

# Binomial Sampling to Estimate Rust Mite (Acari: Eriophyidae) Densities on Orange Fruit

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**ABSTRACT** Binomial sampling based on the proportion of samples infested was investigated for estimating mean densities of citrus rust mite, *Phyllocoptruta oleivora* (Ashmead), and *Aculops pelekassi* (Keifer) (Acari: Eriophyidae), on oranges, *Citrus sinensis* (L.) Osbeck. Data for the investigation were obtained by counting the number of motile mites within 600 sample units (each unit a 1-cm<sup>2</sup> surface area per fruit) across a 4-ha block of trees (32 blocks total): five areas per 4 ha, five trees per area, 12 fruit per tree, and two samples per fruit. A significant ( $r^2 = 0.89$ ), linear relationship was found between  $\ln(-\ln(1 - P_0))$  and  $\ln(\text{mean})$ , where  $P_0$  is the proportion of samples with more than zero mites. The fitted binomial parameters adequately described a validation data set from a sampling plan consisting of 192 samples. Projections indicated the fitted parameters would apply to sampling plans with as few as 48 samples, but reducing sample size resulted in an increase of bootstrap estimates falling outside expected confidence limits. Although mite count data fit the binomial model, confidence limits for mean arithmetic predictions increased dramatically as proportion of samples infested increased. Binomial sampling using a tally threshold of 0 therefore has less value when proportions of samples infested are large. Increasing the tally threshold to two mites marginally improved estimates at larger densities. Overall, binomial sampling for a general estimate of mite densities seemed to be a viable alternative to absolute counts of mites per sample for a grower using a low management threshold such as two or three mites per sample.

**KEY WORDS** *Phyllocoptruta oleivora*, *Aculops pelekassi*, proportions, presence-absence sampling

The citrus rust mite, *Phyllocoptruta oleivora* (Ashmead), is an important pest in Florida citrus (Yothers 1918, Knapp and Fasulo 1983). The lesser known rust mite *Aculops pelekassi* (Keifer) (Acari: Eriophyidae) also commonly infests citrus fruit in Florida (Childers and Achor 1999). Extensive feeding by rust mites on fruit results in a russetting of the skin of the fruit with associated losses in fruit quality and yield (Yothers and Miller 1934, McCoy and Albrigo 1975, McCoy et 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 mite control decisions. Based on Allen (1981) and Yang et al. (1995), economic thresholds for citrus rust mites may vary from fewer than five up to 40 or more mites per cm<sup>2</sup> depending on factors such as the duration of an infestation, time of year, and whether fruit is grown for the fresh or juice markets. How comparable *P. oleivora* and *A. pelekassi* are with respect to their

importance as citrus pests is not known. Currently, the same scouting and management programs are used to manage both species (Childers and Achor 1999).

Research investigations on sampling to estimate citrus rust mite densities on fruit have been published previously (Hall et al. 1991, 1994, 2005), and sampling plan recommendations are available to growers (Childers et al. 2005). Rust mite density estimates are commonly based on direct counts of the number of motile mites per sample unit, with the sample unit being a 1-cm<sup>2</sup> surface area of fruit under the lens-field of a 10 to 20 $\times$  magnifier. However, counting rust mites can be tedious and time-consuming when large densities are present. As an alternative to counting mites per sample, a method of estimating the count per sample has been presented (Rogers et al. 1994). A binomial sampling plan also may be an alternative to counting mites. Statistical relationships between the mean rust mite density per sample unit on fruit and the percentage of sample units containing at least one motile mite have been observed (Hall et al. 1997). Early survey techniques by growers were sometimes based on percentage of samples infested (McCoy et al. 1976). Binomial sampling plans based on the presence or absence of mites have been developed for other

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mite species, including *Tetranychus urticae* Koch (Nachman 1984, Binns and Bostanian 1990), *Phytoseiulus persimilis* Athias-Henriot (Nachman 1984), *Panonychus ulmi* (Koch) (Binns and Bostanian 1990), and *Euseius tularensis* (Congdon) (Grout 1985); for aphids, such as *Diuraphis noxia* (Mordvilko) (Schaalje et al. 1991); and for other arthropod species, such as *Diabrotica longicornis* Say and *Diabrotica virgifera* Le Conte (Gerrard and Chiang 1970). Considerable information is available on relating proportion of samples infested to mean density (Kono and Sugino 1958, Gerrard and Chiang 1970, Nachman 1984, Binns and Bostanian 1990, Schaalje et al. 1991, Jones 1994). Sampling plans based on the frequency of occurrence of individuals in a sample unit (presence or absence) can be based on a tally threshold of 0 (e.g., the presence of at least one motile mite per sample unit), but the fit of the binomial is sometimes improved by using a higher tally threshold (Nachman 1984) (e.g., threshold = 2, the presence of three or more motile stages per sample unit).

Here, we report the results of research on binomial, fixed sample size sampling plans for estimating rust mite densities on citrus fruit.

### Materials and Methods

Binomial sampling to estimate densities of citrus rust mites on oranges was investigated using a data set consisting of 32 subsets of rust mite counts. Each subset was a composite of 600 samples per 4-ha block of orange, *Citrus sinensis* (L.) Osbeck, trees and was originally described by Hall et al. (1994). For each subset, the 600 samples were allocated among five areas per 4 ha, five trees per area, three fruit per compass quadrant of each tree, and two sample units per fruit. The first tree in each area was randomly selected, and the following four trees were sampled systematically as described previously (Hall et al. 1994). A sample unit was a 1-cm<sup>2</sup> surface area per fruit as described previously (Hall et al. 1994), and all motile-stage rust mites within each unit were counted (an estimation procedure was sometimes used if a sample unit contained >100 mites). 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). In addition to the 600 sample sampling plan, a separate sampling plan consisting of 192 samples per 4 ha was studied (two samples per fruit, four fruit per tree, 12 trees along one transect between the northeastern and southwestern corners of the block, and 12 trees along a second transect between the northwestern and southeastern corners of the block) (Hall et al. 1994). Comparisons indicated estimates from each plan were the same 69% of the time and that, among estimates that differed, mean densities were less than one motile mite per cm<sup>2</sup> 70–80% of the time (Hall et al. 1994). In the study presented here, after fitting data from the

sampling plan with 600 samples to the binomial, we used the data from this second, separate sampling plan to validate fit of the binomial.

