

Distribution and Sampling of *Bemisia argentifolii* (Homoptera: Aleyrodidae) and *Eretmocerus eremicus* (Hymenoptera: Aphelinidae) on Cantaloupe Vines

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ABSTRACT Studies were conducted to examine the spatial distribution of immature *Bemisia argentifolii* Bellows & Perring and immature *Eretmocerus eremicus* Rose & Zolnerowich on cantaloupe vines and to develop efficient sampling plans. More *B. argentifolii* eggs were found on the 3rd leaf from the terminal of a cantaloupe vine than on any other leaf. The density of whitefly nymphs peaked at leaf position 8, whitefly pupae (large 4th instars) peaked at leaf position 11, and immature *E. eremicus* peaked at leaf position 14. We looked at 4 parameters to describe the distribution of whitefly and parasitoid life-stages among the various leaf positions as follows: (1) median leaf position, (2) the leaf position with the highest percentage of a particular stage, (3) the leaf position where insect counts were best correlated with counts on the entire vine, and (4) the coefficient of variation (CV). All 4 distribution parameters changed throughout the season. In general the leaf positions described by the 4 distribution parameters increased (i.e., were further from the terminal) until the middle of the season when they began to decline. Across the entire season, the 4 distribution parameters for whitefly eggs were associated with leaf positions 3, 4, and 5; for whitefly nymphs with leaf positions 7, 8, and 10; for whitefly pupae with leaf positions 11 and 12; and for immature parasitoids with leaf positions 11, 13, and 14. Based on considerations of cost and precision, it was most efficient to sample leaf 3 for whitefly eggs, leaf 8 for whitefly nymphs, leaf 11 for whitefly pupae, and leaf 14 for immature parasitoids. Using the Taylor power law, density-dependent minimum sample sizes (number of leaves per field) necessary to achieve a predetermined statistical precision (mean \pm SE) were estimated. Over a broad range of densities, 50 leaves per field are adequate to achieve a precision of 0.20-0.25 for all life stages. We also provide estimates of the optimum number of leaves to collect per vine based on the within-vine and between-vine variability and the costs (time) associated with counting whiteflies and moving to another vine.

KEY WORDS *Bemisia argentifolii*, *Eretmocerus eremicus*, cantaloupe, sampling, distribution

Bemisia argentifolii Bellows & Perring, the B strain of *B. tabaci* (Gennadius), is one of the most significant pests of agricultural production in the southwestern United States (USDA 1995). Early in this decade, *B. argentifolii* assumed key pest status in several crops in Arizona, California, and Texas, including cotton, *Gossypium hirsutum* L., and cucurbits, *Cucurbita* spp. (Butler and Henneberry 1994, Brown et al. 1995). Biological control is considered a promising approach toward ameliorating the whitefly problem, and considerable research is underway to examine the impact of native natural enemies (Naranjo and Hagler 1998) and to introduce exotic parasitoids (e.g., Hoelmer 1996, Kirk and Lacey 1996). Work also is ongoing to develop techniques for augmentative releases of parasitoids in melon crops (Simmons et al. 1996). The development of reliable, cost-effective sampling methods is essential to the study of *B. argentifolii* population dynamics, making management decisions, and the evaluation of biological control. Estimates of

parasitoid activity in melons may affect control treatment thresholds, and such information is critical to evaluation of augmentative release programs.

The within-plant distribution of *Bemisia* has been examined on a number of host crops (reviewed by Naranjo 1996). *Bemisia* females preferentially oviposit on young foliage (Gerling et al. 1980, van Lenteren and Noldus 1990) and distributions of different stages become stratified as the host crop grows as a result of the sessile habit of immatures. Cantaloupe vines grow rapidly, sending out new leaves from the terminal of the vine, and the average number of leaves per vine increases through most of the growing season. Whiteflies and parasitoids of different stages would, therefore, not be expected to be distributed evenly among the leaves on a cantaloupe vine. Thus, the position of leaves sampled from cantaloupe vines will strongly influence the stages that are found. Tonhasca et al. (1994) contrasted distributions of whitefly eggs, nymphs, and pupae (red-eyed nymphs) on crown leaves and terminal portions of vine leaves of cantaloupe, but did not examine distributions within entire vines or distributions of parasitoids. Immature parasi-

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toids inside whiteflies are visible only during a short part of their life cycle, and sampling leaves containing visible parasitoids is critical to evaluating whitefly biological control effectively. Discrimination between crown and terminal leaves is not sufficient to assure scientists interested in parasitoid density that they will select leaves where parasitoids are visible.

