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Optimization of sample unit size for sampling stink bugs (Hemiptera: Pentatomidae) in soybean

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Abstract

Cost-effective and reliable sampling procedures are crucial for integrated pest management. Sweep net sampling is commonly used for stink bugs (Hemiptera: Pentatomidae) in soybean, with sample size being the number of sets of sweeps, and sample unit size the number of sweeps in each set. Sample unit size has received little attention, but can affect sampling parameters. Here, two sample unit sizes (10 vs. 25 sweeps) were compared for the sampling of stink bug taxa. On average, sampling for stink bugs took 3.6 more minutes with the 25-sweep than with the 10-sweep sample unit size. Generally, estimates of the mean number of stink bugs per sweep were similar between the two sample unit sizes for *Euschistus* spp. and *Chinavia hilaris* combined ("combined herbivores") and *Euschistus* spp. The 25-sweep sample unit size had a higher probability of detecting combined herbivores, *Euschistus* spp. and *Podisus* spp., lower standard errors and relative variance for combined herbivores and *Euschistus* spp., lower standard errors for *C. hilaris*, and higher relative net precision [which accounts for sampling cost (i.e., time)] for combined herbivores and *Euschistus* spp. Taken together, the better probability of detection, precision and efficiency of the 25-sweep sample unit size support the continued use of sampling plans developed for that sample unit size. The optimization of sample unit sizes is an important factor that should be accounted for in the development of sampling plans.

Keywords: Efficiency, IPM, Precision, Spatial pattern

1. Introduction

Soybean, *Glycine max* (L.) Merrill (Fabales: Fabaceae) is one of the most valuable crops in the world in terms of area planted, production and end-use (FAO, 2019; Shea et al., 2020). In the United States, the Midwest is the leading soybean-producing region (NASS, 2017). Herbivorous stink bugs (Hemiptera: Pentatomidae) are important pests of soybean; both nymphs and adults feed directly on pods and seeds, reducing yield and seed quality (Koch et al., 2017). Furthermore, stink bugs can cause indirect damage to soybean by transmitting fungal diseases (Clarke and Wilde, 1970, 1971; Daugherty, 1967). However, some predatory species of stink bugs are beneficial, attacking other pests including herbivorous stink bugs (Koch et al., 2017). Sampling soybean for stink bugs in the Midwest is often done with a sweep net because this technique is simple, requires little equipment, and is more cost-effective and easier to do than other sampling methods (Kogan and Pitre, 1980; Koch et al., 2017). However, farmers and consultants are often constrained by time, and integrated pest

management (IPM) can be time-consuming, so optimizing sampling to reduce sampling time would be welcomed (Bueno et al., 2021).

Sampling plans can be used for research or decision-making to guide the collection of samples from fields (Pedigo and Buntin, 1994; Pedigo and Rice, 2009; Radcliffe et al., 2009). In the case of stink bugs in soybean, sample size is the number of sets of sweeps with a net, and sample unit size is the number of sweeps in each set. Sample size is an important and often-studied component of sampling plans for arthropod pests because it affects both precision and cost (Pedigo and Buntin, 1994; Ruesink, 1980). Sample unit size can also affect precision, but has been less intensively studied (Burkness and Hutchison, 1997; Hall and Albrigo, 2007; Hill et al., 1975).

Across the United States, recommended sample unit sizes for stink bugs in soybean range from 10 to 100 sweeps per set (Pezzini et al., 2019a). Stink bug sampling in soybean is usually based on combined counts of nymphs and adults since stink bug thresholds in soybean do not differentiate between stink bug taxa and life stages (Koch et al., 2017). In the Midwest, enumerative and binomial sequential sampling plans for research-based and decision-making purposes in soybean, respectively, were recently developed for stink bugs based on a sample unit size of 25 sweeps (Pezzini et al., 2019a; Aita et al., 2021). A sample unit size smaller than 25 sweeps could potentially benefit farmers by reducing sample effort and overall cost (Pedigo and Buntin, 1994; Ruesink, 1980), potentially increasing the adoption of IPM (Bueno et al., 2021). However, the impact of a smaller sample unit size on sampling precision and cost has not been evaluated for stink bugs in soybean in the Midwest.

Here, a study was done across eight states in the Midwest to compare the probability of detecting stink bugs, mean number of stink bugs per sweep, standard error, relative variance (RV) and relative net precision (RNP) of the recently published sample unit size of 25 sweeps versus a lower sample unit size of 10 sweeps for sampling stink bugs in soybean. RV is a unitless measure of error relative to the mean, and lower values of RV are preferred. RNP is a measure of efficiency as it incorporates both precision and cost (i.e., time), thus higher values of RNP are preferred (Burkness and Hutchison, 1998; Pedigo et al., 1972). The results of this study will help refine sampling recommendations for these pests.

