Developing Sampling Plans for the Invasive Megacopta cribraria (Hemiptera: Plataspidae) in Soybean

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J. Econ. Entomol. 107(6): 2213-2221 (2014); DOI: http://dx.doi.org/10.1603/EC14203

ABSTRACT Since its discovery in the southeastern United States, the invasive plataspid *Megacopta* cribraria (F.) (Hemiptera: Plataspidae) has infested soybean (*Clycine max* (L.) Merrill) fields in often very high numbers. To optimize sampling plans, sweep-net and beat-cloth sampling was conducted in soybean fields in South Carolina during 2012 and 2013. Across all fields, densities averaged 7.2 \pm 0.5 (SEM) adults and 4.5 \pm 0.4 nymphs per 20 sweeps and 5.5 \pm 0.3 adults and 4.5 \pm 0.3 nymphs per 1.83 m of row. Coefficients of Taylor's power law were used to generate sampling plans for population estimates and sequential sampling plans for pest management decision making. At an economic threshold of one nymph per sweep, optimum sample sizes were 184, 48, and 22 within 10, 20, and 30% of the mean with the sweep-net method. At the corresponding threshold for the beat cloth (24.7 nymphs per 1.83 m of row), optimum sample sizes were 239, 62, and 29 within 10, 20 and 30% of the mean, respectively. At all adult and nymph densities, fewer sweep-net samples were required for population estimations compared with the number of beat-cloth samples. Sequential sampling reduced the sample size required to reach a management decision for the sweep net and beat cloth compared with a fixed sampling plan. The sweep-net method was more cost reliable for population estimation at low densities of both life stages, with the beat cloth becoming more cost reliable as populations increased. The beat-cloth method was more cost reliable than sweep-net sampling across all densities and life stages for pest management practices. These results may be used by researchers, county Extension agents, consultants, and farm managers to both facilitate sampling and improve reliability of *M. cribraria* estimates for research purposes and for pest management.

KEY WORDS invasive species, sweep net, beat cloth, Taylor's power law, sequential sampling

The invasive *Megacopta cribraria* (F.) (Hemiptera: Plataspidae), a species endemic to Asia, was first discovered in northern Georgia, United States, in 2009 (Suiter et al. 2010). Initially regarded as a nuisance pest due to high numbers around homes, emissions of unpleasant odors, and the tendency to discolor the skin when handled, it was later elevated to an agricultural pest due to its infestation of soybean, *Clycine max* (L.) Merrill (Suiter et al. 2010, Ruberson et al. 2013). A variety of legumes are reported as host plants from the native range of *M. cribraria* (Eger et al. 2010), but soybean and kudzu, *Pueraria montana* (Loureiro) Merrill variety *lobata* (Willdenow), are the most important hosts in the United States (Suiter et al. 2010, Zhang et al. 2012). By the end of 2013, the range of

³ Department of Entomology, University of Arkansas, Southeast Research and Extension Center, P.O. Box 3508, Monticello, AR 71656. ⁴ Clemson University, School of Agricultural, Forest, and Environmental Sciences, Pee Dee Research and Education Center, 2200 Pocket Rd., Florence, SC 29506. *M. cribraria* had expanded to include most of the southeastern states (Gardner et al. 2013).

Studies in Georgia and South Carolina in 2010 and 2011 showed an average yield loss of 18% from M. cribraria damage (Ruberson et al. 2013). Furthermore, cage trials by Seiter et al. (2013b) showed that soybean yield was reduced by up to 59.6% at high densities of *M. cribraria*. Corresponding reductions in seeds per pod and seed weight were attributed to stress induced by M. cribraria feeding primarily on the stem during the seed fill stage (R5) and full seed development stage (R6; Fehr and Caviness 1977). Existing broad-spectrum insecticides already used in soybean are effective against M. cribraria in the United States (Ruberson et al. 2013) and in their native range (Zhixing et al. 1996). Current management strategies recommend application of insecticide at a threshold of one nymph per sweep or when nymphs can be observed from 10 visual canopy inspections per field (Greene et al. 2012).

Having a method to assess insect population density through sampling plans is a vital component of integrated pest management (IPM). Due to the relatively new incursion of M. *cribraria* into the United States, no comprehensive sampling plans have been developed.

0022-0493/14/2213-2221\$04.00/0 © 2014 Entomological Society of America

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Behavioral aspects of the target species, its developmental stage, cost of sampling equipment, sampling speed, and host-plant stage often affect the sampling method chosen as the most appropriate and effective (Turnipseed and Kogan 1976, Shepard 1980). Additionally, knowledge of insect spatial distributions can aid in the formation of comprehensive sampling plans (Taylor 1984, Binns and Nyrop 1992).

Sampling of insects is used in research to determine population estimates that are as close to the population mean as possible and in pest management to classify an insect population as above or below an economic threshold to enable decisions to be made on applying an intervention (Binns and Nyrop 1992, Hutchison 1994, Wilson 1994). Sequential sampling plans can reduce insecticide applications and overall sampling costs in many crop systems compared with fixed sampling plans (Shepard 1980, Hoffmann et al. 1991, Binns and Nyrop 1992, Schexnayder et al. 2001). The objectives of this study were to 1) determine sample sizes for population estimates for adults and immatures of *M. cribraria*, 2) develop and validate sequential sampling plans for the immature stages of M. cribraria, and 3) determine the relative-cost efficiency of the methods used to sample adults and nymphs in South Carolina soybean fields.

