BEMISIA GROWTH REGULATORS: A FIELD SAMPLING PROTOCOL FOR NYMPHS

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Abstract

We developed a sampling protocol for nymphs of the sweetpotato whitefly for timing the application of insect growth regulators. These plans require counting the number of large, visible nymphs within a 3.88 cm^2 leaf disk on the fifth main stem node leaf down from the terminal. Use of insect growth regulators for whitefly control is recommended when whitefly densities from 30 plants average 0.5 – 1.0 large nymphs per disk and 3 – 5 adults per leaf. We evaluated these sampling and decision-making plans within a large-scale field experiment. Precision was adequate for densities of 1.0 large nymph per leaf and greater, but sample sizes greater than 30 are needed for lower densities. The ability of samplers to detect and categorize nymphal instars and sampler-to-sampler variation in this ability were significant sources of variation. A binomial (presence/absence) sampling plan may diminish sampler-sampler variation while increasing efficiency and accuracy of decision-making.

Introduction

Careful monitoring of pest density for timing control is a key to whitefly management. Since 1994, Arizona pest managers have used a 'leaf-turn' method for sampling whitefly adults (Ellsworth *et al.* 1995; Naranjo *et al.* 1996). This method for sampling adult whiteflies is a reliable and efficient technique for estimating whitefly abundance and timing control activities (Diehl et al. 1995; Ellsworth *et al.* 1996a; Naranjo, 1995; Naranjo *et al.* 1995). However, with the introduction of two new insect growth regulators (IGRs) for whitefly control, sampling needs in Arizona cotton have changed. Because the IGRs Applaud™ and Knack® target immature whiteflies, an estimate of nymph densities in addition to adult densities is needed for timing use of these compounds.

We developed and evaluated sampling plans for whitefly nymphs in cotton. These plans were adapted from Naranjo & Flint (1994) and are outlined in Ellsworth et al. (1996b). A provisional threshold of 0.5 – 1.0 large nymphs per 3.88 cm^2 leaf disk and 3 – 5 adults per fifth main stem leaf was derived from efficacy testing in Arizona (for example, Ellsworth *et al*. 1994) and compared to experience with these IGRs in Israel (Horowitz personal communication). We evaluated these sampling and decision-making plans as part of a commercial-scale whitefly management trial in 1996.

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Methods

Sampling Plans

Large, visible whitefly nymphs are counted within a 3.88 cm^2 (quarter-sized) disk wedged between the central and left-side main veins on the underside of the fifth main stem node leaf down from the terminal. Naranjo and Flint (1994) found this sample unit to be the most accurate and efficient measure of egg and total nymph numbers. Only large nymphs visible to the naked eye are counted, making this method "field friendly"; no microscopes or hand lenses are required. These third and fourth instar nymphs appear as flattened, egg-shaped disks or scales. At least 30 leaves are sampled per field. Samplers start at least 10 rows into the field and choose a plant at random. Sampling continues along a zigzag line moving over several rows and taking 5 – 10 steps before selecting a new plant (see Ellsworth et al. 1995). Individual plants sampled should be 10 – 15 feet apart. After sampling 15 plants, the sampler moves to a new site within the field before sampling 15 more.

The provisional threshold for initiating IGR use is 0.5 – 1.0 large nymphs per leaf disk and 3 – 5 adults per fifth main stem node leaf. If nymphs exceed the threshold level but adults do not, then the grower can wait and re-sample in 3 days or apply Applaud which is effective against nymphs. If the adults exceed the threshold level but nymphs do not, then the grower can wait and re-sample in 3 days, use a conventional insecticide to lower adult counts, or apply Knack which can sterilize adults and developing eggs. If both nymph and adult levels exceed the upper threshold limit, then application of conventional insecticides may be warranted (Ellsworth *et al.* 1996b).

Evaluation

We evaluated sampling plans within a commercial-scale whitefly management trial (Ellsworth *et al.* 1997). Plots (48 total) were sampled at least weekly from June 14 through September 28, 1996 as described in sampling plans, above. In addition, samplers detached leaves after counting and brought them back to the lab for counting under the microscope.

We examined the relationship between mean and variance for the whitefly populations in this trial. Using Taylor's power law, we estimated sample size requirements for a precision (S.E./mean) of 0.25 (see Naranjo and Flint, 1994 for analytical methods). We estimated sample size requirements for whitefly populations before insecticides were applied, after insect growth regulators were applied, and after conventional insecticides were applied.

A presence/absence binomial model was developed by by using an empirical model (Naranjo *et al.* 1996) to describe the relationship between mean density and the proportion of leaf disks with one or more large nymphs.

Sampler-to-sampler variation was examined on July 12 within one of the plots of the commercial-scale trial. Two groups of four samplers sampled the plot simultaneously. In each group, one sampler would detach a leaf, count the number of large nymphs per leaf disk and pass it to the next sampler to count. Nymph densities in this plot were relatively high compared to both threshold levels and overall levels experienced in the trial. Leaves were then brought back to the laboratory for counts under a dissecting scope, and relationship between laboratory and field counts was evaluated using linear regression.

