Dispersion Patterns and Sampling Plans for *Diaphorina citri* (Hemiptera: Psyllidae) in Citrus

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ABSTRACT The abundance and spatial dispersion of *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) were studied in 34 grapefruit (*Citrus paradisi* Macfad.) and six sweet orange [*Citrus sinensis* (L.) Osbeck] orchards from March to August 2006 when the pest is more abundant in southern Texas. Although flush shoot infestation levels did not vary with host plant species, densities of *D. citri* eggs, nymphs, and adults were significantly higher on sweet orange than on grapefruit. *D. citri* immatures also were found in significantly higher numbers in the southeastern quadrant of trees than other parts of the canopy. The spatial distribution of *D. citri* nymphs and adults was analyzed using Iowa's patchiness regression and Taylor's power law. Taylor's power law fitted the data better than Iowa's model. Based on both regression models, the field dispersion patterns of *D. citri* nymphs and adults were aggregated among flush shoots in individual trees as indicated by the regression slopes that were significantly >1. For the average density of each life stage obtained during our surveys, the minimum number of flush shoots per tree needed to estimate *D. citri* densities varied from eight for eggs to four flush shoots for adults. Projections indicated that a sampling plan consisting of 10 trees and eight flush shoots per tree would provide density estimates of the three developmental stages of *D. citri* acceptable enough for population studies and management decisions. A presence–absence sampling plan with a fixed precision level was developed and can be used to provide a quick estimation of *D. citri* populations in citrus orchards.

KEY WORDS *Diaphorina citri*, dispersion, sampling units, sample size

The psyllid *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), one of the vectors of Huanglongbing or citrus greening disease, has invaded two areas in the United States over the past decade, Florida and Texas (French et al. 2001, Halbert and Manjunath 2004). Citrus greening disease was found in Florida during 2005 (Halbert 2005), but it has not yet been reported in Texas. Citrus greening is probably the most important disease of citrus in the world (Aubert 1990, da Graça 1991, Bové 2006). The presence of the disease in Florida is threatening the profitability and sustainability of this state's citrus industry. Citrus is an important agricultural industry in the United States, with ≈500,000 ha in citrus orchards primarily located in the states of Florida, California, Texas, and Arizona, and an annual on-tree value of $1.3 billion (USDA–NASS 2005).

In the absence of the bacterial causal agents of citrus greening disease, *D. citri* does not pose a major threat except for nursery and newly established orchard plants where direct feeding damage by the pest can cause reduced flush growth (Michaud 2004) and sometimes young shoot death. However, the recent discovery of citrus greening disease and its phloem-limited, nonculturable causal agent *Candidatus* Liberibacter asiaticus in Florida (Halbert 2005) has dramatically increased the pest status of *D. citri* and the threat posed by this disease to the U.S. citrus industry. In a recent statewide survey in Texas, da Graça et al. (2006) reported *D. citri* in 40% of the counties where citrus was found throughout the state, with all three counties where commercial citrus is grown being infested. The rapid spread of *D. citri* in Texas is not surprising given its high reproductive potential (Mead 1976, Liu and Tsai 2000), the favorable climatic conditions and availability of its host plants in the state. *D. citri* infests leaves and flush shoots of its host plants, all of which are restricted to the Rutaceae family, including *Citrus* spp. (Aubert 1990). Eggs are laid exclusively on new flush shoots (Hall and Albrigo 2007) of the host plant, and both nymphs and adults preferentially feed on these flushes.

Knowledge of the spatial distribution of insects is important in understanding the biology and ecology of

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a species. In addition, information on the spatial distribution of an insect pest is the basis for the development of a reliable sampling plan (Binns and Nyrop 1992), which is required for the implementation of a successful integrated pest management (IPM) program. Sampling is performed to quantify pest densities, to define treatment thresholds, and to forecast pest populations. Researchers also need reliable sampling procedures to describe pest populations.

Dispersion patterns of and sampling plans for *D. citri* have been investigated in the United States in plantings of orange jasmine, *Murraya paniculata* (L.) Jack (Tsai et al. 2000). No research information on sampling citrus flush in the United States was available. Dharajothi et al. (1989) studied sampling for *D. citri* in citrus in India by using a sample unit of one new flush shoot (4–5 cm in length). Eggs and nymphs were found to follow an aggregated dispersion among sampling units per tree, whereas adults were found to more often follow a random dispersion (Dharajothi et al. 1989). These researchers projected that 40, 38, or 19 sample units per tree for eggs, nymphs or adults would provide a sufficient level of precision for a mean density prediction. Due to 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) relative to *D. citri* infestations in the United States is not known.

The aim of this research was to study the population densities and spatial distribution patterns of *D. citri* on citrus flush and to determine the number of flush samples needed to make reliable estimates of its population densities on citrus. This information is required for the development of management decisions of the pest in citrus orchards.

Materials and Methods

Data Collection. Thirty-four commercial grapefruit, *Citrus paradisi* Macfad., and six sweet orange, *C. sinensis* (L.) Osbeck, citrus orchards were sampled for all development stages of *D. citri* from March to August 2006 in southern Texas. These orchards were located in the Texas citrus belt in the three southern most counties (Hidalgo, Cameron, and Willacy counties) of the Lower Rio Grande Valley of Texas, where ≈11,000 ha of commercial citrus are grown in an area extending ≈50 km from north to south and ≈ 80 km from east to west. The sample of citrus orchards was assumed to be representative of the whole survey area. Each orchard sampled had a minimum acreage of 2 ha (range, of 2–24 ha and an average of 5.2 ha). Selected orchards varied in age but each had profuse new flush growth at the time of sampling. Each orchard was sampled once during the survey. In all of the orchards, cultural practices included flood irrigation and weed management with herbicides, except for one sweet orange and two grapefruit orchards that were organically grown. The selected orchards had not received any pesticide application for a minimum of 30 d before the surveys. In total, 10 trees were randomly sampled per orchard; trees were selected to be representative of the whole orchard. Subsequently, the canopy of the selected trees was divided into four quadrants along the four cardinal points. Five new flushes were randomly sampled in a nondestructive manner for each of the southeast (SE), southwest (SW), northeast (NE) and northwest (NW) canopy quadrants, for a total of 20 flushes per tree. First, flushes were carefully examined and the number of *D. citri* adults per flush was counted and recorded. Using a 10× hand held lens, nymphs and eggs also were counted in situ and recorded per flush. No attempt was made to distinguish between the five instars of the psyllid.