Sampling plans have been developed that reduce sampling effort by concentrating on specific leaf positions that are most likely to contain the stage of interest (e.g., von Arx et al. 1984, Naranjo and Flint 1994). The goals of this study were to describe the spatial distribution of *B. argentifolii* and the parasitoid *Eretmocerus eremicus* Rose & Zolnerowich on cantaloupe vines, define optimal sample units, and develop sampling plans for the precise estimation of density of the various whitefly and parasitoid life stages. Sampling efficiency is a critical consideration because the time that can be devoted to sampling by researchers and pest managers often is limited. Thus, our sampling plans attempt to minimize cost (time) for estimating density with an acceptable level of statistical precision.

Materials and Methods

Field Plots and Sampling. Cantaloupe, *Cucumis melo* L., was planted in 20 plots (0.03 ha each) in Poston, AZ, on 13 April 1995. Individual plots were separated by at least 300 m of bare dirt on all sides to minimize cross-infestation of plots. Individual plots were randomly assigned to 1 of 4 parasitoid release treatments (0, 0.3, 3, and 30 parasitoids per plant), with 5 replicates per treatment. We counted the number of plants in each row of each plot on 19 May. On 26 May, we randomly selected 50 plants from each plot and counted the number of leaves on each of these plants. We also counted the number of adult whiteflies on each of 200 randomly selected leaves in each plot. The plot with the highest density of whitefly adults had an average of 5 whiteflies per leaf. We combined the information on the number of plants per row and the number of leaves per plant with the density of whitefly adults per leaf in each plot to calculate the number of whitefly adults necessary for release to increase the whitefly density in all plots to 5 whiteflies per leaf.

Whiteflies and parasitoids were reared on eggplant in the USDA-APHIS rearing facility in Brawley, CA, and were released by gently tapping the collection vials until the insects flew into the melon foliage. Whitefly pupae were placed in wooden emergence boxes (90 by 35 by 45 cm) with slanted glass tops. Emerging whitefly adults were counted as they were aspirated into 148 cm³ plastic vials. In the evening of 2 June, we released enough whitefly adults evenly throughout each plot to bring the average density of whiteflies to 5 adults per leaf.

Eretmocerus eremicus pupae were placed in emergence cages, with honey streaked on the top for food. Parasitoid adults were aspirated, counted, and evenly released on a weekly basis throughout the plots in the evening, over 3 wk, beginning on 13 June.

We began sampling on 12 June 1995 and continued weekly until 24 July, when the cantaloupe plants began to deteriorate because of the heat. We randomly counted the number of leaves on 2 cantaloupe vines in each of 20 plots on each sampling occasion to characterize the change in the number of leaves per vine over the growing season. We also randomly collected 15 cantaloupe vines each week for 7 wk to determine whitefly distribution. Ten of these vines were collected from the 5 plots with the high parasitoid release rate (2 vines per plot) and 5 vines were collected from the plots where no parasitoids were released (1 vine per plot). The position of each leaf on the vine was marked on the leaf with an indelible marker in the field at the time of collection (leaf 1 is the terminal leaf at the growing tip of the plant). Only leaves >30 mm in diameter were collected. Leaves were placed between paper towels in plastic bags and were brought to the laboratory in ice chests kept cool with ice bricks. The leaves were frozen and analyzed in the winter of 1995. For another study, we randomly collected 50 cantaloupe leaves from each of the 20 plots weekly for 7 wk. The position of each leaf on the vine was written on the leaf, and the number of each insect stage was counted as described below. These data were used as an extra data set when calculating Taylor power law coefficients (see below).

In the laboratory, we removed a 3.88-cm² disk from each leaf half way between the leaf tip and the petiole and half way between the left side of the leaf and the midvein. We assumed that the position of the leaf disk on the leaf was not critical for an accurate assessment of whitefly density because Tonhasca et al. (1994) found that immature *B. argentifolii* were evenly distributed on individual leaves. We counted all individuals of the following stages on each disk using a dissecting microscope: whitefly eggs, whitefly nymphs, whitefly pupae (4th-instar red-eyed nymphs), and immature parasitoids (large larvae and pupae). Parasitoids are visible inside whiteflies only after they have become sufficiently large to displace the whitefly mycetomes and after pupation. Dead individuals were not counted. We also did not count whitefly or parasitoid exuviae because they can accumulate on leaves and then fall off after an unknown period of time, making accurate counts impossible.