Materials and Methods

Field Sampling. Populations of *M. cribraria* were monitored in four soybean fields in 2012 (two at the Clemson University Edisto Research and Education Center [Edisto REC], Blackville, SC, and two at the Clemson University Pee Dee REC [Pee Dee REC], Florence, SC) and four soybean fields in 2013 (one at the Edisto REC and three at the Pee Dee REC). Field size ranged from 2.3 to 6.5 ha with an average of 4.6 ha in both years. In each field, a uniform sampling grid consisted of one sampling location (marked with a 1.8-m fiberglass flag) every 0.12 ha, with flags separated by 35 m. Number of sampling locations within fields ranged from 25 to 40 (2012) and 24 to 58 (2013) due to field shape and size.

At each sampling location, two sampling methods were compared. The first method required a 0.91- by 0.91-m white beat cloth to sample 1.83 m of row at Edisto REC and 3.7 m of row at Pee Dee REC. After placing the beat cloth under the soybean plant canopy between two rows, the plants were vigorously shaken on both sides of the beat cloth to dislodge any M. cribraria. Adults and nymphs were counted in the field and recorded. Adults and nymphs counted in Florence were halved before analysis to standardize densities between Edisto REC and Pee Dee REC. The second method consisted of swinging a 38-cm-diameter sweep net across two soybean rows at each sampling location. Each sample consisted of two subsamples of 10 sweeps which were summed before analyses. Samples were collected in plastic bags (30 by 50 cm) and frozen $(-20^{\circ}C)$ in the laboratory before adults and nymphs were counted. Sampling was conducted biweekly in Blackville, SC, in 2013 and weekly in all other fields in



both years from late vegetative stage (V8) until late reproductive stages (R6) when the plants became too mature to sweep. Number of sampling dates ranged from 7 to 14 in 2012 and from 6 to 12 in 2013. On 16 August 2013, in Blackville, SC, insecticide applications were made with γ -cyhalothrin (Declare, Cheminova, Inc., NC; 0.017 kg [AI]/ha). Sampling resumed the following week. No insecticide was applied in any other field. In selected fields in both 2012 and 2013, a stop watch was used to record time taken to carry out the required tasks of each sampling method, including sampling time in the field, walking time between sample points, and processing time for samples in the laboratory.

Taylor's Power Law. Means and variances of densities of adults and nymphs were calculated for each field at each date. The spatial patterns of *M. cribraria* adults and nymphs were described according to Taylor's power law (Taylor 1961, Taylor 1984) which relates mean density to variance (equation 1):

$$s^2 = ax^b$$
 [1]

where s^2 is the sample variance, *x* is the sample mean, and a and b are Taylor's coefficients. Calculations were performed in SigmaPlot (SigmaPlot 2006) using nonlinear regression rather than simple linear regression after log transformations, which can overestimate variance at low insect densities (Wilson 1994). Outlying observations, as determined by Cook's distance, on the regression estimates were deleted and the regression was repeated (Mendenhall and Sincich 2011) to obtain Taylor's coefficients a and b. Cook's distance values that exceeded the 50th percentile of an F distribution with k + 1 and n - (k + 1) degrees of freedom were deleted, with k = number of terms in regression and n = number of observations. Coefficient b was compared with a value of 1 using a t-test [t = (slope - 1)/(SE of the slope)], with df = n - 2and P = 0.05 (Zar 1999). Variance to mean ratios were calculated from raw data points.

Optimum Sample Size for Population Estimates. Calculated means and variances were also used to determine the sample size (n) required for population estimation within a given level of reliability. The equation of Karandinos (1976) as modified by Wilson and Room (1983) was used:

$$n = t_{\alpha/2}^2 D_x^{-2} s^2 x^{-2} \qquad [2]$$

where $t_{\alpha/2}$ is the standard normal variate for a twotailed confidence interval, D_x is the ratio of half the chosen confidence interval to the mean, and x is the mean population density. The substitution of the sample variance (s^2) from equation 1 in equation 2 gives:

$$n = t_{\alpha/2}^2 D_x^{-2} a x^{b-2}$$
 [3]

Equation 3 was used to obtain estimates with 90% confidence ($\alpha = 0.1$) within 10, 20, or 30% ($D_x = 0.1$, 0.2, and 0.3) of the mean for optimum sample sizes using the sweep net and beat cloth.