A flush shoot was recorded as infested whenever at least one of any developmental stage of *D. citri* was present. The percentage of infested flushes was calculated per tree. The mean number (m) of *D. citri* eggs, nymphs or adults and the related variances (s^2) per flush per tree were calculated for each orchard.

A nested analysis of variance (ANOVA) (PROC NESTED, SAS Institute 1999) (Zar 1999) was conducted to determine the variance components of each hierarchical level of the sampling plan (flushes nested within canopy quadrant nested in trees nested in orchard nested in host plant type) and their relative contribution to the total variance in psyllid infestation and densities on flush shoots. Because different numbers of orchards were sampled for each of the variety and thus the design was unbalanced, PROC NESTED provided only unbiased estimates of variance components. To determine the significant level of each nested factor, a mixed model analysis with the TYPE 3 estimation of mean square was used (PROC MIXED, SAS Institute 1999). Subsequently, the Ryan–Einot–Gabriel–Welsh (REGWQ) test was used to discriminate between means of fixed factors, i.e., host plant and quadrant (Westfall et al. 2003). All *D. citri* counts were log $(x + 1)$-transformed, and percentage of flush infestation per tree was arcsine transformed before analysis to correct for nonadditivity and non-normality, respectively.

Within-Tree Dispersion Analysis. Spatial distribution patterns of the life stages of *D. citri* among flushes per tree were determined using the Iowa's patchiness regression (1968) and Taylor's power law (Taylor 1961) for each of the two citrus types, grapefruit of sweet orange. Taylor's power law model expresses variance–mean relationships and provides a quantitative analysis of the index of aggregation in terms of some ecologically meaningful parameters (Kuno 1991).

Iowa's patchiness regression quantifies the relationship between the mean crowding index M and the sample mean m as follows:

$$M = \alpha + \beta m, \qquad [1]$$

where $M = m + [(s^2/m) - 1]$. The intercept α is an index of basic contagion and the slope β is a density contagiousness coefficient which describes the distribution of individuals in their habitats (Iowa 1970, Taylor 1984), with $0 < \beta < 1$, $\beta = 1$, and $\beta > 1$, showing a uniform, random and aggregated dispersion, respectively.

Taylor's power law relates the variance to the mean density through:

$$s^2 = am^b. \quad [2]$$

Values of the coefficients a and b were estimated using linear least square regression (PROC GLM, SAS Institute 1999) of log-transformed variance $[\ln(s^2)]$ on log-transformed mean density $[\ln(m)]$ for all pairs of means and variances per flush per tree from all citrus trees sampled using the model $\ln(s^2) = \ln(a) + b\ln(m)$. The parameter a is largely a scaling factor related to sample size with no ecological meaning (Southwood 1978), and the slope b is an intrinsic property of the species that describes the degree of aggregation of its population in a particular environment and a given time, with a constant graduation from regular ($b < 1$), through random ($b = 1$), to aggregated ($b > 1$) (Taylor 1961).

The appropriateness of the two regression models for describing the dispersion patterns of *D. citri* was evaluated by the criterion of Downing (1986), which compares the explained variances R^2 and the standard error of the slope. Appropriateness is indicated by a regression with an R^2 value >0.80 and a $SE_b/b < 0.20$, where SE_b is the standard error of the slope.

The dispersion index (b) and the density contagiousness coefficient (β) were tested for departure from 1 (randomness) by using a two-tailed t-test ($t = [\text{slope} - 1]/SE_b$), with df = $n - 1$ and $P = 0.05$) (Zar 1999). In addition, the heterogeneity of the dispersion indices between grapefruit and sweet orange, and between the different stages was tested by a parallel analysis the PROC MIXED (SAS Institute 1999) (Littell et al. 1996).

Determination of Optimum Sample Size. Because a two-level sampling method was used, i.e., sampling of trees and of flush shoots within trees, the precision of the overall mean will depend on the variance between-tree samples (σ_T^2) and the variance between flush shoots within tree samples (σ_u^2), and also on the costs of sampling a flush within the same tree (C_u) or moving to another tree and sampling within it (C_T). The two levels of our sampling scheme were characterized as follows: t, the number of trees sampled per orchard ($t = 10$) and u, the number of flushes sampled per tree ($u = 20$). Considering the budget constraint or cost structure, it was found that it took between 2 and 5 min to select a tree on a random basis in the orchards, with a mean time of 3 min (or 0.05 h). The time to collect and evaluate a flush from each canopy was between 5 s to 60 s. A mean figure of 30 s was considered reasonable for our surveys. On this basis, the optimum number of secondary sampling units per primary unit or number of flushes per tree to minimize sampling variance for any given development stage of *D. citri* is given by Harcourt (1961) as follows:

$$u_{opt} = \sqrt{[(C_T/C_u)/(\sigma_T^2/\sigma_u^2)]} \quad [3]$$

where C_T is the basic cost per tree (i.e., 3 min), and C_u is the additional cost for each secondary unit sampled, i.e., the time spent to sample and evaluate each flush or 30 s in our surveys. σ_T^2 and σ_u^2 are respectively the between-tree and between-flushes within-tree variance components associated to the mean number of *D. citri* eggs, nymphs, or adults. These variance components were obtained by performing a nested ANOVA by using PROC NESTED of SAS (SAS Institute 1999).

Because a nested design was used in our surveys—samples of trees and of flushes within trees—the sample variance of the mean of any developmental stage is determined after White (1978) as follows:

$$V(mean) = \frac{\sigma_u^2}{ut} + \frac{\sigma_T^2}{t} \quad [4]$$

where u and m are the number of flushes and number of trees, respectively, per sample, and σ_T^2 and σ_u^2 the variance components as previously defined. The typical standard error E of mean is the square root of sample variance; generally E is defined as a decimal of the mean (i.e., for a standard error of ±5%, then $E = 0.05$). It was then possible to select the number of trees (t), which must be sampled for each developmental stage to obtain any desired approximate standard error of the sample mean after Southwood and Henderson (2000) as follows:

$$t = \frac{\left(\sigma_T^2 + \frac{\sigma_u^2}{u}\right)}{(\bar{x} + E)^2} \quad [5]$$

where $\bar{x}$ is the sample mean. The required number of trees for each developmental stage of *D. citri* was determined for two precision levels (E = 10% and 25%); 10% allows detection of smaller changes in populations and may be required for some research purposes, whereas 25% permits the detection of doubling or halving of sampling means and is usually applied for general estimates of insect populations (Southwood and Henderson 2000). Also, given the estimated variance components in our survey, the error and the sampling cost could be determined for various combinations of number of trees t (primary units) and flushes per tree u (secondary units).

