

# Evaluation of Sampling Methods and Development of Sample Plans for Estimating Predator Densities in Cotton

ALLEN E. KNUTSON,<sup>1</sup> MARK A. MUEGGE,<sup>2</sup> L. T. WILSON,<sup>3</sup> AND STEVE E. NARANJO<sup>4</sup>

Texas A&M AgriLife Research and Extension Center, 17360 Coit Road, Dallas, TX 75252-6502

J. Econ. Entomol. 101(4): 1501-1509 (2008)

**ABSTRACT** The cost-reliability of five sampling methods (visual search, drop cloth, beat bucket, shake bucket, and sweep net) was determined for four groups of predatory arthropods on cotton plants in Texas. The beat bucket sample method was the most cost-reliable sampling method for *Orius* adults, and the beat bucket and drop cloth were the most cost-reliable methods for *Orius* nymphs. The drop cloth and beat bucket were the most cost-reliable methods for sampling spiders. For sampling adult Coccinellidae, the sweep net and the beat bucket were the most cost-reliable. The visual sample method was the least cost-reliable method for *Orius* adults and nymphs and spiders. No one sampling method was identified as the optimum method for all four predator groups. However, the relative cost-reliability of the beat bucket method ranked first or second among the five sampling methods and this method was chosen for further evaluation in field studies in Texas and Arizona. The relative cost-reliability of 1-, 3-, 5-, and 10-plants per beat bucket sample varied with predator group, but multiple plant sample units were equal to or more cost-reliable than the one plant sample unit. Fixed sample plans for the beat bucket method were developed for *Orius* adults, *Orius* nymphs, spiders, and adult Coccinellidae, and the sum of these groups using the 3-, 5-, and 10-plant sample unit sizes. The greater cost-reliability of the beat bucket sampling method and its ease of use is of particular advantage in assessing predator densities in a commercial cotton field monitoring program.

**KEY WORDS** beneficial arthropods, *Orius*, spiders, cotton, beat bucket

Predatory arthropods have long been recognized as important natural enemies of several major insect pests of cotton, *Gossypium hirsutum* L., especially *Heliothis virescens* (F.), *Helicoverpa zea* (Boddie), *Spodoptera exigua* (Hübner), *Bemisia tabaci* (Gennadius), and *Aphis* spp. (van den Bosch and Hagen 1966, Sterling et al. 1989, Naranjo and Ellsworth 2005). The impact of these and other natural enemies on cotton insect pest abundance becomes most apparent when the use of broad-spectrum insecticides disrupts this natural control and leads to pest resurgence and secondary pest outbreaks (Ewing and Ivy 1943, Leigh et al. 1966, Ridgeway et al. 1967, Adkisson 1971, Stoltz and Stern 1978). McDaniel and Sterling (1979) reported that *Orius insidiosus* (Say), *Geocoris* spp., *Solenopsis invicta* Buren, *Pseudatomoscelis seriatus* (Reuter), some coccinellid spp., and spiders in the families Salticidae, Oxyptoidae, and Thomisidae were important predators of *H. virescens* eggs in east central Texas. Predators and parasitoids are important in suppressing

infestations of *Spodoptera exigua* (Hübner) infestations and disruption of these natural enemy populations have been implicated in outbreaks of this pest after widespread application of malathion to cotton (Eveleens et al. 1973, Ruberson et al. 1994). In Arizona, predators are a major source of whitefly mortality (Naranjo and Ellsworth 2005), and a variety of species, including *Geocoris* spp., *Orius tristicolor* (White), and adults of *Drapetis*, a small predatory fly, commonly feed on whiteflies in cotton (Naranjo and Hagler 1998, Hagler and Naranjo 2005). Predators, especially *Hippodamia convergens* Guérin-Méneville, also suppress populations of *Apis gossypii* Glover (Kidd and Rummel 1997). Although integrated pest management (IPM) programs recognize predators in controlling cotton pests, there are few guidelines on how to use field information on predatory arthropods in making pest management decisions. A major constraint to the development of these guidelines has been the lack of cost-efficient sampling methods for estimating densities of predatory arthropods. An optimum sampling method would ideally be suitable for all important predators, rapid and simple to use, and easily integrated into commercial field monitoring programs. Sampling equipment, if any, should be readily available and easy to carry and use in the field. Furthermore, sampling procedures should be simple

<sup>1</sup> Corresponding author, e-mail: a-knutson@tamu.edu.

<sup>2</sup> Texas A&M AgriLife Extension Center, P.O. Box 1298, Fort Stockton, TX 79735.

<sup>3</sup> Texas A&M AgriLife Research and Extension Center, 1509 Aggie Dr., Beaumont, TX 77713.

<sup>4</sup> USDA-ARS, U.S. Arid-Land Agricultural Research Center, 21881 N. Cardon Lane, Maricopa, AZ 85238.

to understand and conduct and be sampler-independent.

Various methods, including visual search counts, sweep net, drop cloth, and various types of containers in which plants are shaken or beaten, have been evaluated for sampling predatory arthropods in cotton (Smith et al. 1976, Byerly et al. 1978, Pyke et al. 1980, Wilson and Gutierrez 1980, Garcia et al. 1982, Nuessly and Sterling 1984). However, in most cases the cost (time) associated with a particular sample method has not been considered. Other sampling methods using mechanical vacuums and large field cages or bags (Smith et al. 1976, Ellington et al. 1984) are only suited for research programs where a lower premium is placed on cost-efficiency relative to commercial field monitoring. Currently, visual search or in some cases a sweep net is used to estimate densities of predators in commercial programs (Parker et al. 2005, Ohlendorf 1996). However, sampling plans to estimate densities of predatory arthropods on cotton plants have not been developed for either method.

The objectives of this study were to compare the visual search, sweep net, drop cloth, beat bucket, and shake bucket sampling methods for estimating densities of predatory arthropods on cotton plants and determine the optimal sample unit size for the most efficient method. The goal is to develop an efficient and reliable method for estimating abundance of predators in cotton that is suitable for use by a consultant, field scout or producer in a cotton field-scouting program.

