Field Survey of Tahiti Lime, *Citrus latifolia*, for Algal Disease, Melanose, and Greasy Spot in Southern Florida

R. B. MARLATT, Plant Pathologist, KEN POHRONEZNY, Pest Management Specialist, and R. McSORLEY, Assistant Nematologist, University of Florida, Agricultural Research and Education Center, Homestead 33031

ABSTRACT

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A mature lime grove in southern Florida was surveyed biweekly for algal disease, melanose, and greasy spot from April 1980 through November 1981. Algal disease symptoms began with sporulation on branches in April 1980 from long-established infections, then appeared on leaves in October 1980 and continued to spread until February 1981. It began again in July 1981 and continued to March 1982. Greasy spot appeared 3 wk after leaves reached maximum size in May 1980 and occurrence of new infections peaked in August. Greasy spot incidence reached 100% about 1 yr after the survey began. Except when disease incidence was low, use of the random selection of foliar growth flushes was an acceptable sampling method to assess the proportion of leaves with the three diseases, as evaluated by relative variation (standard error-to-mean ratio). Correlation coefficients between midseason greasy spot levels and late-season leaf loss were significant but no significant correlations were found between algal disease or melanose and leaf loss. Methodology is presented for developing statistical sampling plans for the three diseases.

Commercial Tahiti lime production in southern Florida has increased steadily since the 1920s to about 2 million bushels annually (1). Little attention has been given to the three major diseases of this crop, algal disease (*Cephaleuros* sp. Kunze) (4), melanose (*Diaporthe citri* Wolf) (10), and greasy spot (*Mycosphaerella citri* Whiteside) (13). These lime diseases were assumed to have the same epidemiology as described for oranges and other citrus cultivars in central Florida. We believed, however, that the diseases might behave differently on Tahiti lime in southern Florida and that a more comprehensive survey of lime trees, to which no pesticides were applied, was needed to verify the matter.

Disease symptoms on lime and other citrus cultivars were compared, the times of first appearance of disease symptoms and disease progress were recorded, and the effect of each disease on leaf abscission was measured. An analysis of the precision of sampling procedures was made to optimize sample size.

MATERIALS AND METHODS

Ten-year-old Tahiti lime trees on rough lemon rootstock at the Homestead Agricultural Research and Education Center were used for this study. The experimental grove was 1 ha in area, with trees in rows 5 m apart and 3.5 m between trees in a row. Trees were fertilized twice a year with 898 kg/ha of an 8-3-9 analysis granular fertilizer. No pesticides were applied to the trees for 3 yr before the surveys or at any time during our observations. They received supplemental water during the dry season from a drip-irrigation system.

Flushes were examined closely with a ×10 hand lens for appearance of greasy spot, melanose, and algal disease. The first survey began 29 April 1980 before heavy rains started and ended 6 July 1981 when all tagged flushes were defoliated. The second survey was started 2 July 1980 after heavy rains had begun and ended 1 December 1981. The third survey began the following year on 31 March 1981, about 1 mo before the spring rains began and while surveys one and two were continuing.

This last survey was discontinued prematurely on 10 November 1981 because sooty mold covered the leaves, masking disease symptoms. Leaves were examined biweekly when weather permitted until they abscised or fell for any reason. New growth flushes of several adjacent leaves were chosen that could be reached most easily for inspection by an observer standing on the ground. All flushes were examined within a 48-hr period. Labels for future sampling were attached to flushes about 2 wk old that had expanded to nearly full size and were on the outside of the tree canopy. Thirty-six flushes, two per tree on 18 trees, were selected randomly and examined in the first two surveys, and in the third, there were 128 flushes, four per tree on 32 trees. The number of leaves in a flush and those showing each disease were counted. The amount of leaf loss on the various sampling dates was correlated with disease incidence, using the correlation procedures outlined for the Statistical Analysis System (3). Incidence of the three diseases was determined for each date using a formula for unequal cluster size (2):

$$p = \frac{\sum_{i=1}^{n} a_i}{\sum_{i=1}^{n} m_i},$$

where p = proportion of leaves with a given disease, m_i = number of leaves in flush i, and a_i = number of leaves with a given disease in flush i. The variance of the mean $V(p)$ of these proportion measurements was approximated using the formula (2):