Mean–Incidence Relationship and Binomial Sampling. The relationship between the proportions of pest-infested flushes (P_I) and the mean density of psyllids per flush (m) for each tree was studied using two methods. In the first method, the binomial model developed by Wilson and Room (1983) uses parameters from Taylor's power law to describe this relationship as follows:

$$P_1 = 1 - e^{-m\ln\left(\frac{am^{(b-1)}}{am^{(b-1)}-1}\right)} \quad [6]$$

The other method used was the empirical formula proposed by Kono and Sugino (1958) and Nachman (1984), which estimates the mean density of pest (m) from the proportion of sampling units infested (P_I) as follows:

$$\ln(m) = a' + b'\ln(-\ln[1 - P_i]) \quad [7]$$

where a' and b' are parameters of the regression. The fit of the model was assessed using the explained variance of the regression. All regressions were performed using the PROC REG procedure (SAS Institute 1999).

Table 1. Nested analysis of variance[a] of *D. citri* infestation levels and densities as affected by citrus host plant, orchard, tree, and canopy quadrant in the Lower Rio Grande Valley of Texas, 2006

Source of variation	Numerator df	Error df	*F*	*P*	% variance component
		% flush shoot infested by *D. citri*			
Host plant	1	31.32	0.94	0.34	0
Orchard	32	310.22	4.53	<0.001	12.53
Tree	306	1260	2.51	<0.001	21.21
Error	1,260				66.25
		No. *D. citri* eggs per flush shoot			
Host plant	1	30.96	5.26	0.03	0.32
Orchard	32	308.3	1.62	0.02	1.03
Tree	306	7,657	4.59	<0.001	11.20
Canopy quadrant	3	7,657	8.20	<0.001	8.34
Error	7,657				79.11
		No. *D. citri* nymphs per flush shoot			
Host plant	1	31.70	1.73	0.20	1.05
Orchard	32	308.4	4.98	<0.001	6.10
Tree	306	7657	4.46	<0.001	8.33
Canopy quadrant	3	7657	14.08	<0.001	16.11
Error	7,657				68.40
		No. *D. citri* adults per flush shoot			
Host plant	1	31.49	13.81	0.0008	6.41
Orchard	32	306.97	1.40	0.08	1.22
Tree	306	7657	10.92	<0.001	25.85
Canopy quadrant	3	7657	0.87	0.45	7.07
Error	7,657				59.46

[a] *F* values are obtained using the type 3 method of Proc Mixed, whereas variance components are unbiased estimates obtained using Proc Nested of SAS.

Results and Discussion

***D. citri* Infestations on Citrus.** There were no significant differences in flush shoot infestation levels (percentages) between grapefruit and sweet orange, but the host plant type significantly affected densities of psyllid eggs, nymphs, and adults per flush (Table 1). Nested analyses of variance indicated that *D. citri* immature densities varied significantly within and among trees, differed significantly among canopy quadrants and between orchards. *D. citri* flush shoot infestation levels significantly varied between trees and orchards and not among canopy quadrants. The sum of the 1) between-trees variance (or primary sampling unit variance component) and the 2) between-flushes within-trees variation (or secondary sampling unit variance component) accounted for ≥77% of the total variation associated with *D. citri* infestations and densities, but the between-flushes within-trees variance was the major contributor with a percentage of variance component of ≥60%. The ratios of the between flushes within trees and between trees variance components were 7:1 for eggs, 8:1 for nymphs, and 2:1 for adults, and they were was significantly lower for adults than immature stages ($G = 12.70$, df = 2, $P < 0.01$; log-likelihood test). This difference in ratios of variance components between immature and adult stages may be explained by the limited movement of immatures contrasting with the spreading of adults from their sites of emergence in search for mates and feeding sites. Contribution of host plant type and orchard to the total variance component were minimal.

The percentage of flush shoots infested by *D. citri* ranged from 0 to 80 on grapefruit and from 0 to 90% on sweet orange, with mean values of 3.6 and 5.8%, respectively (Table 2). Mean densities of *D. citri* developmental stages were significantly higher on sweet orange than grapefruit. The mean number of 0.24 adult psyllids per flush shoot per tree recorded on sweet orange was similar to that reported on untreated *C.*

Table 2. *D. citri* infestation levels[a] on grapefruit and sweet orange flush shoots selected from different compass quadrants of the plant canopy in the Lower Rio Grande Valley of Texas, 2006

Host plant	Canopy quadrant[b]				Overall[c]
	NE	NW	SE	SW	
	% flush shoot infested by *D. citri*				
Grapefruit	2.6b	2.7b	5.4a	3.9ab	3.6A
Sweet orange	4.7a	6.0a	6.7a	6.0a	5.8A
	No. *D. citri* eggs per flush shoot				
Grapefruit	0.12ab	0.04b	0.16a	0.05b	0.09B
Sweet orange	0.19a	0.03b	0.19a	0.23a	0.16A
	No. *D. citri* nymphs per flush shoot				
Grapefruit	0.31b	0.31b	0.46a	0.28b	0.34B
Sweet orange	1.39ab	0.31b	0.81ab	2.14a	1.16A
	No. *D. citri* adults per flush shoot				
Grapefruit	0.02a	0.02a	0.03a	0.04a	0.03B
Sweet orange	0.44a	0.20a	0.17a	0.18a	0.24A

[a] Analyses were performed on $\log(x + 1)$- of $\arcsin(\sqrt{x})$-transformed data but raw means are shown.

[b] Within each host plant species, means followed by the same lowercase letter are not significantly different (REGWQ test).

[c] Host plant overall means followed by different capital letters are significantly different using the *t*-test.