## Materials and Methods

**Comparison of Sample Methods.** Sweep net, drop cloth, beat bucket, shake bucket, and whole plant visual search sample methods were evaluated for capture efficiency and cost-reliability relative to an absolute sample method as determined from sampling predatory arthropods on cotton plants in Texas. Adults and immatures were recorded separately for all predators except spiders. Predators were identified, counted, and recorded in the field to simulate commercial field monitoring. All study fields were sampled beginning at first bloom.

The sweep net method used a standard 38-cm-diameter sweep net swung in a pendulum-like motion perpendicular to the row and through the top of the canopy while walking down the row (Fleischer et al. 1985). A single sample consisted of five sweeps. Sweep net samples were converted to a per plant basis by dividing by 19 based upon a single sweep intercepting 3.8 plant terminals (Wilson and Gutierrez 1980).

The drop cloth method involved beating five adjacent cotton plants onto a 0.9- by 1.1-m white cloth placed on the soil surface between two adjacent rows of cotton (Nuessly and Sterling 1984). Predators dislodged onto the cloth were identified, counted, and recorded. Predator densities were divided by five to convert counts to a per plant basis.

The shake bucket was 14 cm in depth and 27 cm in diameter and was cut from the bottom of a white,

18-liter plastic pail. The upper 15–20 cm of the plant was placed inside the shake bucket and shaken five times to dislodge predators and the captured predators were counted (Pyke et al. 1980).

The beat bucket method used a common white, 18-liter plastic pail 27 cm in diameter and 37 cm in depth. The beat bucket was held at a 45° angle to the ground and the sample plant was grasped near the base and quickly bent into the bucket. The plant was rapidly beaten against the side of the bucket 12–16 times for 3–4 s. This action dislodged predators, which fell into the bucket. The plant was removed and the bucket held upright to prevent predators from escaping. Predators in the bucket were identified and counted. Leaves or fruit that fell into the bucket were also examined for predators.

The visual search method involved examination of the terminal and all fruiting structures beginning in the terminal and working down through the plant. Blooms and bracts were opened to expose predators hiding in these structures. Predators on leaves and stems also were recorded during the examination, but samplers did not uniformly sample all of these structures.

The absolute sampling method used a 95-liter galvanized metal trash can (38-cm opening at the top and 67 cm in depth) fitted with a large funnel in the bottom. The can was supported in the field by an 18-liter plastic pail 27 cm in diameter and 37 cm in height. One side of the pail was removed to allow access to the bottom of the funnel, which was fitted with a 0.5-liter glass jar containing 50% ethyl alcohol and water. An aerosol can of 0.5% pyrethrin (PT 565 Fogger, Whitmire Labs, St. Louis, MO) was fastened to the outside of the sampling device with the tip of the spray nozzle inserted through a small hole. Exposure to pyrethrin flushed small predators (*Orius* spp. nymphs) from behind bracts and in blooms to more exposed areas of the plant where they were dislodged by beating the plants. The main stem of the sample plant was cut with pruning shears just below the first branch and immediately placed inside the sampling device. The lid was quickly placed on top to seal the sampler and the contents were fumigated by releasing a 1-s spray of pyrethrin inside the sampler. After 1 min, the lid was removed and the plant vigorously shaken and beaten against the side of the sampling device to dislodge predators that were collected in the jar at the bottom of the funnel. All leaves and fruiting forms were removed and examined for predators. Bracts were removed from fruit and blooms were opened to reveal any concealed predators. During this time, the plant was periodically beaten against the side of the sampler and was used to brush any predators from the sampling device sides into the funnel and collection jar below. The collection jar was then removed, sealed and returned to the laboratory. A Buchner funnel was used to concentrate the predators onto a filter paper where they were identified and counted using a dissecting microscope.

In 1997, all sampling methods were compared in a 1.2-ha field of cotton at the Texas A&M AgriLife Re-

search and Extension Center at Dallas and in two large commercial fields located near Hillsboro in central Texas. The field at Dallas was planted to 'Delta Pine 50' cotton on rows spaced 102 cm apart and was not treated with any foliar insecticides. Grain sorghum and corn bordered the field. The Hillsboro fields were planted to Bt cotton 'Delta Pine 33B', and both fields were bordered by grain sorghum fields. Both Hillsboro cotton fields were treated with foliar insecticides for fleahoppers and boll weevils three weeks before the start of the trial. Also, one field received an application of endosulfan and oxamyl on 23 July, and it was not sampled again until 1 August. In 1998, the study was repeated at the Dallas location and only the absolute, sweep net, beat bucket, and visual sampling methods were evaluated. The drop cloth and shake bucket methods were not evaluated in 1998 as experience in 1997 demonstrated that both methods would be unsuited for scouting programs as explained later. In the two Hillsboro fields, a block of cotton 50 rows wide by 165 m long was divided into 30 plots each five rows wide and 55 m long. Each plot was further divided into six subplots five rows wide by 9.2 m long. Because of the smaller field size at the Dallas location, each subplot was five rows by 4.6 m long. Each of the six sampling methods was randomly assigned to one of the subplots within each plot to provide 30 samples per method on each sample date.

The Dallas field was sampled on 11 dates at weekly intervals from 16 June to 21 August 1997. The Hillsboro fields were each sampled on four dates from 3 July to 1 August 1997. Only the Dallas field was sampled in 1998, and samples were taken on six dates at weekly intervals from 15 June to 28 July 1998. Sampling began in the early morning and was completed by noon because many insects readily fly or drop from the plant when the plant is disturbed once temperatures reach 25–30°C (Garcia et al. 1982). Samplers worked in teams with one person sampling, whereas the other person recorded data. The time in minutes for the team to complete each sampling method was recorded.

