Effects of Reducing Sample Size on Density Estimates of Citrus Rust Mite (Acari: Eriophyidae) on Citrus Fruit: Simulated Sampling

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J. Econ. Entomol. 98(3): 1048–1057 (2005)

ABSTRACT The consequence of reducing sample size on the accuracy and precision of estimates of citrus rust mite, *Phyllocoptruta oleivora* (Ashmead), densities on oranges was investigated. The sample unit was a 1-cm^2 surface area on fruit. Sampling plans consisting of 360, 300, 200, 160, 80, 48, 36, or 20 samples per 4 ha were evaluated through computer simulations by using real count data from 32 data sets of 600 sample units per 4 ha. The original and reduced sampling plans were hierarchical with different numbers of sample areas per 4 ha, trees per area, fruit per tree, and samples per fruit. Individual estimates ($n = 100$ simulations per data set) using each plan were sometimes considerably below or above target densities. In an original set of count data with a mean of six mites per cm^2, simulations of 36 samples per 4 ha produced individual estimates ranging from one to 16 mites per cm^2, whereas 80 samples per 4 ha produced estimates ranging from two to 10 mites per cm^2. The plans consisting of 36 or more samples were projected to provide precision levels of 0.25 (SEM/mean) or better at densities of five or more mites per cm^2 based on log-data, a projection that needs to be verified under real-grove situations. Each plan consistently provided mite detection in these sampling simulations except those consisting of 20 or 36 samples, which sometimes failed to detect mites when the target density was less than five mites per cm^2. The study provided insight into the probable precision, accuracy and detection thresholds for eight candidate sampling plans varying from relatively low to high resource input.

KEY WORDS *Phyllocoptruta oleivora*, *Aculops pelekassi*, accuracy, precision, detection

THE CITRUS RUST MITE, *Phyllocoptruta oleivora* (Ashmead), has long been recognized as an important pest of citrus in Florida (Yothers 1918, Knapp et al. 1996). Extensive feeding by the mite on fruit results in russetting of the skin and associated losses in fruit quality and yield (Yothers and Miller 1934, McCoy and Albrigo 1975, McCoy el al. 1976). Models for predicting damage and yield losses (Allen 1976, 1978, 1979, 1981; Yang et al. 1995) at different mite densities can be used in conjunction with scouting estimates of mite densities to make control decisions. Sampling plans for citrus rust mites have been suggested (Yothers and Miller 1934, Knapp et al. 1996) and investigated (Hall et al. 1994). The density of citrus rust mites on fruit at which economic losses occur may vary from fewer than five up to 40 or more mites per cm^2 depending on many factors, such as the duration of an infestation and whether fruit is grown for the fresh or juice markets (Allen 1981, Knapp and Fasulo 1983, Knapp et al. 1996). A lesser known rust mite, *Aculops pelekassi* (Keifer), also commonly infests citrus fruit in Florida (Browning et al. 1995). How comparable the two mites are with respect to their importance as citrus pests is not known. The spatial dispersion of rust mites (Hall et al. 1997) increases the complexity of establishing management thresholds for citrus rust mites.

In selecting or developing a sampling plan for citrus rust mites, decisions must be made regarding the number of samples to take and how these are allocated across a grove. These decisions are primarily dependent on the level of precision required of a density estimate and the resources available for sampling. A number of different sampling plans for citrus rust mites may be similar with respect to precision (Hall et al. 1994). Basic information needed to develop a hierarchical sampling plan for estimating citrus rust mite densities on fruit with a desired level of precision has been presented and was obtained by taking 600 sample units (each unit a 1-cm^2 surface area per fruit) per 4 ha across 32 4-ha blocks of orange trees: five sample areas per 4 ha, five trees per area, 12 fruit per tree (three fruit per compass quadrant), and two samples per fruit (Hall et al. 1994). Variance components associated with each hierarchical level were used to

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project the minimum number of samples to achieve a specified level of sampling precision (Hall et al. 1994). Analyses on $\log_{10}$-transformed data indicated a sample size of $n = 80$ 1-cm^2 samples per 4 ha (20 areas per 4 ha, one tree per area, four fruit per tree, one sample per fruit) would usually provide density estimates with enough precision for commercial purposes at mean rust mite densities of 10 or more per cm^2 (Hall et al. 1994). At mean densities of three to five mites per cm^2, projections indicated estimates with enough precision for commercial purposes would usually be provided by $n = 160$ 1-cm^2 samples per 4 ha (20 areas per 4 ha, one tree per area, eight fruit per tree, one sample unit per fruit) (Hall et al. 1994). The precision levels projected for 80 and 160 samples per 4 ha have not been validated in the field.