**Binomial Analyses on  $P_T$  ( $T = 0$ ).** The binomial relationship between mean density of rust mites per square centimeter (mean) and proportion of samples infested ( $P_T$ ) by greater than  $T$  mites ( $T = 0$ ) was investigated using the following empirical formulae (Kono and Sugino 1958, Gerrard and Chiang 1970):

$$\ln(\text{mean}) = a' + b' \ln(-\ln(1 - P_T)) \quad [1]$$

$$\text{mean} = \exp^{a'} [(-\ln(1 - P_T))^{b'}] \quad [2]$$

The  $a'$  and  $b'$  parameters of equation 1 were determined using simple linear regression. After determining  $a'$  and  $b'$ , these parameters were applied in equation 2 to obtain the prediction on an arithmetic scale. Because the mean prediction from equation 2 [or from antilogs of  $\ln(\text{mean})$ ] is a biased estimate (Nachman 1984, Binns and Bostanian 1990, Schaalje et al. 1991), the prediction can be improved using a factor to correct for the bias. We used the correction factor offered by Nachman (1984):

$$\text{mean}_{\text{adj}} = \text{mean}(\exp(0.5 \text{ mse})) \quad [3]$$

where mse was the residual mean square error associated with the regression of  $\ln(\text{mean})$  on  $\ln(-\ln(1 - P_T))$  (equation 1). With respect to calculating a variance associated with a mean prediction, Schaalje et al. (1991) present a detailed review of variance estimates for a density prediction based on equation 1. Their variance estimate for  $\ln(\text{mean})$  consists of the following four components:

$$c1 = P_T b'^2 / [n(1 - P_T)((\ln(1 - P_T)) (\ln(1 - P_T)))] \quad [4]$$

$$c2 = \text{mse} \{1/N + [\ln(-\ln(1 - P_T)) - p_{\text{avg}}]^2 / \text{SSP}\} \quad [5]$$

$$c3 = \exp[\ln(a) + (b - 2) \cdot (a' + b' \ln(-\ln(1 - P_T)))] / n \quad [6]$$

$$c4 = \text{mse} \quad [7]$$

where  $n$  is the number of samples taken from a population, 600 in our case;  $N$  is the number of population means used to fit equation 1, 32 in our case; mse is the residual mean square error from the regression of  $\ln(\text{mean})$  on  $\ln(-\ln(1 - P_T))$ , i.e., equation 1;  $p_{\text{avg}}$  is the mean value of  $\ln(-\ln(1 - P_T))$  for the populations used in the regression; SSP is the sum of the squared deviations of the  $\ln(-\ln(1 - P_T))$  values from  $p_{\text{avg}}$ ;  $\Sigma [p_{\text{avg}} - \ln(-\ln(1 - P_T))]^2$ ; and  $a$  and  $b$  are the parameters associated with Taylor's power law (Taylor 1961):

$$\ln(\text{variance}) = a + b \ln(\text{mean}) \quad [8]$$

Variance component c1 was originally proposed by Kuno (1986), c2 by Gerrard and Chiang (1970), c3 by Nachman (1984), and c4 by Binns and Bostanian

(1990). Our equations for  $c_1$ ,  $c_2$ , and  $c_3$  were modified versions of those presented by Schaalje et al. (1991) for reasons discussed later. Schaalje et al. (1991) present a discussion of these four variance components and rationale for the following equation for estimating total variance:

$$\text{Var}_s(\ln(\text{mean})) = c_1 + c_2 + (c_4 - c_3) \quad [9]$$

This variance  $\text{Var}_s(\ln(\text{mean}))$  can be converted to arithmetical scale ( $V_{\text{arm}}$ ) by using (from Schaalje et al. 1991):

$$V_{\text{arm}} = \text{mean}^2 \text{Var}_s(\ln(\text{mean})) \quad [10]$$

Jones (1994) presented the following formula for calculating confidence limits around an estimate from equation 1:

$$\ln(\text{mean}) \pm z_{\alpha/2} \text{sqrt}(\text{Var}_s(\ln(\text{mean}))) \quad [11]$$

where  $z_{\alpha/2}$  is the standard normal deviate (for 95% confidence,  $z = 1.96$ ). For minimum bias on the arithmetic scale, these intervals can be added or subtracted to individual  $\ln(\text{mean})$  predictions to obtain minimum and maximum limits based on natural logs and then converted to arithmetical scale using (from Schaalje et al. 1991):

$$\text{limit}_{\text{arm}} = [\exp(\ln(\text{limit}))][1 - 0.5 (\text{Var}_s(\ln(\text{mean})))] \quad [12]$$

Parameters associated with equations 1–12 were determined from our data, and the fit of the binomial was then evaluated using the statistical analysis associated with the regression equation 1, plots of the residuals from this regression, and visual fit of a plot of  $\ln(\text{mean})$  on  $\ln(-\ln(1 - P_T))$ , where  $T = 0$ . The fit of the validation data set to these binomial parameters was then evaluated by comparing observed proportions of samples infested and corresponding mean densities to the means and confidence limits expected based on the fitted binomial parameters.