$$V(p) = \frac{1}{n\bar{m}^2} \cdot \frac{\Sigma a_i^2 - 2p\Sigma a_i m_i + p^2 \Sigma m_i^2}{n-1},$$

where n = total number of flushes sampled and

$$\bar{m} = \frac{\Sigma m_i}{n}$$

(or average number of leaves per flush). Experimental precision of the disease sampling procedure was measured by calculating relative variation (RV) (11), using the formula:

$$\text{RV}(\%) = \frac{\text{SE}}{\bar{x}}(100),$$

where SE is standard error of the mean, equal in these experiments to the square root of $V(p)$, and $\bar{x} = p$. Because of the obvious importance of maximizing sampling efficiency when assessing disease levels in groves, a graphical technique was developed to estimate the number of flushes needed to achieve a relative variation of 25% or less. For a given date, a random numbers table was used to select five flushes ($n = 5$) without replacement, and standard error (SE) and RV of disease incidence were calculated. Five additional flushes were then chosen at random and statistics recalculated on the basis of $n = 10$. Additional calculation

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was done for n = 15, 20, 25, 30, and 36. These calculations were replicated five times for each value of n and the mean RV plotted versus n. The acceptable number of flushes needed to estimate disease incidence for a given date then was determined by inspection of the graph for the 25% RV value.

RESULTS

Algal disease. In the first survey, symptoms appeared in early October; leaves with no disease on 22 September had 36.5% incidence 15 days later (Table 1). Leaves with new infections were recorded monthly through March 1981 but no new symptoms were seen after 31 March. After winter and spring defoliation, 6.3% of labeled leaves still remained on the trees and all showed algal disease in May 1981. The 1981–1982 survey revealed a lower disease incidence of 1.7% in July to only 8.8% in November. On lime leaves, the subcuticular algal thallus rarely attains a diameter greater than 4 mm. On branches measuring up to 8 cm in diameter, algal disease was manifested by bark swelling and reddish fruiting bodies, but no visible thallus, as early as 29 April 1980. Sporangia can appear on branches before the rainy season because branch infections are perennial, in contrast with leaf infections, which are annual.

In Florida, Ruehle (8,9) observed symptoms of algal disease and formation of algal fruiting structures on host tissues soon after the rainy season began. Fruiting bodies in guava groves were usually abundant during May through August 1940 and rare in October or later (9), but disease incidence was highest during August through January (4,6). During 1980–1981, observations of the disease on lime were contrary to those reports; symptoms did not closely follow the onset of the first heavy rains.

Greasy spot. Greasy spot did not appear in central Florida on spring growth until fall of the same year (13,14). In contrast, the disease on lime in southern Florida began in May only 17 days after flushes were labeled and symptoms of new infections continued to appear through March 1981 (Table 1). After winter and spring defoliation in 1981, greasy spot incidence continued at 100% from April through June until all labeled leaves had fallen. Disease incidence in subsequent surveys varied inconsequentially from this pattern.

Melanose. Most melanose infection started before flushes, and succulent twigs were tagged before they attained full size (Table 1). Disease incidence increased long after leaves matured. Because *D. citri* does not infect mature tissue (10), the increasing incidence after the spring season must be attributed to defoliation of normal leaves and retention of those with melanose. Rains spread the pathogen's spores from pycnidia on dead branches to each flush of new foliage and fruit (10) and disease was evident in all surveys.

Melanose symptoms on lime leaves differed in slight but important ways from symptoms on orange and grapefruit. Individual lesions rarely were as raised from surrounding lime leaf tissue and did not feel rough. "Tear-staining" symptoms (streaks of lesions following the paths of rivulets down the sides of fruits, as found on orange and grapefruit) were never observed on limes. Outbreaks of melanose on lime in southern Florida differed very little from reports of the disease on other citrus in central Florida (10).

Relationships of foliar diseases and leaf loss. When correlation coefficients between percent incidence of each disease and percent leaf loss were calculated using only data within the same sampling date, a few significant correlation coefficients were found between greasy spot and leaf loss. None were found between algal disease or melanose and leaf loss. Greasy spot incidence, however, was often significantly correlated with leaf loss at later dates during the 1980 spring flush, based on a correlation matrix relating leaf loss with greasy spot on various dates (Table 2). For example, leaf loss in January and February was highly correlated with greasy spot assessments of the previous September and October (Table 2). Similar results were obtained in the second 1980 summer flush survey.