Characterizing Distributions of Whiteflies and Parasitoids. The seasonal distribution of whiteflies and parasitoids among the various leaf positions was characterized by taking the sum of all individuals found at a given leaf position throughout the season and dividing by the total number of that stage found at all leaf positions. This was done, rather than averaging the proportions on each leaf by date, because the number of leaves per vine, over which the insects were distributed, changed throughout the season.

Four parameters were calculated to describe the distribution of each life stage among the different leaf positions on a vine for each sample date, as follows: (1) the leaf position that contained the highest percentage of the individuals in each stage, (2) the median leaf position, (3) the leaf position where the density of a

given stage was most highly correlated with the density of that stage on the entire vine, and (4) the leaf position where the coefficient of variation (CV) was lowest. Our goal was to determine whether we could select, based on these parameters, a range of leaf positions (sample units) that would give precise and cost-effective estimates of the density of each whitefly and parasitoid stage. Further analysis (described below) was then conducted on these leaf positions.

As the season progressed, the cantaloupe plants had increasingly more leaves per vine and a greater density of whiteflies. These 2 factors had to be taken into account during data analysis so that each sample date and each leaf position received equal weighting. We estimated all 4 parameters by date and then determined the seasonal mean by averaging across all dates. On a given date, the highest leaf positions (those farthest from the vine terminal) were present on only a few vines. Initial analysis indicated that inclusion of these leaves seriously skewed our results. We therefore conducted our calculations only on leaf positions that were equal to or below the mean number of leaf positions on that date, thus eliminating leaf positions represented by only a few leaves and very few whiteflies. This resulted in analysis of $\geq 90\%$ of the leaves we had collected on a given date. Pearson correlations between counts on each individual leaf with the counts on all leaves of the entire vine were calculated using PROC CORR (SAS Institute 1989a).

Development of Sampling Plans. Our first step in developing a sampling plan was to determine the leaf position (sample unit) for which sampling efficiency was the highest for each life stage. The most efficient leaf position for a given life stage was determined as the one which required the fewest sample units to achieve a given level of precision. For each stage, we conducted the following analysis on the leaf positions with the largest percentage of that stage, the median leaf position, the leaf position that best correlated to counts on an entire vine, and the leaf position with the lowest coefficient variation. We estimated the number of leaves to collect per field over a range of life stage densities (1–1,000 per leaf disk) using a modification of Cochran's (1977) formula for sample size (n) that incorporates the relationship between sample mean (m) and sample variance (s^2) (see Naranjo and Flint 1994):

$$n \geq am^{b-2}/D^2, \quad [1]$$

where D is precision (SE/m) and a and b are parameters of the Taylor power law, $s^2 = am^b$ (Taylor 1961, 1984). A precision of 0.25 was arbitrarily chosen for the initial analysis. We regressed $\ln(s^2)$ on $\ln(m)$ to calculate the Taylor power law parameters (PROC REG, SAS Institute 1989b), where the mean and variance of each life stage were calculated for given leaf positions separately for each date. We then estimated the density-dependent sample size for this optimal leaf for each life-stage at fixed precision levels of 0.10, 0.15, 0.20, and 0.25. Analysis of covariance (ANCOVA) (PROC GLM, SAS Institute 1989a) was conducted to determine if the slopes, b , and intercepts, $\ln(a)$, of the

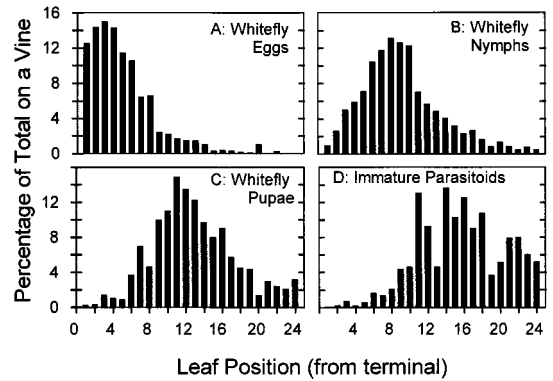


Fig. 1. Percentage of whitefly life stages and immature parasitoids present at each leaf position (all dates combined). Leaf number 1 is the terminal leaf.

Taylor power law were different among the various stages.

