; which is consistent with the estimate of α ($<$ 0) by Iwao's regression (Table 1).

Sampling Plans

The relationships between mean psyllid density and required sample size for fixed precision levels of 10 and 25% are shown in Figure 6. The stop line of the fixed-precision-level of 25% of the mean for sequential sampling is presented in Figure 7. The stop line of the fixed-precision-level of 10% of the mean was not presented because of the requirement for extremely large samples from the field. Based on computer simulation, the performance of the sequential sampling procedures improved with in-

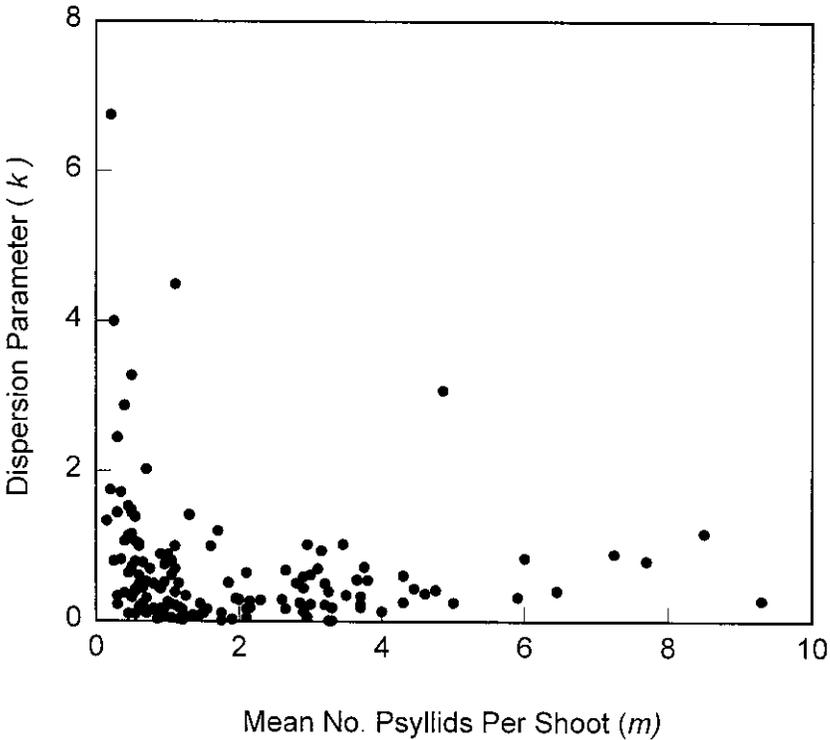


Fig. 5. Scatter plots of k for the NBD over mean (m) for *D. citri* populations on orange jessamine.

creasing psyllid density. Also because the variance-mean regression provided a good description of the data (Table 1), regression variability had only a minor effect at very low mean density.

Figures 6 and 7 show that these sampling plans required quite large sample sizes to obtain relatively precise density estimates. Although such precision in density estimates may be required for research purpose, it will probably not be of practical use in commercial citrus production.

A person sampling *D. citri* could use Figure 7 by plotting T_n (accumulated adults) and n (number of shoots sampled) after each sample was taken. When the plot falls above the line, sampling is stopped and the mean density (m) is within 25% of $m = T_n / n$. This sequential-count plan permits researchers and pest managers to describe the mean density more accurately than before. It may lead to a better determination of the economic threshold in the future.

Sampling small arthropods is operationally difficult and often time consuming. In this paper, we have developed a sequential sampling plan based on counts of psyllids which will be useful to anyone requiring accurate decisions based on mean numbers. As a way of easing this burden presence-absence, (binomial) sampling has been used in place of complete counts for estimating or classifying densities of