2. Material and methods

2.1. Sample sites and insect data collection

A total of 21 soybean fields ranging from 0.3 to 120 ha were sampled for stink bugs in Illinois, Indiana, Michigan, Minnesota, Nebraska, North Dakota, Ohio, and Wisconsin during 2018. Fields were located at university research stations and collaborating commercial farms. Fields with plants in the reproductive stages [beginning bloom (R1) to full maturity (R8)] were sampled weekly by sweeping the upper canopy of plants from two adjacent rows in a pendulum-style swing using a 39-cm diameter net (Kogan and Pitre, 1980; Koch et al., 2017). On each sampling date, each field was sampled with two sampling regimes by taking 15 sample units of 25 sweeps (current recommendation) and 15 sample units of 10 sweeps (reduced effort). Stink bug density and injury to soybean is generally higher at the edge of the field (<10 m into the field) (Koch and Pahs, 2014; Venugopal et al., 2014; Pezzini et al., 2019a). Because of this, all sample units were taken at least 10 m from the field border to avoid edge-effects and because interior samples are more representative of a larger proportion of the field area. Sample units were spaced at least 10 m apart from one another, and the sample unit size alternated for each sample unit until the targeted total number of sample units was achieved. Specimens (nymphs and adults) collected in each sample unit were transferred to individual 20.3 × 25.4-cm zipper-locking plastic bags, frozen, and shipped to the University of Minnesota for later identification. A more detailed description of field sites and insect data collection is provided in Aita et al. (2021), where binomial sampling plans using a sample unit size of 25 sweeps were developed for herbivorous stink bugs.

The analyses described below were done for *Euschistus* spp. and *Chinavia hilaris* combined ("combined herbivores") and also for individual taxa to explore possible effects of the insect biology on sampling parameters and spatial pattern. Individual taxa analyses focused on *Euschistus* spp. (including the brown stink bug, *E. servus*, one-spotted stink bug, *E. variolarius*, and dusky stink bug, *E. tristigma*) and the green stink bug, *C. hilaris*, due to their commonality, and the predatory *Podisus* spp. (including the spined soldier bug, *P. maculiventris*) due to their prevalence and predatory capacity in the

Midwest soybean (Koch and Pahs, 2014; Koch and Rich, 2015; Pezini et al., 2019b; Aita et al., 2021). *Euschistus* spp. were common in the fields sampled in the study, therefore analyses could be done for nymphs, adults, and the combination of nymphs and adults (henceforth nymphs + adults). However, *C. hilaris* and *Podisus* spp. were less common, so analyses were done only for nymphs + adults for these species.

2.2. Sample unit size comparisons

For each sample unit in each field, stink bugs were visually identified and counted. Then, mean number of stink bugs per sweep (m) and standard error of the mean (SEM) were calculated for both 25-sweep and 10-sweep sample unit sizes in each field. Relative variance (RV) was calculated from $SEM \div m$. Relative net precision (RNP) was calculated from $[1 \div (RV \times time)] \times 100$, where $time$ was the total time in hours spent during sampling. The total time spent during sampling was estimated for each sampling regime from $t_{su} + t_c + t_w$, where t_{su} was the time required to complete a sample unit (i.e., set of sweeps), t_c was the time counting stink bugs collected in a sample unit, and t_w was the time walking between sample units. The times required to collect sample units of 10 sweeps and 25 sweeps were estimated in two fields by recording the time spent on each set of sweeps. The time needed to count stink bugs collected in each sample unit depends on the insect abundance in the field (i.e., higher numbers take more time to count), while the time to walk between sample units depends on the distance between them. For this study, a low number of stink bugs per sweep and a fixed distance between sample units were assumed. Therefore, the time needed to count stink bugs collected in each sample unit and the time to walk between sample units were assumed to be the same for each sample unit size and adapted from Aita et al. (2021). In their work, 1.12 min was required to count stink bugs in a sample unit (t_c), and 1.22 min to collect a sample unit and walk between sample units. Thus, t_w was calculated from $1.22 - t_{su}$.

To estimate the probability of detection, only dates where at least one stink bug was collected were used. For the remaining response variables, only dates where both 25-sweep and 10-sweep sample unit sizes collected at least one stink bug were used. Due to the low

abundance of *Podisus* spp. found in this study, only the probability of detection could be estimated for this taxon. Data were analyzed and graphed with R 3.5.1 (R Core Team 2019) and RStudio Desktop 1.1.463 (RStudio Team 2018).

The probability of detecting at least one stink bug was estimated based on presence-absence of stink bugs with a random intercept generalized linear mixed model (package, code: *lme4*, *glmer*; Bates et al., 2015) and a binominal distribution (logit link). Initially, treatments were included as fixed factors, and states and treatments nested within fields as random factors to account for dependencies within fields (i.e., paired samples within fields) and across time (i.e., repeated measures of fields over time). However, the random variation of treatments within fields was close to zero (singular models). Thus, the final model consisted of treatments as fixed factors, and states and fields as random factors. The significance of fixed factors was estimated with a Type II Wald chi-square test (*car*, *Anova*; Fox and Weisberg, 2019). Significant fixed effects were compared using estimated marginal means with *P*-values of pairwise comparisons adjusted with the Tukey method ($\alpha = 5\%$) (*emmeans*, *emmeans*; Lenth et al., 2020).

The mean number of stink bugs per sweep, standard error of the mean, relative variance, and relative net precision were estimated with random intercept linear mixed models (*lme4*, *lmer*; Bates et al., 2015) with treatments as fixed factors, and states and fields as random factors for the same reason described above. The significance of fixed factors was estimated with a Type II Wald chi-square test (*car*, *Anova*; Fox and Weisberg, 2019). Significant fixed effects were compared using estimated marginal means with *P*-values of pairwise comparisons adjusted with the Tukey method ($\alpha = 5\%$) (*emmeans*, *emmeans*; Lenth et al., 2020).