**Reduced Sampling Plans, Binomial Analyses on  $P_T$  ( $T = 0$ ).** The effect of sample size on estimation of mean density from proportions of samples infested was investigated using bootstrap simulations (Efron and Tibshirani 1986, Hutchison 1994, Beers and Jones 2004). Data for sample sizes of 200, 160, 80, and 48 samples per 4 ha were simulated by randomly selecting samples from the 32 data sets of real count data from 600 samples as described previously (Hall et al. 2005). These sampling plans varied with respect to the number of 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 range of sample sizes and allocations generally used by citrus growers in Florida to monitor rust mites were encompassed by these sampling plans. For each sampling plan, 500 bootstrap simulations were run on each of the 32 data sets. A computer program was written in SAS (SAS Institute 1999a) to randomly select areas per 4 ha, trees per area, fruit per tree quadrant, and sample units per fruit from each

**Table 1.** Sample sizes and allocation of data sets used to determine the binomial relationship (600 samples per 4 ha), data sets of real count data used to validate the relationship (192 samples per 4 ha), and data sets of simulated count data from reduced sampling plans

Samples per 4 ha	Areas per 4 ha	Trees per area	Quadrants per tree	Fruit per tree quadrant	Samples per fruit
600 <sup>a</sup>	5	5	4	3	2
192 <sup>b</sup>	48	1	4	1	1
200 <sup>c</sup>	5	5	4	2	1
160 <sup>c</sup>	5	4	4	2	1
80 <sup>c</sup>	5	4	4	1	1
48 <sup>c</sup>	4	3	4	1	1

<sup>a</sup>This was the sampling plan used to obtain data for binomial modeling.

<sup>b</sup>This was the validation data set, real data collected in the same citrus blocks that were sampled using the sampling plan consisting of 600 samples. The plan has previously been referred to as a transect plan (Hall et al. 1994).

<sup>c</sup>These were computer simulated by randomly selecting data from the data set of count data from 600 samples per 4 ha.

original data set. The program relied on a computer routine in which the appropriate number of areas and trees per area were randomly selected using the RANUNI function, and the appropriate number of fruit per tree quadrant and samples per fruit were then randomly selected using PROC SURVEYSELECT (SAS Institute 1999a,b). After these bootstrap simulations, simple linear regression of proportions of samples infested according to each reduced sampling plan on the proportions observed to be infested according to 600 samples was conducted and 95% confidence limit (CL) determined to evaluate the effect of reducing sample size on estimating  $P_0$ . For each reduced sampling plan,  $\ln(\text{mean})$  density estimates were computed using bootstrapped proportions of samples infested ( $P_0$ ) in conjunction with the binomial parameters determined from the complete data set of 600 samples, and the percentage of these  $\ln(\text{mean})$  estimates that fell within the 95% CL expected according to the complete data set was determined. Also, for each reduced sampling plan, plots of  $\ln(\text{mean})$  on  $\ln(-\ln(1 - P_0))$ , and of arithmetic mean ( $\text{mean}_{\text{adj}}$ ) on  $P_0$ , were visually evaluated for similarities/deviations from plots of data associated with the complete data set.

**Binomial Analyses on  $P_T$  ( $T = 2$ ).** The fit of the binomial to the citrus rust mite data based on a tally threshold of two motile mites per sample was investigated. The proportion of samples with  $>2$  mites ( $P_2$ ) was computed for each data set. Parameters associated with equations 1–12 were determined from the data, and the fit of the binomial was evaluated using the same procedures used to evaluate  $P_0$ . The fit of the validation data set to the binomial parameters associated with  $P_2$  was then evaluated by comparing observed proportions of samples infested and mean densities to the means and confidence limits expected based on the fitted binomial  $P_2$  parameters.

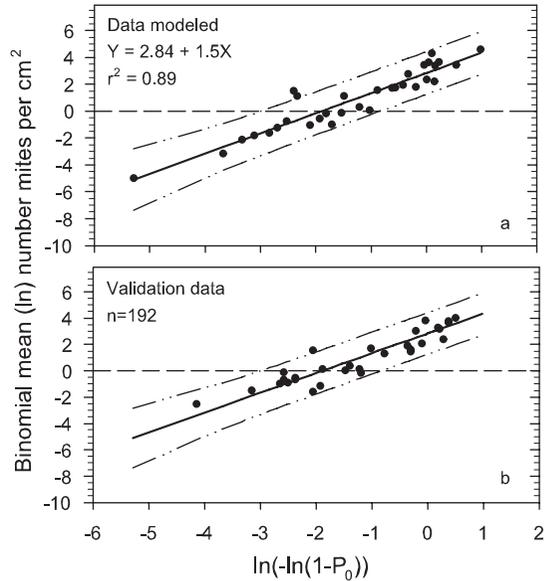
**Table 2.** Observed motile rust mite densities per square centimeter on oranges and proportion of samples infested  $P_T$  for tally thresholds of  $T = 0$  and  $T = 2$  ( $n = 600$  samples per data set)

Grove	Date	Mean no. mites per cm <sup>2</sup>	$P_0$	$P_2$
1	31 May 1989	10.5	0.63	0.28
1	8 June 1989	30.7	0.62	0.56
1	6 July 1989	15.6	0.51	0.42
2	19 July 1989	99.5	0.93	0.91
3	24 May 1991	5.6	0.42	0.23
3	8 July 1991	0.4	0.12	0.04
3	13 Aug. 1991	5.7	0.43	0.29
3	10 Sept. 1991	0.8	0.15	0.08
3	11 Oct. 1991	0.0	0.01	0.00
4	17 June 1991	0.9	0.19	0.08
4	12 July 1991	0.3	0.07	0.03
4	19 Aug. 1991	3.1	0.20	0.14
4	12 Sept. 1991	0.2	0.06	0.02
4	11 Oct. 1991	0.0	0.03	0.01
5	2 July 1991	9.1	0.69	0.49
5	28 Aug. 1991	0.6	0.14	0.05
5	20 Sept. 1991	0.1	0.04	0.01
6	10 July 1991	0.5	0.08	0.03
6	29 Aug. 1991	3.0	0.09	0.07
6	16 Sept. 1991	4.4	0.09	0.06
6	25 Oct. 1991	4.7	0.34	0.25
7	12 Aug. 1991	73.4	0.67	0.49
7	25 Aug. 1991	31.4	0.82	0.61
7	25 Sept. 1991	0.4	0.17	0.04
7	16 Oct. 1991	6.1	0.56	0.35
7	18 Nov. 1991	6.9	0.48	0.27
8	13 Aug. 1991	0.2	0.04	0.01
8	28 Aug. 1991	1.4	0.26	0.09
8	26 Sept. 1991	1.1	0.30	0.11
8	17 Oct. 1991	29.7	0.69	0.55
8	19 Nov. 1991	37.9	0.65	0.49
8	13 Dec. 1991	38.3	0.72	0.54

**Results and Discussion**

Observed mean densities of motile rust mites per square centimeter and corresponding  $P_T$  probabilities for  $T = 0$  and two across the 32 data sets studied are presented in Table 2.

