Standardized Visual Comparison Keys for Rapid Estimations of Citrus Rust Mite (Acari: Eriophyidae) Populations

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ABSTRACT Standardized visual comparison keys were effective in improving accuracy and precision in rapid field estimations of citrus rust mite, *Phyllocoptruta oleivora* (Ashmead), population densities. A standardized key based on the Horsfall-Barratt system for the measurement of plant disease is presented. Errors in mean population estimations were generally <10% using this key when either leaves or fruits were sampled. Effective use of this technique depends on the ability of scouts to adopt a system of grouping instead of counting rust mites. Accurate and precise population measurements are independent of environmental and genetic factors; thus this technique is applicable to leaf or fruit tissue in any geographical location. Use of the modified Horsfall-Barratt system for estimating citrus rust mite populations in an integrated crop management program is described.

KEY WORDS *Phyllocoptruta oleivora*, populations, sampling

ANY APPROACH TO THE quantitative management of citrus rust mite, *Phyllocoptruta oleivora* (Ashmead), populations must involve appropriate sample sizes and accuracy of the estimation method (McCoy et al. 1974, Hall et al. 1991). Citrus rust mite is a small invertebrate parasite of citrus that can cause a brown to black rind discoloration on all commercial cultivars (McCoy et al. 1988). An ideal measurement system should be quick, easy, and accurate over a wide variety of conditions (O'Brien & van Bruggen 1992). Because reliable predictions of damage and losses caused by mite populations are limited by the accuracy of the estimates of population size, rapid and reproducible techniques for estimating abundance are critically important.

Various methods for sampling and analysis of citrus rust mite populations have been described (McCoy et al. 1974, Smith 1980, Allen 1981, Knapp & Fasulo 1983, Mora Morin 1987, Peña & Baranowski 1990, Hall et al. 1991). Mathematical modeling has recently been applied to the analysis of temporal changes in citrus rust mite populations (J.S.R., unpublished data). Of these methods, three general approaches to the measurement of citrus rust mite populations on leaves and fruit commonly are used in commercial industry: (1) percentage infestation measurements, (2) qualitative rating scales, and (3) individual adult mite counts. Percentage infestation measurements, although rapid, are insensitive to subtle variations in mite population density (particularly on fruit), resulting in the application of pesticides when actual numbers may be declining or below injury thresholds (McCoy et al. 1974). Variations in specificities of citrus rust mite infestations and injury, weather effects, and chemical selection pressures suggest that population estimates derived from percentage infestation measurements can be inaccurate except when making general inferences regarding seasonal changes (preferably after infestation-density relationships have been determined for each grove) (J.S.R., unpublished data). Qualitative rating scales for estimating rust mites (i.e., low, medium, and high) are subjective and pose the same problems as percentage infestation measurements. Individual mite counts, although exact and preferred for ecological research (Roush 1987, Hall et al. 1991), are time-consuming and impractical when populations increase rapidly in spring and summer, and when many leaves and fruits must be sampled over large areas each day. Detailed studies of both accuracy and precision of the above three estimation methods over time and for different geographical regions of the citrus industry have not been published. Consequently, more efficient methods of counting citrus rust mites must be developed (Thompson 1992).

In this article, we present a methodology based on the Horsfall-Barratt system for the assessment of plant diseases (Horsfall & Barratt 1945, Rogers 1992) that permits simple, rapid,

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and quantitative assessments of rust mite population densities on the leaves and fruits of citrus trees. The Horsfall-Barratt system has been used in plant pathology for over 40 yr (Redman & Brown 1964, Horsfall & Cowling 1978, Hebert 1982, Hollis 1984, Campbell & Madden 1990) and is often specified for use with logarithmically stratified standardized comparison templates that permit the estimation of the percentage plant tissue sustaining damage from a disease or environmental injury (James 1974, 1980; Gaunt 1987). Standard diagrams have been developed for use with many diseases and host crops worldwide, including apple, *Malus* × *domestica* (Borkhausia); barley, *Horteum vulgare* (L.); lettuce, *Lactuca sativa* (L.); pear, *Pyrus communis* (L.); and wheat, *Triticum aestivum* (L.) (Commonwealth Mycological Institute 1968, Chiarappa 1971, Berger 1980, James 1980, Campbell & Madden 1990, O'Brien & van Bruggen 1992). The Horsfall-Barratt system has also been used at the University of California at Berkeley for the quantitative assessment of freezing injury in citrus and russet injury on pears (Steven E. Lindow, University of California at Berkeley, personal communication).

