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Sequential sampling for adult coccinellids in wheat

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Abstract

Adult aphidophagous coccinellids are important predators of cereal aphids in wheat in the Great Plains of the United States for which sampling methods are needed to facilitate improved management. An equation relating the mean number of adult coccinellids per m^2 in a wheat field to its variance was obtained using Taylor's power law. A sequential sampling procedure was developed to estimate the number of adult coccinellids per m^2 with constant average statistical precision (standard error/mean). The procedure was constructed by an equation relating the number of adult coccinellids per m^2 to the number of adult coccinellids per minute of counting incorporating into the Taylor's power law relationship. The procedure involves conducting a series of 1-min counts while walking through a field at a constant velocity of 10 m per minute. After each 1-min count sequential sampling stop-lines are consulted to determine if the specified level of precision has been achieved. Two methods, a statistical procedure and comparison with independent data, were used to assess the consistency with which the specified level of precision was achieved by the procedure. Results indicated that observed precision was close to that specified by the user over a wide range of adult coccinellid density.

Introduction

Coccinellids (Coleoptera: Coccinellidae) are ubiquitous predators of aphids in wheat fields in the Great Plains of the United States. They play an important role in controlling pest cereal aphids in wheat and other grain crops in the Great Plains (Kring et al., 1985; Rice & Wilde, 1988; RWK and NCE, unpubl.). Because cereal aphids can cause significant yield loss to wheat at relatively low population densities (Wratten et al., 1995), the potential for coccinellids to control aphids below damaging levels is likely to be determined early in the spring, before aphid populations in fields increase to levels stimulating coccinellid oviposition. Thus, predation on cereal aphids in wheat by adult coccinellids is particularly important from a pest management perspective.

Efficient population sampling methods for adult coccinellids would make it possible to incorporate quantitative knowledge of their impact on cereal aphid populations into integrated pest management (IPM) programs. In an IPM context it is desirable to estimate coccinellid densities using relative sampling methods because absolute sampling methods, such as quadrat sampling, are labor intensive and often require specialized equipment (Iperti et al., 1988; Michels et al., 1997). Iperti et al. (1988) developed a sequential sampling procedure for estimating populations of adult *Coccinella septempunctata* L. in wheat fields in Europe. Their method involves counting all *C. septempunctata* observed while walking through a series of 25 m^2 plots for 2 min each and converting estimates of relative population density thus obtained to estimates of absolute density. Counting is continued until estimates attain a fixed average precision, defined as the ratio of the standard error to the mean number of adult *C. septempunctata* per m^2.

Michels et al. (1997) sampled fields of winter wheat in Texas and Oklahoma using both relative and absolute sampling methods. Their objectives were to compare

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several relative sampling methods to determine which were acceptable for sampling coccinellids, in terms of yielding statistically accurate and precise population estimates for an acceptable amount of sampling effort, and to develop regression models to convert estimates of relative population density obtained using these sampling methods to estimates of absolute population density. The objectives of the study reported here were to: (1) use an approach similar to that of Iperti et al. (1988) and a regression model developed by Michels et al. (1997) to construct a sequential sampling procedure for estimating densities of adult coccinellids in wheat fields in the Great Plains; and (2) assess the utility of the sequential sampling procedure. Specifically, we developed a sequential sampling procedure for estimating adult coccinellid population density with fixed statistical precision from results of relative sampling accomplished by counting coccinellids during a series of 1-min walks through a field. A statistical method was developed and used to determine whether density estimates obtained using the sequential sampling procedure were likely to achieve levels of precision close to those specified by the user. In addition, the sequential sampling procedure was tested using coccinellid sampling data obtained independently of data used to construct it.

Materials and methods

Sampling methods. Only a brief description of the sampling methods used in this study will be presented here since a detailed description was presented in Michels et al. (1997). Study plots (30 by 30 m) were established in wheat fields. When a plot was sampled, an observer walked the length of the plot parallel to the rows of wheat at a velocity of 10 m/min. All adult coccinellids seen in an approximately 1-m wide path immediately in front of and along the direction of movement of the observer were counted and recorded. A particular 1-m wide path was traversed only once by the observer. The observer repeated the procedure six times within the plot, so that 18 min were spent counting adult coccinellids in the plot.

Quadrat samples were taken from the plot immediately after the observer finished counting. Quadrat sampling was accomplished within plywood enclosures (1.0 by 1.0 by 0.4 m high). The quadrat was placed at 18 locations within the plot in areas not traversed by the observer making timed counts, and all adult coccinellids trapped inside it were counted.