Reducing sample size is advantageous with respect to minimizing the cost of a research or integrated pest management (IPM) program. However, reducing the number of 1-cm^2 samples taken to estimate rust mite densities can have negative effects on the precision of estimates (Hall et al. 1994) and possibly on the accuracy of estimates. Sample sizes of 80 or 160 1-cm^2 samples per 4 ha were relatively small compared with 600 samples per 4 ha and yet were projected to provide adequate precision given specified constraints (Hall et al. 1994). The influence of reducing sample size on the accuracy of density estimates for citrus rust mites and on detecting mite infestations was not known. Precision refers to the degree of statistical error in making estimates, whereas accuracy refers to the extent to which an estimated density deviates from the true mean (Buntin 1994). The accuracy of an individual density estimate is of utmost importance when an absolute true mean density estimate is desired (Buntin 1994). Some degree of inaccuracy would be expected for each of the two aforementioned sampling plans because they were formulated using variance component estimates. Although sampling precision may usually be a more important issue in an IPM program than estimate accuracy (Buntin 1994), the accuracy of an individual mean density estimate for citrus rust mites is sometimes of importance in an IPM program and often important in a research program.

Here, we report on the effects of reducing sample size on the accuracy and precision of mean density estimates of citrus rust mites on citrus fruit and on detecting rust mites on fruit, based on computer simulations by using real count data. Eight candidate sampling plans varying from relatively low-to-high resource input were evaluated.

Methods and Materials

The effect of reducing sample size on the accuracy and precision of estimates was investigated using a data set consisting of 32 subsets of rust mite counts, each subset a composite of 600 samples per 4-ha block of orange trees (Hall et al. 1994). These 32 subsets of data were from 10 different groves sampled in Florida during 1990–1991, with six groves located in north central Florida, two groves located in central Florida, one grove in east central Florida, and one in south central Florida. Seven of the groves sampled were planted to 'Hamlin' oranges and three were planted to 'Valencia' oranges. Four blocks were sampled once during June–July in 1990, and six blocks were periodically sampled during May–December 1991. Most of the samples were taken during periods that commercial growers make rust mite density estimates for management decisions. None of the groves were treated with hard miticides or insecticides during 1990 or 1991 before taking samples but some received a summer application of copper and oil, in which case sampling was not conducted until at least 6 wk after the treatment. Although one grove sampled was infested by particularly high densities of rust mites (mean 99.5 per cm^2), the count data generally reflected the normal spectrum of mite densities encountered in Florida groves. An average of 13.2 motile citrus rust mites per square centimeter was observed across the 32 blocks sampled, with individual means per block ranging from less than one to 99.5 mites per cm^2.

Table 1. Candidate sampling plans assessed for accuracy and precision of density estimates and for detecting citrus rust mites on citrus fruit

Samples/ 4 ha	Areas/ 4 ha	Trees/ area	Quadrants/ tree	Fruit/tree quadrant	Samples/ fruit
360	3	5	4	3	2
300	5	5	4	3	1
200	5	5	4	2	1
160	5	4	4	2	1
80	5	4	4	1	1
48	4	3	4	1	1
36	3	3	4	1	1
20	5	1	4	1	1

For each of the 32 data subsets, the 600 samples were allocated among five sample areas per 4 ha, five trees per area, three fruit per compass quadrant of each tree, and two sample units per fruit. A sample unit was a 1-cm^2 surface area per fruit as described previously (Hall et al. 1994), and all motile stage rust mites within each unit were counted. The data were originally collected to investigate the spatial dispersion of rust mites; evaluate the precision of the sampling plan; compare the statistical variation among the hierarchical levels to optimize sample allocation; and project the precision of sampling plans consisting of fewer than 600 samples (Hall et al. 1994). For the research presented here, the observed spectrum of rust mite counts per square centimter and mean density estimate associated with each of the 32 data subsets were assumed representative of the universal mite population within each 4-ha block, with the mean density assumed to be the universal mean.

Eight reduced sampling plans were evaluated in this study. These smaller sampling plans varied with respect to number of sample areas per 4 ha, number of trees per area, number of fruit per tree quadrant (all four quadrants always included), and number of samples per fruit (Table 1). The plans encompassed and exceeded the range of sample sizes and allocations generally used by citrus growers in Florida to monitor