**Evaluation of Sample Unit Size for Beat Bucket Method.** The 1997–1998 data were used to identify the best sample method and then further studies to identify optimum sample size were conducted in Arizona and Texas in 1999–2000. Four sample unit sizes were evaluated with the beat bucket method: 1, 3, 5, and 10 plants per beat bucket sample unit. In Arizona, predatory arthropods were sampled at four field sites on the University of Arizona, Maricopa Agricultural Center, Maricopa, AZ. The Arizona fields were divided into a total of 20 subplots each four rows wide by 51.8 m long. In Texas, samples were collected from a study field located at the Texas A&M Research and Extension Center at Dallas. The Texas field was divided into 24 subplots each four rows wide and 11.2 m long. One sample unit was collected from a different row in each plot, yielding 24 and 20 replications for each sample unit size per date at the Texas and Arizona sites, respectively. Multiple plants within a sample unit (3, 5, or 10) were spaced two paces ( $\approx 2$  m)

apart within the same row of cotton. The time necessary to collect a sample unit was recorded with a stopwatch for ten samples on each sample date. Plots were sampled at weekly intervals on six dates from 1 July to 5 August 1999 and on five dates from 5 July to 2 August 2000 in Texas and at 2-wk intervals on six dates from 1 July to 9 September 1999 in Arizona.

Individual cotton plants were sampled with a beat-bucket constructed from an 18-liter, white plastic pail (37 cm by 27 cm in diameter). The bottom of the bucket was removed, and a large plastic funnel (P-06121-20, Cole Parmer Co., Vernon Hills, IL) was fastened to the bottom with metal brackets to direct insects into a small 120-ml plastic jar at the base of the funnel. The jar could be detached and capped. The bucket was fitted with a drawer handle attached to the side so it could be easily held and tilted for sampling. When sampling, the bucket was held at a 45° angle to the ground and a single cotton plant was carefully grasped by the lower stem and then quickly bent into the bucket. The plant was beaten against the sides of the bucket for a 3–4 s period ( $\approx 12$ –16 beats) to dislodge the insects and spiders. The plant was then removed, the bucket was held upright and the sides of the bucket were sharply tapped a few times with the hand until all the arthropods had fallen through the funnel into the collecting jar. For multiple plant sample units this procedure was repeated for each plant. Predators on leaves or fruit that fell into the bucket also were dislodged and collected. Once the sampling bout was completed, the collection jar was removed and alcohol was added to kill and preserve the arthropods. A Buchner funnel was used to concentrate the predators onto a filter paper where they were identified and counted using a dissecting microscope.

**Data Analysis.** An optimal sampling method or sample unit size provides the most cost-reliable estimate of density for a given expenditure of sample time (Wilson 1994). A consideration of cost, expressed as time to collect the sample, is especially important in developing sampling methods and plans for use in commercial field monitoring programs. Relative-cost reliability is the ratio of the costs of two or more sampling methods or sample unit sizes and was computed as follows:

$$C_1/C_2 = n_1(T_1 + t_1)/n_2(T_2 + t_2) \quad [1]$$

where  $C$  is cost per sample for each sample method or sample unit size,  $n$  is required number of samples needed to provide a density estimate with a specified level of precision,  $T$  is time required to collect a sample for each sample method or sample unit size, and  $t$  is time to move from sample unit to sample unit (Wilson 1994). The time in minutes to move from one sample unit to the next was set at  $t = 0.5$  m for all sample methods. Relative cost-reliability was used to select the optimum sample method and sample unit size. The absolute sample method was used as the denominator in equation 1. The lowest relative cost reliability value represents the optimum sample method.

Minimum sample size,  $n$ , in equation 1 was determined from Wilson (1994) as follows:

$$n = t_{\alpha/2}^2 D_x^{-2} a x^{b-2} \tag{2}$$

where  $t_{\alpha/2}$  is standard normal variate for a two-tailed confidence interval,  $D_x$  is a fixed level of precision and is defined as a proportion of the ratio of half the desired confidence interval to the mean,  $x$  is sample mean density, and  $a$  and  $b$  are Taylor's coefficients. For this study,  $D = 0.35$  and was chosen to reduce sampling cost while maintaining a reasonable level of precision for estimating predator groups in a commercial scouting program. Taylor's coefficients were determined by fitting Taylor's power law to each predator group by sample method and sample unit size. Taylor's power law is defined as follows:

$$S^2 = am^b \tag{3}$$

where  $S^2$  is sample variance,  $m$  is sample mean, and  $a$  and  $b$  are coefficients (Taylor 1961). Taylor's coefficients were calculated by iterative nonlinear regression using JMP IN 4.0 (SAS Institute 2001).

Relative capture efficiency reflects the effectiveness of arthropod recovery by one sample method or sample unit size relative to a standard, and for this reason can be a very useful sample method evaluation criterion. Relative capture efficiencies were computed from field data for each sample method and sample unit size by

$$\text{Relative Capture Efficiency} = \bar{X}_i / (\bar{X}_s \omega_i) \tag{4}$$

where  $\bar{X}_i$  is the mean predator density of the  $i$ th sample method or sample unit size,  $\bar{X}_s$  is the mean predator density of the absolute sample method or the one plant sample unit size, and  $\omega$  is the number of plants sampled for the  $i$ th sample method or sample unit size.

Based on the relationship between Taylor's power law and minimum sample size (equation 2) relative cost-reliability values for each sample method and sample unit size were determined (equation 1) for 30 mean predator densities at 0.05 intervals between 0.10 and 1.55 predators per plant. This range of densities was chosen to reflect the range of predator densities found in the absolute samples taken in the four study fields in 1997 and 1998.