Sequential Sampling. Equation 4, which incorporates both type I (α) and type II (β) errors, was used

to develop sequential sampling plans for nymphs for the beat-cloth and sweep-net methods (Wilson 1994):

$$n = t^{2}_{\alpha-\beta} |x - T|^{-2} a x^{b} \qquad [4]$$

where *n* is sample size, *t* is the standard normal variate for a one-tailed confidence interval, α is the type I error rate of declaring that an insect population is above a threshold when it is not, β is the type II error rate of declaring that an insect population is below a threshold when it is not, x is the population density, T is the economic threshold expressed as the mean density per sample unit, and a and b are Taylor's coefficients. The current economic threshold used for the sweep-net method is one nymph per sweep (Greene et al. 2012). A linear regression relating beat-cloth data to sweep-net data collected from the same sampling location was used to determine the equivalent economic threshold for the beat-cloth method corresponding to this threshold (PROC REG; SAS Institute 2010). Sequential plans were developed using error rates $\alpha = \beta = 0.1$ and $\alpha = \beta = 0.2$.

Cost Reliability. A ratio of costs for beat-cloth and sweep-net methods was used to compare the two sampling methods (Wilson 1994). This identified the better method for estimation of insect population (equation 5):

$$C_{bc}/C_{sn} = n_{bc}(\theta_{bc} + \varphi_{bc}) / [n_{sn}(\theta_{sn} + \varphi_{sn})]$$
 [5]

where C_{bc} and C_{sn} are the cost per sample in time for a given level of reliability for the beat-cloth and sweep-net methods, n_{bc} and n_{sn} are the number of sampling units required either for 1) a population estimate with a given level of reliability (D_r) or 2) classification of an insect population above or below the economic threshold for a given level of reliability for the corresponding sampling method, θ_{bc} and θ_{sn} are the times required to carry out the required sampling (both sampling and enumerating) for the beat cloth and sweep net, respectively, and φ_{bc} and φ_{sn} are the times required to move between each sampling location. It was assumed that the time required to move between sampling location was the same for either method, hence $\varphi_b = \varphi_{sn}$. Equation 5 calculates the relative cost reliability of the sweep-net method compared with the beat-cloth method based on sample units and time required to carry out the corresponding method. Using equation 3 to replace n in equation 5 and including the previously calculated parameters of the linear regression equation determines equation 6:

$$\frac{C_{bc}}{C_{sn}} = a_{bc}(A + Bx)^{(b_{bc}-2)}(\theta_{bc} + \varphi_{bc}) / [(a_{sn}x^{(b_{sn}-2)})(\theta_{sn} + \varphi_{sn})] [6]$$

where C_{bc}/C_{sn} is the relative cost reliability of the sweep-net compared with the beat-cloth method, a_{bc} and b_{bc} are Taylor's coefficients for the beat-cloth method, a_{sn} and b_{sn} are Taylor's coefficients for the sweep-net method, A and B are the intercept and slope of the linear regression relating beat-cloth to sweepnet counts, and x is mean population density expressed in number of *M. cribraria* per sample unit (20 sweeps).



When n is replaced in equation 5 with equation 4 for pest management sampling and with addition of the linear regression equation (Table 2), we obtain:

$$\frac{C_{bc}'}{C_{sn}'} = B^{-2}a_{bc}(A + Bx)^{bbc}(\theta_{bc} + \varphi_{bc}) / (a_{sn}x^{bm})(\theta_{sn} + \varphi_{sn}) \quad [7]$$

where C'_{bc}/C'_{sn} is the relative cost reliability of the beat-cloth method compared with the sweep-net method. Equations 6 and 7 were used to determine cost reliability of both methods for commercial pest management and population estimation, respectively.

Comparison of Fixed and Sequential Samplings Plans. To compare sequential sampling plans with a fixed sampling plan, we used n = 10 as a fixed sample size, where each sample equates to 20 sweeps. Two additional fields were sampled creating a total of 20 new field-date combinations (14 in Florence in 2012 and six in Blackville in 2013). The number of M. cribraria nymphs caught after each sample unit was recorded. Numbers were compared with the corresponding sequential sampling plans developed for beat-cloth and sweep-net methods. Both error rates were used ($\alpha = \beta = 0.1$ and $\alpha = \beta = 0.2$) and sample size and decision as to whether an intervention was required, not needed, or whether sampling must continue were recorded. Mean sample sizes for each sequential plan at each error rate ($\alpha = \beta = 0.1$ and $\alpha =$ $\beta = 0.2$) were calculated. Fixed and sequential sampling plans were compared using a one-sample *t*-test (SAS Institute 2010). Percentage of sample size reduction for sequential sampling plans with respect to the fixed sample plan was calculated.

Results

Across both years of this study, 23,760 adults and 18,048 nymphs were collected with sweep-net sampling. Densities of adults and nymphs per 20 sweeps averaged 7.2 \pm 0.5 (SEM) (range, 0–698) and 4.5 \pm 0.4 (range, 0–276), respectively. Across both years of study, 15,828 adults and 12,564 nymphs were counted with beat-cloth sampling. Densities of adults and nymphs per 1.83 m of row averaged 5.5 \pm 0.3 (range, 0–435) and 4.5 \pm 0.3 (range, 0–325), respectively. The corresponding economic threshold for the beat cloth was calculated as 24.7 nymphs per 1.83 m of row (Table 1).