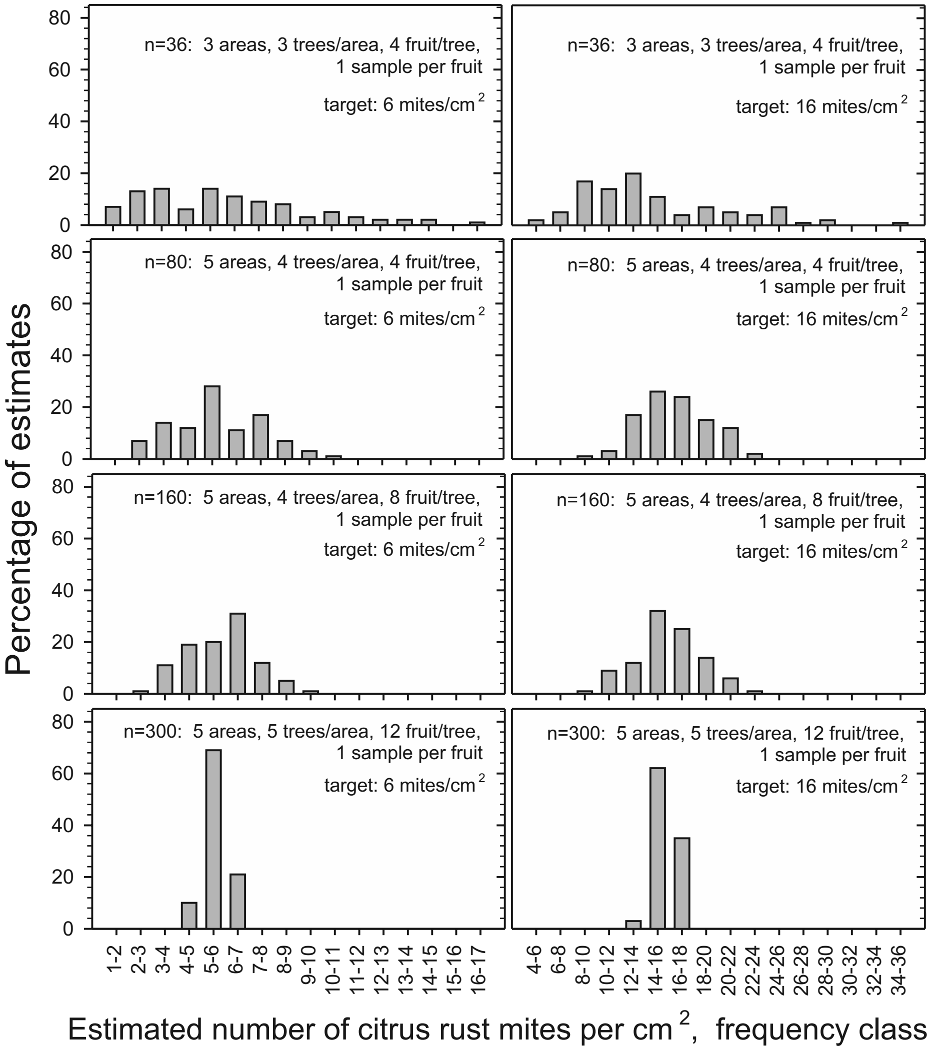

Fig. 1. Four candidate sampling plans for citrus rust mites and projected accuracy of estimates using each plan at a target rust mite density of six or 16 mites per cm^2. The graphs present frequency histograms of the percentage of 100 estimates that fell across different class intervals (class minimum $\leq$ estimate $<$ class maximum) for each target density.

rust mites. Estimates based on the eight reduced sampling plans were simulated by randomly selecting samples from the complete data set of 600 samples. For each sample size, 100 computer simulations were run on each of the 32 data subsets. The accuracy and precision of mean estimates from computer trials by using these reduced sample sizes, and the percentage of simulations in which mites were successfully detected, was investigated.

For the computer simulations of each reduced sampling plan, a computer program (SAS Institute 1999a) was used to randomly select areas per 4 ha, trees per area, fruit per tree quadrant, and sample units per fruit from the original data set. The program relied on a macro in which the appropriate numbers of areas and trees per area were randomly selected using the RANUNI function (computer-clock driven, INT function), and the appropriate numbers of fruit per tree quadrant and samples per fruit were then randomly selected using PROC SURVEYSELECT (SAS Institute 1999a, b).

Accuracy of the smaller sampling plans was evaluated by comparing individual estimates (n = 100) to the mean estimate from 600 samples (target density) for each of the 32 subsets. The accuracy of estimates using four of the reduced plans (n = 36 [3, 3, 4, 1], n = 80 [5, 4, 4, 1], n = 160 [5, 4, 4, 2], and n = 300 [5, 5, 12, 1] (numbers in brackets refer to allocation, indicating numbers of areas per 4 ha, trees per area, fruit per tree, and samples per fruit, respectively) was elucidated by examining frequency histograms for two of the 32 subsets, one with a mean of six mites per cm^2 and one with a mean of 16. Class intervals for these frequency histograms were arbitrarily chosen. Bias