General Linear model analysis of variance (ANOVA) (SAS Institute 2004) was used to compare sample time among sample methods. Mixed model ANOVA (SAS Institute 2004) was used to compare relative cost-reliability and relative capture efficiency among sample methods and beat bucket sample unit size. Year and location were combined into a single variable to represent potential variation over different times and sites ( $n = 4$  for sample methods,  $n = 3$  for sample units). This single variable and its interaction with method or unit size were entered as random effects in the model. To account for non-normality and heteroscedasticity, the GLIMMIX procedure (SAS Institute 2006) was used for all analyses. Exponential distributions were specified for all species groups in sample method analyses except for *Orius* spp. nymphs, where the distribution was log-normal. Log-normal

**Table 1. Percentage of beneficial arthropods recovered in samples by using the absolute sample method from four cotton fields in Texas**

Predator group	Location				% total <sup>a</sup> (total no.)
	Dallas A	Dallas B	Hill A	Hill B	
	1998	1997	1997	1997	
<i>Orius</i> adults	20	28	63	56	41.8 (937)
<i>Orius</i> nymphs	13	33	8	20	18.5 (483)
Spiders	30	18	9	12	17.3 (372)
Lady beetles	10	4	0.5	0.2	3.7 (74)
Lacewing larvae	1	2	11	2.5	4.1 (93)
<i>Geocoris</i> adults	5	5	2	0.5	3.1 (69)
<i>Geocoris</i> nymphs	3	3	0.2	0.6	1.7 (41)
Fleahopper adults	19	6	6	8	9.8 (195)

<sup>a</sup> Percentage of total represents total predator groups collected across all four fields.

distributions were specified for all species groups in analyses of sample unit size. The Kenwardroger method for estimating degrees of freedom was used in all analyses. Analyses did not include the standards (unity) used to calculate relative cost-reliability and relative capture efficiency as the variance of these means was zero. Confidence intervals (95%) were constructed around back-transformed means in GLIMMIX and used to test the hypotheses that relative cost-reliability and relative capture efficiency of sample methods or sample units differed from unity. Significant differences among relative cost-reliability and capture efficiency means were identified using Tukey's studentized range (honestly significant difference [HSD]) procedure (SAS Institute 2004).

### Results and Discussion

**Sample Method Selection.** Numerous species of beneficial arthropods were collected and identified during the course of this study; however, the density of many species was too low for meaningful analysis. Thus, we selected four predator groups for developing sample plans based on their abundance in the absolute data and research indicating their importance in suppressing populations of major cotton pests (McDaniel and Sterling 1981, McDaniel et al. 1981, Kidd and Rummel 1997, Sansone and Smith 2001, Diaz et al. 2004). These four predator groups were *Orius* spp. nymphs, *Orius* spp. adults, all spiders (primarily Thomisidae, Oxyopidae, and Salticidae) and all adult Coccinellidae (primarily *H. convergens*), except *Scymnus* spp. These four predator groups constituted >80% of all predatory arthropods collected by the absolute sample method (Table 1). Red imported fire ants, *Solenopsis invicta* Buren, are also important predators of noctuid pests of cotton (McDaniel and Sterling 1979, Diaz et al. 2004). However, because fire ants forage primarily in the cotton canopy at night, early morning and evening, they were not consistently sampled in this study and therefore were not considered in these analyses.

The average time required to collect and record the 30 samples with each sampling method is shown in

**Table 2.** Mean time required to collect a sample size of 30 for each sample method

Sample method	<i>n</i>	Time (min) ± SE
Visual	22	42.27a ± 2.89
Drop cloth	19	35.53b ± 2.38
Sweep net	20	35.10b ± 2.07
Beat bucket	20	25.15c ± 1.96
Shake bucket	20	22.60c ± 1.71

Sample method study, 1997–1998. Means in a column followed by the same letter are not significantly different ( $P = 0.05$ ; Tukey’s HSD).

Table 2. These sample times include 0.5 min for walking between each of 30 samples sites per sample method. The beat bucket method and the shake bucket method required significantly less time than the other three methods and the visual search method required significantly more time than all other methods ( $F = 34.59$ ;  $df = 6, 94$ ;  $P < 0.0001$ ).

The relative capture efficiency of the beat bucket sample method was significantly greater than the other four methods when sampling *Orius* adults and spiders (Table 3). The sweep net method recovered significantly fewer *Orius* adults and nymphs relative to the other four sample methods, and except for the visual sample method, the sweep net collected significantly fewer spiders than the other methods. The beat bucket and visual sample methods captured significantly more coccinellids than the sweep net. Many predator species are not uniformly distributed on the plant (Wilson and Gutierrez 1980), and this can result in reduced capture efficiency for methods that sample only the terminal, such as the sweep net and shake bucket. Wilson and Gutierrez (1980) reported the sweep net recovered an average of 10–12% of the predatory arthropods in cotton in California, similar to the results reported herein (Table 3). Also, small predators such as *Orius* adults and nymphs, small lacewing larvae and other small beneficial arthropods often shelter or search for prey behind bracts and in blooms and subsequently are not readily dislodged by the sweep net whereas the repeated beating of the plant during beat bucket sampling increases the chance that

**Table 3.** Relative capture efficiency of five sample methods for predator groups in cotton<sup>a</sup>

Sample method	Predator group			
	<i>Orius</i> adults	<i>Orius</i> nymphs	Spiders	Coccinellidae
Beat bucket	1.0740a	0.3455a	1.0080a	1.1463a
Drop cloth	0.2226d	0.0990b	0.4061bc	0.4324ab
Shake bucket	0.5100b	0.1683ab	0.5195b	0.4578ab
Sweep net	0.0471e	0.0165c	0.0686d	0.1331b
Visual	0.3803c	0.2282ab	0.2271cd	0.5796a

Sample method study, 1997–1998.

<sup>a</sup> Capture efficiency values in table are relative to the capture efficiency of the absolute sample method = 1.0000. Means in a column followed by the same letter are not significantly different ( $P = 0.05$ ; Tukey’s HSD). Lower values of relative capture efficiency indicate reduced predator group recovery relative to the absolute sample method.