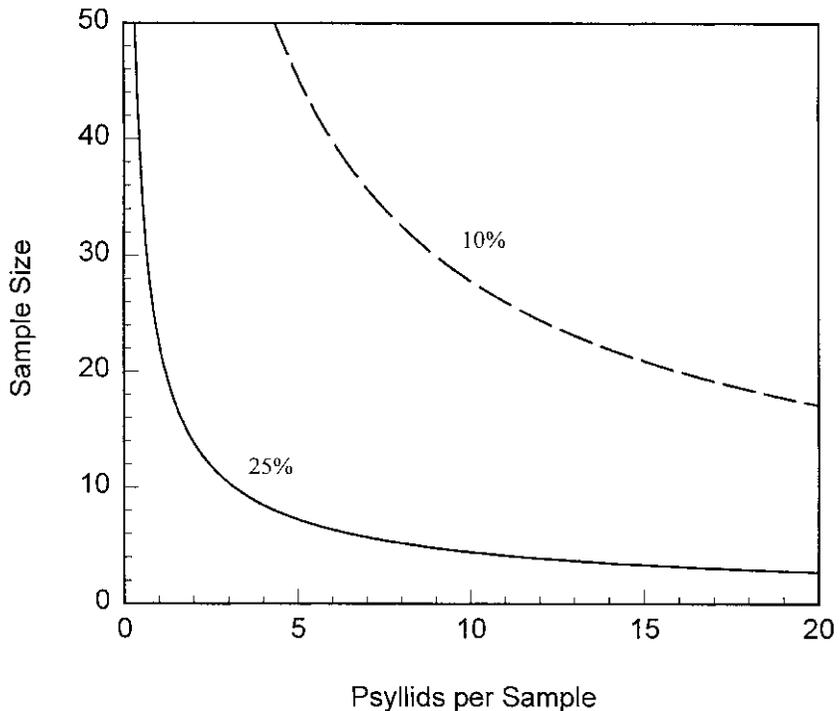


Fig. 6. Relationship between required sample size and mean density for achieving a fixed precision level of 10 and 25% for *D. citri* populations on orange jessamine.

these organisms (Nyrop et al. 1989). Binomial sampling is appealing because it is often easier to determine whether one or more animals reside on a sample unit than it is to make a complete count. It is usually faster and therefore less costly on a per-sample-unit basis. In addition, there are organisms, such as psyllids, for which binomial sampling is the only feasible field-sampling method. Sequential sampling plans can result in saving up to 75% of the time compared with fixed sample size procedures having comparable error rates (Harcourt 1966). When sampling is used for decision making, it often suffices to classify a population density as opposed to obtaining an estimate. Many sequential sampling programs are based on this premise. However, due to the new pest status and vector ability of *D. citri*, further research on the biology, ecology, disease transmission and integrated management are needed.

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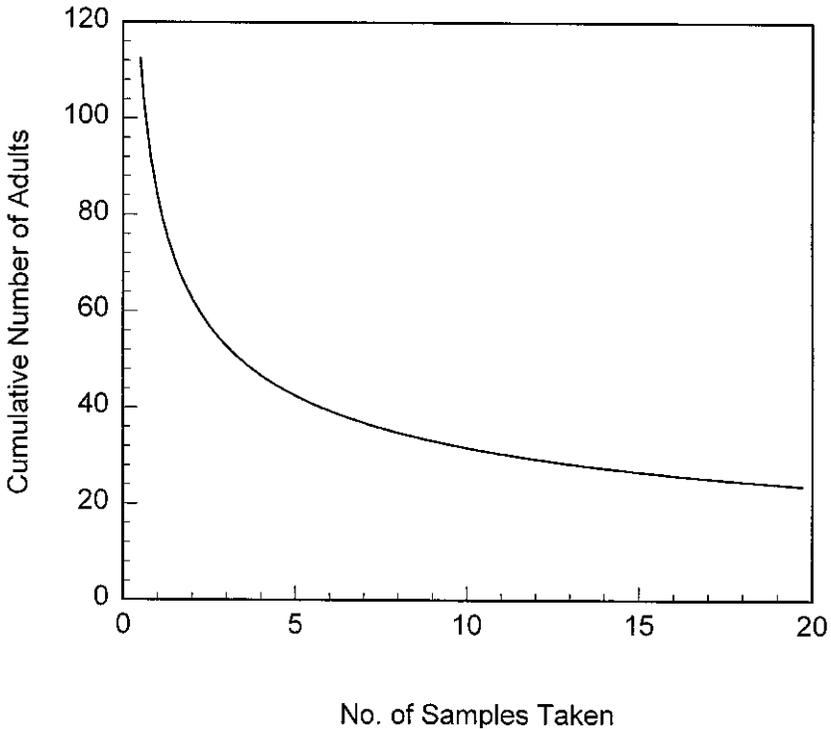


Fig. 7. Sequential count plan for *D. citri* populations on orange jessamine, showing the stop line at a fixed precision level of 25%.

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