The spatial pattern of nymphs, adults and nymphs + adults of *Euschistus* spp., and of nymphs + adults of *C. hilaris* were compared between treatments using Taylor's power law $s^2 = am^b$, where s^2 is the sample variance, m is the sample mean, a is a parameter associated with sampling size, and b is an aggregation parameter (Taylor, 1961). Values of $b < 1$, $b = 1$, and $b > 1$ indicate that the spatial patterns are likely uniform, random, and aggregated, respectively. The parameters a and b of Taylor's power law can be estimated by regressing the log transformed sample variance (y-axis) on the log transformed sample

mean (x-axis), where the antilog of the intercept and the regression slope correspond to the parameters a and b , respectively (Clark et al., 1996; Hall and Albrigo, 2007; Pezzini et al., 2019a). Thus, a random intercept linear mixed model (lme4, lmer; Bates et al., 2015) was fitted with \log_{10} variance as the response variable, and \log_{10} mean and treatments as fixed effects. States and fields were included as random factors for the same reason described above. The significance of fixed factors was estimated with a Type II Wald chi-square test (car, Anova; Fox and Weisberg, 2019), and significant fixed effects were compared with estimated marginal means ($\alpha = 5\%$) (emmeans, emmeans; Lenth et al., 2020). The spatial patterns were also obtained by comparing each slope (parameter b of Taylor's power law) to 1 (emmeans, emtrends; Lenth et al., 2020).

3. Results

For the *Euschistus* spp. complex, which included *E. servus servus*, *E. servus euschistoides*, *E. servus* hybrid, *E. tristigmus luridus*, *E. tristigmus tristigmus* and *E. variolarius*, a total of 2,856 individuals (618 adults and 2,238 nymphs) were collected. For *C. hilaris*, a total of 334 individuals (137 adults and 197 nymphs) were collected. For the predatory *Podisus* spp., a total of 56 individuals (42 adults and 14 nymphs) were collected. The average times (\pm standard error) required to complete a set of 10 or 25 sweeps in a sample unit were 9.41 ± 0.09 and 23.87 ± 0.13 s, respectively. The estimated times required to sample a soybean field for stink bugs using 15 sets of 10 sweeps or 15 sets of 25 sweeps were 31.66 and 35.26 min, respectively, including a fixed time of 50 s walking between sample units.

3.1. *Euschistus* spp. and *Chinavia hilaris* combined

The probability of detecting at least one stink bug with the 25-sweep sample unit size was significantly higher than for the 10-sweep sample unit size. This held true for nymphs ($\chi^2 = 10.99$, $df = 1$, $P = 0.001$), adults ($\chi^2 = 9.20$, $df = 1$, $P = 0.002$) and nymphs + adults ($\chi^2 = 11.20$, $df = 1$, $P < 0.001$) (Fig. 1). In contrast, the mean number of stink bugs per sweep collected with the 25-sweep sample unit size was significantly