**Table 4.** Mean relative cost-reliability of five sample methods for predator groups in cotton

Method	Predator group			
	<i>Orius</i> adults	<i>Orius</i> nymphs	Spiders	Coccinellidae
Beat bucket	0.1803a	0.6127a	0.1562a	0.2115c
Drop cloth	0.2934b	0.6336a	0.0796b	0.5322a
Shake bucket	0.2645b	1.2301b	0.2208c	0.2870b
Sweep net	0.3218b	1.2196bc	0.2517c	0.1448d
Visual	0.8934c	1.3314c	1.0612d	0.3069b

Sample method study, 1997–1998.

<sup>a</sup> Cost-reliability values are relative to the cost-reliability value for the absolute method (=1.0000). Lower values for relative cost-reliability indicate a more optimal sample method. Means in a column followed by the same letter are not significantly different ( $P = 0.05$ ; Tukey’s HSD).

small predators are dislodged from these sites. Low relative capture efficiency of the drop cloth likely occurred because predators did not fall on the drop cloth or escaped from the cloth before being counted by the sampler. This is less likely to occur for the beat bucket method because the deep recess of the bucket contained predators until they could be counted. In contrast, the shake bucket was only 14 cm deep, and predators were often observed to fly out or be blown out of the bucket by wind before they could be identified and counted. The low relative capture efficiency of spiders by the visual search method probably resulted from spiders escaping to adjacent plants or spinning silk lines and escaping to the ground once the sampler began handling the plant terminal. The low relative capture efficiency of the visual search method for *Orius* adults and nymphs is attributed to their small size and secretive habits.

The relative cost-reliability of the visual search method was significantly greater (more costly) than all other sample methods for *Orius* adults and spiders and significantly greater than three of the four other sample methods for *Orius* nymphs and coccinellidae (Table 4). The low relative capture efficiency of the visual search (Table 3) and the high cost of sampling time (Table 2) contributed to the high relative cost-reliability (poor performance) of the visual search method. These results are attributed to the difficulty in seeing small predators such as *Orius* spp. within the plant canopy, the opportunity for spiders to escape given the long sampling time needed to visually search all plant parts, and variations in the visual acuity of the sampler. Thus, although the visual search method is routinely used for sampling pest species, this method was not cost-reliable for sampling these four predator groups.

The relative cost-reliability of the sweep net and beat bucket methods were significantly less than that of the other three methods for sampling Coccinellidae and therefore were the optimum sampling methods for this group (Table 4). The behavior of many coccinellid species to congregate in the plant terminal where they prey on aphids no doubt contributes to the favorable cost-reliability of the sweep net and beat bucket methods for sampling this predator group.

Table 5. Mean relative capture efficiency for 3-, 5-, and 10-plant sample unit sizes using the beat bucket for predator groups in cotton

Sample unit size <sup>a</sup>		Predator group			
		<i>Orius</i> adults	<i>Orius</i> nymphs	Spiders	Coccinellidae
3		0.8633a	0.6604a	1.0500a	0.7365a
5		0.7802a	0.6211a	0.8027a	0.8292a
10		0.6718a	0.4620a	0.8141a	0.9193a
Unity <sup>b</sup> vs. sample unit size					
1	3	$P = 0.1972$	$P = 0.0191$	$P = 0.8378$	$P = 0.0946$
1	5	$P = 0.0666$	$P = 0.0136$	$P = 0.2323$	$P = 0.3582$
1	10	$P = 0.0193$	$P = 0.0018$	$P = 0.2029$	$P = 0.3126$

Sample unit size study, 1999–2000.

<sup>a</sup> Sample unit size is 1, 3, 5, and 10 plants, with the 1 plant sample unit (unity) serving as the standard. Means in a column followed by the same letter are not significantly different ( $P = 0.05$ ; Tukey's HSD). Lower values of relative capture efficiency indicate reduced predator group recovery.

<sup>b</sup> Approximate *t*-tests constructed by the GLIMMIX procedure to test the hypothesis that relative capture efficiency of the 3, 5, and 10 plant sample unit sizes are equal to the capture efficiency of the standard 1 plant sample unit (unity).

However, the beat bucket was significantly more cost-reliable (lower value) than the sweep net for *Orius* adults and nymphs, and spiders (Table 4). Byerly et al. (1978) concluded that sweep net sampling failed to reflect actual population trends of predators throughout the season in cotton.

The drop cloth method was significantly more cost-reliable (lowest relative cost-reliability value) than the other four methods for sampling spiders and was not significantly different from the beat bucket for *Orius* nymphs (Table 4). However, the drop cloth method was the most physically tiring as it required the sampler to get on hands and knees to beat the plants and observe and record predators on the cloth. High soil temperatures, threat of stinging by red imported fire ant, and the still air and high humidity within the canopy added to the discomfort. Sampling with the drop cloth was not considered suited to a commercial scouting program due to these disadvantages and the lack of a significant benefit in cost-reliability relative to the beat bucket method.

The relative cost-reliability analysis indicated that the beat bucket sample method performed significantly better (least cost-reliability value) for *Orius* adults and, with the exception of the drop cloth, significantly better for *Orius* nymphs relative to the other sample methods tested (Table 4). With the exceptions of the drop cloth for spiders and the sweep net for coccinellids, the beat bucket was significantly more cost reliable for sampling spiders and coccinellids than the other three methods tested (Table 4). For most predator groups, the beat bucket sample method yielded a higher relative capture efficiency (Table 3) and required less sample time than most other methods (Tables 2) which together contributed to a more favorable relative cost-reliability value. Thus, although no one sampling method was identified as the optimum method for all four predator groups, the beat bucket method ranked first or second among the five sampling methods. Because of the overall superior cost-reliability of the beat bucket method for the predator groups evaluated herein, this method was chosen for further refinement by evaluating the effect of sample unit size on relative capture efficiency and relative

cost-reliability for the predator groups identified in this study.