**Binomial Analyses on  $P_T$  ( $T = 0$ ).** A significant ( $r^2 = 0.89$ ), linear relationship was found between  $\ln(-\ln(1 - P_0))$  and  $\ln(\text{mean})$  (Fig. 1a), where  $P_0$  was the proportion of samples infested. No model lack of fit was detected in plots of residuals from the regression equation 1 (analyses not presented). Two data points fell just outside of the 95% CL expected for the binomial relationship (Fig. 1a). Parameters for predicting a mean density from a proportion of samples infested based on the sampling plan of  $n = 600$  were as follows:  $a' = 2.837$ ,  $b' = 1.500$ ,  $a = 3.147$ ,  $b = 1.567$ ,  $p_{\text{avg}} = -1.311$ ,  $\text{SSP} = 65.129$ ,  $\text{mse} = 0.6266$ ,  $n = 600$ , and  $N = 32$ . Of 30 sets of real count data (192 samples per 4 ha) not included in determining the binomial parameters, the  $\ln(\text{mean})$  estimate for each of 29 data sets fell within the confidence intervals expected by the fitted binomial relationship (Fig. 1b). Two of the original 32 validation data sets could not be tested for fit, one in which no samples contained any mites (mean of 0.01 mites per cm<sup>2</sup> in the corresponding data set modeled) and one which averaged 112.5 mites per cm<sup>2</sup>, and every sample contained mites (mean of 99.5 mites per cm<sup>2</sup> in the corresponding data set modeled).



**Fig. 1.** Fit of rust mite count data to the binomial relationship between  $\ln(\text{mean})$  and  $\ln(-\ln(1 - P_0))$  (tally threshold of 0 mites per cm<sup>2</sup>). The solid line depicts mean predictions, dashed lines depict upper and lower 95% CL for individual predictions, and data points show the observed values. (a) Fit of original data modeled. (b) Fit of validation data to expected means and confidence intervals.

On the arithmetic scale, observed means of 29 of the 32 data sets modeled fell within the binomial confidence limits (Fig. 2a). The observed arithmetic mean of one of 30 validation data sets fell just outside the expected binomial confidence limits (Fig. 2b). The arithmetic data supported fit of the binomial model to rust mite count data. However, for arithmetic means derived from the binomial log means, confidence limits increased dramatically as the proportion of samples infested increased (Fig. 2a and b). This indicated that binomial sampling using a tally threshold of 0 would have less value for estimating the density of motile rust mites when the proportion of samples infested is large. Variability in mean densities at larger proportions of samples infested might be related to normal progression of a rust mite infestation up to and past a peak density. After a population of rust mites reaches a peak density, densities per sample might decline faster than the proportion of samples infested. A possible example is reflected by the means and proportions of samples infested associated with samples taken in grove 7 on 12 and 25 August (Table 2).

Confidence limits for mean estimates based on binomial sampling were narrower at lower probability levels. At  $P_0 = 0.20$ , the binomial relationship indicated an arithmetic mean of 2.5 motile rust mites per cm<sup>2</sup> with a maximum of 6.0 and minimum of 0.2 per cm<sup>2</sup> (Fig. 2a). At  $P_0 = 0.10$ , the binomial relationship indicated an arithmetic mean of 0.8 motile rust mites per cm<sup>2</sup> with a maximum of 2.0 and minimum of 0.1 per cm<sup>2</sup>. The data indicated the binomial relationship would not allow one to declare significant differences

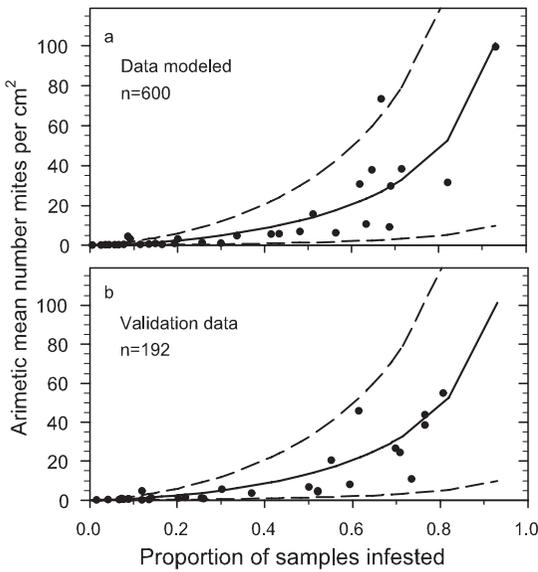


Fig. 2. Arithmetic mean number of motile citrus rust mites per cm<sup>2</sup> observed and predicted over different proportions of samples infested ( $P_0$ ) based on the binomial relationship between  $\ln(\text{mean})$  and  $\ln(-\ln(1 - P_0))$  (tally threshold of 0 mites per cm<sup>2</sup>). The solid line depicts mean predictions from the original data set, dashed lines depict upper and lower 95% CL for these mean predictions, and data points show the observed mean densities. (a) Fit of original data modeled. (b) Fit of validation data.