To determine the optimum number of leaves to sample per vine, we balanced the within-plant versus the between-plant variation and weighed these against the cost of sampling within the same vine versus moving to another vine. We used the formula of Cochran (1977; pp. 281, 288) to estimate the optimal number of leaves to sample per cantaloupe vine. A nested ANOVA (PROC NESTED, SAS Institute 1989a) was used to partition the total variance of counts into within-vine and between-vine components. The optimal number of leaves to sample per vine (n) was calculated as:

$$n = (s_w^2/s_b^2)^{1/2}(C_b/C_w)^{1/2}, \quad [2]$$

where s_w^2 is within-vine variance of counts, s_b^2 is between-vine variance of counts, C_w is time to collect and count a leaf disk, and C_b is time to move to next sample vine. We counted whiteflies and parasitoids on 3.88-cm² leaf disks on all of the leaves of 14 vines collected on the 7 dates (2 vines per date) to estimate the time necessary to count whiteflies. All vines were collected from the same plot. The time taken to count a given stage was estimated as the time necessary to count all stages on a leaf disk multiplied by the proportion of the individuals on that disk that were in a given stage. Given the experience of our counters, we assume that this approach accurately measured laboratory sampling cost. Naranjo and Flint (1994) estimated that it took an average of 0.7 min to move between sampling sites in commercial cotton fields in central Arizona. We used this value as a reasonable estimate of our between-vine sampling costs for cantaloupe fields.

Results and Discussion

The highest percentage of whitefly eggs was found on leaf 3 when data were combined across all dates (Fig. 1). Eighty-nine percent of the eggs were found on the 1st 8 leaves. The greatest percentage of nymphs