From 4 to 8 plots were sampled at each of five phenological stages of wheat development: tillering, stem elongation, boot, head emergence-flowering, and the soft dough stage of grain filling. In total, 34 plots were sampled as described above.

The sequential sampling procedure. It is desirable to express the abundance of insects on a measurement scale that is invariant to changes in environmental conditions. Population density, the number of individuals per unit area, is such a measurement scale, and is the one we adopted in this study. Thus, the quantity of direct interest to us was the number of adult coccinellids per m^2 (y).

Taylor's power law (Taylor, 1961) expresses the relationship between the population variance (σ^2) and mean (μ) of counts of organisms for a particular sample unit. In our case, the power law relates the mean number of adult coccinellids per m^2 to the variance of that number:

$$\sigma_y^2 = a\mu_y^b,$$

where σ_y^2 and μ_y represent the population variance and mean of the number of coccinellids per m^2, respectively, and a and b are parameters. In practice, parameters a and b are usually estimated by transforming the power law equation to logarithms and performing linear least squares regression of the sample variance (s_y^2) versus the sample mean ($\bar{y}$) for samples from a series of fields (or plots established in fields) taken using a particular sampling protocol.

We used Taylor's power law to relate the sample mean and variance of counts of adult coccinellids for the 18 m^2 quadrats sampled in each plot. Thus,

$$s_y^2 = \hat{a}\bar{y}^{\,\hat{b}}, \tag{1}$$

where $\hat{a} = e^{\widehat{\ln a}}$. Estimates of $\hat{a}$ and $\hat{b}$ of Equation (1) were obtained for species individually and for combined species. Tests of the equality of slopes and intercepts of Taylor's power law regressions for different species were conducted using analysis of covariance (SAS Institute, 1988).

Green (1970) presented an equation for the required sample size to attain population estimates with fixed average precision, where precision is defined as the standard error divided by the sample mean ($s_{\bar{y}}/\bar{y}$):

$$n = s_y^2/\bar{y}^{\,2}c^2. \tag{2}$$

In Equation (2), n is the required sample size to estimate the number of adult coccinellids per m^2 with precision equal to c. Green (1970) substituted the right-hand side of Equation (1) for s_y^2 in Equation (2) to obtain an equation for sample size involving a single variable ($\bar{y}$),

$$n = \hat{a}\bar{y}^{\,\hat{b}-2}/c^2. \tag{3}$$

Michels et al. (1997) developed a regression model to relate the number of adult coccinellids per m^2 in a wheat field to the number of adult coccinellids observed per minute while walking through the field at a velocity of 10 m/min:

$$\hat{\mu}_y = \hat{\alpha}\hat{\mu}_x, \tag{4}$$

where $\hat{\mu}_x$ is the mean number of adult coccinellids per minute of counting and $\hat{\alpha} = 1.14$.

Since $\bar{x}$ and $\bar{y}$ are unbiased estimators of μ_x and μ_y, they can be substituted in Equation (4) for $\hat{\mu}_x$ and $\hat{\mu}_y$, followed by substitution of the right-hand side of Equation (4) for $\bar{y}$ in Equation (3), to yield an expression for the sample size required to estimate with μ_y fixed precision from a series of 1-min counts:

$$n = \hat{a}(\hat{\alpha}\bar{x})^{\hat{b}-2}/c^2, \tag{5}$$

Equation (5) was used to develop sequential sampling stop-lines for estimating adult coccinellid density with fixed average precision.

Results

Taylor's power-law regressions. Four coccinellid species were commonly observed in quadrat samples: *Hippodamia convergens* Guerin-Meneville, *H. sinuata* Mulsant, *Coleomegilla maculata lengi* (DeGeer), and *Coccinella septempunctata* L. Other species were recorded, but they occurred too sporadically in samples to facilitate meaningful regressions. Estimates of ln(*a*) and *b* of Taylor's power law were obtained for each species (Table 1). Estimates of ln(*a*) ranged from -0.12 for *C. maculata lengi* to 0.40 for *H. sinuata*. Estimates of *b* varied from 0.96 for *C. maculata lengi* to 1.24 for *H. sinuata*. Analysis of covariance indicated that neither *b* ($F = 1.73$; df=3, 72; P > 0.05) nor ln(*a*) ($F = 0.66$; df=3, 72; P > 0.05) differed significantly among species. Thus, a single power-law regression was used to relate s_y^2 to $\bar{y}$ for data on all species combined. The intercept of the regression did not differ significantly from zero ($t = 1.52$; P > 0.05); therefore a regression was calculated in which the intercept was forced through the origin; this simplified development and evaluation of the sampling procedure without jeopardizing its accuracy. For the regression through the origin, $\hat{b} = 1.24$ (SE $= 0.065$) and $r^2 = 0.92$.

