Sticky Trap and Stem–Tap Sampling Protocols for the Asian Citrus Psyllid (Hemiptera: Psyllidae)

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J. Econ. Entomol. 103(2): 541–549 (2010); DOI: 10.1603/EC09360

ABSTRACT Sampling statistics were obtained to develop a sampling protocol for estimating numbers of adult *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) in citrus by using two different sampling methods: yellow sticky traps and stem–tap samples. A 4.0-ha block of mature orange trees was stratified into 10 0.4-ha strata and sampled using each method seven times over a 7-mo period. One sticky trap was deployed per tree on each of 16 trees randomly selected in each stratum, and numbers of adults on the traps were counted 1 wk later. One stem–tap sample in which the number of adults falling into a pan after three rapid taps to a branch was taken per tree on each of 16 trees randomly selected in each stratum. A sampling protocol of one yellow sticky trap on each of 20 trees, or of one stem–tap sample on each of 30 trees, distributed uniformly across an area up to 4.0 ha (excluding block edges) was projected to provide an average sampling precision rate of ≤25% (SEM/mean × 100) at means of one or more adults per trap or stem–tap sample. Validation sampling indicated 20 sticky trap samples consistently provided the desired precision level at means of approximately two or more adults per trap but not at means of 1.0–1.5 per trap. A sample size of 30 stem–tap samples consistently provided the desired average precision level, but the precision of some individual estimates was >25% at means of around one adult per tap sample.

KEY WORDS citrus greening disease, huanglongbing, monitoring

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) is an important pest of citrus in the United States because it is a vector of *Candidatus* Liberibacter asiaticus (Halbert and Manjunath 2004). This and some other species of *C.* Liberibacter are phloem-limited, nonculturable bacteria responsible for citrus greening (huanglongbing) disease (Halbert and Manjunath 2004, Hung et al. 2004). Citrus greening is considered one of the world's most serious diseases of citrus (Bové 2006). *D. citri* was first found in Florida during June 1998 (Tsai and Liu 2000) and has since spread throughout the state's citrus-growing regions (Michaud 2004). Citrus greening was found in southern Florida during August 2005 (Bové 2006). Subsequent surveys by the State of Florida and USDA–APHIS revealed the disease was already present in many residential areas and commercial citrus groves, especially in southern areas of the state.

Simple and efficient sampling procedures for *D. citri* are vital to the development of a successful integrated pest management (IPM) program aimed at controlling citrus greening disease. Growers can sample trees for psyllids to determine whether they are present, to monitor population levels over time, and to evaluate psyllid management tactics. Some information is available on methods of monitoring population levels of *D. citri.* For example, citrus flush can be sampled to detect and count eggs, nymphs, and adults (Hall and Albrigo 2007, Setamou et al. 2008). A tap sampling method and yellow sticky traps can also be used to detect and monitor infestations of adults in citrus (Hall et al. 2007, Hall 2009). Although citrus researchers and growers are aware of these different sampling methods, guidelines for using yellow sticky traps or stem–tap samples are lacking particularly with respect to numbers of samples required to obtain precise estimates.

A good sampling protocol for estimating the relative abundance of *D. citri* in citrus would define how many samples should be taken across a group of trees to obtain a specific level of statistical precision in a mean density estimate. This statistical precision is often based on the standard error being a specified percentage of the mean (Southwood 1978). A precision level of 25% has been regarded as adequate for general estimates because it enables the detection of a doubling or halving of a population over a time interval

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(Southwood 1978). Research may sometimes require a precision level of 10% or smaller. When working in a group of trees, a sampling protocol should define the number of sample units per tree and number of trees to examine. When sampling across a large acreage of trees, a hierarchical (two or more stages) sampling plan is often a good choice because the area can be stratified and a protocol developed specifying numbers of strata, trees per stratum, and samples per tree (Hall et al. 1994). Nested analysis of variance (ANOVA) can be used to determine variance components associated with these different hierarchical levels, which in turn can be used to project the number of samples required at each level (Snedecor and Cochran 1967).

The objective of research presented here was to develop sampling protocols for using yellow sticky card traps and stem–tap sampling to make relative estimates of infestation levels of adult *D. citri* in citrus.

Methods and Materials

Sampling statistics associated with sticky trap and stem tap sampling were obtained from a four ha block (3.7-m tree spacing, 7.6-m row spacing) of mature 'Valencia' sweet orange, *Citrus* × *sinensis* (L.) Osbeck, trees (2.4 m in height) in St. Lucie County, FL. Ten individual 0.4-ha plots (seven rows per plot with 20 trees per row) were established within this large block of trees with no buffer trees between plots. The study area was therefore stratified, and plots were considered strata. The trees ran north to south. All strata were situated within the interior of the block, with at least two buffer rows from the outer edge of the block and three buffer trees from the end of each row.