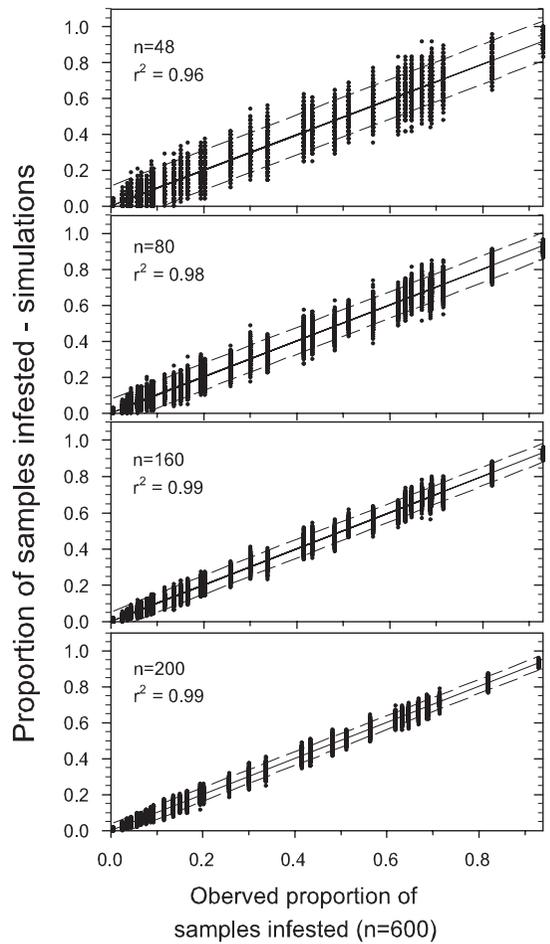


Fig. 3. Effect of reducing sample size on estimating proportions of samples infested. The solid line depicts mean predictions, dashed lines depict upper and lower 95% CL for individual predictions, and data points show means of bootstrapped data for  $n = 48, 80, 160,$  and  $200$  samples per 4 ha.

among mean predictions across a range of small densities. Noted was that at a  $P_0$  probability of 0.09 with an upper 95% confidence limit of 1.6 mites per cm<sup>2</sup>, two observed mean densities (3.0 and 4.4 mites per cm<sup>2</sup>) fell above the expected range. Overall, however, binomial sampling to make general density estimates for rust mites seemed to be a suitable alternative to absolute counts of mites per sample for a grower using a low management threshold such as two or three motile mites per sample.

**Reduced Sampling Plans, Binomial Analyses on  $P_T$  ( $T = 0$ ).** Regression analyses on data from the reduced sampling plans showed significant relationships ( $r^2 = \geq 0.96$  and greater) between estimated proportions of samples infested by rust mites and observed proportions based on the complete sampling plan (Fig. 3). As sample size was reduced from 200 to 160 to 80 to 48 samples per 4 ha, error margins (95% confidence intervals) associated with individual estimates of the proportion of samples infested increased from  $\pm 0.038$  to  $\pm 0.051$  to  $\pm 0.074$  to  $\pm 0.111$ . Inaccuracies associated with estimates of  $P_0$  would lead to inaccuracies in mean density predictions derived using the binomial parameters. The number of bootstrap  $\ln(\text{mean})$  densities that fell outside of the 95% CL of the fitted binomial relationship generally increased as sample size was reduced. Totals of 5.1, 5.7, 6.0, and 6.9% of the  $\ln(\text{mean})$  densities bootstrapped for sample sizes of 200, 160, 80, and 48 samples per 4 ha, respectively, fell outside the expected binomial confidence limits. Plots

of  $\ln(\text{mean})$  on  $\ln(-\ln(1 - P_0))$  according to each reduced sampling plan resembled Fig. 1, and plots of  $\text{mean}_{\text{adj}}$  on  $P_0$  resembled Fig. 2 (plots for reduced sampling plans not presented). Overall, the projections indicated that the relationship between mean density and proportion of samples infested based on the reduced sampling plans in real situations would be similar to the relationship based on 600 samples per four ha, and that the same constraints to binomial sampling noted for the sampling plan consisting of 600 samples would apply to reduced sampling plans.

**Binomial Analyses on  $P_T$  ( $T = 2$ ).** A significant ( $r^2 = 0.93$ ), linear relationship was found between  $\ln(-\ln(1 - P_2))$  and  $\ln(\text{mean})$  (Fig. 4a), where  $P_2$  was the probability of greater than two motile mites in a sample. No curvature bias was detected in plots of residuals from the regression equation 1 (analysis not presented). Two data points fell outside the 95% confidence intervals expected for relationship between  $\ln(\text{mean})$  and  $\ln(-\ln(1 - P_2))$  (Fig. 4a). Parameters

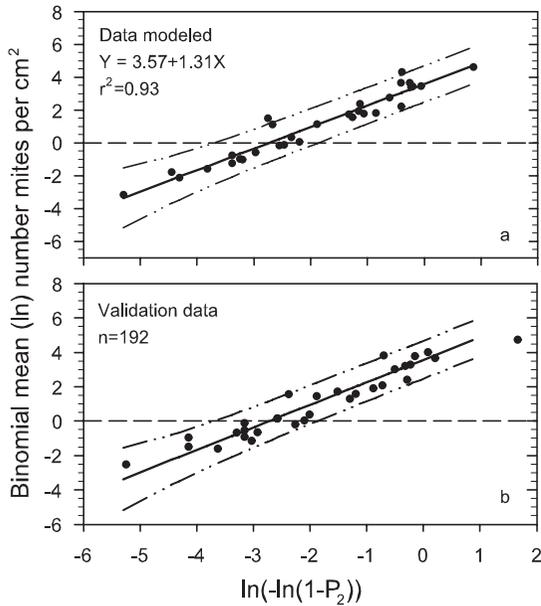


Fig. 4. Fit of rust mite count data to the binomial relationship between  $\ln(\text{mean})$  and  $\ln(-\ln(1 - P_2))$  (tally threshold of two motile mites per  $\text{cm}^2$ ). The solid line depicts mean predictions, dashed lines depict upper and lower 95% CL for individual predictions, and data points show the observed values. (a) Fit of original data modeled. (b) Fit of validation data to expected means and confidence intervals.