Results and Discussion

These sampling plans were easily integrated with adult whitefly sampling. Adults are counted first and then the leaf may be detached and scored for nymph density. A 7/8 in inner diameter washer, a U.S. quarter, or a card with a 3.88 cm^2 hole cut into it (a No. 14 cork-borer also may be used) can be used to locate the area of the leaf to sample.

A sample size of 30 leaves was adequate at moderate nymph densities (figure 1). We obtained a precision of 0.27 before IGRs were applied and 0.322 after IGRs were applied with a sample-size of 30 at a mean whitefly density of 1.0 large nymph per leaf disk. Overall, the required number of samples increases after either IGRs or conventional insecticides are used (figure 1). This is probably a result of the difficulty in distinguishing live from insecticide-killed nymphs in the field. There were no differences in sample-size requirements for IGR treated and conventional insecticide treated whitefly populations. For mean densities less than 1, it may be necessary to count additional leaf disks to achieve a moderate level of precision (0.25).

Many samplers expressed difficulty in distinguishing whether a nymph was “large” (i.e., 3rd or 4th instars) or not. This difficulty was evident in a comparison of lab and field counts (figure 2). In this comparison, field samplers counted on average about twice as many large nymphs as did lab counters. The opposite trend was observed in analyses from other dates.

Sampler difficulty in detecting and categorizing nymphs was also evidenced by a significant sampler-to-sampler variation with most of the variation attributed to a single sampler. The mean number of large nymphs counted by samplers in the sampler variation experiment ranged from 0.96 to 3.96 (table 1). All counts were above threshold levels.

A presence/absence binomial model reduces the frequency of nymph-size determinations that are necessary. As a result, sampler variation was nonsignificant when the proportion of disks with one or more large nymph was examined (table 2). A mean of 0.5 and 1.0 large nymph per disk is approximately equal to proportions of 0.28 and 0.44 disks with one or more large nymph, respectively. Using an action threshold of 0.5 – 1.0 large nymphs per leaf disk, control decisions based on the proportion of disks with one or more large nymph were in agreement with decisions based on numerical counts 88.8 percent of the time (figure 3). In practice, actual numbers of “wrong” decisions made using the proportion infested to predict numerical counts would probably be reduced. Ellsworth *et al.* (1997) found a threshold of up to 1.5 large nymphs per disk to be adequate for timing IGR use. A mean of 1.5 large nymphs per leaf disk is approximately equal to a proportion of 0.57 disks with one or more large nymphs. Using this expanded threshold of 0.5 – 1.5, control decisions based on sampling observations from the commercial-scale trial were in agreement 91.8 % of the time (figure 4). Furthermore, the recommended threshold is based on both adult and nymphal counts. Combining these measures to time control decisions increases the accuracy of the decision.

Conclusions

Overall, these sampling plans are of adequate precision for estimating whitefly nymph densities at the levels for which control decisions are critical. Because sampler-to-sampler variation can be significant, ample training of scouts is recommended. Nymphal sampling plans are being updated to include the binomial sampling scheme. These binomial plans diminish sampler error, should reduce the amount of time required for sampling, and provide an accurate means of classifying pest population density.

Acknowledgments

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Table 1. Mean number of large nymphs per disk from the fifth main stem node leaf counted by each sampler (n = 30). Sampler error is significant (P=0.003).

	Replicate	
Sampler	1	2
1	1.97	0.96
2	1.73	2.43
3	2.13	3.96
4	3.43	2.00
avg.	2.32 ±0.38	2.00 ±0.62

Table 2. Mean proportion of disks with ≥ 1 large nymph counted by each sampler (n = 30) . Sampler error is nonsignificant (P=0.119).

	Replicate	
Sampler	1	2
1	0.70	0.57
2	0.77	0.83
3	0.70	0.80
4	0.83	0.60
avg.	0.75 ±0.03	0.70 ±0.07

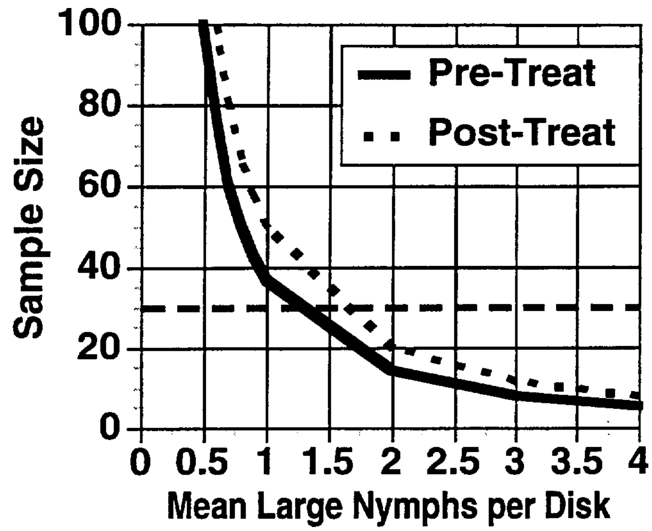

Figure 1. Required number of samples to achieve a precision of 0.25 (S.E./mean). Post-treatment includes observations after plots were treated with either conventional insecticides of IGRs. The horizontal dashed line indicates the currently recommended sample size of 30.