Sticky Trap Samples. Yellow (a bright yellow hue similar to S-G-390 by Behr Process Corp., Santa Ana, CA) sticky card traps (7.62 by 12.7 cm; OL-010MS35, Great Lakes IPM, Vestaburg, MI) were used in the studies presented here. The double-sided sticky traps were suspended 1–1.5 m above ground near the outside of the canopy from a branch using a twist tie (18 cm in length; 91734L3, Consolidated Plastics Company, Stow, OH). A hole was placed near the center of the upper edge of each trap to hang the traps. When traps were retrieved, they were placed in reclosable plastic bags (20 by 20 cm, accepts two traps per bag; 90051L3, Consolidated Plastics Company).

Relative infestation levels of the psyllid were monitored in each stratum over a 1-wk period by using one sticky trap per tree on each of 16 randomly chosen trees. Traps were located on the east side of eight of the trees in each stratum and on the west side of the other eight trees. A total of seven 1-wk periods of trapping data were obtained from the trees during July 2008 through February 2009. The time required to deploy traps (including tying a trap to a tree and removing wax paper to expose both sticky surfaces) was recorded for 20 traps per sample period. The time required to count adults captured on each trap was recorded for 50 traps from each trapping period.

Stem–Tap Samples. Relative infestation levels of the psyllid were monitored in each stratum by taking one tap sample per tree on each of 16 trees (randomly chosen). Tap samples were taken on the east side of eight of the trees in each stratum and on the west side of the other eight trees. A white metal pan (20.32 by 20.32 by 10.16 cm; length by width by depth, respectively) was held several cm under a random branch (1–1.5 m above ground near the outside of the canopy), and a polyvinyl chloride pipe (0.6 m in length, 2.13 cm o.d.) was used to forcefully tap the branch three times in rapid succession. All adult psyllids falling in the pan were immediately counted. The block of trees was tap sampled on seven different dates from July 2008 through February 2009. The number of seconds required to take a tap sample (position the pan, tap a branch, count and record the number of adults) was recorded for 20 tap samples on each sample date.

Analyses. Mean numbers of adult *D. citri* captured on traps deployed on the east and west sides of the trees, and mean numbers observed during tap sampling on the east and west sides of the trees, were compared using a *t*-test on log-transformed data. Dispersion of adults based on numbers per sticky trap per tree or on numbers per stem tap sample per tree was assessed using Taylor's power law (Taylor 1961) by converting means (m) and variances (s^2) to logs and subjecting them to a linear regression of $\log(s^2)$ on $\log(m)$. The slope of the resulting equation indicates a random dispersion when the regression slope = 1.0, a uniform dispersion when the slope <1.0, and an aggregated dispersion when the slope >1.0 (Taylor 1961, Southwood 1978). Three different analyses using Taylor's power law were conducted: means and variances for data collected within each stratum for each trapping period or stem–tapping date; means and variances among the ten strata for each trapping period or stem–tapping date; and simple means and variances across all strata for each trapping period or stem–tapping date.

The data from sticky trap and stem–tap samples were subjected to nested analyses of variance (Snedecor and Cochran 1967), with trees ($n = 16$) nested in strata ($n = 10$) nested in sample periods ($n = 7$). Nested analyses of variance (ANOVAs) were also conducted on log-transformed data [$\log(x + 1)$]. The value of stratifying a 4.0-ha block of trees for sampling purposes was determined based on variance components.

The precision of mean estimates for the number of adult *D. citri* per sticky–trap sample or per stem–tap sample was investigated based on the standard error (s_e) and mean (x) from log-transformed data: relative variation = $s_e/x \times 100$. The optimal number of trees to sample per 0.4-ha stratum or per 4.0-ha block of trees (n_t) by using one sticky trap per tree or one tap sample per tree was calculated across the observed range of means with a target precision level of 25%: $n_t = (s/0.25x)^2$, where s was the standard deviation and x was the mean number of adults per sample per sample date. The relationships between mean number of psyllids per sample and relative variation, and mean

Table 1. Results of Taylor's power law analyses on dispersion of adult *D. citri* based on numbers per sticky trap or numbers per stem tap sample

Variance source	df_{error}	*F*	*P*	r^2	*a*	*b*	*b* SEM
Sticky trap sampling							
Within strata	69	571	<0.0001	0.89	0.46*	1.60***	0.07
Among strata	6	70	0.0004	0.93	−1.78**	2.16**	0.26
Overall	6	44	0.0012	0.90	0.64^{ns}	1.79**	0.27
Stem tap sampling							
Within strata	69	434	<0.0001	0.86	0.79***	1.41***	0.07
Among strata	6	5	0.08	0.48	-1.27^{ns}	1.67^{ns}	0.78
Overall	6	30	0.0029	0.86	1.0**	1.86**	0.34

Significantly different than 0.0: $*P < 0.05$; $**P < 0.01$; $***P < 0.0001$; ^{ns}, not significant at $\alpha = 0.05$.

number of psyllids per sample and optimal sample sizes, were investigated visually by graphing. Based on the results of these visual investigations, the average optimal number of samples (Y) needed at different means per sample (X) was projected based on nonlinear regression using a two parameter hyperbolic decay model [$Y = (a \times b)/(b + X)$].

t-Tests (PROC TTEST), nested analyses of variance (PROC NESTED), and linear regressions (PROC GLM) were conducted (SAS Institute 2008). Nonlinear regressions were conducted using SigmaPlot (Systat Software, Inc. 2008). Based on the sampling statistics derived from the above-mentioned analyses, a sampling protocol was identified for each sampling method that was projected to provide mean estimates with an average precision level of 25% relative variation at or above a mean of one adult per sample.