for predicting a mean density from the proportion of samples infested by three or more motile mites based on the sampling plan of  $n = 600$  were as follows:  $a' = 3.575$ ,  $b' = 1.311$ ,  $a = 3.147$ ,  $b = 1.567$ ,  $n = 600$ ,  $N = 31$  (data set 3–28 was dropped from the analysis because it had no samples of three or more mites per  $\text{cm}^2$ ),  $p_{\text{avg}} = -1.917$ ,  $\text{SSP} = 70.347$ , and  $\text{mse} = 0.2998$ . Of 32 sets of real count data (192 samples per 4 ha) used to validate the  $P_2$  binomial parameters, the mean estimate for each of 30 data sets fell within the confidence limits for the fitted binomial relationship (Fig. 4b). The mean from one data set fell outside of the observed range of  $\ln(-\ln(1 - P_2))$  in the data set modeled, but this mean fell within extrapolated 95% CL. These data validated general fit of the  $P_2$  binomial model to rust mite count data.

On the arithmetic scale, observed means of 29 of the 32 data sets modeled fell within the binomial confidence limits expected for a tally threshold of two motile mites per sample (Fig. 5a). The observed arithmetic means of 30 of the 32 validation data sets fell within the expected binomial confidence limits (Fig. 5b). The arithmetic data supported general fit of rust mite count data to the binomial with a tally threshold of 2. However, similar to the binomial with a tally threshold of 0, confidence limits increased dramatically as the proportion of samples infested increased (Fig. 5a, 5b). Comparisons of  $P_0$  and  $P_2$  with respect to confidence limits indicated little difference between tally thresholds of 0 and 2. For example,  $P_0$  and  $P_2$  tally thresholds at  $P_T$  levels of 0.33 and 0.20, respectively,

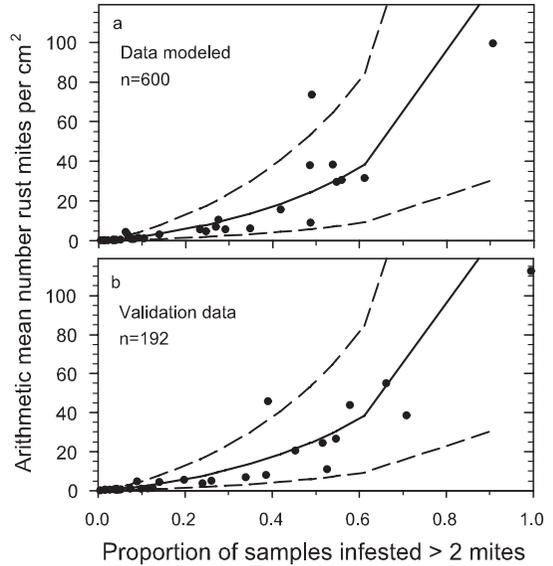


Fig. 5. Arithmetic mean number of motile rust mites per square centimeter observed and predicted over different proportions of samples infested with  $>2$  mites ( $P_2$ ) based on the binomial relationship between  $\ln(\text{mean})$  and  $\ln(-\ln(1 - P_2))$ . The solid line depicts mean predictions from the original data set, dashed lines depict upper and lower 95% CL for individual predictions, and data points show the observed mean densities. (a) Fit of original data modeled. (b) Fit of validation data.

each indicated a mean density of approximately six mites per sample; the upper 95% confidence limit associated with these tally thresholds was 13.9 and 12.8, respectively. Marginal reductions in confidence limits associated with  $P_2$  may not be worth the increased labor associated with making sure there are at least three mites in individual samples.

Our binomial analyses were based on the relationship between mean density and proportion of samples infested ( $P_T$ ) by greater than  $T$  mites ( $T = 0$  or 2) as defined by Gerrard and Chiang (1970). Others have conducted similar binomial analyses but on the relationship between mean density and proportion of samples not infested ( $P_T$ ) (Nachman 1984, Binns and Bostanian 1990, Schaalje et al. 1991). If binomial analyses are based on the proportion of samples not infested,  $(1 - P_T)$  should be changed to  $(P_T)$  in equations 1, 2, 4, 5, and 6 and  $P_T b'^2$  changed to  $(1' - P_T) b'^2$  in equation 4.

Many reports on binomial sampling of insects and mites include figures showing the relationship between mean per sample plotted on proportion of samples infested (Wilson et al. 1983, Elliott et al. 1990, Wright et al. 1990, Jones 1991, Dornan et al. 1995, Boeve and Weiss 1997, Hodgson et al. 2004). Working on binomial sampling of *Euseius tularensis* (Congdon), Grout (1985) presented a plot of  $\ln(\text{mean} + 1)$  on the proportion of samples infested. Nachman (1984) presented for *Tetranychus urticae* Koch and *Phytoseiulus persimilis* Athias-Henriot means per sample plotted on  $\ln(-\ln(P_T))$ , where  $P_T$  was the proportion of samples