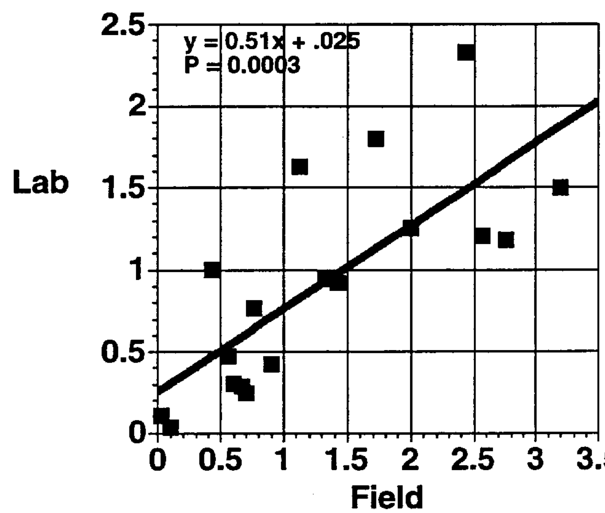

Figure 2. Regression of lab and field counts of mean large whitefly nymphs per 3.88 cm^2 leaf disk (n=60 disks).

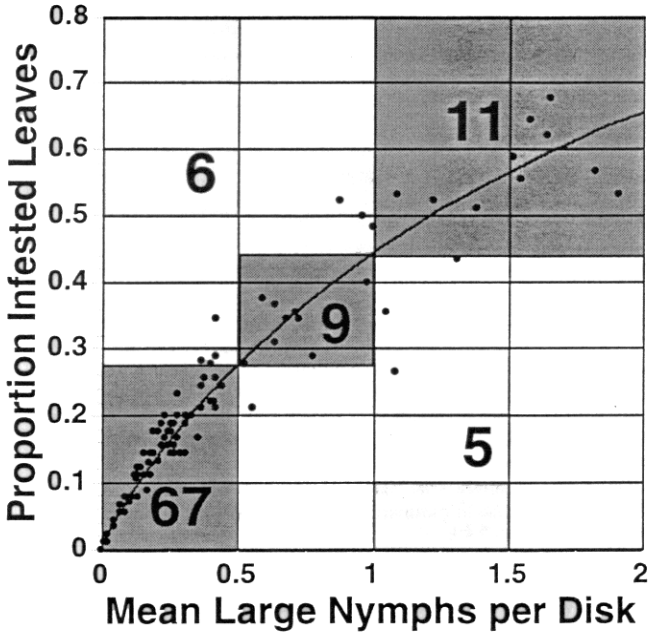

Figure 3. Relationship between proportion of infested leaf disks and mean large nymphs per disk. The line indicates predicted values and the dots indicate observed values. Numbers indicate number of control decisions falling within the given category for a threshold of 0.5 - 1.0 large nymphs per leaf disk. For example, in 67 cases both measures of nymph density would lead to a decison of no spray, in 9 cases, both would lead to a decision to spray IGRs, and in 11 cases, both would lead to a decision to spray with conventional insecticides. In a total of 11 cases the decisions from the two measures would differ.

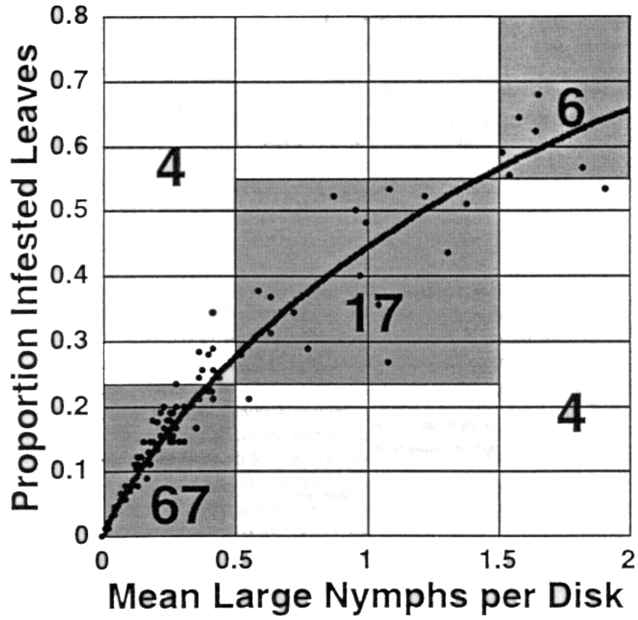

Figure 4. Relationship between proportion infested leaf disks and mean large nymphs per disk. The line indicates predicted values and the dots indicate observed values. Numbers indicate number of control decisions falling within the given category for the expanded threshold of 0.5 - 1.5 large nymphs per leaf disk.