Materials and Methods

Tests were conducted to determine the ability of scouts using a modified Horsfall-Barratt system to rapidly and accurately estimate numbers of citrus rust mites on plant surfaces. A standardized key for assessing citrus rust mites on citrus was constructed based on models of standardized keys for disease assessment on plants. Seven categories, instead of 12 used in the original Horsfall-Barratt system (Horsfall & Barratt 1945), were used in our key. Thus, we refer to this key as the truncated Horsfall-Barratt key.

Groves of different ages located in central to eastern and southwestern Florida with a total of ≈324 ha of commercial citrus were used for this study. At least 10 rootstock-scion combinations under a variety of environmental conditions and production regimes were represented in the area. Leaf and fruit samples were selected randomly from around the perimeter of citrus trees from the interior and exterior of the canopy. Height ranged from 0.5 to ≈2 m, and samples included orange, *Citrus sinensis* (L.) Osbeck; grapefruit, *C. paradisi* (L.) Macfayden; specialty fruit, *C. reticulata* (Blanco) × *C. paradisi*; and leaf tissues. Sample observation areas were chosen randomly on each leaf or fruit, except that surfaces exposed to full sunlight were excluded from consideration.

Thirty scouts trained in the use of the standard diagram participated in laboratory and field visual key calibration trials. Citrus rust mites were monitored over a period of nearly 12 mo to include population densities ranging from 0 to >100 mites per square centimeter.

Table 1. Truncated Horsfall-Barratt rating scale for rapidly determining the numbers of citrus rust mites viewed under a hand-held magnifying lens

Parameters	Rating scale						
Estimated numbers	0	0–3	3–6	6–12	12–25	25–50	>50
HB code (modified)	0	1	2	3	4	5	6
Conversion value	0	1.5	4.5	9.0	18.5	37.5	75.0

Live citrus rust mite populations were estimated by viewing one lens view area on each of 20 individual leaf or fruit surfaces with a 10× magnifying hand lens and assigning a numerical code ranging from 0 to 6 based on the standardized key. Scouts were asked not to *count* but to rapidly *group and compare* the mite numbers with the key, then assign the appropriate code. For example, if the numbers of observed mites per lens field were ≈10, then the coded value of 3 was recorded onto a data sheet. For subsequent linear regression analyses of actual versus estimated citrus rust mite numbers, mites from the exact same 1-cm^2 surface areas were then counted individually, and the actual count for each lens view area was recorded directly below the coded data. The number of estimated/actual citrus rust mite population data-pairs ranged from 100 to 400 for each of the individual scouts. Twenty observations were included in each sample group. Mean mite population densities were calculated for the actual counts by dividing the total number of live mites counted per sample by 20. These data were then normalized by further dividing by the number of square centimeters under each lens view area. Average population densities for the 20 coded estimations per sample were obtained by taking their conversion value counterparts from Table 1 and dividing the total of those values by 20 and normalizing for lens view area. Actual mite counts were recorded for calibration purposes only but need not be taken in large-scale commercial practice. Laboratory calibration trials were conducted with flash cards specifically constructed to simulate the appearance of citrus rust mites on plant surfaces (Forbes & Jeger 1987).

Twenty observations per sample were replicated five to 20 times for the nine experienced and 16 novice scouts, and the accuracy and precision of each scout were analyzed by linear regression (Sokal & Rohlf 1981). Accuracy is represented by the closeness of the slope of the linear regression line to one in a zero-intercept model. Precision is represented by the correlation coefficient of the data in their fit to the calculated curve (Sherwood et al. 1983). This coding, conversion, and analysis process was described in detail by Rogers (1992). Statistical analyses were performed using Statview Version 4.0.2 (Sager & Rocco 1992).