not infested. Gerrard and Chiang (1970) presented for *Diabrotica longicornis* Say and *Diabrotica virgifera* Le Conte plots of mean per soil sample on the proportion of samples infested and plots of mean per soil sample on  $-\ln(1 - P_T)$ . Kuno (1986) presented a plot of proportion of samples infested on mean density (the latter on log scale) for the planthopper *Nilaparvata lugens* Stål in rice (*Oryza* spp.). Binns and Bostanian (1990) presented a plot of  $\ln(-\ln(P))$  on  $\ln(\text{mean})$  for *T. urticae* in strawberry, where  $P$  was the proportion of samples not infested. Because our binomial analyses began with analyses on the relationship between  $\ln(\text{mean})$  and  $\ln(-\ln(1 - P_T))$  (Jones 1994), we thought it prudent to graphically present this relationship (Figs. 1 and 4). We then present plots of means per sample on proportions of samples infested along with confidence limits (Figs. 2 and 5) because 1) we were interested in using the binomial relationship to predict a mean density from the proportion of samples infested (the latter thus being the independent variable), and 2) visual assessment of the relationship was more practical based on raw means and proportions. Most of the published reports on binomial sampling we cite present some information on sampling precision and confidence limits for a mean prediction, but few reports present such information graphically for a mean density prediction. An exception was that Nachman (1984) graphically presented 95% CL for estimated mean densities of *T. urticae* and *P. persimilis* plotted on proportion of sampling units without mites (estimated means plotted on log scale). Presenting these limits gives immediate insight into expected precision across a range of mean density estimates.

This is the fourth of a series of reports on sampling to estimate rust mite densities on oranges. Hall et al. (1991) reported on estimating densities of citrus rust mites in individual citrus trees. Sampling statistics associated with estimating mean densities of motile citrus rust mites on oranges across a 4-ha block of trees were investigated using a hierarchical sampling plan consisting of 600 sample units per 4 ha (Hall et al. 1994). A sampling plan consisting of 192 samples per 4 ha taken along two transects provided estimates usually as accurate as the plan consisting of 600 samples (Hall et al. 1994). A plan consisting of 160 samples per 4 ha was projected to be large enough for commercial estimates at average rust mite densities of three or more mites per sample (Hall et al. 1994). The research indicated some flexibility in how the samples could be allocated but that more emphasis should be placed on the number of locations sampled than the number of trees per location or number of fruit per tree. The number of samples needed for the same level of precision was projected to decrease as mite densities increased, with 80 samples projected to be enough for commercial estimates at average mite densities of 10 or more per sample. In a separate study, sampling plans consisting of 360, 300, 200, 160, 80, 48, 36, or 20 sample units per 4 ha were evaluated using bootstrapped data (Hall et al. 2005). The analyses indicated sampling plans consisting of 80 or 160 samples per 4 ha

would provide better levels of precision than previously projected and that sampling plans consisting of as few as 36–48 samples per 4 ha could be used commercially to estimate mean density with minimal loss in accuracy and precision (Hall et al. 2005). The precision of reduced sampling plans in practice remains to be verified.

The research presented here showed that rust mite densities can be estimated based on the proportion of samples infested rather than actual mite counts. Mathematical parameters associated with binomial sampling were determined using data from the sampling plan consisting of 600 samples per 4 ha, and these parameters were shown to adequately describe the observed relationship between proportion of samples infested and mean density per sample based on the sampling plan consisting of 192 samples per 4 ha. Projections indicated the fitted binomial parameters could be applied to sampling plans consisting of as few as 48 samples per 4 ha, but reducing sample size was projected to result in an increase of estimates falling outside the confidence limits expected based on 600 samples. Although rust mite count data followed the binomial model, confidence limits for mean predictions increased dramatically as the proportion of samples infested increased, narrowing its usefulness as a substitute for actual counts to situations where mite populations are just beginning to increase. Binomial sampling for rust mites seemed to be a suitable alternative to absolute counts of mites per sample for a grower sampling on a regular basis and using a low management threshold such as two or three motile mites per sample. A logical next research step would be to evaluate in real grove situations several of the reduced sampling plans presented here and by Hall et al. (2005) for precision of density estimates based on both mite counts and binomial sampling. Ideally, future sampling plan recommendations for commercial purposes would include several plan choices (varying in input costs) along with information for each plan on the expected precision of estimates.

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### References Cited

- Allen, J. C. 1976. A model for predicting citrus rust mite damage on Valencia orange fruit. *Environ. Entomol.* 5: 1083–1088.
- Allen, J. C. 1978. The effect of citrus rust mite damage on citrus fruit drop. *J. Econ. Entomol.* 71: 746–750.
- Allen, J. C. 1979. The effect of citrus rust mite damage on citrus fruit growth. *J. Econ. Entomol.* 72: 195–201.
- Allen, J. C. 1981. The citrus rust mite game: a simulation model of pest losses. *Environ. Entomol.* 10: 171–176.
- Beers, E. H., and V. P. Jones. 2004. Fixed precision sampling plans for white apple leafhopper (Homoptera: Cicadellidae) on apple. *J. Econ. Entomol.* 97: 1752–1755.