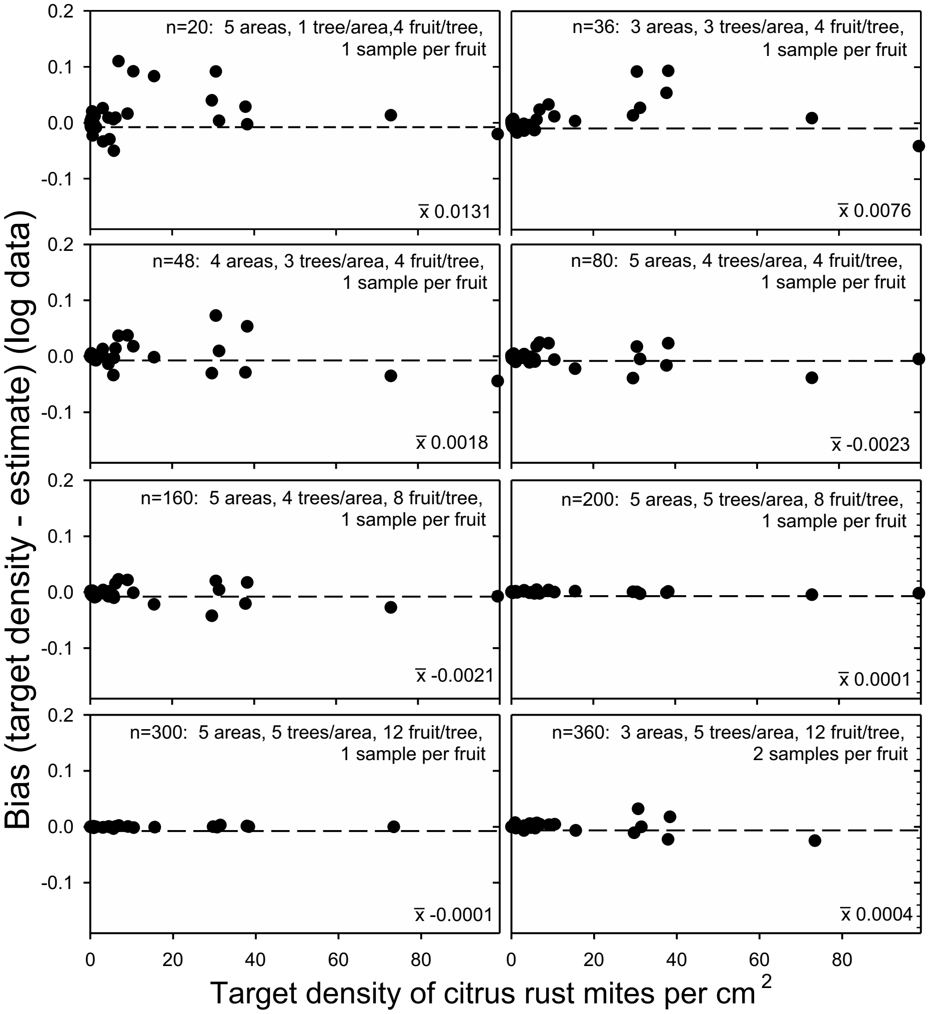

Fig. 2. Effect of reducing sample size on the accuracy of density estimates of citrus rust mites based on bias measurements from $\log_{10}$-transformed data (each data point an average from 100 estimates; positive measurements indicate an underestimate; dashed lines indicate a one-to-one, nonbiased target-to-estimate relationship).

measurements were made for estimates ($\log_{10}$ data) from each of the sampling plans in Table 1 by using the equation $B = u - m$ (Legg and Moon 1994), where u was the target density, and m was the average estimate over 100 simulations for each data set. Minimum and maximum estimates observed among the 100 simulations of each reduced sampling plan were examined as the likely inaccuracy range of estimates. Accuracy was further evaluated by calculating 95% fiducial limits for each estimate from each sampling plan and, if the limits overlapped the target density from 600 samples per 4 ha, the estimate was declared accurate (analyses for each of the 32 data sets of count data, log-transformed data). The precision of each of the reduced sampling plans was evaluated based on relative variation (SEM/mean*100) (Pedigo et al. 1972, Karandinos 1976) of raw and log-transformed counts. Relative variation was calculated for each of the 100 simulations for each sampling plan except those that produced an estimate of zero mites per cm^2. The percentage of 100 simulations in which mites were detected in at least one sample was computed to assess the probability that mites would have been detected using each sampling plan. For graphical presentations of results of the analyses, the upper range of target densities presented was sometimes truncated to magnify results pertaining to smaller mite densities.

Results and Discussion

Frequency histograms associated with citrus rust mite density estimates showed that some of the sampling plans produced more accurate density estimates than others (Fig. 1). The magnitude of estimate inaccuracies increased as sample sizes were reduced. For example, in the case of one set of original count data with a mean of six mites per cm^2, simulations using only 36 samples per 4 ha produced estimates ranging