**Sample Unit Size Selection.** Relative cost-reliability was again used to identify the optimum sample unit size based on 1-, 3-, 5-, and 10-plants per sample unit. As expected, the mean time to collect each sample unit increased as the number of plants per beat bucket sample increased. The mean ( $\pm$ SE) time to collect a single sample unit from the field was  $0.87 \pm 0.23$ ,  $1.17 \pm 0.30$ ,  $1.75 \pm 0.45$ , and  $2.25 \pm 0.58$  min for the 1-, 3-, 5-, and 10-plants per beat bucket sample unit, respectively. However, the time required on a per plant basis decreased as sample unit size increased making larger sample unit sizes more time efficient than smaller sample sizes.

The 3-, 5-, and 10-plants per beat bucket sample recovered similar proportions of all four predator groups (Table 5). When compared with the single plant sample, sample unit size for multiple plant samples did not significantly influence relative capture efficiency for spiders and coccinellids, and except for the 10-plant sample unit size did not influence *Orius* adults. For *Orius* nymphs, the 1-plant sample unit size was significantly more efficient than the other sample unit sizes tested (Table 5). This decline in relative capture efficiency of sample unit sizes greater than one plant could result from beating of the cotton plant in the beat bucket which could damage the minute and fragile nymphs to the point that they become difficult to see and recognize.

Relative cost-reliability varied significantly by sample unit size regardless of predator group (Table 6). The 5-plant sample unit was significantly more cost reliable (least relative cost-reliability value) for *Orius* adults and nymphs, compared with the three and 10-plant sample unit sizes. Also, the 5-plant sample unit size was significantly more cost reliable than the 1-plant sample unit size for *Orius* adults but not nymphs. For spiders, the relative cost-reliability of the 10-plant sample unit size was significantly less than that for the 3- and 5-plant sample size. However, the cost reliability of the 3-, 5-, and 10-plant sample unit sizes for spiders were not significantly different from the cost reliability of the 1-plant unit sample size. For

**Table 6.** Mean relative cost-reliability for 3-, 5- and 10-plant sample unit sizes using the beat bucket by predator group

Sample unit size <sup>a</sup>		Predator groups			
		<i>Orius</i> adults	<i>Orius</i> nymphs	Spiders	Coccinellidae
3		0.5525a	0.8604a	0.5491a	0.3263a
5		0.3382b	0.7536b	0.6367b	0.6005ab
10		0.4357a	0.9360c	0.4620c	0.7149b
Unity <sup>b</sup> vs. sample unit size					
1	3	$P < 0.0001$	$P = 0.2035$	$P = 0.1696$	$P = 0.0432$
1	5	$P < 0.0001$	$P = 0.0931$	$P = 0.2159$	$P = 0.0623$
1	10	$P < 0.0001$	$P = 0.5541$	$P = 0.1176$	$P = 0.0961$

<sup>a</sup> Cost-reliability values in table are relative to the cost-reliability value for the 1 plant sample unit (unity) = 1.0000. Lower values for relative cost-reliability indicate a more optimal sample method. Means in a column followed by the same letter are not significantly different ( $P = 0.05$ ; Tukey's HSD).

<sup>b</sup> Approximate *t*-tests constructed by the GLIMMIX procedure to test the hypothesis that relative capture efficiency of the 3, 5 and 10 plant (sample unit sizes) are equal to the capture efficiency of the 1 plant sample unit (unity).

coccinellids, the 3-plant sample unit size was significantly more cost-reliable than the 1- and 10-plant sample unit sizes.

The relative cost-reliability of multiple plant sample unit sizes was significantly less (more cost-effective) than the 1-plant sample unit for *Orius* adults and coccinellids. In addition, the cost-reliability of the 1-plant sample unit size was never significantly more cost-reliable than the multiple sample unit sizes. For these reasons the 1-plant sample unit size was not considered as cost-reliable as the multiple plant unit sizes for these four predator groups and was consequently not selected for sample plan development.

As an optimum sample unit size for all predator groups was not identified, fixed sample plans were developed for the 3-, 5-, and 10-plant sample unit sizes by using the beat bucket sample method. Taylor's parameters were derived for all the predator groups and total predators (the sum of the four predator groups) for the 3-, 5-, and 10-plant sample units (Table 7). Minimum sample size needed to estimate density of each predator group was determined using Taylor's coefficients as described previously (Table 8). Threshold densities for these predator groups have not been determined in cotton. Therefore, we arbitrarily chose 0.5 predators per plant or 0.75 total predators per plant to develop the fixed sample size plans.

The predator groups evaluated in this study are generalist predators and therefore, potentially impact a variety of cotton pests. However, estimating the density of a specific predator group may be of interest when the predator group is of primary importance in

making a management decision for that pest. For example, a sampling plan specific for *Orius* spp. could be used when *Helicoverpa zea* (Boddie) oviposition is anticipated to increase. Estimating densities of adult coccinellids, which feed primarily on aphids, would be important when making decisions regarding aphid management in cotton. Thus, the sampling plans presented in Table 8 allow the pest manager to select the sampling plan for the predator group of most interest and which requires the least amount of time. These plans are valid for predator densities of 0.5 or greater per plant; however, a greater number of samples would be required to estimate mean densities <0.5 per plant. For example, 45 beat bucket samples of three plants each would estimate densities of Coccinellidae with a precision level of 0.35 and require 52.5 min. This same sample plan would be more than sufficient to estimate densities of spiders and *Orius* adults as only 30 and 34 3-plant samples are required, respectively, for these two predator groups.

The sampling plan for total predators can be used when the objective of the scouting program is to estimate the mean density of these generalist predators. This information is currently the most commonly recorded in cotton pest management programs (Parker et al. 2005, Ohlendorf 1996). The 3- and 10-plant sample unit sample plans would require the least sampling effort (28 and 27 min, respectively) for total predators at a precision level of 0.35 and at a density of 0.75 predators per plant (Table 8).