No significant correlations were found between percent leaf loss on any dates in the other surveys and algal disease or melanose incidence. Correlations of leaf loss with disease by these methods are not necessarily causally related but strongly indicate that greasy spot of Tahiti lime in southern Florida is more likely to cause premature leaf abscission than are algal disease and melanose.

Analysis of sampling design and precision. Random selection of 36 flushes in the grove very often gave estimates of disease levels within acceptable limits of precision. For example, in the spring 1980 survey, the RV determinations for the proportion of leaves with greasy spot

Table 1. Estimates of precision, expressed as relative variation (RV[a]), in sampling surveys for proportion (p[b]), of lime leaves with three common foliar diseases[c]

Date	Greasy spot		Melanose		Algal disease	
	p	RV	p	RV	p	RV
Spring 1980 survey						
16 May 1980	0.047	42.6	0	...	0	...
29 May 1980	0.16	26.9	0.026	61.0	0	...
13 June 1980	0.17	27.0	0.039	51.0	0	...
25 June 1980	0.17	27.0	0.034	56.8	0	...
11 July 1980	0.17	27.0	0.034	56.8	0	...
24 July 1980	0.27	20.0	0.034	56.8	0	...
7 Aug. 1980	0.50	12.0	0.034	56.8	0	...
4 Sept. 1980	0.61	9.6	0.035	56.6	0	...
22 Sept. 1980	0.65	9.7	0.037	56.6	0	...
7 Oct. 1980	0.75	7.9	0.037	56.6	0.365	17.8
23 Oct. 1980	0.82	5.9	0.028	68.9	0.47	14.1
18 Nov. 1980	0.86	6.3	0.032	68.1	0.67	8.2
5 Dec. 1980	0.88	5.5	0.034	78.0	0.75	7.6
29 Dec. 1980	0.90	5.8	0.039	67.2	0.805	6.4
29 Jan. 1981	0.72	19.4	0.035	72.0	0.73	9.8
4 Feb. 1981	0.80	14.7	0.049	91.5	0.90	5.4
24 Feb. 1981	0.87	13.4	0.057	90.2	0.93	5.1
11 Mar. 1981	0.88	11.5	0.070	87.1	0.98	2.6
31 Mar. 1981	0.92	7.0	0.12	83.8	0.96	1.8
Summer 1980 survey						
2 July 1980	0.020	70.8	0.60	2.3	0	...
16 July 1980	0.039	58.9	0.68	10.1	0	...
29 July 1980	0.030	55.5	0.74	8.8	0	...
14 Aug. 1980	0.040	48.0	0.77	8.1	0	...
9 Sept. 1980	0.062	46.3	0.77	8.5	0	...
22 Sept. 1980	0.057	47.6	0.77	8.5	0	...
7 Oct. 1980	0.079	41.8	0.77	8.6	0.016	74.0
23 Oct. 1980	0.11	33.1	0.75	9.2	0.042	68.0
18 Nov. 1980	0.27	22.6	0.80	8.4	0.48	16.6
5 Dec. 1980	0.54	16.1	0.85	8.6	0.70	11.8
12 Dec. 1980	0.67	13.0	0.89	7.9	0.81	7.6
21 Jan. 1981	0.73	9.8	0.85	6.1	0.80	8.2
4 Feb. 1981	0.98	1.5	0.90	5.4	0.80	9.6
24 Feb. 1981	0.96	3.7	0.80	12.0	0.81	10.6
3 Nov. 1981	1.0	0	0.74	17.1	0.84	13.3

[a] RV (%) = $\frac{SE}{\bar{x}}(100)$, where x = estimate of p for given date and SE is standard error.

[b] $p = (\sum_{i=1}^{n} a_i)/(\sum_{i=1}^{n} m_i)$, where p = proportion of leaves with at least one disease lesion, m_i = numbers of leaves in flush i, and a_i = numbers of leaves with a given disease in flush i.

[c] Thirty-six random flushes examined periodically for greasy spot, melanose, and algal disease.

were below 25%, a level considered acceptable in entomological and nematological surveys (7,12), on 14 of 19 sampling dates (Table 1). In the summer 1980 survey, seven of 15 dates had greasy spot RV values below 25% (Table 1). The greasy spot RV values were apparently related to the estimates of mean p for that date; in both surveys, RV $\leqslant 25\%$ was calculated on all dates where p was equal to or more than 26% (Table 1).