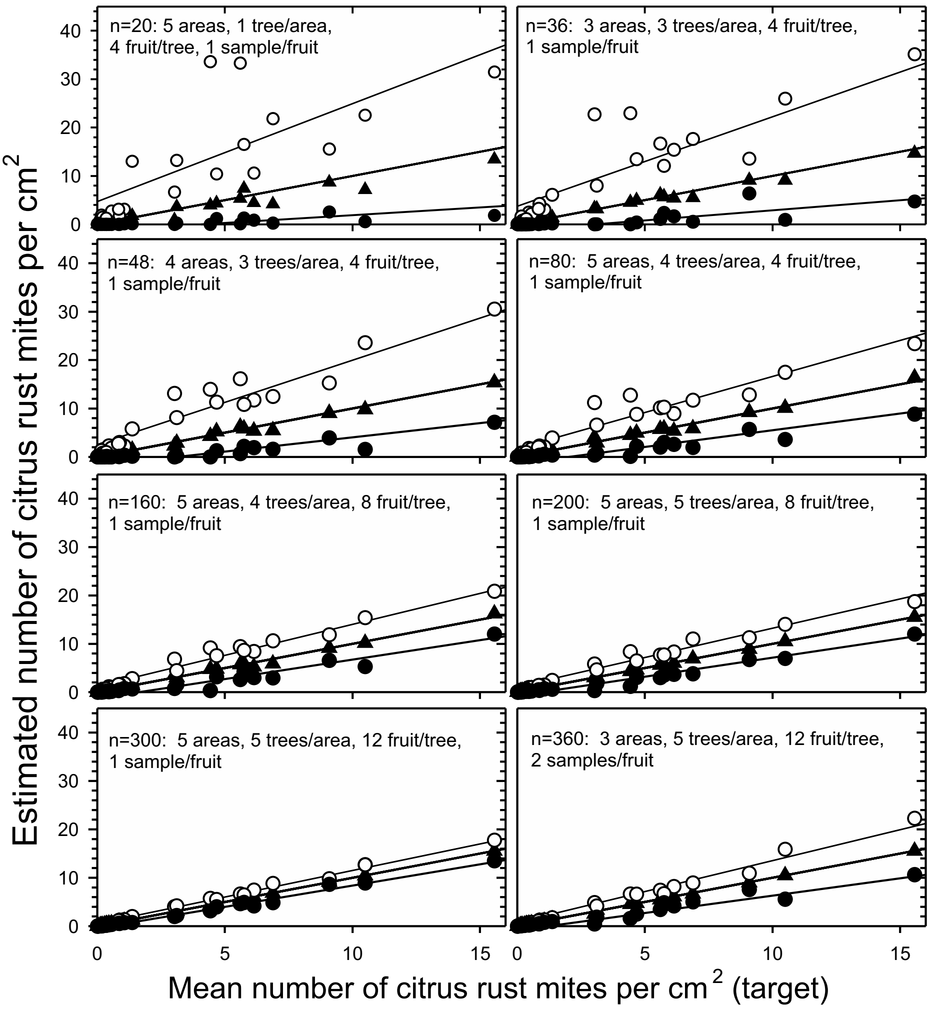

Fig. 3. Reduced sampling plans for citrus rust mites and sample accuracy: mean (▲), maximum (○), and minimum (●) estimates for number of citrus rust mites per square centimeter on fruit compared with the mean density based on 600 samples (target density). In each graph, the middle line is a simple linear regression of all estimates regressed against target density, and the lower and upper lines are simple linear regressions of minimum and maximum estimates, respectively, regressed against target density.

from one to 16 mites per cm^2, whereas simulations using 300 samples per 4 ha produced estimates ranging from four to less than seven mites per cm^2 (Fig. 1). In the case of a set of original data with a mean of 16 mites per cm^2, simulations using only 36 samples produced estimates ranging from five to 36 mites per cm^2, whereas 300 samples per 4 ha produced estimates ranging from 12 to <18 mites per cm^2. Frequency histograms of mean estimates associated with 36 samples per 4 ha were visually skewed toward densities below target densities of six or 16 mites per cm^2, indicating a bias toward underestimates (Fig. 1). Bias measurements supported that the incidence of underestimates increased as sample size was reduced (Fig. 2). In light of the mathematical probability distribution associated with rust mite counts (Hall et al. 1997), underestimates occurred because reducing sample size decreased the probability of selecting sample units with large counts. Also, in light of mite aggregation (Hall et al. 1994), reducing sample size and in particular the number of sample areas per 4 ha increased the probability of not sampling trees with larger mite infestations, which would have led to underestimates. Guarding against a gross underestimate of the mean mite density may be more important than a gross overestimate with respect making management decisions to prevent damage, but a gross overestimate may prompt a needless emergency control tactic.

Individual estimates from each sampling plan were sometimes considerably below or above mean estimates (Figs. 1 and 3). For example, estimates using the plan with 36 samples per 4 ha were projected to have an average range of from one to 13 mites per cm^2 when the target density was five mites per cm^2, and some