Many consultants, field scouts and growers find it convenient to simultaneously record predator densi-

**Table 7.** Taylor's coefficients and coefficient of determination for each predator group and all groups combined for the 3-, 5- and 10-plant sample unit sizes using the beat bucket method in cotton

Predator group	3-plant sample unit			5-plant sample unit			10-plant sample unit		
	<i>a</i> <sup>a</sup>	<i>b</i> <sup>a</sup>	<i>r</i> <sup>2</sup>	<i>a</i>	<i>b</i>	<i>r</i> <sup>2</sup>	<i>a</i>	<i>b</i>	<i>r</i> <sup>2</sup>
<i>Orius</i> adults	1.2688	1.2139	0.938	0.7586	1.5687	0.846	1.9359	1.0181	0.865
<i>Orius</i> nymphs	1.8508	1.0052	0.766	1.6109	1.0055	0.875	2.6100	0.7884	0.667
Spiders	1.0835	1.5844	0.867	0.6957	1.8489	0.834	0.6948	1.5967	0.886
Coccinellidae	1.2932	1.2705	0.976	1.8948	1.6948	0.989	1.3914	1.1358	0.714
Total predators	1.1447	1.2700	0.700	1.5460	1.1160	0.600	0.8312	1.4223	0.783

<sup>a</sup> *a* and *b* are coefficients from Taylor's power law  $S^2 = am^b$ .

**Table 8.** Sample size and time required to estimate density of a given predator group at a mean of 0.5 per plant, or total predators at a mean density of 0.75 per plant for 3-, 5- and 10-plant sample units using the beat bucket method in cotton

Predator group	3-plant sample unit		5-plant sample unit		10-plant sample unit	
	Samples	Time (min) <sup>a</sup>	Samples	Time (min)	Samples	Time (min)
<i>Orius</i> adults	34	39.7	19	33.2	21	47.2
<i>Orius</i> nymphs	60	70.0	38	66.5	35	78.7
Spiders	30	35.0	20	35.0	14	31.5
Coccinellidae	45	52.5	52	91.0	16	36.0
Total predators	24	28.0	22	38.5	12	27.0

<sup>a</sup> Includes 0.5 min to walk between sites of each sampling bout.

ties while visually inspecting the plant for pest species. However, the analyses reported herein found the visual search method was relatively inefficient at recovering common predators, required the greatest sample time, and yielded a high and therefore undesirable cost-reliability value. In addition, visual examination of the plant is especially subject to the sampler's ability to see and detect predators on plants. Although time required to sample is a significant constraint to visual search, Garcia et al. (1982) concluded that sampler bias is the principal disadvantage of the visual search method. We believe the beat bucket sample method has the potential to reduce sampler bias as the captured predators are retained on the white bottom of the bucket where they are easily observed, identified and counted. Movement by the predators in the beat bucket also aids in detection and identification. Also, plastic pails for use as beat buckets are readily available and inexpensive. These advantages and the low cost-reliability of the beat bucket method suggest it is well suited for commercial field scouting programs. However, devoting time specifically to estimating predator densities is an additional cost. Weekly assessment of predator densities, which is the typical schedule for assessing pest densities, is probably not warranted as predator densities do not change as rapidly as those of many pest species. Rather, investing time to accurately assess predator densities may be most valuable if made 1–2 wk before pests are expected to increase to economic thresholds, such as oviposition events by heliothines. Data on predator densities would then be available on a field-by-field basis for use in management decisions regarding economic thresholds and insecticide selection, if needed. Sampling predators as needed during the growing season when predator information is most useful in making management decisions can maximize the return on investing additional time to accurately assess predator densities in a commercial scouting program. Although other foliage active predators (*Nabidae*, *Drapetis* spp., *Geocoris* spp., Formicidae, larval Chrysopidae, and *Scymnus* spp.) are readily collected by beat bucket sampling, the utility of the beat bucket method for sampling other predators not evaluated in this study will need to be determined in production areas where they are important.

The beat bucket method used in conjunction with the sample plan presented herein should prove useful for rapidly and reliably estimating densities of *Orius* adults and nymphs, spiders, and adult coccinellids in a commercial field scouting program for both irrigated (i.e., AZ) and dryland (i.e., Texas) cotton. Taylor's parameters are presented herein so that sample plans may be developed to estimate total and individual densities of *Orius* spp., spiders, and adult coccinellids. This method and plan has been adopted by the Texas Cooperative Extension for use in IPM programs in cotton (Parker et al. 2005). Research to identify threshold densities of predators necessary to maintain target pests below economic thresholds would increase the value of predator density information in making management decisions and also increase sampling efficiency. Sample sizes needed to determine if a predator density is above or below a threshold density (decision or classification sampling) are usually much smaller than those necessary to estimate predator density (enumeration sampling) (Wilson 1994). Decision sampling plans would further reduce sample size and cost and facilitate integrating the impact of predation in pest management decisions for cotton.

#### Acknowledgments

Thanks to Marty Jungman (Texas Cooperative Extension) and Bill Langston (Texas Agricultural Experiment Station) for assistance with field studies. This research was supported in part through grants from the National Biological Control Institute, Texas Department of Agriculture IPM Program, and Texas State Support Committee and Cotton Inc.

#### References Cited

- Adkisson, P. L. 1971. Objective use of insecticides in agriculture, pp. 43–51. In J. E. Smith [ed.], *Agricultural chemicals: harmony or discord for food, people, environment*. University of California, Division of Agricultural Sciences Publications, Davis, CA.
- Byerly, K. F., A. P. Gutierrez, R. E. Jones, and R. F. Luck. 1978. A comparison of sampling methods for arthropod populations in cotton. *Hilgardia* 48: 257–282.
- Diaz, R., A. E. Knutson, and J. S. Bernal. 2004. Effect of the red imported fire ant on cotton aphid population density and predation of bollworm and beet armyworm eggs. *J. Econ. Entomol.* 97: 222–229.
- Ellington, J., K. Kiser, G. Ferguson, and M. Cardenas. 1984. A comparison of sweepnet, absolute, and Insetovac sampling method in cotton ecosystems. *J. Econ. Entomol.* 77: 599–605.
- Eveleens, K. G., R. van den Bosch, and L. E. Ehler. 1973. Secondary outbreak inductions of beet armyworm by experimental insecticide application in cotton in California. *Environ. Entomol.* 2: 497–503.
- Ewing, E. P., and E. E. Ivy. 1943. Some factors influencing bollworm populations and damage. *J. Econ. Entomol.* 36: 602–606.
- Fleischer, S. J., M. J. Gaylor, and J. V. Edelson. 1985. Estimating absolute density from relative sampling of *Lygus lineolaris* and selected predators in early to mid-season cotton. *Environ. Entomol.* 14: 709–717.