Validation of Sampling Protocols. Five 4.0-ha blocks of trees located in three counties (Highlands, Martin, and St. Lucie) and eight 0.4-ha blocks located in four counties (Highlands, Indian River, Martin, and St. Lucie) were sampled using the sticky–trap and stem–tap protocols. There was among these blocks a mix of varieties—'Hamlin' sweet orange, 'Valencia' sweet orange (three blocks), 'Rio Red' grapefruit, *Citrus* × *paradisi* Macfad., 'Flame' grapefruit, and 'Temple' orange trees, and one block with a mix of 'Honeybell' and Temple orange trees. The blocks ranged in tree height from 1.5 to 3.6 m. Tree rows ran north to south in all except one block. Half of the sticky trap or stem tap samples were taken on the west side of trees, and half were taken on the east side. For the one block in which trees ran west to east, there was enough space between trees to take samples on the east and west sides of the trees. The objective of validation research was to compile a good range of mean estimates using the protocols for each sampling method and to evaluate achieved precision levels associated with these means. Each block was sampled at least once using sticky traps and stem–tap samples on trees uniformly selected across 4.0 ha and at least once on trees uniformly selected across a 0.4-ha area within each block. To obtain the desired range of means, some of the 4.0-ha blocks (and a 0.4-ha area within the block) were sampled an additional one or two times with each sampling method, and a 0.4-ha area within one block was tap–sampled an additional three times. For blocks sampled more than once, there was usually 3 to 4 wk between sample dates. Validation sampling was conducted from June through September 2009. The original block of trees in St. Lucie County from which sampling statistics were obtained and used to develop the sampling protocols was not among any of the blocks in which validation sampling was conducted. For the evaluation of achieved precision levels, relative variation (based on both raw data and log-transformed data) was graphed on nontransformed mean estimates and visually examined to determine whether a relative variation value of 25% was consistently achieved at means of one or more psyllids per sticky trap or stem–tap sample. To assess average achieved precision levels for each protocol, observed levels of precision (based on both raw and log-transformed data) (Y) across untransformed means (X) for sticky trap and stem–tap validation samples were subjected to nonlinear regression by fitting the data to the afore mentioned decay model using SigmaPlot (Systat Software, Inc. 2008).

Results

Sticky Trap Samples. An overall mean (SEM) of 3.9 (1.2) adult *D. citri* per trap per wk was observed during the study. The largest and smallest mean (SEM) observed among the seven sample periods was 10.0 (2.5) and 0.7 (0.1) adults per trap, respectively. The largest mean (SEM) observed per stratum over the seven sample periods was 26.1 (11.2) and the smallest was 0.3 (0.1). The mean (SEM) number of psyllids per trap over all trapping periods was significantly greater on traps deployed on the east side of trees [4.2 (0.3)] than on the west side of trees [3.6 (0.4)] ($t = 2.6$; df = 1,118; $P = 0.01$), even though no significant difference between the east and west sides of trees was found in data from six of the seven trapping periods.

Taylor's power law analyses on trap counts indicated that adults were aggregated among trees within individual strata, among strata and over all samples taken across the block of trees (Table 1). The results of analyses on dispersion within a 0.4-ha stratum and across the entire block indicated the data were adequately described by Taylor's power law (Fig. 1). Nested ANOVA over all sample dates indicated stratum was a significant source of variation in the trap counts (Table 2). These results supported that adult *D.*

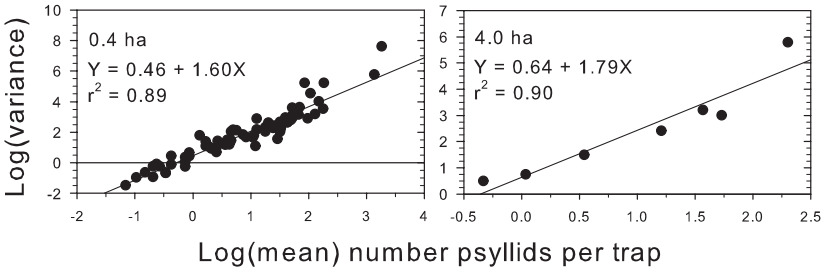

Fig. 1. Dispersion of adult *D. citri* in citrus based on captures of adults on yellow sticky traps (left) within a 0.4-ha stratum of trees and (right) across a 4.0-ha block of trees: results of Taylor's power law analyses.

citri were aggregated within and among strata. However, between stratum variation accounted for a relatively small percentage of total variation (7–11%; Table 2). Overall sample periods, variation among trees in individual strata accounted for most (74%) of the variation in the trap count data. Analyses on data from each individual sample period indicated stratum was a nonsignificant ($\alpha = 0.05$) source of variation in three of the seven data sets, contributing 0–5% to total variation. Among the four sample periods in which between strata variation was significant, variation within-strata accounted for 81–93% of total variation.