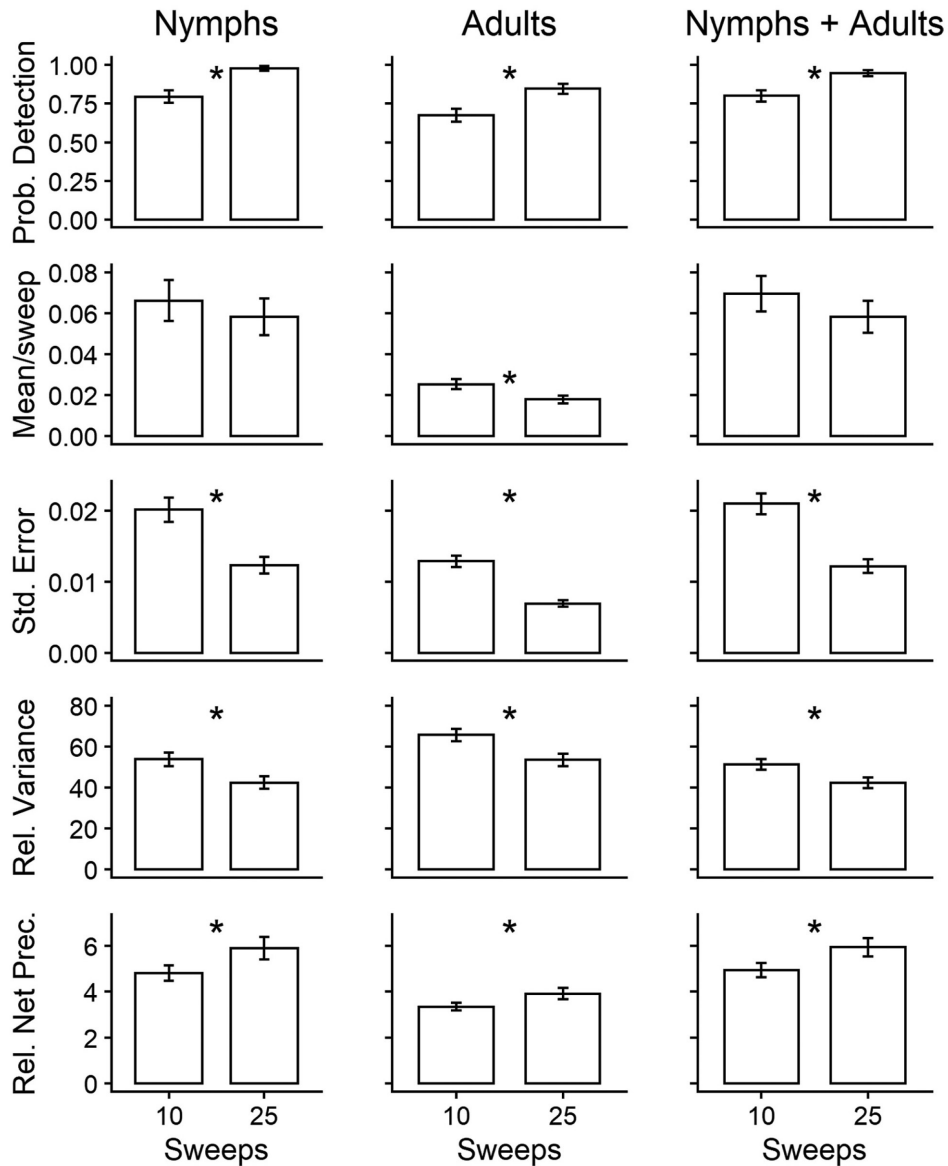


Fig. 1. Sampling parameters for nymphs, adults or nymphs + adults of *Euschistus* spp. and *Chinavia hilaris* combined in Midwest soybean for two sample unit sizes, 10 or 25 sweeps: mean \pm standard error for the probability of detection (Prob. Detection), mean number of insects per sweep (Mean/ sweep), standard error of the mean (Std. Error), relative variance (Rel. Variance), and relative net precision (Rel. Net Prec.). Asterisks in each graph indicate significant differences between sample unit sizes ($P < 0.05$).

lower than with the 10-sweep sample unit size for adults ($\chi^2 = 6.69$, $df = 1$, $P = 0.010$), but no significant difference was observed between the two sample unit sizes for nymphs ($\chi^2 = 0.53$, $df = 1$, $P = 0.465$) and

nymphs + adults ($\chi^2 = 1.36$, $df = 1$, $P = 0.242$) (Fig. 1). The mean standard error for the 25-sweep sample unit size was significantly lower than that of the 10-sweep sample unit size for nymphs ($\chi^2 = 19.48$, $df = 1$, $P < 0.001$), adults ($\chi^2 = 49.41$, $df = 1$, $P < 0.001$) and nymphs + adults ($\chi^2 = 30.65$, $df = 1$, $P < 0.001$) (Fig. 1). Similarly, the mean relative variance for the 25-sweep sample unit size was significantly lower (i.e., less variable) than that of the 10-sweep sample unit size for nymphs ($\chi^2 = 8.63$, $df = 1$, $P = 0.003$), adults ($\chi^2 = 8.85$, $df = 1$, $P = 0.003$) and nymphs + adults ($\chi^2 = 7.24$, $df = 1$, $P = 0.007$) (Fig. 1). Furthermore, the mean relative net precision for the 25-sweep sample unit size was significantly higher (i.e., more efficient) than that of the 10-sweep sample unit size for nymphs ($\chi^2 = 4.79$, $df = 1$, $P = 0.029$), adults ($\chi^2 = 4.08$, $df = 1$, $P = 0.043$) and nymphs + adults ($\chi^2 = 5.90$, $df = 1$, $P = 0.015$) (Fig. 1).

The sample variance (\log_{10} variance) increased with an increase in the mean number of stink bugs per sweep (\log_{10} mean) for nymphs, adults and nymphs + adults (**Table 1**). On average, the sample variance of the 25-sweep sample unit size was significantly lower than that of the 10-sweep sample unit size for nymphs, adults and nymphs + adults (Table 1). The overall regression slopes (\log_{10} mean) were similar between the two sample unit sizes for nymphs, adults and nymphs

Table 1 Analysis of deviance (Type II Wald χ^2 tests) of Taylor's power law regressions (linear mixed models) for nymphs, adults, and nymphs + adults of *Euschistus* spp. and *Chinavia hilaris* combined.

Variables	χ^2	df	P^\dagger
Nymphs			
Log ₁₀ mean	1600.47	1	<0.001
Sample unit size	155.01	1	<0.001
Log ₁₀ mean x Sample unit size	0.14	1	0.703
Adults			
Log ₁₀ mean	736.23	1	<0.001
Sample unit size	174.45	1	<0.001
Log ₁₀ mean x Sample unit size	0.03	1	0.850
Nymphs + adults			
Log ₁₀ mean	2279.68	1	<0.001
Sample unit size	234.58	1	<0.001
Log ₁₀ mean x Sample unit size	0.07	1	0.793

† Significant effects are boldfaced.

Table 2 Taylor's power law log₁₀ mean slopes \pm standard error of the mean (95% confidence interval) for sample unit sizes of 10 and 25 sweeps, and t-tests comparing each slope to 1 (H_0 : slope = 1) for nymphs, adults, and nymphs + adults of *Euschistus* spp. and *Chinavia hilaris* combined.

Sweeps	Slope \pm SEM (CI)	df ^a	t value	p [‡]
Nymphs				
10	1.10 \pm 0.04 (1.02–1.19)	139	2.430	0.016
25	1.12 \pm 0.04 (1.05–1.20)	134	3.345	0.001
Adults				
10	1.07 \pm 0.06 (0.95–1.19)	114	1.184	0.239
25	1.06 \pm 0.05 (0.95–1.16)	116	1.097	0.275
Nymphs + adults				
10	1.11 \pm 0.04 (1.04–1.18)	188	3.109	0.002
25	1.12 \pm 0.03 (1.07–1.18)	187	4.152	<0.001

‡ Significant effects are boldfaced.

a. Degrees of freedom estimated using the Kenward-Roger method.