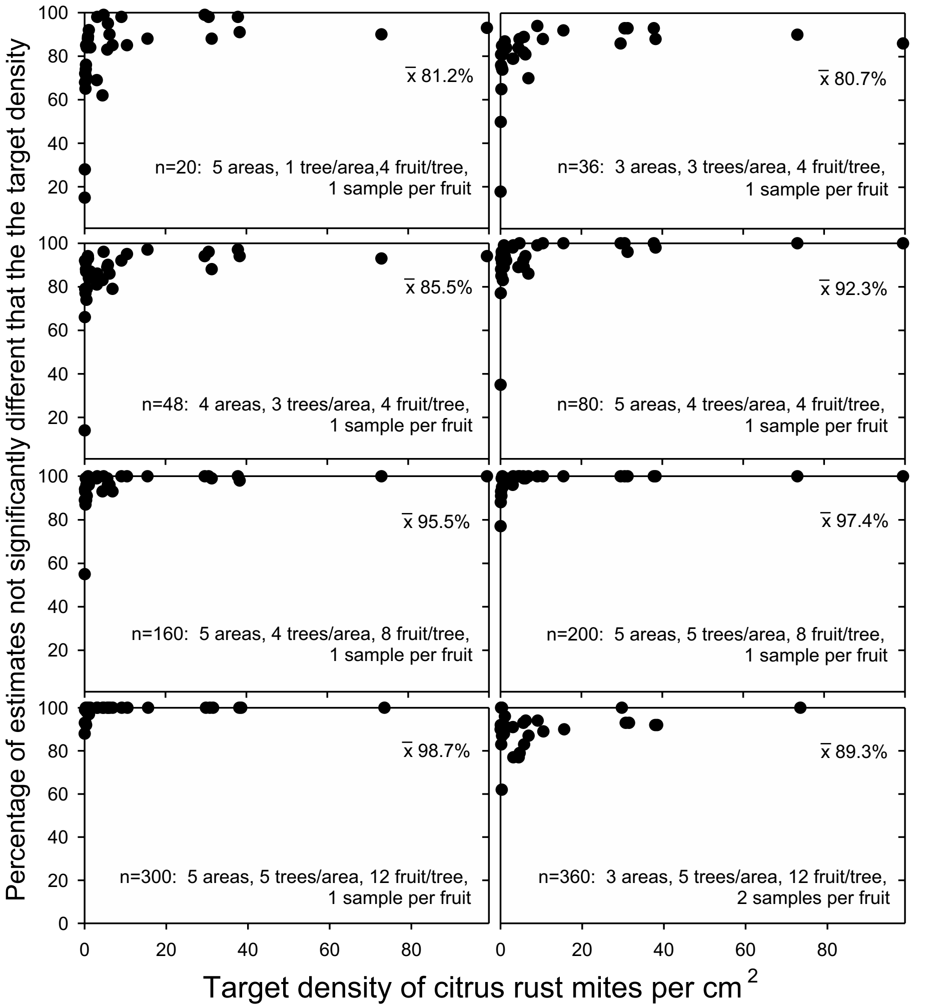

Fig. 4. Reduced sampling plans for citrus rust mites and sample accuracy: percentage of density estimates not statistically different than the target density based on 600 samples.

individual estimates exceeded this average upper range (Fig. 3). Minimum and maximum differences between individual estimates and target densities increased as mean mite densities increased regardless of sample size, but at any target density the magnitude of inaccuracies increased as sample size was decreased. For example, at a target density of five mites per cm^2, the average range of individual estimates from 80 versus 36 samples spanned from as low as three or one mite per cm^2, respectively, to as high as nine or 13 mites per cm^2, respectively. The simulations indicated that reducing the number of sample areas per 4 ha generally increased estimate inaccuracies more than reducing the number of samples per area. Previous research indicated that aggregation by mites decreased as population densities increased (Hall et al. 1994), thus including as many sample areas as possible in a reduced sampling plan should help guard against gross inaccuracies particularly when mite densities are small.

No significant difference was found for any of the reduced sampling plans between the mean estimate over all 32 data sets and the mean of the target densities based on 600 samples per 4 ha (analyses not presented), which was reflected in plots of mean density estimates against target densities (Fig. 3). However, for each of the 32 data sets taken individually, significant differences were often found between the mean estimate and the target density using some of the reduced sampling plans (Fig. 4). Based on 95% fiducial limits overlapping target densities, the most accurate sampling plans were those with 200 or 300 samples per 4 ha (Fig. 3). The fiducial limits associated with mean estimates from these two sampling plans encompassed target densities for 97.4 and 98.7%, respectively, of the 32 data sets (Fig. 4). Estimates from the sampling plan