Table 1. Estimates of Taylor's power-law parameters for four species of adult coccinellids sampled using m^2 quadrats

Species	Sample size	ln(*a*)	*b*	r^2
H. convergens	33	0.19 ± 0.07	1.21 ± 0.04	0.95
H. sinuata	13	0.40 ± 0.17	1.24 ± 0.10	0.91
C. septempunctata	22	0.27 ± 0.16	1.16 ± 0.07	0.92
C. maculata lengi	12	-0.12 ± 0.24	0.96 ± 0.10	0.91

The sequential sampling procedure. In our sampling procedure we propose to count the number of adult coccinellids observed during 1-min walks at a velocity of 10 m/min through a wheat field. Equation (5) was used to develop the sequential sampling stop-lines for estimating coccinellid density with fixed average precision from such counts. Parameter estimates $\hat{a} = 1.0$ ($e^0 = 1$), $\hat{b} = 1.24$, and $\hat{\alpha} = 1.14$ (from Michels et al., 1997) were used in developing the stop-lines. Stop-lines for estimating adult coccinellid density in wheat fields with precision levels of $c = 0.10$, 0.25, and 0.40 are illustrated in Figure 1. For $c = 0.10$ sample sizes are very large; over 50 min of counting would be required to estimate a population of $\mu_x = 2$ adult coccinellids per minute of counting. Sample sizes become increasingly larger as μ_x decreases below 2 adult coccinellids per minute. For $c = 0.25$ sample size requirements are reasonably small except at low adult coccinellid densities. Ten or fewer min of counting are required for $c = 0.25$ when $\mu_x = 1.5$, and sample sizes do not become excessive until μ_x drops below 1 adult coccinellid per minute. Thus, $c = 0.25$ appears to be a good compromise between sampling effort and precision of estimates.

Evaluating the sequential sampling procedure. For a particular value of *c* in Equation (5), *n* is the expected value of a random variable with unknown distribution. However, it is perhaps more useful for our purpose to consider *n* in Equation (5) as fixed and precision, *c*, as random.

When a sample of size *n* is chosen using Equation 5, the actual level of precision achieved will differ from that specified for several reasons. First, the mean and variance of a random sample of size *n* from a population will vary by chance. In this instance, $\bar{x}$ and s_x^2 are unbiased estimates of μ_x and σ_x^2, respectively, but vary from their true values due to sampling error. Second, error arises from the nature of the sequential sampling process in which sampling is terminated upon contacting or exceeding stop-lines. Bias in *c*

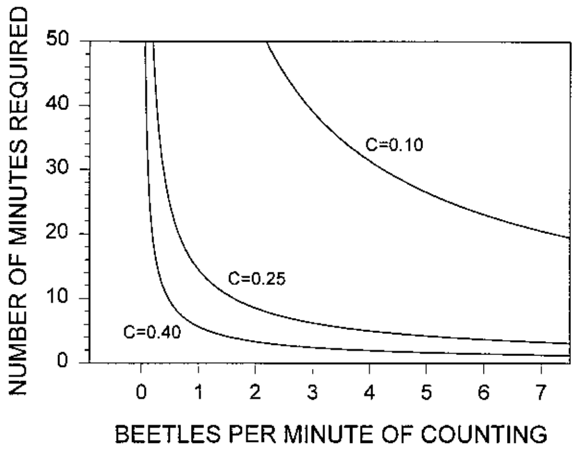

Figure 1. Sample size requirements for 1-min counts of adult coccinellids to achieve precision levels (c) of 0.40, 0.25, and 0.10.

results because stop-lines are usually exceeded rather than contacted directly when the last sample is taken. Anscombe (1952) found that bias from this source was negligible and usually can be ignored. Third, actual precision will vary from that specified due to error in estimates of α and b of Equation (5). This results in bias in c that depends in an unknown way on the magnitude of differences between the parameter estimates and their true values. Thus, once α and b are estimated and a sampling procedure is developed from the estimates, average sample size requirements to achieve a particular level of precision will be biased.