Relative variation associated with estimates of the mean number of psyllids per sticky trap and optimal numbers of traps to deploy in individual 0.4-ha strata and across the 4.0-ha block of trees are presented in Fig. 2. The optimal number was large at small mean numbers per trap but decreased as the mean number per trap increased. The relationship between optimal number of samples (Y) and mean number of adults per sample (X) was described by the following model: for 0.4-ha areas, $Y = (89.6 \times 0.289) / (0.289 + X)$; $F = 369$, $P < 0.0001$, $r^2 = 0.84$, df = 69; and for 4.0-ha areas, $Y = (223.9 \times 0.097) / (0.097 + X)$; $F = 223$, $P < 0.0001$, $r^2 = 0.98$, df = 6. Based on these models, the following protocol would provide an average precision level of 25% at means of one or more psyllids per trap: 20 trap samples (one trap per tree) distributed uniformly across an area of trees up to 4.0 ha.

Means (SEM) of 18.2 (0.5) and 26.5 (0.6) s were required to deploy and count the number of adults per trap, respectively. Therefore on the average 45 s was required to deploy and later count psyllids on each trap at densities observed in this study.

Stem–Tap Samples. An overall mean (SEM) of 1.5 (0.3) adult *D. citri* was observed per tap sample among the seven sample dates. The largest and smallest mean (SEM) observed among the seven sample dates was 2.5 (1.0) and 0.4 (0.1) adults per sample, respectively. The largest mean (SEM) observed per stratum over the seven sample periods was 10.6 (4.0). The mean (SEM) number of psyllids per tap sample over all sample dates was 1.4 (0.1) for samples taken on the east side of trees and 1.5 (0.2) for samples taken on the west side of trees. There was no significant difference between these means ($t = 0.5$; df = 1,118; $P = 0.6$).

Taylor's power law analyses on numbers of adult *D. citri* per tap sample indicated that adults were aggre-

Table 2. Results of nested ANOVA on numbers of adult *D. citri* per yellow sticky trap per tree or stem tap sample per tree[a]

Variance source	df_{error}	Sum of squares	*F*	*P*	Error term	Mean square	Variance component	% total
Sticky trap samples—raw data analysis								
Sample period	6	10,281	10.4	<0.0001	Stratum	1,713	9.68	15
Stratum	63	10,370	3.4	<0.0001	Tree	165	7.22	11
Tree	1,050	51,560				49	49.11	74
Sticky trap samples—log data analysis								
Sample period	6	301	34.4	<0.0001	Stratum	50.2	0.305	35
Stratum	63	92	2.9	<0.0001	Tree	1.5	0.060	7
Tree	1,050	532				0.5	0.506	58
Stem tap samples—raw data analysis								
Sample period	6	549	3.6	0.004	Stratum	91	0.411	5
Stratum	63	1,619	3.3	<0.0001	Tree	26	1.125	12
Tree	1,050	8,085				8	7.700	83
Stem tap samples—log data analysis								
Sample period	6	47	7.2	<0.0001	Stratum	8	0.042	9
Stratum	63	69	2.7	<0.0001	Tree	1	0.043	9
Tree	1,050	434				1	0.413	83

[a] Data were obtained using a hierarchical sampling plan consisting of one sticky trap per tree (1-wk trapping period) or one tap sample per tree, 16 trees per 0.4-ha stratum of trees, and 10 strata within 4.0-ha of trees.

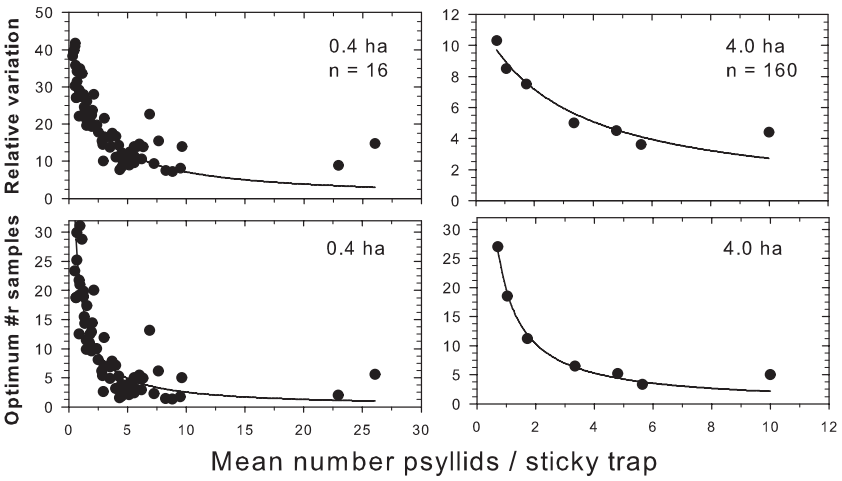

Fig. 2. Sampling statistics associated with estimating relative infestation densities of adult *D. citri* in citrus using yellow sticky traps within a 0.4-ha stratum of trees and across a 4.0-ha block of trees. Relative variation and optimal sample sizes are based on log-transformed data; results are plotted on untransformed means.