Estimates of algal disease were usually within acceptable limits (Table 1). This disease appeared abruptly and rapidly infected or became visible on more than 30% of the inspected leaves.

Melanose surveys were adequate only for the 1980 summer flush, when weather conditions favored dispersal of pycnidiospores (10). RV values for the proportion of leaves with melanose were well above 25% throughout the spring flush survey (Table 1).

The graphical approach to determining the minimum sample size needed to assess the proportion of leaves infected with a disease proved useful (Fig. 1). For two dates (24 July and 23 October) of the 1980 spring flush survey, the RV values for the proportion of leaves with greasy spot were determined over various values of n (sample size). Mean RV values over five replications were plotted against n (Fig. 1). The resultant curves were of an exponential form, with $y = 146\,x^{-0.567}$ and $y = 74.3\,x^{-0.723}$ for 24 July and 23 October, respectively, when $y = \text{RV}$ and $x = n$. The fit of the equations to the actual points from the original data base was excellent ($r^2 = 0.968$ for 24 July and $r^2 = 0.978$ for 23 October, both significant at $P \leqslant 0.01$). Inspection of the graph for an RV of 25% gives a corresponding n of about 24 on 24 July and 4 on 23 October.

Table 2. Correlation coefficients between percent greasy spot infection of lime flushes and percent leaf loss in the 1980 spring flush survey

Percent greasy spot	Percent leaf loss 22 Sept.	7 Oct.	23 Oct.	18 Nov.	5 Dec.	29 Dec.	21 Jan.	4 Feb.
7 Aug.	0.089	0.110	0.234	0.165	0.219	0.222	0.364	0.183
4 Sept.	0.108	0.127	0.265	0.173	0.207	0.222	0.364	0.596**[a]
22 Sept.	0.151	0.157	0.396*	0.349*	0.329	0.376*	0.445*	0.594**
7 Oct.	···	0.182	0.341*	0.365*	0.412*	0.420*	0.452*	0.602**
23 Oct.	···	···	0.395*	0.377*	0.415*	0.446**	0.576**	0.632**
18 Nov.	···	···	···	0.325	0.344*	0.376*	0.622**	0.710**
5 Dec.	···	···	···	···	0.312	0.361*	0.553**	0.659**
29 Dec.	···	···	···	···	···	0.351*	0.589**	0.725**
21 Jan.	···	···	···	···	···	···	0.355	0.553*
4 Feb.	···	···	···	···	···	···	···	0.590*

[a]* = Significant correlation coefficient at $P = 0.05$, ** = significant correlation coefficient at $P = 0.01$.