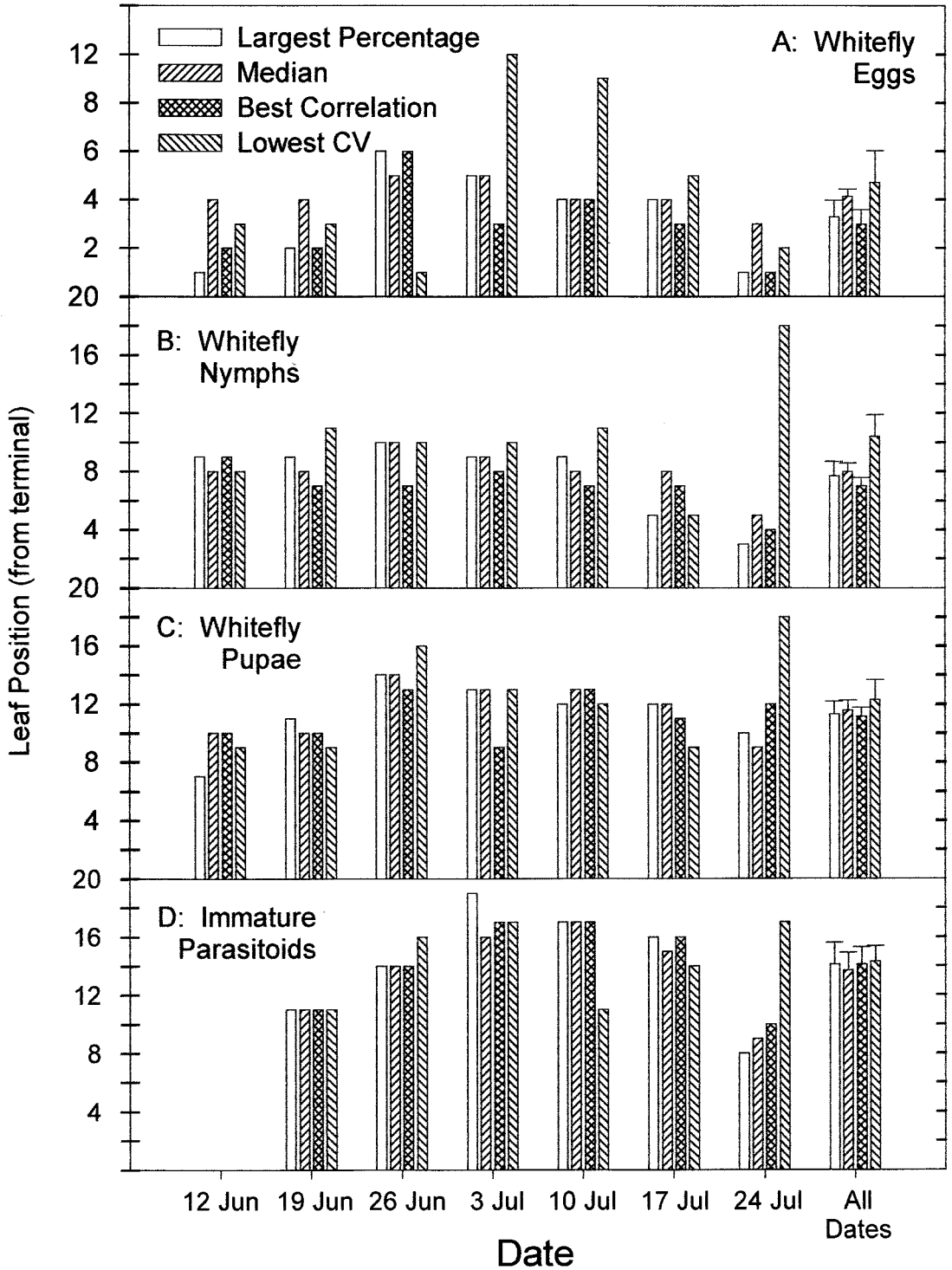


Fig. 2. Median leaf position, leaf position with the largest percentage of each life stage, leaf position where insect counts showed the best correlation with the total number on the vine, and leaf position with the lowest coefficient variation for whitefly life stages and immature parasitoids on 7 dates.

Table 1. Growth of cantaloupe vines in Poston, AZ, 1995

Date	No. leaves per vine ^a		No. leaves added in preceding week
	Mean \pm SEM	Range	
12 June	9.4 \pm 0.5	6–12	—
19 June	11.6 \pm 0.5	8–16	2.3
26 June	17.0 \pm 1.2	11–28	5.3
3 July	19.0 \pm 0.7	14–26	1.9
10 July	21.3 \pm 1.2	16–30	2.4
17 July	23.2 \pm 1.4	17–36	1.9
24 July	18.1 \pm 1.6	10–33	-5.1

^a $n = 40$ vines per week.

and pupae were found on leaves 8 and 11, respectively. No leaf contained >16% of the individuals of a particular stage that occurred on a given vine. Tonhasca et al. (1994) found more eggs on terminal cantaloupe leaves (positions 1–4) than on crown leaves (positions ≥ 5) and more red-eyed nymphs (pupae) on crown leaves than on terminal leaves, but they did not detect differences in the distribution of 1st–4th instars between terminal and crown leaves.

Immature parasitoids were found on a greater number of leaf positions than was typical for whitefly stages (Fig. 1), and the peak in their distribution was less pronounced. In general, more parasitoids were found on older leaves (89% of immature parasitoids were found on leaf 10 or higher). This distribution probably results from the fact that *Eretmocerus* sp. usually oviposit in >1 whitefly instar (Gerling 1990), which would be distributed among a large number of leaf positions. Parasitoids deposited in younger instars would become visible on relatively younger leaves and parasitoid eggs deposited in older instars would become visible on older leaves, leading to a broad distribution of immature parasitoids.

All 4 distribution parameters (median leaf position, leaf position with the highest percentage of a particular stage, leaf position with the best correlation to vine counts, and the leaf position with the lowest coefficient variation) changed throughout the season (Fig. 2). In general, the median leaf position changed least. Pearson correlation coefficients ranged from 0.66 to 0.96. The optimal leaf position got progressively older for all 4 parameters (with a few exceptions for coefficient variation) until a peak from 26 June to 10 July, after which it declined again to younger leaves. We hypothesize that this is caused by the rapid growth of cantaloupe at the beginning of the season, followed by a slowing of growth. The peak growth of cantaloupe occurred between 19 and 26 June, when the plants added an average of 5.3 leaves during 1 wk (Table 1). The most efficient leaf to sample probably changes throughout the season. Without detailed knowledge of plant and insect phenology, however, it was not possible for us to design a sampling regime where the recommended leaf position changes over time. We chose, therefore, to concentrate on selecting leaf positions that would give reliable and efficient estimates throughout the season.