+ adults (Table 1). Furthermore, slopes were significantly higher than 1 for nymphs and nymphs + adults, indicating that the spatial pattern of nymphs and nymphs + adults of the combined herbivores in the field is possibly aggregated (**Table 2**). On the other hand, slopes did not differ from 1 for adults, indicating that the spatial pattern of adults of the combined herbivores in the field is possibly random (Table 2).

3.2. *Euschistus* spp.

The probability of detecting at least one stink bug with the 25-sweep sample unit size was significantly higher than for the 10-sweep sample unit size. This held true for nymphs ($\chi^2 = 10.81$, $df = 1$, $P = 0.001$), adults ($\chi^2 = 17.04$, $df = 1$, $P < 0.001$) and nymphs + adults ($\chi^2 = 14.42$, $df = 1$, $P < 0.001$) (**Fig. 2**). However, the mean number of stink bugs per sweep did not differ significantly between the two sample unit sizes for nymphs ($\chi^2 = 0.58$, $df = 1$, $P = 0.447$), adults ($\chi^2 = 0.19$, $df = 1$, $P = 0.660$) and nymphs + adults ($\chi^2 = 0.15$, $df = 1$, $P = 0.698$) (Fig. 2). The mean standard error for the 25-sweep sample unit size was significantly lower than that of the 10-sweep sample unit size for nymphs ($\chi^2 = 18.32$, $df = 1$, $P < 0.001$), adults ($\chi^2 = 27.05$, $df = 1$, $P < 0.001$) and

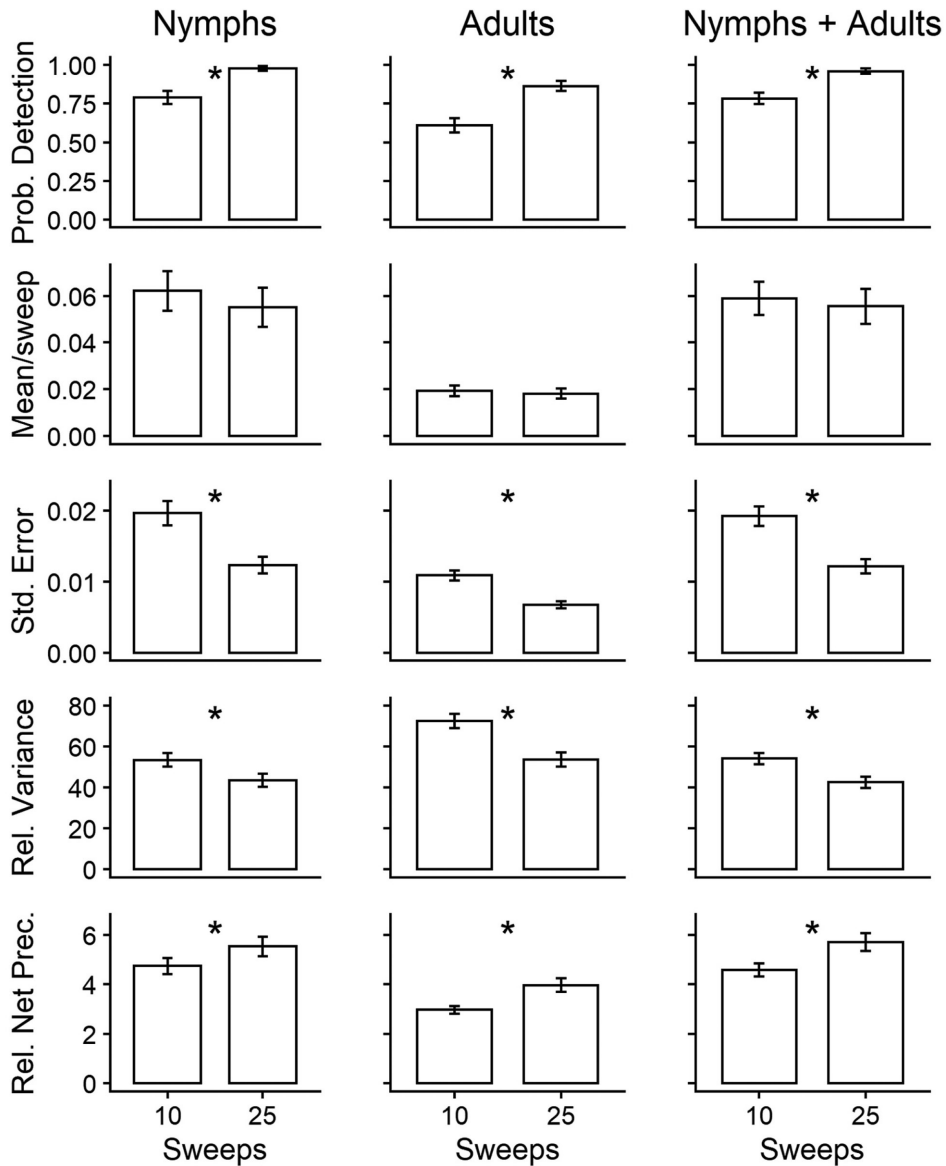


Fig. 2. Sampling parameters for nymphs, adults or nymphs + adults of *Euschistus* spp. in Midwest soybean for two sample unit sizes, 10 or 25 sweeps: mean \pm standard error for the probability of detection (Prob. Detection), mean number of insects per sweep (Mean/sweep), standard error of the mean (Std. Error), relative variance (Rel. Variance), and relative net precision (Rel. Net Prec.). Asterisks in each graph indicate significant differences between sample unit sizes ($P < 0.05$).