- Garcia, A., D. Gonzalez, and T. F. Leigh. 1982. Three methods for sampling arthropod numbers on California cotton. *Environ. Entomol.* 11: 565–572.
- Hagler, J. R., and S. E. Naranjo. 2005. Use of a gut content ELISA to detect whitefly predator feeding activity after field exposure to different insecticide treatments. *Biocontrol Sci. Technol.* 15: 321–339.
- Kidd, R. W., and D. R. Rummel. 1997. Effect of insect predators and a pyrethroid insecticide on cotton aphid, *Apis gossypii* Glover, population density. *Southwest. Entomol.* 22: 381–393.
- Leigh, T. F., H. Black, C. E. Jackson, and V. E. Burton. 1966. Insecticides and beneficial insects in cotton fields. *Calif. Agric.* 20: 4–6.
- McDaniel, S. G., and W. L. Sterling. 1979. Predator determination and efficiency on *Heliothis virescens* eggs in cotton using  $P^{32}$ . *Environ. Entomol.* 8: 1083–1087.
- McDaniel, S. G., and W. L. Sterling. 1981. Predation of *Heliothis virescens* eggs on cotton in east Texas. *Environ. Entomol.* 11: 60–66.
- McDaniel, S. G., W. L. Sterling, and D. A. Dean. 1981. Predators of tobacco budworm larvae in Texas cotton. *Southwest. Entomol.* 6: 101–108.
- Naranjo, S. E., and J. R. Hagler. 1998. Characterizing and estimating the impact of heteropteran predation. pp. 170–197. *In* M. Coll and J. Ruberson [eds.], *Predatory Heteroptera: their ecology and use in biological control*. Thomas Say Symposium. Proceedings of the Entomological Society of America, Lanham, MD.
- Naranjo, S. E., and P. C. Ellsworth. 2005. Mortality dynamics and populations regulation in *Bemisia tabaci*. *Entomol. Exp. Appl.* 16: 93–108.
- Nuessly, G. S., and W. L. Sterling. 1984. Comparison of D-Vac and modified drop cloth methods for sampling arthropods in cotton. *Southwest. Entomol.* 9: 95–103.
- Parker, R., D. D. Fromme, A. Knutson, and M. Jungman. 2005. Managing cotton insects in the southern, eastern and Blackland areas of Texas. *Texas Coop. Ext. Bull.* E-5.
- Pyke, B., W. L. Sterling, and A. Hartstack. 1980. Beat and shake sampling of cotton terminals for cotton fleahoppers and other pests and predators. *Environ. Entomol.* 9: 572–576.
- Ridgeway, R. L., P. D. Lingren, C. W. Cowan, and J. W. Davis. 1967. Populations of arthropod predators and *Heliothis* spp. after applications of systemic insecticides to cotton. *J. Econ. Entomol.* 60: 1012–1016.
- Ruberson, H. R., G. A. Herzog, W. R. Lambert, and W. J. Lewis. 1994. Management of the beet armyworm (Lepidoptera: Noctuidae) in cotton: role of natural enemies. *Fla. Entomol.* 77: 440–453.
- Ohlendorf, B. 1996. Integrated pest management for cotton in the western region of the United States. Publication 3305. The Regents of the University of California, Oakland, CA.
- Sansone, C. G., and J. W. Smith, Jr. 2001. Natural mortality of *Helicoverpa zea* (Lepidoptera: Noctuidae) in short-season cotton. *Environ. Entomol.* 30: 112–122.
- SAS Institute. 2001. JMP Start statistics. SAS Institute, Cary, NC.
- SAS Institute. 2004. SAS/STAT, version 9.1, User's guide. SAS Institute, Cary, NC.
- SAS Institute. 2006. The GLIMMIX procedure, June 2006. SAS Institute, Cary, NC.
- Smith, J. M., E. A. Stadelbacher, and C. W. Gantt. 1976. A comparison of techniques for sampling beneficial arthropod populations associated with cotton. *Environ. Entomol.* 5: 435–444.
- Sterling, W. L., L. M. El-Zik, and L. T. Wilson. 1989. Biological control of pest populations, pp. 155–189. *In* R. E. Frisbie, K. El-Zik, and L. Ted Wilson [eds.], *Integrated pest management systems and cotton production*. John Wiley, New York.
- Stoltz, R. L., and V. M. Stern. 1978. Cotton arthropod food chain disruption by pesticides in the San Joaquin Valley California. *Environ. Entomol.* 7: 703–707.
- Taylor, L. R. 1961. Aggregation, variance and the mean. *Nature (Lond.)* 189: 732–735.
- van den Bosch, R., and K. Hagen. 1966. Predaceous and parasitic arthropods in California cotton fields. *Calif. Agric. Exp. Stn.* 820.
- Wilson, L. T., and A. P. Gutierrez. 1980. Within-plant distribution of predators on cotton: comments on sampling and predator efficiencies. *Hilgardia* 48: 3–11.
- Wilson, L. T. 1994. Estimating abundance, impact, and interactions among arthropods in cotton agroecosystems, pp. 475–514. *In* L. P. Pedigo and G. D. Buntin [eds.], *Handbook of sampling methods for arthropods in agriculture*. CRC, Boca Raton, FL.

Received 31 December 2007; accepted 3 May 2008.