gated among trees within individual strata and over all samples taken across the four ha block of trees on each sample date (Table 1). However, no significant aggregation was found among strata over the seven sample dates. The distribution of adults among strata based on tap samples was not significantly different than random. The results of analyses on dispersion within a 0.4-ha stratum and across the entire block indicated the data were adequately described by Taylor's power law (Fig. 3). Nested ANOVA indicated sample date and stratum were each significant sources of variation in stem-tap counts (Table 2). Although between-stratum variation was significant over all sample dates, it accounted for a relatively small percentage of total variation (Table 2). Overall sample periods, variation among trees in individual strata accounted for most (83%) of the variation in tap–sample data. The results supported that adult *D. citri* were aggregated within strata. Analyses on data from each individual sample period indicated between-stratum variation was a nonsignificant ($\alpha = 0.05$) source of variation in four of the seven datasets, contributing only 0–2.7% to total variation. Among the three sample periods in which between strata variation was significant, within-stratum variation still accounted for 80–86% of total variation.

The range in mean estimates per tap sample per sample date, the precision of these estimates as indicated by relative variation, and optimal sample sizes are presented in Fig. 4. The optimal number was large at small mean numbers of adults per sample but decreased as the mean number per sample increased. The relationship between optimal number of samples (Y) and mean number of adults per tap sample (X) was described by the following model: for 0.4-ha areas, $Y = (675 \times 0.037)/(0.037 + X)$; $F = 510$, $P < 0.0001$, $r^2 = 0.88$, df = 69; and for 4.0-ha areas, $Y = (100.3 \times 0.385)/(0.385 + X)$; $F = 16$, $P = 0.011$, $r^2 = 0.76$, df = 6. Based on these models, the following protocol would provide an average precision level of 25% at means of one or more psyllids per tap sample: 30 tap samples (one tap sample per tree) distributed uniformly across an area of trees up to 4.0 ha.

A mean (SEM) of 11.7 (0.2) s was required to take a tap sample and record the number of adults at densities observed in this study.

Validation of Sampling Protocols. Means (SEMs) of 5.7 (0.6) and 3.7 (0.5) adult *D. citri* were captured per sticky trap during validation sampling in the 0.4- and 4.0-ha sample areas, respectively. Observed mean (SEM) number of adults per trap per trapping period in the 0.4 ha sample areas ranged from 0.1 (0.1) in a

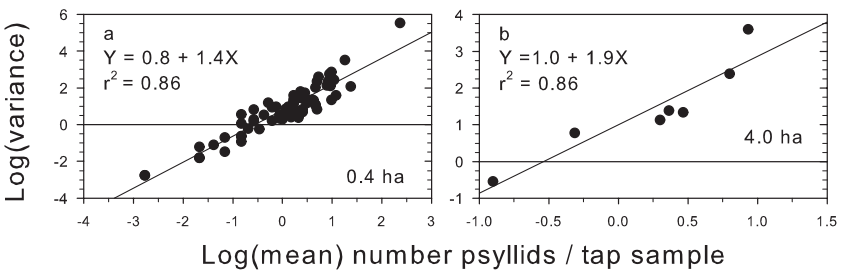

Fig. 3. Dispersion of adult *D. citri* in citrus based on numbers of adults observed using the stem–tapping sampling method (a) within a 0.4-ha stratum of trees; (b) across a 4.0-ha block of trees: results of Taylor's power law analyses.

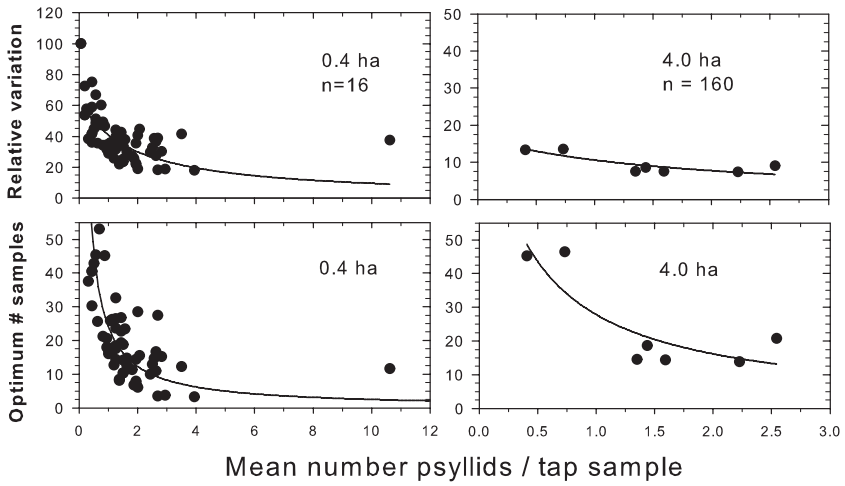

Fig. 4. Sampling statistics associated with estimating relative infestation densities of adult *D. citri* in citrus using the stem–tapping sampling method within a 0.4-ha stratum of trees and across ten 0.4-ha strata of trees. Relative variation and optimal sample sizes are based on log-transformed data; results are plotted on untransformed means.