Table 3. Dispersion patterns of *D. citri* eggs, adult, and nymph counts on grapefruit and sweet orange flush shoots for individual trees as determined by Iowa patchiness regression and Taylor power law in the Lower Rio Grande Valley of Texas, 2006

Host plant	*D. citri* stage	*n*	Intercept	Slope	SE_b	r^2	*P*	Test for H_0: slope = 1	
								t	*P*
				Iowa's patchiness regression					
Grapefruit	Eggs	73	1.26^{ns}	12.39	0.77	0.78	<0.0001	14.75	<0.0001
	Nymphs	218	2.94**	5.16	0.27	0.63	<0.0001	15.58	<0.0001
	Adults	133	−0.38**	7.33	0.24	0.87	<0.0001	30.19	<0.0001
Sweet orange	Eggs	13	9.87*	1.35^{ns}	1.34	0.08	0.33	0.26	0.80
	Nymphs	32	7.61^{ns}	6.48	1.37	0.43	<0.0001	4.00	0.0004
	Adults	26	1.43*	1.45	0.34	0.43	0.0003	1.32	0.20
				Taylor's power law					
Grapefruit	Eggs	73	2.61**	1.81	0.04	0.96	<0.0001	19.02	<0.0001
	Nymphs	218	8.91**	1.72	0.02	0.96	<0.0001	31.32	<0.0001
	Adults	133	3.31**	1.40	0.04	0.92	<0.0001	11.09	<0.0001
Sweet orange	Eggs	13	2.0**	1.04	0.24	0.61	0.002	0.13	0.93
	Nymphs	32	13.80**	1.76	0.04	0.98	<0.0001	17.13	<0.0001
	Adults	26	3.80**	1.33	0.10	0.87	<0.0001	3.16	0.004
Pooled host plants	Eggs	86	2.36**	1.58	0.06	0.88	<0.0001	8.95	<0.0001
	Nymphs	250	2.26**	1.73	0.02	0.96	<0.0001	34.76	<0.0001
	Adults	159	1.20**	1.38	0.03	0.90	<0.0001	10.34	<0.0001

n is the number of data points used for estimating the regression parameters; intercepts are *a* and α for Taylor's power law and Iowa'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 is regression coefficient as defined in equations 1 and 2; *P* is the significance level of the *t*-value.

sinensis'Madame Vinous' sweet orange in Florida (Hall et al. 2007), and it was eight-fold higher than that recorded on grapefruit (Table 2). Although significantly more *D. citri* eggs and nymphs were recorded on sweet orange than grapefruit, their mean numbers in our surveys were lower than those reported by Hall et al. (2007) in Florida. The higher densities of *D. citri* on sweet orange than grapefruit may be due to higher oviposition preferences and/or higher immature survival on the former host plant. *D. citri* is known to exhibit host plant preferences. The African citrus psyllid, *Trioza erytreae* Del Guercio, also preferentially selects young leaves of lemon (*Citrus lemon* L.) over leaves of other host plants for oviposition and feeding (Moran and Buchan 1975). The reasons of the higher abundance of *D. citri* on sweet orange relative to grapefruit observed in the current study are not fully understood but might be related to the availability of young flush shoots on which *D. citri* develops on its host plants. Because psyllids reproduce almost exclusively on young flush shoots, Catling (1969) stated that their population fluctuations were positively correlated with flushing rhythms and flush quality. In Florida, *D. citri* population levels were positively related to the availability of new shoot flushes (Tsai et al. 2002, Hall et al. 2007). Although no information is recorded on flush shoot densities and developmental stages during our surveys, empirical observations in south Texas suggest that sweet orange produces more profuse flush shoots than grapefruit. Hall and Albrigo (2007) also reported two major flushing peaks on 'Temple' sweet orange compared with one on 'Marsh' grapefruit from February to May in Florida. In addition, sweet orange flush shoots remain juvenile for a longer period, whereas grapefruit flush shoots mature very quickly (J. E. Fucik, personal communication). Seasonal flushing patterns and abundance on citrus vary with region, variety, tree age and heath, and the environment (Knapp et al. 1995, Hall and Albrigo 2007). In south Texas, the first two major flushing periods of mature trees are observed between March and July and our field surveys were conducted during these major flushing periods. Moreover, only orchards with young flush shoots were sampled, thus suggesting that the differential abundance of *D. citri* between grapefruit and sweet orange may be a result of host plant characteristics.

Densities of *D. citri* immatures varied significantly among canopy quadrants (Table 1). In grapefruit, nymphs were most abundant on flush shoots in the southeastern quadrant, but no significant differences were observed for the mean number of adult psyllids per flush between the four quadrants (Table 2). A possible explanation may be the way flush growth patterns react to changing light conditions. At our latitude in southern Texas, the south side of trees in east-to-west–oriented rows are exposed to full sunlight all day, whereas the north side is shaded (List 1971). This differential exposure to sunlight intensity and direction in the spring would induce more vigorous and compact flush shoot growth in the south side of the tree canopy than the north side (Fucik 1982), thus leading to higher *D. citri* abundance and infestation on the south half of the canopy at the time the surveys were conducted. Nonrandom distribution of the psyllid *Cardiaspina densitexta* Taylor between canopy sectors on pink gum (*Eucalyptus fasciculosa* F.v.M.) also was reported in Australia (White 1978). Similarly, Dharajothi et al. (1989) did not find significant differences in the mean population density of *D. citri* among the four canopy directions of citrus trees in India.

Within-Tree Dispersion Analysis. Dispersion indices calculated using Iowa's patchiness regression and Taylor's power law are presented in Table 3. A positive

and significant 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. The intercept of the Iowa's patchiness regression was negative for adults on grapefruit ($\alpha = -0.38$, $t = 4.77$, $P < 0.0001$) and was equal to one for sweet orange ($\alpha = 1.43$; Table 3). The slope of the regressions or the density contagiousness coefficients (Iowa 1970) were positive and significantly >1 for all three developmental stages on grapefruit and for nymphs on sweet orange (Table 3), suggesting a contagious distribution of all *D. citri* populations on grapefruit and of nymphs on sweet orange. Because the slope of the regression for each developmental stage significantly varied with host plant ($P < 0.0001$) as determined by parallel line analysis of PROC MIXED of SAS, no attempt was made to determine pooled Iowa's patchiness regression coefficients across host plants.