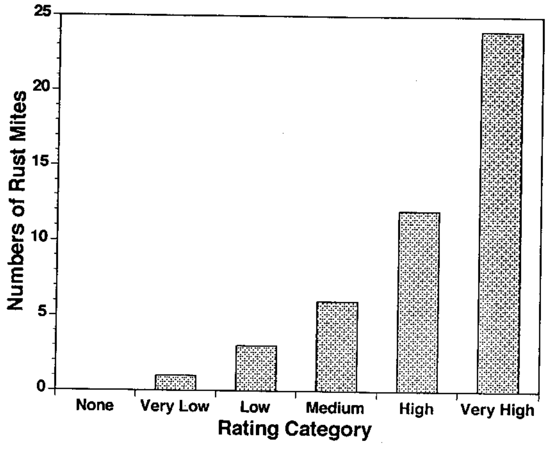

Fig. 1. Visual perception of citrus rust mite numbers as related to increases in the numbers of citrus rust mites derived from a professional grove manager's rating scale. For every perceived linear increase in the categorical numbers of mite individuals, there is a corresponding exponential increase in the actual numbers of mites.

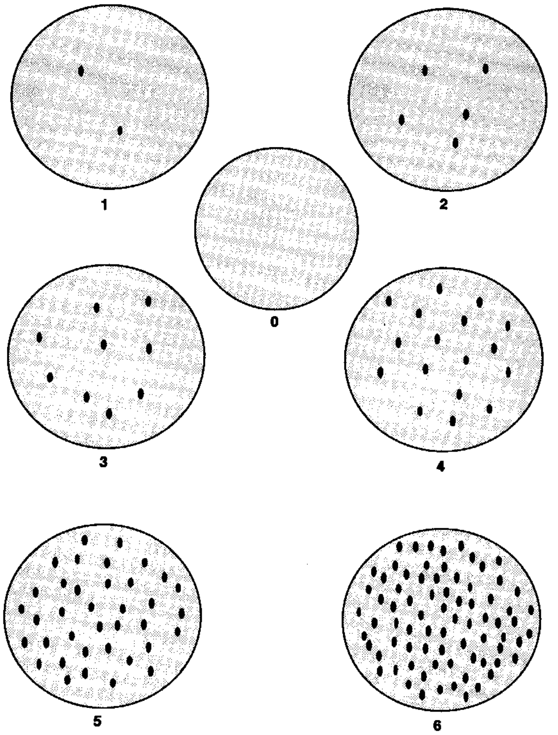

Fig. 2. Standardized visual comparison key for the quantitative measurement of citrus rust mite population densities. The shaded circles represent views of the plant surface through a 10× hand-held magnifying lens. The small oval black spots represent mites on the plant surface as seen through the magnified lens view. The coded values 0 through 6 are based on the Horsfall-Barratt system for the assessment of plant disease (except that 7 instead of 12 rating levels are used). Range of numbers of citrus rust mites per lens view area represented by the seven different categories: 0, 0; 1, 0–3; 2, 3–6; 3, 6–12; 4, 12–25; 5, 25–50; and 6, >50. Population densities within lens view areas were matched with this comparison template, and conversion codes were then assigned. Average estimated numbers and conversion values for each of the coded categories are presented in Table 1.

Results and Discussion

Interviews conducted with professional crop consultants and grove managers showed that the measurement of citrus rust mite population densities on citrus is most often a subjective rating scale divided into none, low, medium, or high. Generally, however, a rating of medium by one individual was not equivalent to a rating of medium by other individuals. One individual might interpret medium population densities as 10–15, but another might interpret the same densities as high. Similar discrepancies were detected for the other rating categories. The manner in which different individuals categorized mite populations was variable. Each person interviewed thus used a different citrus rust mite assessment scale. For one grove manager, the number of citrus rust mites for each low to high category plotted against the category designation itself permitted the construction of a chart depicting the relationship between the numbers of citrus rust mites and each category (Fig. 1). By drawing an imaginary line through the apices of the histogram bars of Fig. 1, a large exponential change in the numbers of mites is perceived for every corresponding linear categorical change in citrus rust mite numbers. This finding suggested that a standardized key based on the Horsfall-Barratt system might be appropriate for estimating mite populations.