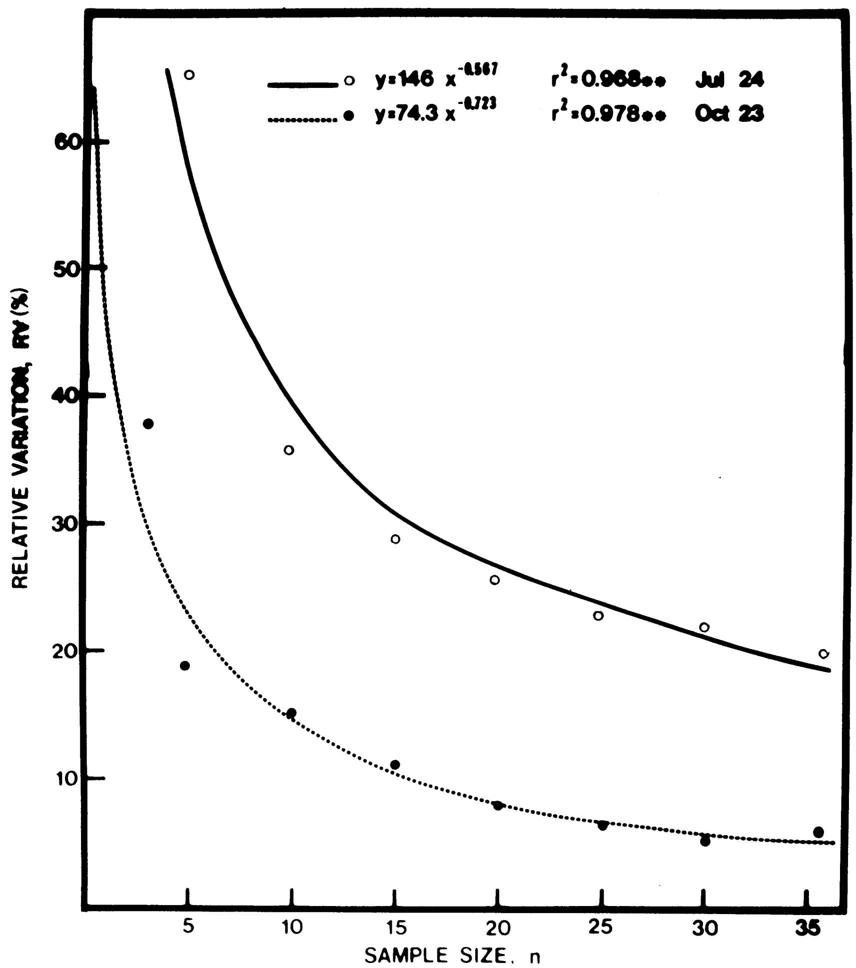

Fig. 1. Plot of relative variation (RV) vs. sample size (n) for 24 July 1980 (solid line) and 23 October 1980 (dotted line). Mean RV values (five replicates) were plotted for each n using a random numbers table to select individual flushes from the initial data base.

DISCUSSION

Lime, oranges, and other citrus respond similarly to algal disease. On lime, it began later than anticipated and continued to spread throughout the winter, in contrast with previous reports of its epidemiology on guava (9). This might be explained by a different species or race of the alga on guava. No taxonomic studies of *Cephaleuros* in Florida have been made. Symptoms on guava and lime are markedly different. Very early in their development, lesions extend entirely through a guava leaf. No thallus is evident on the adaxial surface and sporulation occurs on the abaxial lesion surface. In contrast, adaxial surfaces of lime leaf lesions are covered with thalli and sporulation occurs on the same surface. Lime lesions completely penetrate the lamina only after several months.

Melanose lesions on limes are not as elevated from surrounding leaf tissue so they do not feel as rough as reported on some other citrus (10). Tear-staining was not found on lime fruits.

Greasy spot symptoms appeared on lime leaves in southern Florida as early as 17 days after full leaf expansion, in contrast with previous reports of its incubation period of about 12 wk in central Florida (13). There may be several explanations. In addition to the host difference, climate and grove conditions were dissimilar.

M. citri hyphae grow six times as fast at 25–30 C as at 20 C (14); thus, southern Florida's higher temperature may partially account for the shorter incubation period. Hyphal growth was also enhanced by sucrose on host leaves and it is possible that insect honeydew can also speed the pathogen's growth (14). The pesticide-free trees in our experiment had honeydew on their leaves during the surveys. Also, early symptoms of greasy spot consist of subtle hypertrophy of spongy mesophyll and chlorosis. Possibly, other unidentified factors caused similar symptoms, resulting in diagnostic errors.

RV is a ratio that compares the

distribution of observations around an observed mean and can be used to determine the reliability of a given mean estimate (11). Analysis of the sampling data showed that the sampling procedures and frequency were often within acceptable limits for estimating the proportion of leaves with greasy spot (RV $\leqslant$25%). Indeed in many cases, considerably fewer samples could have been taken with considerable savings in time and money. For example, during the 1980 spring flush survey, acceptable estimates of the mean proportion of leaves with greasy spot on 24 July could have been obtained by inspecting 24 flushes instead of 36.

By 23 October, when greasy spot levels were very high, as few as four flushes would have been adequate. At low incidence of greasy spot (such as 16 May to 11 July 1980), the maximum 36 flushes inspected resulted in substantial error in the mean proportion estimate. If it is critical for precise estimates of greasy spot incidence to be obtained early in the development of a flush, considerably more effort must be devoted to inspection of trees at that time.

Frequency of sampling for some diseases may have to be varied with season. For instance, observing 36 flushes was not sufficient for estimates of melanose in the spring flush when the proportion of leaves with this disease was low (usually less than 10%). The frequency was usually adequate, however, for the summer flush survey, when melanose incidence was higher, because of melanose-favorable environmental conditions.

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