Taylor's power law regression showed highly significant positive relationships between the ln(variance) and ln(mean) of *D. citri* adults, nymphs, or eggs per flush per tree in both grapefruit and sweet orange. In contrast to Iowa's patchiness regression which showed a poor fit with low R^2 values for some models (Table 3), the Taylor's power law model was appropriate and provided an adequate description of variance–mean relationships of all *D. citri* developmental stages on both host plants. The data quality was appropriate according to the criteria of Downing (1986) criteria of high explained variance ($R^2 > 0.80$) and low ratio of $SE_b/b < 0.20$, except for eggs on sweet orange. The lower fit of Taylor's power law regression for eggs on sweet orange may be due to few data points available (Table 3). The parallel line analysis also revealed that the degrees of aggregation on grapefruit and sweet orange were similar for adults ($F = 0.88$; df = 1, 155; $P = 0.35$) and nymphs ($F = 0.67$; df = 1, 246; $P = 0.41$); thus, pooled Taylor's power law regression coefficients were determined for each of these developmental stages across host plants (Table 3; Fig. 1). Although the degree of aggregation of *D. citri* eggs significantly varied with host plant ($F = 37.49$; df = 1, 82; $P < 0.0001$), no strong statistical inference could made because of the low number of data points. For simplicity, data of both host plants were pooled and common regression coefficients for the distribution of *D. citri* eggs also were determined (Table 3; Fig. 1).

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 both grapefruit and sweet orange (Table 3; Fig. 1). However, the parallel line analysis indicated that the slopes for the three developmental stages of *D. citri* were unequal as shown by the significant ln(mean) × developmental stage interaction ($F = 63.30$; df = 3, 446; $P < 0.0001$) (Fig. 1). Eggs and nymphs had a similar contagion, which is stronger than that of adults.

Both regression methods showed that *D. citri* populations have a contagious distribution but the negative (on grapefruit) and unity (on sweet orange) index of basic contagion suggests that *D. citri* adults do not form colonies on grapefruit and sweet orange. Therefore, a single individual is the basic component of *D. citri* population on citrus as reported by Tsai et al. (2000) on orange jasmine. Similar observations were made for *D. citri* adults on acid lime (Dharajothi et al. 1989) and for *Psylla pyricola* Förster (Burts and Brunner 1981). Because the mean crowding index is the mean number of other individuals per sample unit per individual and thus expresses the interaction between individuals (Southwood and Henderson 2000), the lack of colonies of adult *D. citri* may be a means by which competition is limited, specifically for the progeny of this highly reproductive species. The Taylor's power law aggregation index for *D. citri* adults on citrus (Fig. 1) was significantly higher than the value of 1.30 reported by Tsai et al. (2000) on orange jasmine ($t = 2.32$, df = 1,157; $P = 0.03$), suggesting that *D. citri* are more clumped on the former host plant than the latter. The aggregated behavior of *D. citri* on citrus flush can be attributed to their preference for new flushes for feeding, oviposition, and development. *D. citri* eggs are laid in groups or clusters on new flush

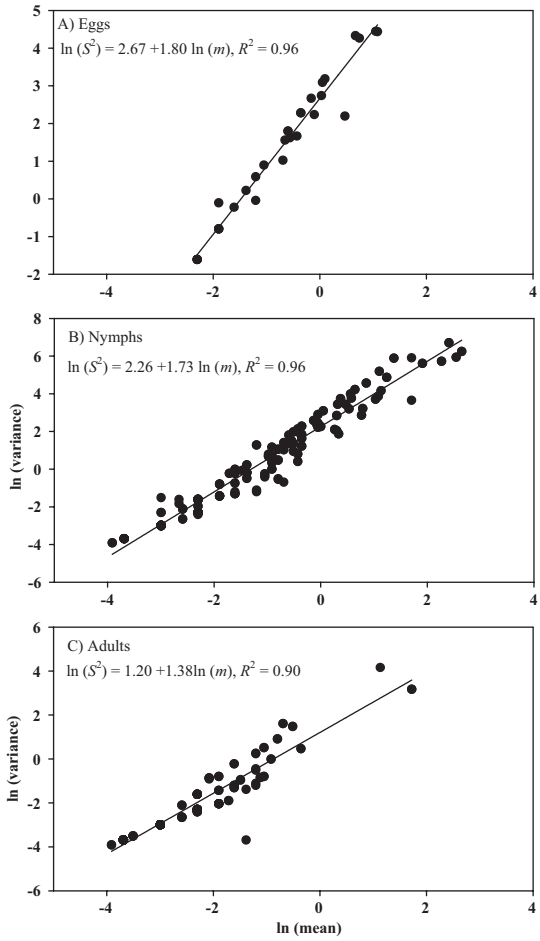

Fig. 1. Taylor power law ANOVA against mean number of *D. citri* counted per citrus flush shoots (A, eggs; B, nymphs; and C, adults).

shoots where nymphs feed during their developmental stage. The decreased aggregation of *D. citri* from immature to adult stage, as also shown by the decrease in ratios of variance components (ratios of between-flushes within-trees and between-trees variance components) can be attributed to an increased mobility and inter-habitat movement of individuals as they mature and increase mortality with developmental stages. Similar decreases in degree of clumping with insect age have been reported for many insect pests (Wilson and Room 1983), and this stage-specific clumping behavior of insects affects the number of samples required for estimating the population of a particular insect stage for a given level of reliability.