Techniques for measuring insect and mite populations include mechanical trapping devices, marking living insects, and mathematical estimation procedures (Kuno 1991). Morris (1959) classified the various techniques for measuring insect populations but did not describe standardized keys for directly measuring insect numbers. Although Sen (1966) described the use of a standardized approach to measuring injury caused by insects to tea leaves, visual comparison keys for measuring the number of insects were not mentioned. Previous use of the Horsfall-Barratt system for measuring numbers of insects has not been described. Thus, the standardized key presented in Fig. 2 represents a use in directly assessing the numbers of insects on plants (see also Strickland [1961]).

Linear regression analyses of mite population data obtained from citrus leaves using the key showed an accuracy and precision that generally varied <10% (i.e., 1.03 and 0.99, respectively [Fig. 3]). An accuracy (slope of the linear regression line) of 1.03 indicates that this particular

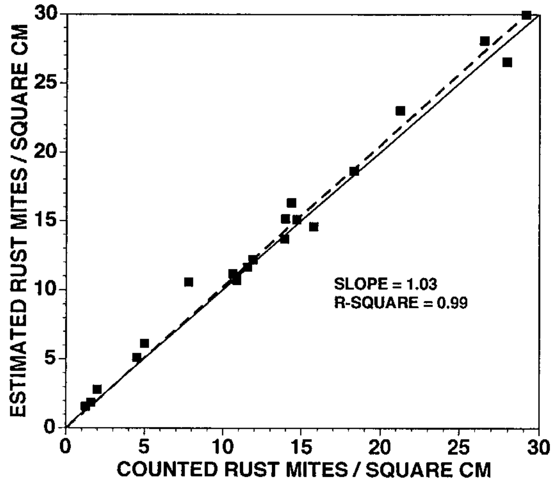

Fig. 3. Graphical analysis of the numbers of citrus rust mites on tangerine leaves as estimated by one scout using the truncated Horsfall-Barrett system. The solid line represents a slope of 1.00 and is the line upon which estimates would fall if estimates were 100% accurate and precise. The dashed line bisects the experimental data and has a slope of 1.03. Data were fit to the curve with a correlation coefficient (R^2 value) of 0.99. The closer the dashed line to the solid line, the more accurate is the estimation of the scout. The closer the individual data points to the dashed line, the greater the precision of the scout.

scout overestimated numbers of mites by ≈3%. Similar tests conducted with fruits resulted in high accuracy and precision with plant tissues other than leaves (Table 2). In calibration trials conducted on leaf and fruit tissues in geographical locations distributed across Florida, nearly all experienced scouts (87.5%) obtained accuracy and precision within our specified targets of 0.90–1.10 and 0.80–1.00, respectively. Means and 95% confidence intervals for the accuracy and precision of the experienced scouts were 1.00 ± 0.07 and 0.97 ± 0.04, respectively. Accuracy of estimates of mite populations obtained with standardized keys thus appears independent of both physical environment and genetic complexity of the host-parasite system.

Table 2. Accuracy and precision of experienced scouts using the truncated Horsfall-Barrett system to rapidly estimate citrus rust mite population densities

Scout[a]	Accuracy	Precision	Target[b]	Calibration[c]
1	1.01	0.99	*	Orange
2	1.04	0.99	*	Orange
3	1.10	1.00	*	Orange
4	0.81	0.96		Orange
5	1.09	0.97	*	Orange
6	0.95	0.86	*	Grapefruit
7	0.96	0.98	*	Grapefruit/Orange
8	1.01	0.99	*	Grapefruit/Orange
9	1.03	0.99	*	Tangerine leaves
Means	1.00 ± 0.07[d]	0.97 ± 0.04[e]		

Within a column, 95% confidence intervals represent the errors in estimations of the means. Statistics calculated using values carried to three decimal places.

[a]Experienced scouts were scouts with at least 1 yr of training in the field monitoring of citrus rust mites.

[b]Scouts in rows designated with an asterisk achieved accuracy and precision between the targets of 0.90–1.10 and 0.80–1.00, respectively.

[c]Citrus rust mite populations were assessed on the fruits of orange and grapefruit trees. Populations were assessed on the leaves of tangerine trees.