To evaluate the potential magnitude of sampling error and error resulting from estimating α and b on the accuracy with which c is achieved using Equation (5) we calculated approximate 95% confidence intervals for c. To accomplish this we solved Equation (5) for c,

$$c = \{[\hat{\alpha}\bar{x}]^{(\hat{b}-2)}/n\}^{1/2}$$

and derived the variance of c using the Δ-method (Seber, 1982). By the Δ-method, the variance of a function of random variables is approximated by a 1st-order Taylor series expansion of the moments of the random variables distributions (Kendall & Stuart, 1977, p. 246–248). In our case,

$$\text{var}(c) = (\partial c/\partial\hat{\alpha})^2\ \text{var}(\hat{\alpha}) + (\partial c/\partial\hat{b})^2\text{var}(\hat{b}) + (\partial c/\partial\bar{x})^2\ \text{var}(\bar{x}). \quad (6)$$

Because $\hat{b}$ and $\hat{\alpha}$ were estimated by linear least-squares regression, estimation of var($\hat{b}$) and var($\hat{\alpha}$) can be accomplished using standard methods (Neter & Wasserman, 1974, p. 157):

$$\text{var}(\hat{b}) = MSE_t/\sum_i^{n_t}\ \bar{y}_i^{\,2} \text{ and}$$

$$\text{var}(\hat{\alpha}) = MSE_d/\sum_i^{n_d}\ \bar{x}_i^{\,2},$$

where $\bar{y}_i$, and $\bar{x}_i$ are the mean the number of adult coccinellids per m^2 and per minute of counting, respectively, obtained from sampling the ith plot, and the summations are over the total number of such samples (i.e., n_t and n_d) used in calculating linear regressions to estimate b and α. MSE_t and MSE_d are error mean squares of the respective regressions. The variance of $\bar{x}$ is estimated by $s_{\bar{x}}^2 = s_x^2/n$. For the purpose of estimating var(c) we replaced s_x^2 by its Taylor's power-law equivalent,

$$s_x^2 = 0.20\bar{x}^{\,1.35},$$

parameters of which were estimated by a linear regression of ln(s_x^2) on ln($\bar{x}$) which had a coefficient of determination of $r^2 = 0.86$.

The first two terms in Equation 6, involve variation resulting from having estimated α and b using linear least-squares regression. Reducing this source of variation could only be accomplished by collecting more data from which to estimate these parameters. The third term in Equation (6) involves the variance of $\bar{x}$ (i.e., sampling error incurred when estimating the number of coccinellids per minute from a sample of size n). The variance of c, and the relative contributions of sampling error and error caused by parameter estimation are illustrated in Figure 2. The variance of c is greatest for very low values of μ_x (the true mean number of adult coccinellids per 1-min count) but decreases rapidly as μ_x increases, and reaches a minimum when $\mu_x = 0.81$ adult coccinellids per 1-min count. The variance of c then increases gradually as μ_x increases further. When μ_x is small, variance due to uncertainty in the values of the regression parameters dominates the total variance, with sampling error contributing very little to total variance. As μ_x increases, the contribution to total variance from sampling error increases, and becomes the dominant source of variation for $\mu_x > 0.40$.

Using estimates of var(c) obtained from Equation (6), approximate 95% confidence limits for $c = 0.25$ were generated for μ_x ranging from 0.03 to 7.5 adult coccinellids per minute of counting:

$$\text{confidence limits} = c \pm z_{0.975} \cdot \text{var}(c)^{1/2},$$

where, $z_{0.975}$ is the 97.5 percentile of the standard normal distribution.