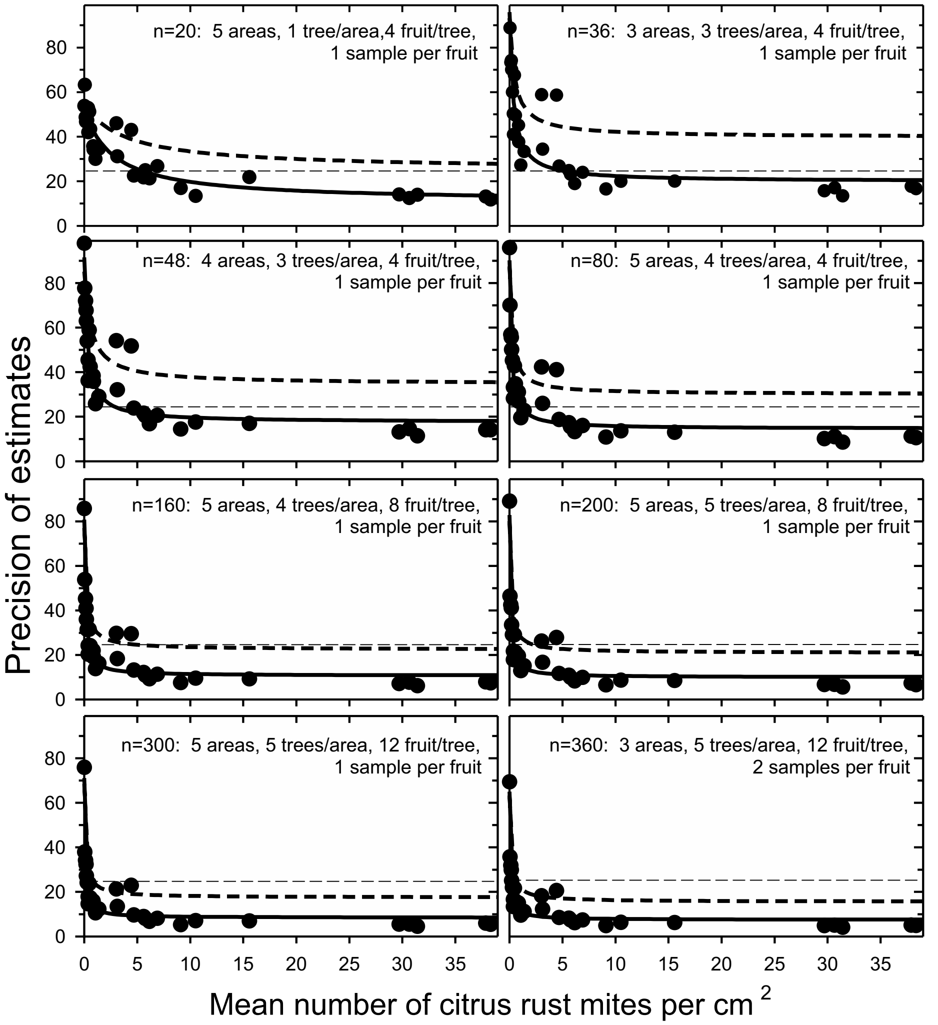

Fig. 5. Projected effect of reducing sample size on the precision (SEM/mean*100) of density estimates of citrus rust mites. Each circle represents the mean precision level of up to 100 estimates at the indicated mite density based on $\log_{10}$-transformed data. Solid lines represent average precision levels across mite densities based on $\log_{10}$-transformed data. The heavy, short-dashed line depicts average precision levels based on raw data (data points not shown). The light, short-dashed straight line references a precision level of 25%.

consisting of 360 sample units were among the most accurate of the plans studied based on the magnitude of minimum and maximum estimates (Fig. 3); however, 95% fiducial limits associated with estimates from this sampling plan overlapped the target density for 89.3% of the data sets, less often than did estimates from the sampling plans consisting of 80, 160, 200, or 300 sample units (Fig. 4). This was usually a consequence of making an underestimate in combination with reduced statistical variation associated with 360 samples (i.e., smaller confidence intervals), and the underestimates were generally a consequence of sampling only three of the original five sample areas.

Sampling precision increased as mite densities increased (Fig. 5). Reducing the number of sample areas per 4 ha generally reduced precision of estimates more than reducing the number of samples per area. For example, the precision of estimates projected for the plan consisting of 20 samples taken from five sample areas was generally better than plans consisting of 36 or 48 samples taken from fewer sample areas, indicating that a sampling plan should emphasize the number of areas sampled. These results complimented those from variance component analyses on relative variation (Hall et al. 1994) that indicated greater precision in estimates would be achieved by placing more emphasis on the number of sample areas per 4 ha than on the number of samples taken per area. The sampling plans consisting of 160 or more samples per cm^2 were projected to provide average precision levels of 25% or better at mean densities of five or more mites per cm^2 based on raw data counts. When the count data were