nymphs + adults ($\chi^2 = 20.57$, $df = 1$, $P < 0.001$) (Fig. 2). Similarly, the mean relative variance for the 25-sweep sample unit size was significantly lower than that of the 10-sweep sample unit size for nymphs

($\chi^2 = 6.84$, $df = 1$, $P = 0.009$), adults ($\chi^2 = 18.22$, $df = 1$, $P < 0.001$) and nymphs + adults ($\chi^2 = 11.16$, $df = 1$, $P < 0.001$) (Fig. 2). Furthermore, the mean relative net precision for the 25-sweep sample unit size was significantly higher than that of the 10-sweep sample unit size for nymphs ($\chi^2 = 4.00$, $df = 1$, $P = 0.046$), adults ($\chi^2 = 12.84$, $df = 1$, $P < 0.001$) and nymphs + adults ($\chi^2 = 8.69$, $df = 1$, $P = 0.003$) (Fig. 2).

The sample variance (\log_{10} variance) increased with an increase in the mean number of stink bugs per sweep (\log_{10} mean) for nymphs, adults and nymphs + adults (**Table 3**). On average, the sample variance of the 25-sweep sample unit size was significantly lower than that of the 10-sweep sample unit size for nymphs, adults and nymphs + adults (Table 3). The overall regression slopes (\log_{10} mean) were similar between the two sample unit sizes for nymphs, adults and nymphs + adults (Table 3). The slopes were significantly higher than 1 for nymphs and nymphs + adults, indicating that the spatial pattern of nymphs and nymphs + adults of *Euschistus* spp. in the field is possibly aggregated (**Table 4**). On the other hand, the slopes did not differ from 1 for adults, indicating that the spatial pattern of *Euschistus* spp. adults in the field is possibly random (Table 4).

Table 3 Analysis of deviance (Type II Wald χ^2 tests) of Taylor's power law regressions (linear mixed models) for *Euschistus* spp. nymphs, adults and nymphs + adults.

Variables	χ^2	df	P^\dagger
Nymphs			
Log ₁₀ mean	1802.59	1	<0.001
Sample unit size	151.02	1	<0.001
Log ₁₀ mean x Sample unit size	1.00	1	0.317
Adults			
Log ₁₀ mean	991.37	1	<0.001
Sample unit size	236.93	1	<0.001
Log ₁₀ mean x Sample unit size	0.009	1	0.922
Nymphs + adults			
Log ₁₀ mean	2469.68	1	<0.001
Sample unit size	239.71	1	<0.001
Log ₁₀ mean x Sample unit size	1.21	1	0.271

† Significant effects are boldfaced.

Table 4 Taylor's power law log₁₀ mean slopes ± standard error of the mean (95% confidence interval) for sample unit sizes of 10 and 25 sweeps, and t-tests comparing each slope to 1 (H₀: slope = 1) for *Euschistus* spp. nymphs, adults and nymphs + adults.

Sweeps	Slope ± SEM (CI)	df ^a	t value	P [‡]
Nymphs				
10	1.11 ± 0.04 (1.03–1.19)	132	2.673	0.008
25	1.16 ± 0.03 (1.09–1.23)	128	4.637	<0.001
Adults				
10	1.05 ± 0.06 (0.94–1.16)	99.7	0.922	0.359
25	1.06 ± 0.04 (0.97–1.15)	95.2	1.332	0.186
Nymphs + adults				
10	1.10 ± 0.04 (1.03–1.17)	182	2.764	0.006
25	1.15 ± 0.03 (1.09–1.21)	180	5.051	<0.001

‡ Significant effects are boldfaced.

a. Degrees of freedom estimated using the Kenward-Roger method.

3.3. *Chinavia hilaris*

The probability of detecting at least one stink bug was similar between the 10- and the 25-sweep sample unit size ($\chi^2 = 0.59$, $df = 1$, $P = 0.441$) for nymphs + adults (**Fig. 3**). However, the mean number of stink bugs per sweep collected with the 25-sweep sample unit size was significantly lower than with the 10-sweep sample unit size ($\chi^2 = 3.98$, $df = 1$, $P = 0.046$) for nymphs + adults (Fig. 3). Similarly, the mean standard error for the 25-sweep sample unit size was significantly lower than that of the 10-sweep sample unit size ($\chi^2 = 13.84$, $df = 1$, $P < 0.001$) for nymphs + adults (Fig. 3). On the other hand, no significant difference was observed between the 10- and the 25-sweep sample unit sizes for the mean relative variance ($\chi^2 = 0.51$, $df = 1$, $P = 0.474$) and mean relative net precision ($\chi^2 = 0.005$, $df = 1$, $P = 0.946$) for nymphs + adults (Fig. 3).