Taylor's coefficients and variance/mean ratios are reported for each life stage (Table 2). The *b* parameter of Taylor's power law was significantly different from 1 (P < 0.05) for nymphs with either method (Table 2), indicating aggregated distributions. The number of sample units required to arrive at an estimate within a given reliability for different population estimates was expressed as the number of *M. cribraria* per 20 sweeps (sweep net) and 1.83 m of row (beat cloth) for both nymphs and adults (Fig. 1). To obtain a population estimate within 10, 20, or 30% ($D_x = 0.1, 0.2$ or 0.3) of the mean for both the sweep-net and beat-cloth methods, the number of sample units was higher at

Table 1. Equation relating counts of *M. cribraria* nymph using sweep-net sampling (independent variable) and beat-cloth sampling (dependent variable) to determine economic thresholds for development of sequential sampling plans for beat-cloth sampling

Stage	Intercept \pm SEM	Slope \pm SEM	F	Р	r^2	n	BC economic threshold \pm SEM
Adult	1.881 ± 0.778	0.714 ± 0.088	62.62	< 0.0001	0.483	68	-
Nymph	1.672 ± 0.944	1.155 ± 0.119	22.89	< 0.0001	0.263	66	24.8 ± 4.606

A threshold of one nymph per sweep was used to determine threshold. BC, beat-cloth.

lower densities compared with higher densities. At levels of reliability of 10% ($D_x = 0.1$) for the sweep-net method, the number of samples exceeded 5,000 in the case of adults (Fig. 1A), in comparison with ≈ 600 samples for nymphs (Fig. 1B). At levels of 30% reliability $(D_r = 0.3)$, the number of samples required was considerably smaller; at economic threshold densities of one nymph per sweep (20 nymphs per 20 sweeps), optimum sample sizes were 184, 48, and 22 within 10, 20, and 30% of the mean, respectively (Fig. 1B). Similar results were found for population estimation using the beat-cloth method. At the corresponding threshold for nymphs with the beat-cloth method (24.7 nymphs per 1.83 m of row), optimum sample sizes were 239, 62, and 29 within 10, 20, and 30% of the mean, respectively (Fig. 1D). Population estimates required fewer sweep-net samples compared with beat-cloth samples at all densities for both adults and nymphs. For example, at a density of 20 adults per 20 sweeps, 24 sweep net sample units would be required for population estimation whereas 32 beat cloth sample units would be required at the same density of adults $(D_r = 0.3).$

Sequential sampling plans are shown for M. cribraria nymphs (Fig. 2). When the cumulative number of nymphs for the corresponding sample unit number falls in the "Stop sampling, intervention not needed" area, a management intervention is not required, and the sampling can stop at that time. When the cumulative number of nymphs falls in the "Stop sampling, intervention needed" area, a management intervention is needed, and sampling can stop at this point. Finally, when the cumulative number of nymphs falls in the "Continue sampling" area, sampling must continue until a decision on whether to intervene can be made. A minimum sample size of four was selected as the minimum number of sample units required as suggested by Shepard (1980) for sampling other arthropods in soybean.

Table 2. Coefficients for Taylor's power law for *M. cribraria* (adults and nymphs) sampled in eight fields in South Carolina during 2012 and 2013

Sampling method	Stage	а	b	r^2	t value for $b = 1$	Var: mean ratio
Sweep net	Adult	18.998	0.901	0.251^{a}	0.39NS	27.00
Beat cloth	Adult Nymph	2.120 28.804 2.973	$1.615 \\ 0.871 \\ 1.620$	0.993 0.557^{a} 0.970^{a}	20.20 1.03NS 7.72 ^b	42.76 48.33

NS, not significant. $^{a}P < 0.0001$.

^b t-test indicates b is different from a value of 1 at $\alpha = 0.05$.

The average times needed to sample and count both adults and nymphs of M. cribraria for the sweep-net and beat-cloth methods were 159.0 \pm 8.0 s (n = 202) and 25.9 ± 0.7 s (n = 332), respectively. Time for the sweep-net method was significantly greater than that for the beat-cloth method (t = 16.58; df = 204; P <0.001). The time taken to walk between sampling locations averaged 28.7 \pm 0.1 s (n = 70). The sweepnet method was more cost reliable at values above one (Fig. 3). The beat-cloth method was more cost reliable at values below one. For population estimation at low densities, the sweep net was the more cost reliable of the two methods for both life stages. For adults, the beat-cloth method became more reliable at densities of 2.8 adults per 20 sweeps. For nymphs, the beat-cloth method became more reliable at densities of 2 nymphs per 20 sweeps. For pest management, the beat-cloth method was most reliable across all population densities for both life stages.

Sample size required to reach a decision using the 0.1 error rate with sweep net sequential sampling plans was significantly smaller compared with the fixed sample plan of 10 sample units (t = 85.73; df = 19; P <0.001). The average number of 20 sweep units required with the sequential sampling method was 4.1 \pm 0.1 compared with the 10 sample size requirement for the fixed plan method. This illustrates a 59% reduction in sample size using the sequential plan for sweep nets (Table 3). The sample size required using an error rate of 0.2 with the sweep-net sequential sampling plan for all the 20 field-date combinations was four (Table 3). This was also the case when using the beat-cloth sampling plan ($\alpha = \beta = 0.1$ and $\alpha = \beta = 0.2$). This illustrates a 60% reduction in sample size using the sequential sampling plan compared with using the fixed sampling plan.