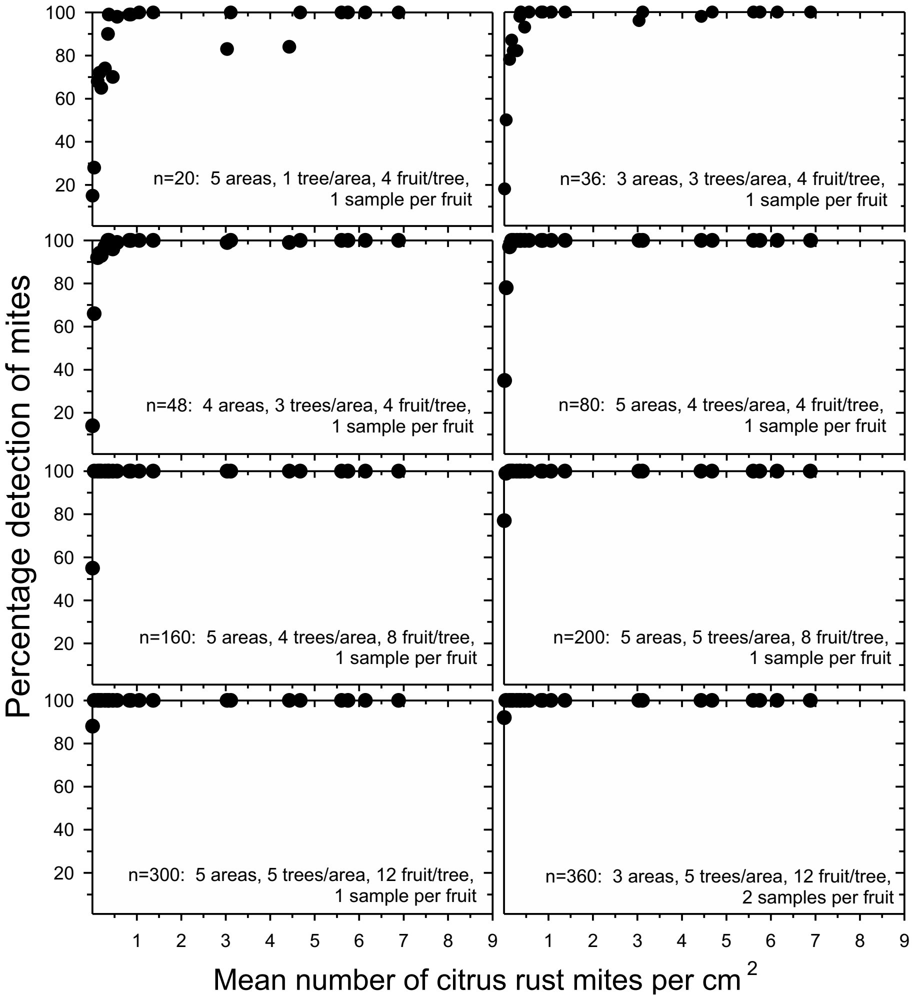

Fig. 6. Effect of reducing sample size on detecting citrus rust mites on fruit. Each circle represents the percentage of estimates (n = 100) within which at least one mite would have been detected based on computer simulations.

transformed to $\log_{10}$, each sampling plan including the plan with only 20 samples was projected to provide average precision levels of 25% or better at mite densities of five or more mites per cm^2. These results were in general disagreement with previous projections based on log-data that >80 samples would be needed to achieve these precision levels at a mean density of five mites; that >48 samples would be needed for these precision levels at a mean density of 20 mites; and that at any given mite density, marked improvement in precision would occur as sample size was increased (Hall et al. 1994). Research to verify the precision of reduced sampling plans for citrus rust mites in real grove situations is needed.

The computer simulations indicated that each sampling plan studied, including the plan with only 20 sample units per 4 ha, consistently facilitated mite detection at target densities above approximately five mites per cm^2 (Fig. 6). At densities from one to five mites per cm^2, simulations indicated 36 samples per 4 ha would usually be enough to detect mites, but not always. If management thresholds for citrus rust mites are low (for example, five or fewer mites per cm^2) and a sampling plan with acceptable levels of accuracy and precision too costly for a commercial program, then there may be no choice but to base management decisions on a sampling plan designed to detect the presence of mites. However, as pointed out by Venette et al. (2002), a sampling plan with a limited number of samples may lead to the erroneous conclusion that a pest is absent. Our data indicated that sampling plans with 36 or fewer samples per 4 ha would sometimes be inadequate for detecting mites when fewer than five were present per cm^2. The cost of a sampling plan consisting of larger sample sizes could be reduced using estimation keys (Rogers et al. 1994) or by recording if mites are present or absent in each sample unit, rather than counting each mite. With respect to presence or absence sampling, previous research indicated a good quantitative relationship between the percentage of sample units with at least one mite and the mean density per square centimeter (Hall et al. 1997).

With respect to error margins for an estimated rust mite density based on a hierarchical sampling plan like those discussed in this report, the standard error of the mean presented in a nested analysis of variance (ANOVA) (PROC NESTED; SAS Institute 1999a, b) can be computed using a variance component formula (Snedecor and Cochran 1967, Hall et al. 1994) to compare different sample sizes and allocations. This standard error is the same as the standard error associated with the mean density per area (i.e., compute the mean density within each area and then compute the standard error among areas). We thought it prudent to relay the standard error calculation method for the hierarchical sampling plans for the benefit of those who may be unaware of the method.

In summary, the results of our simulated rust mite sampling provide growers, extension agents, and researchers insight into the accuracy, precision, and detection thresholds projected for eight candidate sampling plans varying from low resource input (20 1-cm^2 sample units per 4 ha) to relatively high resource input (300 1-cm^2 sample units per 4 ha). Based on the research, the accuracy and precision of density estimates for the mean number of citrus rust mites per square centimeter on fruit will decrease as the number of samples included in a sampling plan is reduced. Reducing sample size will increase the likelihood of underestimates. The research supported recommendations by Knapp et al. (1996) that representative areas throughout an entire block of trees should be sampled, as our research indicated that reducing the number of areas sampled reduced the accuracy and precision of density estimates. When the management threshold for citrus rust mites is relatively low, i.e., below five per cm^2, utilizing a commercial sampling plan consisting of fewer than $\approx$48 samples per 0.4 ha may lead to erroneous management decisions due to reduced estimate accuracy and precision. We elected not to make specific sampling plan recommendations in this report because, ultimately, the choice of a sampling plan will be a consequence of varying factors such as the reason for sampling, the desired precision or accuracy of estimates, and resources available for sampling. The simulations indicated sampling plans consisting of 48–80 samples per 4 ha would provide better levels of precision than previously projected, thus research is needed to clarify the precision of reduced sampling plans under real-grove situations. If a sampling plan is used solely to determine if mites are present, results of the computer simulations indicated each of the sampling plans would consistently provide mite detection except the plans consisting of 20 or 36 samples per 4 ha, which sometimes did not provide mite detection when the mean density was less than approximately five mites per cm^2.

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Received 17 November 2004; accepted 17 February 2005.