[d]$t = 33.79$, df = 8, $P < 0.0001$.

[e]$t = 67.31$, df = 8, $P < 0.0001$.

Table 3. Accuracy and precision of novice scouts using the truncated Horsfall-Barratt system to rapidly estimate citrus rust mite population densities

Scout[a]	Accuracy	Precision	Target[b]	Calibration[c]
10	0.98	0.99	*	Flash cards
11	1.10	0.99	*	Flash cards
12	1.09	1.00	*	Flash cards
13	1.10	0.99	*	Flash cards
14	1.18	1.00		Flash cards
15	1.15	0.99		Flash cards
16	0.92	0.96	*	Flash cards
17	1.13	0.99		Flash cards
18	0.98	1.00	*	Flash cards
19	1.12	0.99		Flash cards
20	1.19	1.00		Flash cards
21	0.97	0.99	*	Flash cards
22	1.25	0.99		Flash cards
23	1.09	0.98	*	Flash cards
24	1.08	0.99	*	Flash cards
25	1.12	0.98		Flash cards
Means	1.09 ± 0.05[d]	0.98 ± 0.01[e]		

Within a column, 95% confidence intervals represent the errors in estimations of the means. Statistics calculated using values carried to three decimal places.

[a]Novice scouts were scouts with no prior training in the recognition or field monitoring of citrus rust mites.

[b]Scouts in rows designated with an asterisk achieved accuracy and precision between the targets of 0.90–1.10 and 0.80–1.00, respectively.

[c]Flash cards were constructed to simulate the appearance of citrus rust mites on plant surfaces.

[d]$t = 48.86$, df = 15, $P < 0.0001$.

[e]$t = 468.60$, df = 15, $P < 0.0001$.

In calibration trials conducted with flash cards designed to simulate citrus rust mites on plant surfaces, about half of the novice scouts (56.3%) obtained accuracies and precisions within the defined targets (Table 3). Means and 95% confidence intervals for the accuracy and precision of the novice scouts were 1.09 ± 0.05 and 0.98 ± 0.01, respectively. Because trials with experienced scouts were calibrated with leaf and fruit tissues and those with the novice scouts were calibrated with flash cards, direct comparisons of the accuracy and precision between groups are not appropriate. However, the greater accuracy on the part of the experienced scouts suggests that accuracy of novice scouts could improve with practice. Errors in population estimations for the five professional grove managers were <10%.

Comparisons with observed increases in crowding densities of citrus rust mites on leaf and fruit tissues indicate that the proposed standardized key contains sufficient intervals to represent the major stages in the development of economically damaging mite populations. Time studies showed that surveillance periods are reduced 50–75% below that required for counting individual mites with negligible loss in accuracy and precision (Philip 1947). Quantitative comparisons of estimated population densities derived with the proposed system are adaptable to commercial-level population and pesticide efficacy studies, and increase the probability of detecting small changes in miticide resistance.

Effective implementation of the truncated Horsfall-Barratt system into an integrated crop management program depends upon the completion of three training criteria: recognition, calibration, and interpretation. Recognition training teaches scouts to recognize the morphological characteristics of citrus rust mite individuals at different stages in the mite life cycle. Calibration training teaches scouts to estimate citrus rust mite populations using the truncated Horsfall-Barratt system standardized keys and the principles of visual perception upon which the system is based (see also Stevens & Galanter 1957, Cornsweet 1970). Scouts are then monitored as they perform sample observations obtained under both laboratory and field conditions, and their accuracy and precision are quantified. Standardized comparison keys are most appropriate during the training phase of applying the Horsfall-Barratt system to the measurement of mite populations on citrus, and continued use of this system improves the performance of the scouts. Effective performance of this technique depends on the ability of individual scouts to adopt a system of grouping instead of counting the mite populations.

Results of these experiments have demonstrated that objective comparison keys can improve simplicity, accuracy, and precision in rapid estimations of citrus rust mite population densities on citrus. However, these studies represent preliminary steps in determining an accurate and precise commercial-level citrus rust mite population assessment. The population estimation method described in this article may contribute to an increased awareness of the basic ecological interactions occurring in citrus and permit growers to more effectively time miticide applications.

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