Discussion

Most insect species show clumped spatial distributions over a wide range of population densities (Taylor et al. 1978). Parameter *b* in Taylor's power law represents the aggregation index of a particular life stage of an insect in a given environment, with b = 1 highlighting a random (Poisson) distribution, b < 1 representing a uniform or overdispersed distribution, and b > 1 denoting a clumped or aggregated distribution (Taylor 1961, Wilson 1985). Our calculated *b* parameter (Table 2) suggests that nymphs of *M. cribraria* have an aggregated distribution with both the sweepnet (as shown also in Seiter et al. [2013b]) and beat-



Fig. 1. Optimum sample size required to obtain population estimates within 10, 20, and 30% of the mean for (A) adult *M. cribraria* with the sweep-net method, (B) nymph *M. cribraria* with the sweep-net method, (C) adult *M. cribraria* with the beat-cloth method, and (D) nymph *M. cribraria* with the beat cloth method. Mean population density is expressed as number of *M. cribraria* per 20 sweeps or as number of *M. cribraria* per 1.83 m of row. Dashed vertical line represents the current recommended threshold of one nymph of *M. cribraria* per sweep (20 nymphs per 20 sweeps). Solid vertical line represents predicted economic threshold using the beat cloth (24.7 nymphs per 1.83 m of row).



Fig. 2. Sequential sampling plan for nymphs of *M. cribraria* using (A) the beat-cloth method for an economic threshold of 24.7 nymphs per 1.83 m of row and (B) the sweep-net method for an economic threshold of one nymph per sweep (20 nymphs per 20 sweeps). Two sets of error rates were used $\alpha = \beta = 0.1$ and $\alpha = \beta = 0.2$.





A - Population Estimation

Fig. 3. Relative cost reliability of the beat-cloth method for (A) population estimation and (B) pest management sampling with respect to the sweep-net sampling in soybean. Mean population density is expressed as the number of *M. cribraria* per 20 sweeps. The straight line represents relative cost reliability value of one, where the sweep net and the beat cloth have the same cost reliability. The beat-cloth method is more cost reliable below the line (at y = 1); the sweep net is more cost reliable above the line.

cloth methods (P < 0.05). Although the *b* parameter was not significantly different from one for adults, the variance/mean ratio suggests there is a degree of aggregation associated with this life stage at certain den-

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sities (Table 2). Wilson (1985) highlights that a small value of b (<1) and a high value of a (>1), can describe a variance/mean ratio by which distribution is aggregated at low densities and regularly distributed at higher densities. Furthermore, Myers (1978) suggests a variance/mean ratio >1 can indicate an aggregated distribution. Spatial aggregations of nymphs and adults are consistent with findings from previous studies conducted both in the insect's native and invasive range. Adults and nymphs of M. cribraria have been shown to have an aggregated distribution in India on pigeonpea, Cajanus cajan (L.) Millspaugh, based on Taylor's power law and Iwao's patchiness regression (Borah et al. 2002), and in Japan on soybean, based on the goodness-of-fit to the Poisson distribution (Kono 1990). Seiter et al. (2013a) also showed a clumped pattern of M. cribraria in South Carolina soybean fields, with a greater abundance along field edges.

The aggregated distribution of *M. cribraria* nymphs can be explained by the behavior of females ovipositing in clusters of two or three parallel rows with ≈ 16 eggs per mass (Zhang et al. 2012). This implies that nymphs would initially be more aggregated before potential dispersal. This is also supported by visual observations of nymphs of *M. cribraria* aggregating at growing points and nodes of various field crops (Thippeswamy and Rajagopal 2005). Adults of *M. cribraria* have been observed in mating aggregations on host plants (Hibino and Ito 1983) but have the ability to disperse with strong flying ability (Ruberson et al. 2013). This could explain the more random distribution associated with adults in this study.

The development of sampling plans is affected by spatial distributions of insects as well as sampling method (Espino et al. 2008, Knutson et al. 2008, Reav-Jones et al. 2009). Greater numbers of samples are required to obtain accurate population estimates at higher levels of aggregation (Karandinos 1976). For both sampling methods and life stages, determining population estimates at low densities required intensive sampling even at lower levels of reliability (Fig. 1). At low mean population densities and for all levels of precision for both sampling methods, more sample units were required for adults compared with nymphs, with the reverse at higher densities. For example, at 30% reliability, 573 and 66 sample units were required for adults and nymphs, respectively, when sampling at a density of one insect per 20 sweeps, but 16 and 20 sample units were required for adults and nymphs,

Table 3. Comparison of mean sample size (\pm SEM) required to reach a management decision for the sweep-net and beat-cloth methods for both error rates ($\alpha = \beta = 0.1 = 0.2$) using the sequential sampling plan versus the fixed sampling plan in South Carolina soybean fields

Method	Error rate $(\alpha = \beta)$	Mean sample size (± SEM)	% reduction	Decisions						
					Fixed		Sequential			
				No intervention	Continue sampling	Intervention required	No intervention	Continue sampling	Intervention required	
Sweep net	0.1	4.1 ± 0.1	59	18	2	0	20	0	0	
-	0.2	4.0 ± 0.0	60	20	0	0	20	0	0	
Beat cloth	0.1	4.0 ± 0.0	60	20	0	0	20	0	0	
	0.2	4.0 ± 0.0	60	20	0	0	20	0	0	

respectively, when sampling at a density of 30 insects per 20 sweeps. This difference is explained by Wilson (1985), where a low b parameter value does not necessarily equate to a uniform spatial distribution but could determine different levels of aggregation at different densities.