block of mature Hamlin orange trees up to 18.0 (2.9) in a block of mature Rio Red grapefruit trees. For the 4.0-ha block samples, the mean (SEM) per trap ranged from 0.1 (0.1) in a block of mature Valencia orange trees up to 11.4 (2.5) in another block of mature Valencia trees. The complete range of observed means for the 0.4- and 4.0-ha sample areas are presented in Fig. 5 along with achieved levels of precision based on both untransformed and log-transformed data. Data from 0.4-ha sample areas indicated the relationship between achieved precision levels (Y) and untransformed means per trap (X) was described by $Y = (93.7 \times 0.56) / (0.56 + X)$; $F = 72.6$, $P < 0.0001$, $r^2 = 0.88$, df = 11; and data from 4.0-ha sample areas was described by $Y = (128.1 \times 0.34) / (0.34 + X)$; $F = 268.1$, $P < 0.0001$, $r^2 = 0.98$, df = 7. These models indicated that average precision levels would fall above 25% at means of ≈1.0–1.5 adults per trap. Among the samples taken within 0.4-ha areas (after data transformation), the precision of two mean estimates were in excess of 25%—a level of 29.0% was associated with a mean estimate of 1.2 per trap, and 29.8% was associated with a mean estimate of 1.5 per trap. Based on the standard deviations associated with these two mean estimates, a precision level of 25% would have been achieved using a sample size of ≈28 traps. Precision levels for 0.4-ha sample areas were <25% for all other mean estimates above 1.0 per trap and generally decreased as means increased (to as low as ≈5% at a mean of 18 per trap). With respect to 4.0-ha sample areas, two

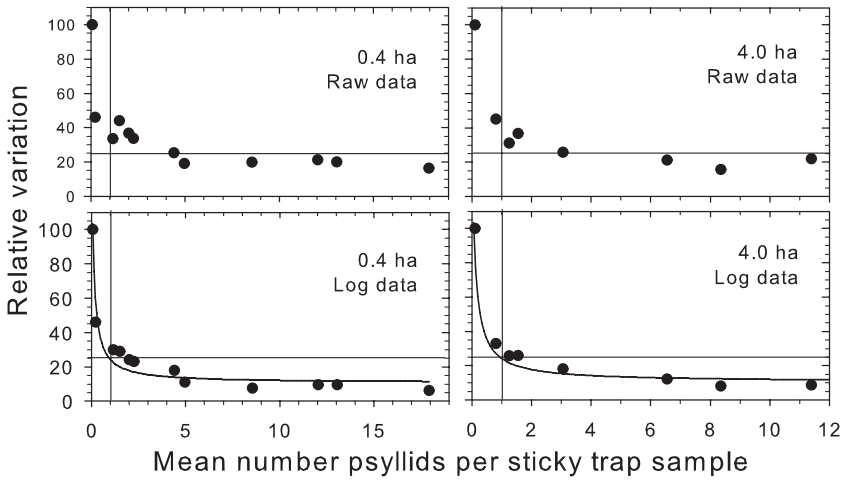

Fig. 5. Relative variation associated with estimating mean numbers of adult *D. citri* in citrus using a sample size of 20 yellow sticky card traps per 0.4- or 4.0-ha: validation study. This particular sample size was projected to be enough to achieve an average relative variation level of 25% at or above a mean number of 1.0 adult per sticky trap. The figure presents relative variation derived from log-transformed [$\log(x + 1)$] data counts plotted on untransformed means per sample. Horizontal reference lines denote 25% relative variation and vertical reference lines denote 1.0 psyllid per sample.

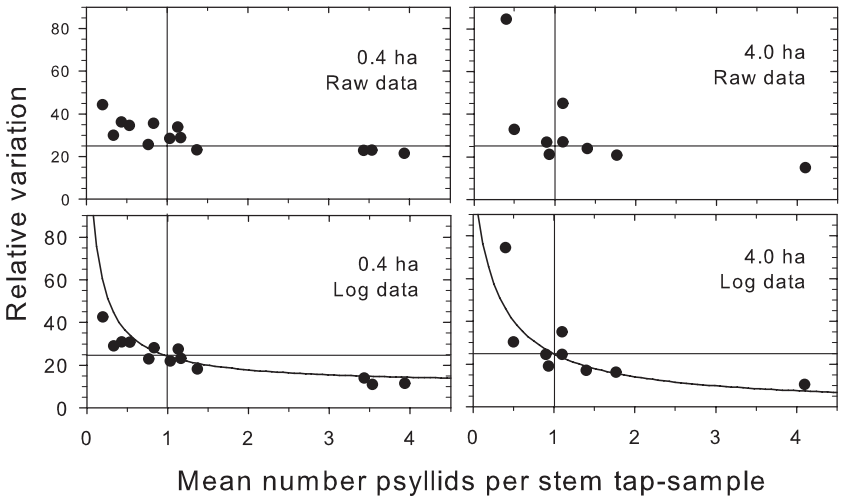

Fig. 6. Relative variation associated with estimating mean numbers of adult *D. citri* in citrus using a sample size of 30 stem–tap samples per 0.4- or 4.0-ha: validation study. This particular sample size was projected to be large enough to achieve an average relative variation level of 25% at or above a mean number of 1.0 adult per stem–tap sample. The figure presents relative variation derived from log-transformed [$\log(x + 1)$] data counts plotted on untransformed means per sample. Horizontal reference lines denote 25% relative variation and vertical reference lines denote 1.0 psyllids per tap sample.

mean estimates fell just above the target maximum of 25%: 25.7% was associated with a mean of 1.3 per trap and 26.0% was associated with a mean of 1.6 per trap. Precision levels for 4.0-ha samples were <25% for all other mean estimates above 1.0 per trap and generally decreased as means increased (to as low as ≈10% at a mean of 11.5 per trap).