Optimum Sample Size. Using the variance components for the primary (trees) and secondary (flushes) sampling units, and the mean times for sampling trees and flushes, the optimum number of citrus flush shoots required per tree for estimating *D. citri* densities were 7.6 (we round to 8) for eggs, 5.8 (we round to 6) for nymphs, and 3.6 (we round to 4) for adults, respectively. These sample sizes are lower than the 20 flushes actually sampled per tree during our surveys. Therefore, estimates of mean *D. citri* densities per tree in the present studied were adequate. These optimum numbers of flushes (u_{opt}) 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 1). The number of citrus trees to be sampled at a precision level of 25% and 10% for the calculated optimum number of flushes per tree (u_{opt}) and for the actual number of flushes sampled ($u = 20$) were determined (Table 4). The optimum allocation of sampling effort to adequately estimate populations of *D. citri* in an orchard and to achieve a relative precision of 25% is a total of 24 (eight flushes per tree and three trees), 60 (six flushes per tree and 10 trees), and eight (four flushes per tree and two trees) flush shoots for the observed densities for eggs (overall mean = 0.11 per flush), nymphs (overall mean = 0.40 per flush) and adults (overall mean = 0.05 per flush), respectively. Using equation 5, we observed that a decrease of *D. citri* mean densities will lead to an increase in sample sizes and vice versa. But for practical reasons, a sampling scheme that uses eight flushes per tree and 10 trees per orchard would provide *D. citri* density estimates for all developmental stages with a percentage relative precision of 25% (or less) required for field studies under the average densities observed in our studies (Table 5). 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 the respective development stages for a sampling plan that is based on a single tree per orchard. For the 20 flushes sampled per tree during our survey, to achieve a 25% precision level, the sampling requirements are two, seven, and one tree per orchard for *D. citri* eggs, nymphs, and adults, respectively. These numbers of trees are considerably lower than the actual 10 trees sampled during our survey.

Table 4. Optimum number of citrus trees (t) and flush shoots (u) per tree and time[a] required to achieve a given precision level while sampling for *D. citri* for the observed densities in citrus in the Lower Rio Grande Valley of Texas

Developmental stage	Sampling unit	Standard error of mean, SE (mean)		
		0.05	0.10	0.25
	Using optimum no. of flushes per tree (u_{opt})[b]			
Eggs	Trees	18	10	3
	Flushes	8	8	8
	Time	58 min	34 min	13 min
Nymphs	Trees	21	17	10
	Flushes	6	6	6
	Time	1 h 6 min	54 min	33 min
Adults	Trees	13	6	2
	Flushes	4	4	4
	Time	43 min	22 min	10 min
	Using no. of flushes actually sampled per tree ($u = 20$)			
Eggs	Trees	12	7	2
	Flushes	20	20	20
	Time	46 min	31 min	16 min
Nymphs	Trees	14	11	7
	Flushes	20	20	20
	Time	52 min	43 min	31 min
Adults	Trees	10	5	1
	Flushes	20	20	20
	Time	40 min	25 min	13 min

[a] A mean figure of 30 s per flush and of 3 min per tree were considered reasonable and used for computing the time required to sample for *D. citri* in citrus orchards in the Lower Rio Grande Valley of Texas.

[b] u_{opt} is calculated after Greenwood and Robinson (2006).

To facilitate the calculation of sample sizes based on the primary and secondary variance components, sampling error defined in equation 4 was computed with a range of trees (t) from 1 to 20 and flush shoots (u) from 1 to 100, which seem reasonable for an operational sampling perspective in citrus orchards. Generally, the error decreases more with an increase in the number of trees than with an increase in number of flush shoots per tree. For the 200 total number of flush shoots sampled per orchard during our surveys (i.e., $t = 10$ trees with $u = 20$ flushes per tree), the error is 5, 11, and 5% for eggs, nymphs, and adults, respectively. However, if 20 trees with 10 flushes per tree (i.e., $t = 20$ trees with $u = 10$ flushes per tree for the same total of 200 flushes per orchard) are sampled, the respective errors will be 2.3, 0.6, and 2.5% for eggs, nymphs, and adults. This second option has a smaller error for the same number of flushes, but it involves additional time (an increase of 25 min; Table 5) for selecting trees within each orchard and may be cost inefficient for this reason. Thus, to achieve a given precision level, the best combination of number of flushes per tree and trees per orchard also will be determined by the cost (time) limitation. The time required for various combinations of number trees and flushes are presented in Table 5.

Mean–Incidence Relationship and Binomial Sampling. Determining the actual numbers of *D. citri* on citrus flushes can be costly, but recording if the insect is simply present or absent on a shoot can be quickly

Table 5. Standard error of mean and cost associated with sample size options for different developmental stages of *D. citri*

Trees (t)	Flushes (u)	Total	Cost (min)	Eggs	Nymphs	Adults
1	1	1	3.5	1.251	3.517	0.425
1	2	2	4	0.897	2.543	0.343
1	4	4	5	0.662	1.906	0.295
1	6	6	6	0.565	1.650	0.277
1	8	8	7	0.511	1.510	0.268
1	10	10	8	0.476	1.420	0.263
1	20	20	13	0.400	1.226	0.251
1	40	40	23	0.356	1.120	0.245
1	100	100	53	0.329	1.053	0.241
2	1	2	6.5	0.853	2.369	0.288
2	2	4	7	0.603	1.680	0.230
2	4	8	8	0.437	1.230	0.196
2	6	12	9	0.368	1.050	0.183
2	8	16	10	0.330	0.950	0.177
2	10	20	11	0.305	0.887	0.173
2	20	40	16	0.251	0.750	0.165
2	40	80	26	0.221	0.675	0.160
2	100	200	56	0.201	0.627	0.158
5	1	5	15.5	0.500	1.351	0.166
5	2	10	16	0.342	0.915	0.129
5	4	20	17	0.237	0.631	0.108
5	6	30	18	0.194	0.516	0.100
5	8	40	19	0.169	0.454	0.096
5	10	50	20	0.154	0.413	0.093
5	20	100	25	0.120	0.327	0.088
5	40	200	35	0.100	0.279	0.085
5	100	500	65	0.088	0.249	0.084
10	1	10	30.5	0.323	0.838	0.104
10	2	20	31	0.211	0.530	0.078
10	4	40	32	0.136	0.329	0.063
10	6	60	33	0.106	0.248	0.058
10	8	80	34	0.088	0.203	0.055
10	10	100	35	0.077	0.175	0.053
10	20	200	40	0.053	0.114	0.049
10	40	400	50	0.040	0.080	0.047
10	100	1000	80	0.031	0.059	0.046
20	1	20	60.5	0.197	0.475	0.061
20	2	40	61	0.118	0.257	0.043
20	4	80	62	0.065	0.115	0.032
20	6	120	63	0.043	0.058	0.028
20	8	160	64	0.031	0.026	0.026
20	10	200	65	0.023	0.006	0.025
20	20	400	70	0.006	0.000	0.022
20	40	800	80	0.003	0.000	0.021
20	100	2000	110	0.001	0.000	0.020

assessed and thus less time-consuming than counting individuals. The use of this presence-absence method will depend on the relationship between the proportion of flushes with at least one *D. citri* or incidence (P_I) and the mean number of *D. citri* (m) per flush. Derivation of the proportion of flush shoots infested from the mean number of *D. citri* per shoot per tree was obtained for each developmental stage by using the respective Taylor's power law coefficients (Fig. 2). According to Wilson and Room (1983), the more clumped an insect is distributed the smaller is the proportion of infested sample units for a given mean density. Hence, for the less contagious adult stage, 100% flush shoot infestation level corresponds to a mean density of ≥15 psyllids per flush per tree, whereas for the same infestation level with immature stages will require a mean of ≥100 eggs and ≥200 nymphs per flush per tree, respectively. A mean density of 15 *D. citri* eggs and nymphs per citrus flush will