The association between sweep-net and beat-cloth counts varied by life stage (Table 1), with a better fit for regression models for adults compared with nymphs based on r^2 values. Sweep-net samples in soybean have shown a bias toward adults and late instar nymphs (Seiter et al. 2013a) which would reflect this better fit. We can predict that this bias may also occur with the beat-cloth method. First instars average 1.1 ± 0.1 mm in length (Zhang et al. 2012). Because the beat-cloth method requires enumeration on the floor under the soybean canopy, small newly hatched nymphs are difficult to visually count.

Sequential sampling plans have been developed for pest sampling in many crop systems (Hoffmann et al. 1991, Espino et al. 2008, Reay-Jones et al. 2009). Decision making using IPM requires classification of a population above or below a threshold rather than absolute population density estimation. Hence, sequential sampling plans for estimating densities for IPM generally require a lower number of sample units to reach the desired outcome. Sample size needed to reach a decision is greater when densities are close to the economic threshold. Sequential sampling plans are most efficient when infestation levels are well below or above economic thresholds. Plans were designed only for the immature stage of the insect because presence of nymphs in soybean fields has been associated with yield loss (Seiter et al. 2013b), and nymphs are currently the targeted life stage for intervention (Greene et al. 2012). No thresholds relating to adult M. cribraria have been recommended.

A type I error would result in an unnecessary insecticide application to the crop, while a type II error would result in no intervention occurring when there should be, which could result in economic yield loss. It is important to balance α and β by determining sampling and insecticide application costs (α) and the damage cost due to yield loss (β) (Wilson 1994). Two error rates (0.1 and 0.2) in this study give a level of flexibility to each sampling plan. Different circumstances may require a more liberal or conservative approach to management of M. cribraria. For example, a high value crop or a sudden increase in insecticide application cost may require a lower risk strategy, with error rates $\alpha = \beta = 0.1$. Consequently, this increases the number of sample units required and, hence, the time costs associated with sampling. Other consequential costs associated with insecticide treatment, such as insecticide resistance development, environmental costs, and reinfestation of fields are also taken into account by α . In the short term, effective chemical applications are the most widely used, immediate option for control of exotic species, such as *M. cribraria*. Natural enemies often do not exist, resistant varieties are not available, and biological information concerning the pest at the time of invasion is often insufficient

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Cost reliability analyses of various sampling methods of predatory arthropods on cotton, Gossypium hirsutum L., have been used to successfully enhance IPM programs (Parker et al. 2005, Knutson et al. 2008). Our cost-reliability analysis incorporated the time required to count the number of insects, which, as expected, increased as population density increased. When comparing the beat-cloth method with the sweep-net method, the beat-cloth method was the most cost-reliable method for all life stages and all densities for pest management. For population estimation, the sweep-net method was most effective at low densities of adults and nymphs, but, as densities increased, the beat-cloth method became more cost effective. Cost reliability depends on sampling costs, insect density, and corresponding variance but does not rely on the economic threshold or error rates. Espino et al. (2008) enhanced their cost-reliability model comparing sweep-net sampling to visual sampling of Oebalus pugnax (F.) in rice, Oryza sativa L., by the addition of a variable for "adoption of sampling" strategy." This is particularly important if scouts and consultants have an aversion to a particular method of sampling, even if it is the most cost-reliable method. In South Carolina, beat cloths are recommended for sampling many other soybean pests, with specific thresholds available for scouts, growers, and consultants for both hemipterans and lepidopterans (Greene 2014); hence, adoption of beat cloths to sample M. cribraria would not prove a new challenge. Beat cloths, however, are not suitable for sampling in narrow-row soybeans (Pitre et al. 1987), so adoption of sweep-net sampling would be more practical in this circumstance.

Developing a sequential sampling plan with validation is crucial if it is to be used in the field. Currently, the sample size recommendation for *M. cribraria* in soybean is at least 10 sweeps at 10 locations in a field (Greene et al. 2012). A comparison of the fixed sample size plan with the sequential sampling plans developed in this study shows that significant savings in time are possible when using sequential plans. Field–date combinations sampled (n = 20) with a fixed sample size of n = 10 had low densities of *M. cribraria* nymphs, and up to 60% reduction was achieved by using sequential sampling with the sweep-net and beat-cloth methods. These results echo other comparisons between fixed and sequential sampling plans with stink bugs in cotton in South Carolina and Georgia (Reay-Jones et al. 2009) and *O. pugnax* in rice in Texas (Espino et al. 2008).