Means (SEMs) of 1.2 (0.1) and 1.1 (0.1) adult *D. citri* were observed per stem–tap sample during validation sampling in the 0.4- and 4.0-ha sample areas, respectively. Observed mean (SEM) number of adults per tap sample in the 0.4 ha sample areas ranged from 0.2 (0.1) in a block of mature Rio Red grapefruit trees up to 3.9 (0.8) in a block of young Valencia orange trees. For the 4.0-ha block samples, the mean (SEM) per tap sample ranged from 0.4 (0.3) up to 4.1 (0.6). The complete range of observed means for the 0.4- and 4.0-ha sample areas are presented in Fig. 6 along with achieved levels of precision based on both untransformed and log-transformed data. Validation results for stem–tap sampling were similar to the results for sticky–trap sampling. Nonlinear regression of transformed data indicated that, on the average, the 25% target precision level was achieved in both 0.4- and 4.0-ha areas. Data from 0.4-ha sample areas indicated the relationship between achieved precision levels (Y) and untransformed means per tap sample (X) was described by $Y = (43.2 \times 1.26) / (1.26 + X)$; $F = 80.4$, $P < 0.0001$, $r^2 = 0.88$, df = 12; data from 4.0-ha sample areas were described by $Y = (326.6 \times 0.07) / (-0.07 + X)$; $F = 19.5$, $P = 0.0031$, $r^2 = 0.74$, df = 8. These models confirmed that precision levels averaged 25% at means of one or more adults per tap sample using the protocol. Among the samples taken within 0.4-ha areas (after data transformation), the precision of one individual mean estimate (1.1 per tap sample) was 27.4%. Precision levels for 0.4-ha sample areas were <25% for all other mean estimates above 1.0 per trap and generally decreased as means increased (to as low as 11% at a mean of 3.9 per trap). With respect to 4.0-ha sample areas, the precision level of one individual mean (1.1 per tap) was 35%, well above the target maximum of 25%. This mean was observed in a block of mature grapefruit trees. Based on the variation associated with this particular estimate, nearly 60 samples would have been needed to achieve 25%. There was another mean estimate of 1.1 that had a precision level of 24.4%. Precision levels for 4.0-ha samples were <25% for all other mean estimates above 1.0 per sample and generally decreased as means increased (to as low as ≈10% at a mean of 4.1 per tap sample).

No significant differences were observed during validation sampling in mean numbers of adult *D. citri* per sticky trap sample between the east and west side of trees ($t = 1.2$, df = 418, $P = 0.22$). Also, no significant difference in mean numbers of adults per stem–tap sample were found between the east and west side ($t = 0.4$, df = 778, $P = 0.7$).

Discussion

Adult *D. citri* were aggregated among citrus trees according to both sticky trap samples and stem tap sampling. Aggregation complicates sampling, increasing the numbers of samples and sample locations needed across a block of trees to make precise estimates. Aggregation of *D. citri* among citrus trees based on dispersion of adults on flush shoots has been noted (Dharajothi et al. 1989, Setamou et al. 2008). Although these researchers noted among-tree aggregation, they reported no significant within-tree aggregation by adults on flush.

Data from both sticky trap and stem-tap sampling indicated adult *D. citri* were aggregated among trees

in individual 0.4-ha strata and among trees across a 4.0-ha area of trees. For an individual 4.0 ha block of trees, adults were usually aggregated among 0.4-ha strata according to sticky trap data but not stem-tap sample data. Biological reasons for this discrepancy could not be identified. The value of the *b* parameter from the Taylor's power law analysis on among stratum dispersion based on tap sample counts was 1.67, larger than the *b* parameter associated with some of the other dispersion analyses; variability precluded declaring it >1.0. Regardless, variance component analyses indicated variation among strata contributed little to the overall variation in psyllid counts by using either sampling method.

We found no difference between the east and west sides of trees with respect to mean numbers of adults per stem–tap sample in the block sampled to develop sampling protocols, but significantly greater numbers of adults were captured on sticky traps deployed in this block on the east side of trees (over all trapping periods and during one individual trapping period). No significant differences were observed during validation sampling with respect to mean numbers of adults on the east and west side of trees. To guard against possible differences between captures of adults on traps on the west and east sides of trees, sticky trap sampling could be conducted with all traps positioned on either the east or west side of trees, or an equal number of traps could be deployed on each side. The sampling protocols developed and validated here applied almost entirely to blocks of trees in which rows ran north to south. For blocks of trees with rows running east to west, if the trees are in a hedgerow situation, 50% of the samples could be taken on the north side and 50% on the south side of the trees.