When averaged across the entire season, the 4 distribution parameters were associated with leaf posi-

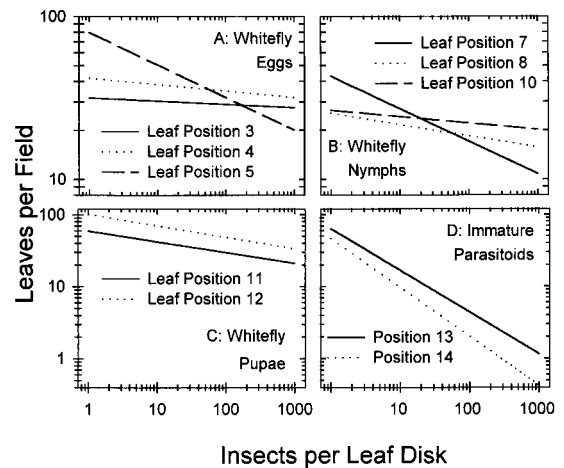


Fig. 3. Minimum sample size (leaves per field) over a range of insect densities for whitefly life stages necessary to achieve a precision of 25%.

tions 3, 4, and 5 (whitefly eggs), leaf positions 7, 8, and 10 (whitefly nymphs), leaf positions 11 and 12 (whitefly pupae), and leaf positions 13, and 14 (immature parasitoids) (Fig. 2). We plotted the minimum number of leaves to collect per field at these positions to achieve a 25% level of precision based on equation 1 (Fig. 3). Over a wide range of densities, fewer leaves at position 3 would need to be collected to estimate density of whitefly eggs, and leaves 11 and 14 were most efficient for sampling whitefly pupae and immature parasitoids, respectively. The lines for leaf positions 7 crossed the lines for positions 8 and 10 for whitefly nymphs, but we would recommend leaf 8 because the number of leaves necessary to achieve 25% precision was lower and more constant over a broader range of nymphal densities. We recommend sampling leaf 3 for whitefly eggs, leaf 8 for whitefly nymphs, leaf 11 for whitefly pupae, and leaf 14 for immature parasitoids.

The variability in counts of all stages was higher within vines than between vines (Table 2). We used equation 2 to calculate the optimal number of leaves to sample per vine in relation to the time needed to collect and count whiteflies and parasitoids on single leaf disks (Fig. 4). One should collect only 1 leaf from each vine when the time to count insects on leaf disks exceeds 2 min. It would be more efficient to collect ≥ 2 leaves per vine when count time is below 2 min. In these instances, we would recommend choosing the leaf position with the 2nd highest efficiency (Fig. 3). It took our skilled personnel ≈ 1 min or less to count the various life stages (Table 3). However, this average was calculated using vines collected throughout the season at one particular site. The time to count leaf disks depends on many factors including the skill level of personnel, the density of whiteflies and parasitoids, and so on. Our recommendation would be for investigators to determine the length of time to count a leaf disk given their situation and to use Fig. 4 as a guide

Table 2. ANOVA of *B. argentifolii* and *E. eremicus* counts on leaves collected within and between cantaloupe vines

Stage	Source	df	MS	Variance component	% total variance
Whitefly egg	Between vine	97	12.18	0.62	22.89
	Within vine	1,617	1.98	1.98	73.42
Whitefly nymph	Between vine	97	6.45	0.25	7.52
	Within vine	1,617	2.39	2.39	73.08
Whitefly pupa	Between vine	97	2.97	0.14	15.62
	Within vine	1,617	0.61	0.61	67.39
Immature parasitoid	Between vine	97	0.59	0.03	12.50
	Within vine	1,617	0.16	0.16	76.39

to determining the optimum number of leaves to collect per vine.

Estimates of the Taylor power law parameters and equation 1 were used to determine the density-dependent number of sample units needed to estimate density at 4 levels of precision. Taylor power law coefficients (\pm SE) were as follows: whitefly eggs (leaf 3): $\ln(a) = 0.68 \pm 0.25$, $b = 1.98 \pm 0.08$, whitefly nymphs (leaf 8): $\ln(a) = 0.46 \pm 0.37$, $b = 1.93 \pm 0.10$, whitefly pupae (leaf 11): $\ln(a) = 1.31 \pm 0.17$, $b = 1.85 \pm 0.11$, and immature parasitoids (leaf 14): $\ln(a) = 1.06 \pm 0.13$, $b = 1.33 \pm 0.09$, where $\ln(a)$ is the intercept and b is the slope of the linear regression. In all cases, both a and b were significantly different from 0 ($P < 0.05$) and significantly different among the various stages ($F = 445.8$; $df = 7, 47$; $P < 0.01$). Taylor b is an index of dispersion, with higher values indicating a more clumped distribution. We found b values