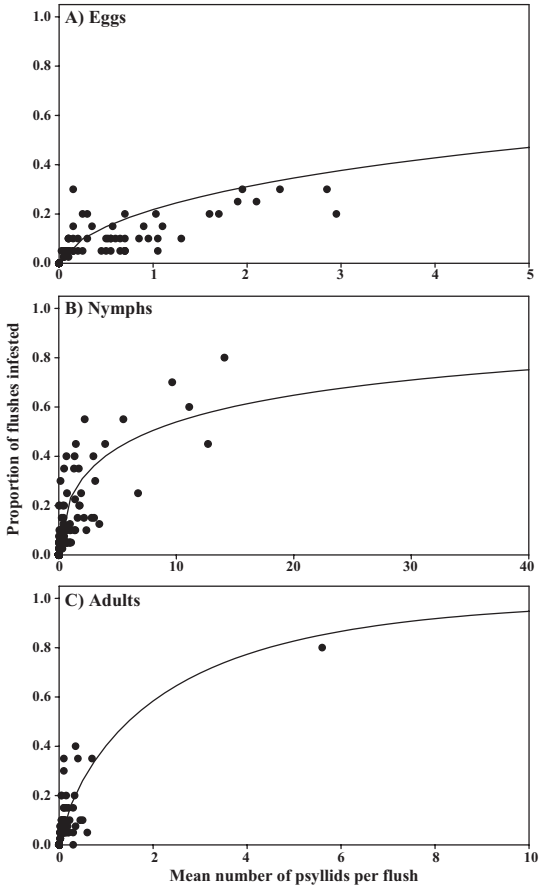

Fig. 2. Relationship between the proportion of infested flush shoots and the mean densities of *D. citri* per flush by using Wilson and Room's model (A, eggs; B, nymphs; and C, adults).

correspond to ≈70 and ≈60% of flush shoots infested by the respective developmental stages. Mean–incidence relationships reported for *D. citri* adults on orange jasmine (Tsai et al. 2000) suggested a higher proportion of shoots infested on that host plant compared with citrus for the same mean density, possibly because of the higher aggregative distribution observed on the latter host plant. Moreover, mature citrus trees have a bigger canopy and produce numerous new flush shoots ranging from 1,700 to 3,800 per tree per year (Garcia-Marí et al. 2002) compared with the orange jasmine shrub. It is not thus surprising that for the same mean *D. citri*, more flush shoots are infested on orange jasmine than citrus plants.

Using Nachman (1984) model the mean number of psyllid per flush per tree was significantly and positively related to the percentage of flush shoots infested—for adults ($y = 0.84x - 0.31, P < 0.001, R^2 = 0.42$), for nymphs ($y = 1.20x + 0.029, P < 0.001, R^2 = 0.52$), and for eggs ($y = 1.22x + 1.71, P < 0.001, R^2 = 0.46$). This suggests that population densities of *D. citri* on citrus flushes can be estimated using flush infestation levels as determined by the presence–absence

method. But the moderate fit of the models as shown by the low explained variances ($R^2 \leq 0.52$) indicates that accurate estimates of psyllid densities cannot be obtained using this method. Because *D. citri* is a small insect generally present in high densities, the binomial sampling method based on the presence or absence of its developmental stages on shoots is however of practical use, specifically for determining psyllid control thresholds in orchards, nurseries, and other ornamental host plants. This sampling method presents a viable alternative to the tedious enumerative sampling plan for growers who can quickly have an estimate of psyllid infestation level during scouting for pest management purposes. In addition, because *D. citri* is economically important mainly as a vector of the deadly citrus greening pathogen, complete estimates of its population densities may not be required before the implementation of control measures. In citrus growing areas such as California, Arizona, Louisiana, and Alabama, where *D. citri* is not presently known to occur the quick and inexpensive binomial sampling method will be useful in early detection surveys of *D. citri* and therefore facilitates eradication efforts in case of accidental introductions of this pest in these areas. Early detection is important for deploying rapid control tactics to prevent population outbreaks and subsequent disease transmission. However, for detailed studies of *D. citri* populations on different host plants, the presence-absence sampling will be of limited use, and complete estimates through enumerative sampling will be required.

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References Cited

Aubert, B. 1990. Integrated activities for the control of huanglongbing-greening and its vector *Diaphorina citri* Kuwayama in Asia, pp. 133–144. *In* Proceedings of the 4th Acia Pacific International Conference on Citriculture, 4–10 February 1990, Chiang-Mai, Thailand.

Binns, M. R., and J. P. Nyrop. 1992. Sampling insect population for the purpose of IPM decision making. Annu. Rev. Entomol. 37: 427–453.

Bové, J. M. 2006. Huanglongbing: a destructive, newly-emerging, century-old disease of citrus. J. Plant Pathol. 88: 7–37.

Burts, E. C., and J. F. Brunner. 1981. Dispersion statistics and sequential sampling plan for adult pear *Psylla.* J. Econ. Entomol. 74: 291–294.

Catling, J. D. 1969. The bionomics of the South African citrus psylla, *Trioza erytreae* (del Guercio) (Homoptera: Psyllidae): 1. the influence of the flushing rhythm of citrus and factors which regulate flushing. J. Entomol. Soc. South. Afr. (Lond.) 32: 191–208.

da Graça, J. V. 1991. Citrus greening disease. Annu. Rev. Phytopathol. 29: 109–136.

da Graça, J. V., J. V. French, M. Skaria, and M. Sétamou. 2006. Citrus psyllid and greening survey. Citrus Center Newsl. 24(6): 1–2.