The sample variance (\log_{10} variance) increased with an increase in the mean number of stink bugs per sweep (\log_{10} mean) for nymphs + adults (**Table 5**). On average, the sample variance of the 25-sweep sample unit size was significantly lower than that of the 10-sweep sample unit size (Table 5). The overall regression slopes (\log_{10} mean)

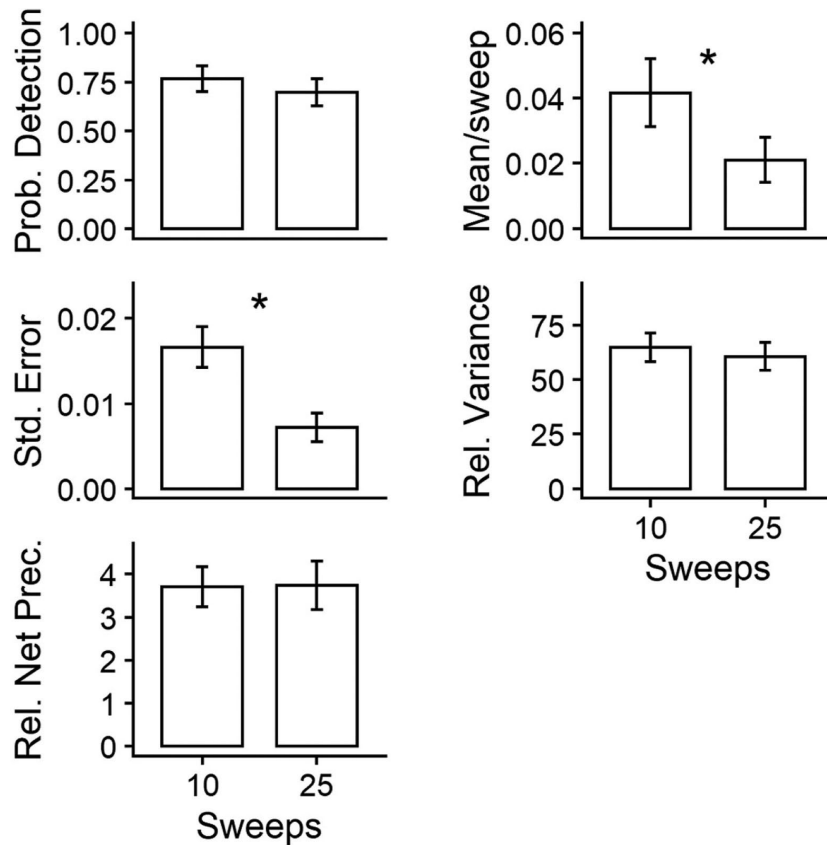


Fig. 3. Sampling parameters for *Chinavia hilaris* nymphs + adults in Midwest soybean for two sample unit sizes, 10 or 25 sweeps: mean \pm standard error for the probability of detection (Prob. Detection), mean number of insects per sweep (Mean/sweep), standard error of the mean (Std. Error), relative variance (Rel. Variance), and relative net precision (Rel. Net Prec.). Asterisks in each graph indicate significant differences between sample unit sizes ($P < 0.05$).

Table 5 Analysis of deviance (Type II Wald χ^2 tests) of Taylor's power law regressions (linear mixed models) for *C. hilaris* nymphs + adults.

Variables	χ^2	df	P^\dagger
Log ₁₀ mean	459.19	1	<0.001
Sample unit size	55.56	1	<0.001
Log ₁₀ mean x Sample unit size	0.002	1	0.966

\dagger Significant effects are boldfaced.

Table 6 Taylor's power law log₁₀ mean slopes ± standard error (95% confidence interval) for sample unit sizes of 10 and 25 sweeps, and t-tests comparing each slope to 1 (H_0 : slope = 1) for *C. hilaris* nymphs + adults.

Sweeps	Slope ± SEM (CI)	df ^a	t value	P
10	1.14 ± 0.08 (0.97–1.30)	29.3	1.707	0.098
25	1.13 ± 0.08 (0.97–1.29)	28.3	1.665	0.107

a. Degrees of freedom estimated using the Kenward-Roger method.

were similar between the two sample unit sizes (Table 5). Furthermore, the slopes were numerically, but not statistically higher than 1 for both the 10-sweep and 25-sweep sample unit sizes, indicating that the spatial pattern of *C. hilaris* in the field is possibly random (Table 6).

3.4. *Podisus* spp.

The mean probability of detecting at least one stink bug with the 25-sweep sample unit size was significantly higher than that of the 10-sweep sample unit size ($\chi^2 = 6.87$, $df = 1$, $P = 0.009$) for nymphs + adults (Fig. 4).

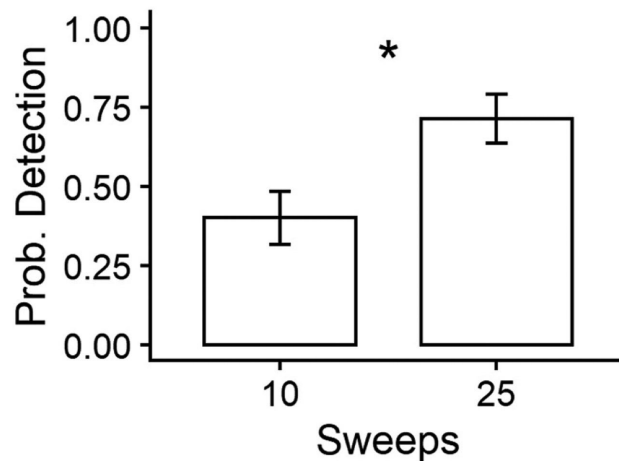


Fig. 4. Mean ± standard error for the probability of detection (Prob. Detection) of *Podisus* spp. nymphs + adults in Midwest soybean for two sample unit sizes, 10 or 25 sweeps. The asterisk indicates a significant difference between sample unit sizes ($P < 0.05$).