Sampling plans for population estimation and pest management were developed for a new insect pest of soybean in the United States, which integrate spatial distributions, economic thresholds, and designated levels of reliability. Our sequential sampling plans can improve efficiency with regards to the number of sample units required to reach management decisions in South Carolina. The beat cloth was the most costreliable sampling method to sample both adults and nymphs of *M. cribraria* across most population densities. These results may be used by researchers, county Extension agents, consultants, and farm managers to both facilitate sampling and improve reliability of *M. cribraria* estimates for research purposes and for IPM decision making.

Acknowledgments

The authors thank James Smoak, Dan Robinson, Grant Pilkay, Terry Teal, and William Griggs for help with data collection and field preparation and maintenance. Appreciation is also expressed to William Bridges (Clemson University) for help with data analysis. This is technical contribution number 6,295 of the Clemson University Experiment Station. This manuscript is based upon work supported by the National Institute of Food and Agriculture–U.S. Department of Agriculture, under project numbers SC-1700470, SC-1700441, and SC-1700455. Partial funding was provided by grants from the South Carolina Soybean Board.

References Cited

- Bansal, R., T.-H. Jun, M.A.R. Mian, and A. P. Michel. 2013. Developing host-plant resistance for hemipteran soybean pests: lessons from soybean aphid and stink bugs. *In* H. El-Shemy (ed.), Soybean - Pest Resistance. InTech. DOI: 10.5772/54597.
- Binns, M. R., and J. P. Nyrop. 1992. Sampling insect populations for the purpose of IPM decision making. Annu. Rev. Entomol. 37: 427–453.
- Borah, B. K., S. K. Dutta, and K. K. Sarmah. 2002. Dispersion pattern of *Megacopta cribrarium* (Fab.) nymhs on pigeonpea. Res. Crops 3: 441–445.
- Eger, J. E. Jr., L. M. Ames, D. R. Suiter, T. M. Jenkins, D. A. Rider, and S. E. Halbert. 2010. Occurrence of the Old World bug *Megacopta cribraria* (Fabricius) (Heteroptera: Plataspidae) in Georgia: a serious home invader and potential legume pest. Insecta Mundi 0121: 1–11.
- Espino, L., M. O. Way, and L. T. Wilson. 2008. Sequential sampling plans for sweep net and visual sampling of *Oebalus pugnax* in rice. Southwest. Entomol. 33: 53–64.
- Fehr, W. R., and C. E. Caviness. 1977. Stage of soybean development. Iowa Agricultural and Home Economics Experiment Station Special Report, pp. 3–11. Iowa State University, Ames, IA.
- Gardner, W. A., H. B. Peeler, J. LaForest, P. M. Roberts, A. N. Sparks, J. K. Greene, D. Reisig, D. R. Suiter, J. S. Bacheler, K. Kidd, et al. 2013. Confirmed distribution and occurrence of *Megacopta cribraria* (F.) (Hemiptera: Heteroptera: Plataspidae) in the southeastern United States. J. Entomol. Sci. 48: 118–127.

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- Greene, J. K., P. M. Roberts, W. A. Gardner, F.P.F. Reay-Jones, and N. Seiter. 2012. Kudzu bug - identification and control in soybeans. Clemson University, The University of Georgia, South Carolina Soybean Board.
- Hibino, Y., and Y. Ito. 1983. Mating aggregation of a stink bug, Megacopta punctatissimum (Montandon) (Heteroptera: Plataspidae). Res. Popul. Ecol. 25: 180–188.
- Hoffmann, M. P., L. T. Wilson, F. G. Zalom, and R. J. Hilton. 1991. Dynamic sequential sampling plan for *Helicoverpa* zea (Lepidoptera: Noctuidae) eggs in processing tomatoes: parasitism and temporal patterns. Environ. Entomol. 20: 1005–1012.
- Hutchison, W. D. 1994. Sequential sampling to determine population density, pp. 207–244. *In L. Pedigo and G. B. Buntin (eds.)*, Handbook of Sampling Methods for Arthropods in Agriculture. CRC, Boca Raton, FL.
- Karandinos, M. G. 1976. Optimum sample size and comments on some published formulae. Bull. Entomol. Soc. Am. 22: 417–421.
- Knutson, A. E., M. A. Muegge, L. T. Wilson, and S. E. Naranjo. 2008. Evaluation of sampling methods and development of sample plans for estimating predator densities in cotton. J. Econ. Entomol. 101: 1501–1509.
- Kono, S. 1990. Spatial distribution of three species of stink bugs attacking soybean seeds (English (abstr.). Jpn. J. Appl. Entomol. Zool. 34: 89–96.
- Mendenhall, W., and T. Sincich. 2011. A second course in statistics: regression analysis 5th ed. Prentice Hall, Upper Saddle River, NJ.
- Myers, J. 1978. Selecting a measure of dispersion. Environ. Entomol. 7: 619–621.
- Parker, R., D. Fromme, A. E. Knutson, and M. Jungman. 2005. Managing cotton insects in the southern, eastern and Blackland areas of Texas. Texas Coop. Ext. Bull. E-5.
- Pitre, H. N., L. G. Thead, and J. L. Hamer. 1987. Prediction of field populations of soybean insects from sweep-net samples in narrow-row soybean plantings. J. Econ. Entomol. 80: 848–853.
- Reay-Jones, F.P.F., J. K. Greene, M. D. Toews, and R. B. Reeves. 2009. Sampling stink bugs (Hemiptera: Pentatomidae) for population estimation and pest management in southeastern cotton production. J. Econ. Entomol. 102: 2360–2370.
- Ruberson, J. R., K. Takasu, G. D. Buntin, J. E. Eger, Jr., W. A. Gardner, J. K. Greene, T. M. Jenkins, W. A. Jones, D. M. Olson, P. M. Roberts, et al. 2013. From Asian curiosity to eruptive American pest: *Megacopta cribraria* (Hemiptera: Plataspidae) and prospects for its biological control. Appl. Entomol. Zool. 48: 3–13.
- SAS Institute. 2010. SAS version 9.3 user's manual, SAS Institute, Cary, NC.
- Schexnayder, H. P., T. E. Reagan, and D. R. Ring. 2001. Sampling for the sugarcane borer (Lepidoptera: Crambidae) on sugarcane in Louisiana. J. Econ. Entomol. 94: 766–771.
- Seiter, N. J., F.P.F. Reay-Jones, and J. K. Greene. 2013a. Withinfield spatial distribution of *Megacopta cribraria* (Hemiptera: Plataspidae) in soybean (Fabales: Fabaceae). Environ. Entomol. 42: 1363–1374.
- Seiter, N. J., J. K. Greene, and F.P.F. Reay-Jones. 2013b. Reduction of soybean yield components by *Megacopta cribraria* (Hemiptera: Plataspidae). J. Econ. Entomol. 106: 1676–1683.
- Shepard, M. 1980. Sequential sampling plans for soybean arthropods, pp. 79–93. In M. Kogan and D. Herzog (eds.),