- Boeve, P. J., and M. J. Weiss. 1997. Binomial sequential sampling plans for cereal aphids (Homoptera: Aphididae) in spring wheat. *J. Econ. Entomol.* 90: 967-975.
- Binns, M. R., and N. J. Bostanian. 1990. Robustness in empirically based binomial decision rules for integrated pest management. *J. Econ. Entomol.* 83: 420-427.
- Childers, C. C., and D. S. Achor. 1999. The eriophyid mite complex on Florida citrus (Acari: Eriophyidae and Dip-tilomopidae). *Proc. Fla. State Hort. Soc.* 112: 79-87.
- Childers, C. C., C. W. McCoy, H. N. Nigg, and P. A. Stansly. 2005. Rust mites, spider mites, and other phytophagous mites. SP-43, 2005 Florida Citrus Pest Management Guide. (<http://edis.ifas.ufl.edu/CG002>).
- Dornan, A. P., M. K. Sears, and J. G. Stewart. 1995. Evaluation of a binomial model for insecticide application to control lepidopterous pests in cabbage. *J. Econ. Entomol.* 88: 302-306.
- Efron, B., and R. Tibshirani. 1986. Bootstrap methods for standard errors, confidence intervals, and other measures of statistical accuracy. *Stat. Sci.* 1: 54-77.
- Elliott, N. C., R. W. Kieckhefer, and D. D. Walgenbach. 1990. Binomial sequential sampling methods for cereal aphids in small grains. *J. Econ. Entomol.* 83: 1381-1387.
- Gerrard, D. J., and H. C. Chiang. 1970. Density estimation of corn rootworm egg populations based upon frequency of occurrence. *Ecology* 51: 237-245.
- Grout, T. G. 1985. Binomial and sequential sampling of *Euseius tularensis* (Acari: Phytoseiidae), a predator of citrus red mite (Acari: Tetranychidae) and citrus thrips (Thysanoptera: Thripidae). *J. Econ. Entomol.* 78: 567-570.
- Hall, D. G., C. C. Childers, and J. E. Eger. 1991. Estimating citrus rust mite (Acari: Eriophyidae) levels on fruit in individual citrus trees. *Environ. Entomol.* 20: 382-390.
- Hall, D. G., C. C. Childers, and J. E. Eger. 1994. Spatial dispersion and sampling of citrus rust mite (Acari: Eriophyidae) on fruit in 'Hamlin' and 'Valencia' orange groves in Florida. *J. Econ. Entomol.* 87: 687-698.
- Hall, D. G., C. C. Childers, and J. E. Eger. 2005. Effect of reducing sample size on density estimates of citrus rust mite (Acari: Eriophyidae) on citrus fruit: simulated sampling. *J. Econ. Entomol.* 98: 1048-1057.
- Hall, D. G., C. C. Childers, J. E. Eger, and J. C. Allen. 1997. Citrus rust mite (Acari: Eriophyidae) counts on fruit and the negative binomial distribution. *Fla. Entomol.* 80: 1-10.
- Hodgson, E. W., E. C. Burkness, W. D. Hutchison, and D. W. Ragsdale. 2004. Enumerative and binomial sequential sampling plans for soybean aphid (Homoptera: Aphididae) in soybean. *J. Econ. Entomol.* 97: 2127-2136.
- Hutchison, W. D. 1994. Sequential sampling to determine population density. In L. P. Pedigo and G. D. Buntin [eds.], *Handbook of sampling methods for arthropods in agriculture*. CRC, Press, Boca Raton, FL.
- Jones, V. 1991. Binomial sampling plans for tentiform leaf-miner (Lepidoptera: Gracillariidae) on apple in Utah. *J. Econ. Entomol.* 84: 484-488.
1994. Sequential estimation and classification procedures for binomial counts. In *Handbook of sampling methods for arthropods in agriculture*, L. P. Pedigo and G. D. Buntin [eds.], *Handbook of sampling methods for arthropods in agriculture*. CRC, Press, Boca Raton, FL.
- Knapp, J. L., and T. R. Fasulo. 1983. Citrus rust mite. In *Florida citrus integrated pest and crop management handbook*. SP-14, 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 stem infested by the rice stem borer. *Japan. J. Appl. Entomol. Zool.* 2: 184-188.
- Kuno, T. 1986. Evaluation of statistical precision and design of efficient sampling for the population estimation based on frequency of occurrence. *Res. Popul. Ecol. (Kyoto)* 28: 305-319.
- McCoy, C. W., and L. G. Albrigo. 1975. Feeding injury to the orange caused by the citrus rust mite, *Phyllocoptruta oleivora* (Protstigmata: Eriophyoidea). *Ann. Entomol. Soc. Am.* 68: 289-297.
- McCoy, C. W., R. F. Brooks, J. C. Allen, and A. G. Selhime. 1976. Management of arthropod pests and plant diseases in citrus agroecosystems. *Proc. Tall Timbers Conf. Ecol. Animal Control Habitat Manage.* 6: 1-17.
- Nachman, G. 1984. Estimates of mean population density and spatial distribution of *Tetranychus urticae* (Acarina: Tetranychidae) and *Phytoseiulus persimilis* (Acarina: Phytoseiidae) based on the proportion of empty sampling units. *J. Appl. Ecol.* 21: 903-913.
- Rogers, J. S., C. W. McCoy, and M. M. Manners. 1994. Standardized visual comparison keys for rapid estimations of citrus rust mite (Acari: Eriophyidae) populations. *J. Econ. Entomol.* 87: 1507-1512.
- SAS Institute. 1999a. SAS/STAT user's guide, version 8. SAS Institute, Cary, NC.
- SAS Institute. 1999b. SAS procedures guide, version 8. SAS Institute, Cary, NC.
- Schaalje, F. B., R. A. Butts, and T. J. Lysyk. 1991. Simulation studies of binomial sampling: a new variance estimator and density predictor, with special reference to the Russian wheat aphid (Homoptera: Aphididae). *J. Econ. Entomol.* 84: 140-147.
- Taylor, L. R. 1961. Aggregation, variance and the mean. *Nature (Lond.)* 189: 732-735.
- Wilson, L. T., C. Pickel, R. C. Mount, and F. G. Zalom. 1983. Presence-absence sequential sampling for cabbage aphid and green peach aphid (Homoptera: Aphididae) on Brussels sprouts. *J. Econ. Entomol.* 76: 476-479.
- Wright, L. C., W. W. Cone, G. W. Menzies, and T. E. Wildman. 1990. Numerical and binomial sequential sampling plans for the hop aphid (Homoptera: Aphididae) on hop leaves. *J. Econ. Entomol.* 83: 1388-1394.
- Yang, Y., J. C. Allen, J. L. Knapp, and P. A. Stansly. 1995. Relationship between population density of citrus rust mite (Acari: Eriophyidae) and damage to 'Hamlin' orange fruit. *Environ. Entomol.* 24: 1024-1031.
- Yothers, W. W. 1918. Some reasons for spraying to control insect and mite enemies of citrus trees in Florida. *U.S. Dep. Agric. Bull.* 645.
- Yothers, W. W., and R. L. Miller. 1934. Methods for determining rust mite abundance. *Proc. Fla. State Hort. Soc.* 47: 53-55.

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