The research indicated there was little value in stratifying a block of trees for an estimate of the mean number of adults per sample using either sticky trap or stem–tap samples. Hierarchical sampling schemes based on stratification were therefore not deemed necessary. For a precise mean estimate, the data indicated that sample sites should be distributed throughout an area of trees up to 4.0 ha because significant spatial variation in psyllid densities (aggregation) can occur. Such variation could be a consequence of immigrating psyllids populating some areas in the block before others. Nonuniformity in the development of flush across a block of trees could also contribute to some areas having higher numbers of adult psyllids. Even within a relatively small area of trees that are similar with respect to size and flush abundance, some trees are sometimes infested by greater numbers of adult psyllids than others (D.G.H., unpublished). This may be a result of attraction of adult *D. citri* to conspecifics of the same or opposite sex either due to aggregation or sex pheromones. Wenninger et al. (2008) reported behavioral evidence of a female-emitted sex pheromone that attracted males.

Validation samples indicated precision levels associated with a sample size of 20 sticky trap samples across an area of up to 4.0 ha would average 25% or less at around two or more adults per trap sample, but >20 traps would be needed to achieve the target precision level at means of one to two adults per trap. With respect to individual estimates, the protocol failed to consistently provide the desired precision level at means of 1.0–1.5 adults per tap sample. Based on standard deviations associated with means of 1.0–1.5 adults per trap, 28 sticky traps would have been needed to achieve the desired precision level.

With respect to stem–tap samples, validation samples indicated that precision levels associated with a sample size of 30 stem–tap samples would average 25% or less at means of one or more adults per tap sample. However, the sampling protocol failed to consistently provide the desired precision level for individual estimates when of the mean number of adults per stem–tap sample was near 1.0. Based on the standard deviation associated with an observed mean of 1.1 adults per tap sample per 4.0 ha, as many as 60 samples would have been required to achieve the desired precision level. This particular mean per tap sample was associated with samples taken in a mature block of Rio Red grapefruit during late August. The protocol consistently provided the desired precision level for individual estimates at means of 1.4 or more adults per tap sample.

Boina et al. (2009) reported that significantly greater numbers of adult *D. citri* were captured on sticky traps deployed in trees along the edges of blocks adjacent to fallow ground than on traps deployed on trees in the interior areas of the blocks. Because edges of blocks were avoided during our research, the sampling protocols developed are not applicable for estimating means per sample for an entire block of trees including edges, nor for an area of trees at the edge of a block.

Comparisons of sticky trap counts of adult *D. citri* among different areas within a block of trees should be made with caution if the trees in these areas differ with respect to sunlight and temperature conditions (due to differences such as tree size and density of leaves within tree canopies). Comparisons of numbers of adults captured on traps in different blocks widely separated in space should also be made with caution. This is because sticky traps can be inconsistent indicators of absolute densities due to the influence of sunlight and temperature on flight activity by adults and resulting numbers of adults captured on traps (Hall 2009).

Taking one stem tap sample per tree was 33 s faster on the average than taking one sticky trap sample per tree (not including the extra time required to take a trap off a tree and place it in a bag, labeling time if traps are individually labeled, and the fact that sticky trapping required two trips to a block of trees). The 20 sticky-trap sampling protocol would have required on the average a total of ≈15 min worth of deploying and counting psyllids on traps. In contrast, the 30 stem–tap sampling protocol would have required on the average a total of ≈6 min of actual tap sampling and only one visit to a grove. The time required to move from tree to tree was assumed to be the same whether running

sticky traps or taking stem–tap samples. In addition to the extra time associated with sticky card trapping, sticky traps, plastic bags and twist ties had to be purchased (US$253.45 per 1,000 traps, US$84.50 per 1,000 plastic bags, and US$14.25 per 2,000 twist ties).

Sticky traps and stem–tap samples were each previously shown to be effective for detecting adult *D. citri* in trees, at least when appreciable numbers were present (for example, means >1.5 adults per trap per week or >1.0 adults per tap sample) (Hall et al. 2007, Hall 2009). However, when adult densities are low, adults are sometimes detected using sticky traps but not using stem–tap samples. This occurred during the validation research presented here. Another advantage of the sticky traps is that they can be used in young trees (e.g., trees less than a meter in height). The tap sampling method does not lend itself very well to trees of this height. Sticky traps serve as a record of what has been collected and are useful for training individuals to identify psyllids. A drawback to stem–tap sampling may be the potential variation between individuals taking the samples—less variation may occur among individuals deploying and retrieving sticky traps. Another drawback to tap sampling is that, when large numbers of adults fall into the pan, it can be difficult to count all individuals before some escape.

Irrespective of whether the data are from sticky traps or stem–tap sampling, transforming count data to logs was advantageous with respect to assessing the precision of estimates and should facilitate better statistical comparisons of means over time. According to Southwood (1978), data from distinctly aggregated or contagious populations will usually be adequately transformed using logarithms. We note this for the benefit of growers and extension personnel not familiar with the benefits of data transformations.

Acknowledgments

We thank Kathy Moulton and Anthea Diamondis (USDA–ARS, U.S. Horticultural Research Laboratory) for assistance during the project. This research was funded by a grant from the Florida Citrus Production Research Advisory Committee.

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Received 19 October 2009; accepted 5 December 2009.