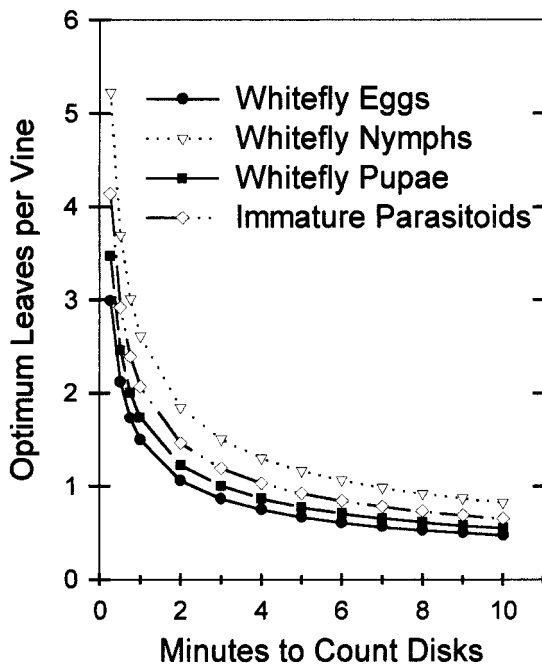


Fig. 4. Optimal number of leaves to sample per vine in relation to the time required to collect and count insects on a single leaf.

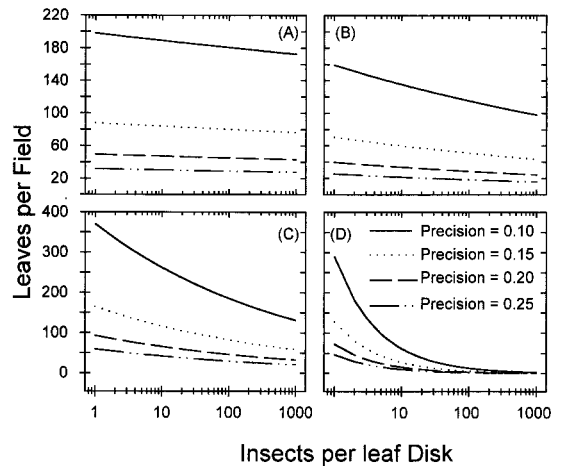


Fig. 5. Minimum sample size (leaves per field) at 4 levels of precision for whitefly life stages. (A) Whitefly eggs. (B) Whitefly nymphs. (C) Whitefly pupae. (D) Immature parasitoids.

for whitefly eggs and nymphs that were between 1.93 and 1.98. Naranjo and Flint (1994) calculated values for Taylor b of 1.76 for whitefly eggs and 1.69 for nymphs on cotton, indicating that these stages have a slightly more contagious distribution in cantaloupe. Tonhasca et al. (1994) also calculated b values of <1.9 when they considered crown leaves and terminal leaves as separate groups.

The minimum number of leaves to sample per field to achieve a fixed level of precision decreased with increasing insect density for all stages (Fig. 5). We typically have been collecting 50 leaves per field in our sampling programs. This sample size would be adequate to achieve a precision of 0.20–0.25 for all life stages of whiteflies and immature parasitoids over a broad range of densities. At densities of >10 whitefly eggs or nymphs per disk, the sample sizes in cantaloupe and cotton (Naranjo and Hutchison 1997) were similar. However, at densities <10 per disk, fewer samples were necessary in cantaloupe than in cotton.

The sampling plans presented here should prove useful to researchers interested in estimating the relative density of immature *B. argentifolii* and in evaluating and assessing levels of biological control provided by *E. eremicus* in cantaloupe. Figs. 4 and 5 should provide useful guides for determining the optimum

Table 3. Time (mean \pm SEM) necessary to count whitefly and parasitoid life stages at various leaf positions

Stage	Leaf position	Count time, (min:s)	Min.	Max.
Whitefly egg	3	1:01 \pm 0:11	0:18	2:03
Whitefly nymph	6	1:16 \pm 0:23	0:00	5:01
Whitefly pupa	11	0:06 \pm 0:02	0:00	0:19
Parasitoid immature	10	0:03 \pm 0:02	0:00	0:21

$n = 14$.

number of leaves per field and leaves per vine to sample at varying whitefly and parasitoid densities and with differing levels of precision.

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