Sampling methods in soybean entomology. Springer, New York, NY.

- SigmaPlot. 2006. SigmaPlot for windows, version 10, Systat Software, Point Richmond, CA.
- Suiter, D. R., J. E. Eger, Jr., W. A. Gardner, R. C. Kemerait, J. N. All, P. M. Roberts, J. K. Greene, L. M. Ames, G. D. Buntin, T. M. Jenkins, et al. 2010. Discovery and distribution of *Megacopta cribraria* (Hemiptera: Heteroptera: Plataspidae) in northeast Georgia. J. Integr. Pest Manage. 1: F1–F4.
- Taylor, L. R. 1961. Aggregation, variance and the mean. Nature 189: 732–735.
- Taylor, L. R. 1984. Assessing and interpreting the spatial distributions of insect populations. Annu. Rev. Entomol. 29: 321–357.
- Taylor, L. R., I. P. Woiwod, and J. N. Perry. 1978. The density-dependence of spatial behaviour and the rarity of randomness. J. Anim. Ecol. 47: 383–406.
- Thippeswamy, C., and B. K. Rajagopal. 2005. Comparative biology of *Coptosoma cribraria* Fabricius on field bean, soybean and redgram. Karnataka J. Agric. Sci. 18: 138–140.
- Turnipseed, S. G., and M. Kogan. 1976. Soybean entomology. Annu. Rev. Entomol. 21: 247–282.
- Whalon, M., D. Mota-Sanchez, and R. Hollingworth. 2008. Analysis of global pesticide resistance in arthropods, pp. 5–31. In M. Whalon, D. Mota-Sanchez and R. Holling-

worth (eds.), Global Pesticide Resistance in Arthropods. Cromwell Press, Trowbridge, United Kingdom.

- Wilson, L. T. 1985. Estimating the abundance and impact of arthropod natural enemies in IPM systems, pp. 303–322. In M. A. Hoy and D. C. Herzog (eds.), Biologial Control in IPM Systems. Academic, Orlando, FL.
- Wilson, L. T. 1994. Estimating abundance, impact, and interactions among arthropods in cotton agrosystems, pp. 475–514. *In L. Pedigo and G. D. Buntin (eds.)*, Handbook of Sampling Methods for Arthropods in Agriculture. CRC, Boca Raton, FL.
- Wilson, L. T., and P. M. Room. 1983. Clumping patterns of fruit and arthropods in cotton, with implications for binomial sampling. Environ. Entomol. 12: 50–54.
- Zar, J. 1999. Biostatistical analysis. Prentice Hall, Upper Saddle River, NJ.
- Zhang, Y., J. L. Hanula, and S. Horn. 2012. The biology and preliminary host range of *Megacopta cribraria* (Heteroptera: Plataspidae) and its impact on kudzu growth. Environ. Entomol. 41: 40–50.
- Zhixing, W., W. Huadi, C. Guihua, Z. Zi, and T. Caiwen. 1996. Occurence and control of *Megacopta cribraria* (Fabricius) on soybean (English (abstr.)). Plant Prot. 22: 7–9.

Received 15 May 2014; accepted 18 September 2014.





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