4. Discussion

A key constraint limiting implementation of scouting is the time associated with sampling. For soybean IPM, there is a need for simple, efficient and rapid sampling procedures that are field-tested (Bueno et al., 2021). Currently, sample unit sizes ranging from 10 to 100 sweeps per set are recommended for stink bug sampling in the United States (Pezzini et al., 2019a). Sampling with a lower sample unit size is less time-consuming and therefore should reduce the overall cost of sampling (Pedigo and Buntin, 1994; Ruesink, 1980). This is desirable for practical purposes and it could increase the adoption of sampling plans by growers (Bueno et al., 2021; Radcliffe et al., 2009). However, a potential reduction in precision due to using smaller sample unit sizes is a potential drawback that must be carefully addressed. In this study, sampling parameters and the spatial pattern of stink bug taxa were compared using two different sample unit sizes (i.e., 25 and 10 sweeps).

Unsurprisingly, we found that the probability of detecting stink bugs increased with a higher sample unit size for the herbivorous *Euschistus* spp., the predatory *Podisus* spp., and the combined herbivores. However, the same was not observed for *C. hilaris*. Higher sampling effort is usually expected to result in a higher chance of encountering insects (Elmouttie et al., 2010; Økland et al., 2012; Venette et al., 2000). However, other factors like abundance and spatial distribution of a species, and placement of sampling units can affect the detection of organisms (Walker et al., 2016). Although consistent results were observed between the two tested sample unit sizes (i.e., 10 and 25 sweeps) for the estimation of the mean number of nymphs, adults, and nymphs + adults of *Euschistus* spp., and nymphs and nymphs + adults of the combined herbivores, dissimilarities were found for *C. hilaris* and adults of the combined herbivores. The effects of smaller sample unit sizes on the estimation of abundance are variable. Sample means have been shown to increase or decrease with an increase in sample unit size for different insect species (Hill et al., 1975; Pieters, 1978).

Sampling programs for management decisions must be precise and cost-effective because imprecision in sampling programs can lead to either unnecessary treatments or crop loss if pests are underestimated. In many ways, the latter type of error is the worst possible

outcome as it results in loss of crop revenue in addition to the resources used for sampling and a potential loss of confidence in the proposed sampling approach (Pedigo and Buntin, 1994). In terms of population sampling, Ruesink (1980) defined efficiency as an increase in reliability per unit cost. In other words, the cost associated with a higher number of samples are justified by a corresponding increase in information and precision. As expected, an increase in variability and a reduction in precision was observed with the adoption of a lower sample unit size in this study, and that often resulted in lower relative net precision. This decrease in relative net precision with the 10-sweep sample unit size indicates that the reduction in precision is proportionally higher than the reduction in cost. Thus, a sample unit size of 25 sweeps may provide a better balance between precision and cost in such cases. However, this did not hold true for nymphs + adults of *C. hilaris*; the relative net precision did not differ between the 10- and 25-sweep sample unit sizes, which indicates that either sample unit size can be used for this species. This is not without precedent; smaller sample unit sizes are more efficient for some insect pests and sampling methods (Burkness and Hutchison, 1997; Guppy and Harcourt, 1973; Hall and Albrigo, 2007; Hill et al., 1975; Pieters, 1978). However, larger sample unit sizes have also been found to result in higher cost-effectiveness in other cases (Cho et al., 1995). This demonstrates that the biology of the pest and crop must be investigated empirically before generating recommendations regarding the optimization of sample unit size for pest sampling. Furthermore, we emphasize that the results found in this study were obtained assuming similar times to count relatively low numbers of stink bugs in each sample unit and a fixed distance between sample units. These two factors have a direct impact on total sampling time and, therefore, can change the relative importance of the time spent sweeping (the only time factor that varied in this study) in the total cost. Additionally, the time to count stink bugs in each sample unit could also vary depending on the amount of bycatch (e.g., Japanese beetle adults) in the sweeps. Thus, further studies investigating the effects of sampling time components on the relative net precision of different sample unit sizes are highly recommended.

In this study, different sample unit sizes did not affect the inference of spatial patterns within life stages (nymphs or adults). The spatial

pattern of *Euschistus* spp. nymphs was aggregated (i.e., Taylor's power law slopes greater than 1), but for adults it was random (i.e., Taylor's power law slope close to 1). An aggregated distribution is commonly observed among insects (Pedigo and Buntin, 1994; Taylor, 1984) and differences in spatial pattern between stink bug life stages is not unusual (Pilkay et al., 2015; Reay-Jones, 2014; Reay-Jones et al., 2010; Souza et al., 2013; Weber et al., 2018), related to behavioral and physiological characteristics of the insect species (Banerjee, 1976; Pilkay et al., 2015). We hypothesize that the aggregated pattern of *Euschistus* nymphs is partially associated with the oviposition behavior and differential dispersal capacity among life stages of stink bugs. Stink bug eggs are laid in clusters of multiple eggs (Koch et al., 2017) and dispersal is lower during early stages compared to late instars and winged adults (Lockwood and Story, 1986). For *C. hilaris*, the fact that nymphs and adults were combined in our analysis may have resulted in spatial pattern being slightly aggregated (i.e., Taylor's power law slope marginally greater than 1).

In summary, the higher probability of detection and precision achieved with a sample unit size of 25 sweeps, supports the continued use of previously developed sampling plans for stink bugs in soybean (Pezzini et al., 2019a; Aita et al., 2021). However, other sample unit sizes are recommended in some states (Pezzini et al., 2019a), so studies comparing additional sample unit sizes may be needed.



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