Dharajothi, B., A. Verghese, and P. L. Tandon. 1989. Ecological studies on citrus psylla, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) with special reference to its spatial distribution and sampling plan. Entomon 14: 319–324.

Downing, J. A. 1986. Spatial heterogeneity: evolved behavior or mathematical artifact? Nature (Lond.) 323: 255–257.

French, J. V., C. J. Khalke, and J. V. da Graça. 2001. First record of the Asian citrus psylla, *Diaphorina citri* Kuwayama (Homoptera: Psyllidae) in Texas. Subtrop. Plant Sci. 53: 14–15.

Fucik, J. E. 1982. Hedging mature Valencia orange trees in South Texas. J. Rio Grande valley Hortic. Soc. 35: 139–147.

Garcia-Marí, F., C. Granda, S. Zaraagoza, and M. Agustí. 2002. Impact of *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) on leaf area development and yield of mature citrus trees in the Mediterranean area. J. Econ. Entomol. 95: 966–974.

Halbert, S. 2005. The discovery of Huanglongbing in Florida, p. 50. *In* Second International Citrus Canker and Huanglongbing Research Workshop, 7–11 November 2005, Florida Citrus Mutual, Orlando, FL.

Halbert, S. E., and K. L. Manjunath. 2004. Asian citrus psyllid (Sternorrhyncha: Psyllidae) and greening disease of citrus: a literature review and assessment of risk in Florida. Fla. Entomol. 87: 330–353.

Hall, D. G., and L. G. Albrigo. 2007. Estimating the relative abundance of flush shoots in citrus, with implications on monitoring insects associated with flush. HortScience 42: 364–368.

Hall, D. G., S. L. Lapointe, and E. K. Wenninger. 2007. Effects of particle film on biology and behavior of *Diaphorina citri* (Hemiptera: Psyllidae) and its infestations in citrus. J. Econ. Entomol. 100: 847–854.

Harcourt, D. G. 1961. Design of a sampling plan for studies on the population dynamics of the diamondback moth, *Plutella maculipennis* (Curt.) (Lepidoptera: Plutellidae). Can. Entomol. 93: 820–831.

Iowa, S. 1968. A new regression method for analyzing the aggregation pattern of animal populations. Res. Popul. Ecol. 10: 1–20.

Iowa, S. 1970. Problem in spatial distribution in animal population ecology, pp. 117–149. *In* G. Patil [ed.], Random counts in models and structures, 1. Pennsylvania State University Press, University Park, PA.

Knapp, J. L., L. G. Albrigo, H. W. Browning, R. C. Bullock, J. B. Heppner, D. G. Hall, M. A. Hoy, R. Nguyen, J. E. Peña, and P. A. Stansly. 1995. Citrus leafminer, *Phyllocnistis citrella* Stainton: current status in Florida—1995. Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL.

Kono, T., and T. Sugino. 1958. On the estimation of the density of rice stems infected by the rice stem borer. Jpn. J. Appl. Entomol. Zool. 2: 184–188.

Kuno, E. 1991. Sampling and analysis of insect populations. Annu. Rev. Entomol. 36: 285–304.

List, R. J. 1971. Smithsonian meteorological tables, 6th revised ed. Smithsonian Institution Press, Washington, DC.

Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS system for mixed models. SAS Institute, Cary, NC.

Liu, Y. H., and J. H. Tsai. 2000. Effects of temperature on biology and life table parameters of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Homoptera: Psyllidae). Ann. Appl. Biol. 173: 201–206.

Mead, F. W. 1976. The South African psyllid, *Trioza erytreae* (Del Guercio) (Homoptera: Psyllidae). Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Entomol. Circ. No. 168.

Michaud, J. P. 2004. Natural mortality of Asian citrus psyllid (Homoptera: Psyllidae) in central Florida. Biol. Control 29: 260–269.

Moran, V. C., and P. R. Buchan. 1975. Oviposition by the citrus psylla, *Trioza erytreae* del Guercio (Homoptera: Psyllidae) in relation to leaf hardness. Entomol. Exp. Appl. 18: 96–104.

Nachman, G. 1984. Estimates of mean population density and spatial distribution of *Tetranychus urticae* (Acarina: Tetranychidae) and *Phytoseiulus persimilis* (Acarina: Phytoseiidae) based upon the proportion of empty sampling units. J. Appl. Ecol. 21: 903–913.

SAS Institute. 1999. SAS procedures guide, version 8. SAS Institute, Cary, NC.

Southwood, T.R.E. 1978. Ecological methods, 2nd ed. Wiley/Halsted, New York.

Southwood, T.R.E., and P. A. Henderson. 2000. Ecological methods. Blackwell, London, United Kingdom.

Taylor, L. R. 1961. Aggregation, variance and the mean. Nature (Lond.) 189: 732–735.

Taylor, L. R. 1984. Assessing and interpreting the spatial distribution of insect population. Annu. Rev. Entomol. 29: 321–358.

Tsai, H. T., J. J. Wang, and Y. H. Liu. 2000. Sampling of *Diaphorina citri* (Homoptera: Psyllidae) on orange jasmine in southern Florida. Fla. Entomol. 83: 446–458.

Tsai, H. T., J. J. Wang, and Y. H. Liu. 2002. Seasonal abundance of the Asian citrus psyllid *Diaphorina citri* (Homoptera: Psyllidae) in southern Florida. Fla. Entomol. 85: 446–451.

[USDA–NASS] U.S. Department of Agriculture–National Agricultural Statistics Service. 2005. Citrus crop value. (http://www.nass.usda.gov/QuickStats).

Westfall, P. H., R. D. Tobias, D. Rom, R. D. Wolfinger, and Y. Hochberg. 2003. Multiple comparisons and multiple tests using SAS. SAS Institute, Cary, NC.

White, T.C.R. 1978. A method of sampling populations of psyllids living on the leaves of *Eucalyptus* trees. Pac. Insects 19: 39–44.

Wilson, L. T., and P. M. Room. 1983. Clumping patterns of fruit and arthropods in cotton, with implications for binomial sampling. Environ. Entomol. 12: 50–54.

Zar, J. H. 1999. Biostatistical analysis. Prentice Hall, Upper Saddle River, NJ.

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