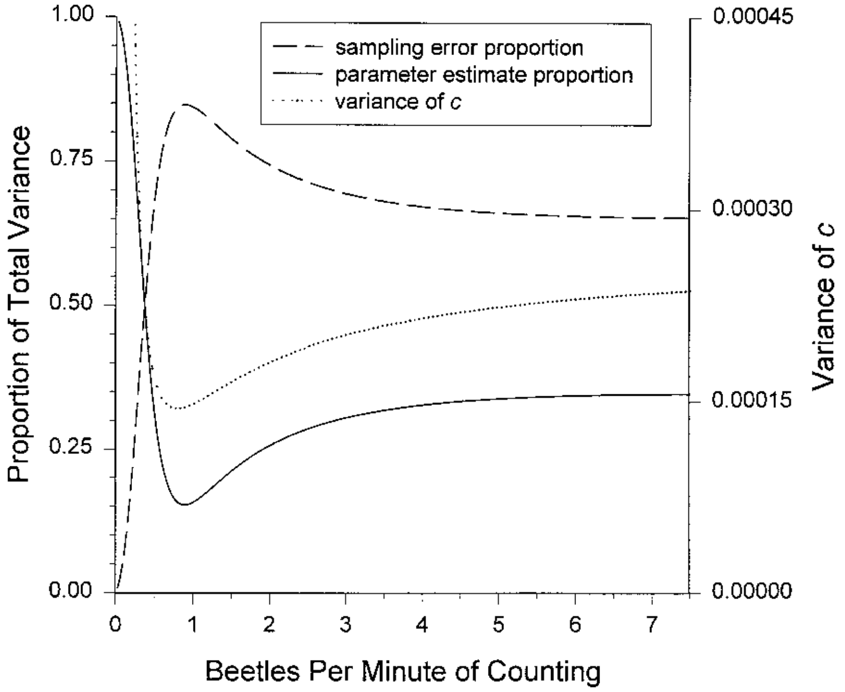

Figure 2. Proportion of the variance of *c* accounted for by sampling error in estimating and sampling error in estimating parameters of Equation (5).

Upper and lower confidence limits are illustrated in Figure 3, in which the solid line represents the specified value of *c* and the dashed lines represent upper and lower 95% confidence limits for *c*. The confidence limits can be interpreted as follows: for any particular estimates of α and *b* and any estimate of μ_x achieved by a sample of size *n* determined from Equation (5), *c* will be included within the interval defined by the dashed lines 95% of the time. Two observations can be made regarding Figure 3. First, for particular estimates of α and *b*, and $\bar{x}$, there is uncertainty regarding the value of *c* that will be achieved by the procedure. Second, the amount by which *c* varies is not constant but varies with μ_x. Thus, even though the specified value of *c* is constant over all values of μ_x, the average value of *c* achieved in practice may vary from that specified, and the amount by which it varies will not necessarily be constant. Inspection of Figure 3 indicates that for $0.10 < \mu_x < 7.5$ the deviation of the observed average value of *c* from that specified will be less than 0.02 in 95% of cases. For example, when $\mu_x = 6.0$ adult coccinellids per 1-min count, the lower and upper 95% confidence limits for *c* are 0.267 and 0.232, respectively. However, for small values of μ_x the deviation could be much greater. Overall, the analysis indicates that a sequential sampling procedure based on an empirical regression model such as ours will usually ($\cong$ 95% of the time) yield average values of *c* that are close to those specified by a user over a broad range of population densities. This analysis, although encouraging, does not provide a conclusive answer to the question of whether the particular sampling procedure reported in this paper has the level of precision that would be achieved 95% of the time when such a procedure is developed and used.

To further explore the utility of the sequential sampling procedure we obtained data consisting of 27 samples of adult coccinellids from wheat fields collected in eastern South Dakota during 1988 and 1989. Each sample consisted of 18 counts of the number of coccinellids seen by an observer in a 1-min walk at a velocity of 10 m/min through a wheat field. For these data $\bar{x}$ ranged from 0.08 to 7.2 adult coccinellids per minute of counting. One way to use these data to assess the validity of the sequential sampling method is to solve Equation (5) for $\bar{x}$ and regress values of $\bar{x}$ expected based on Equation 5 against observed values of $\bar{x}$ for the series of samples. To assomplish this *n* ($n = 18$ for all samples) and *c* are determined for each sample and used to calculate the predicted value

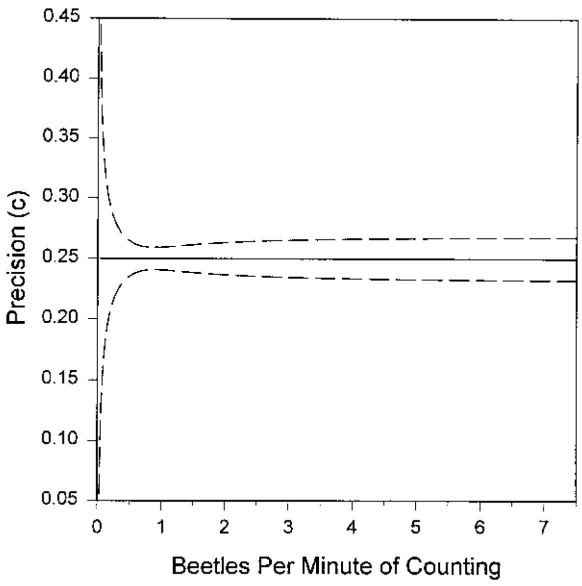

Figure 3. Approximate 95% confidence limits (dashed curves) for a precision level of $c = 0.25$ (solid line).

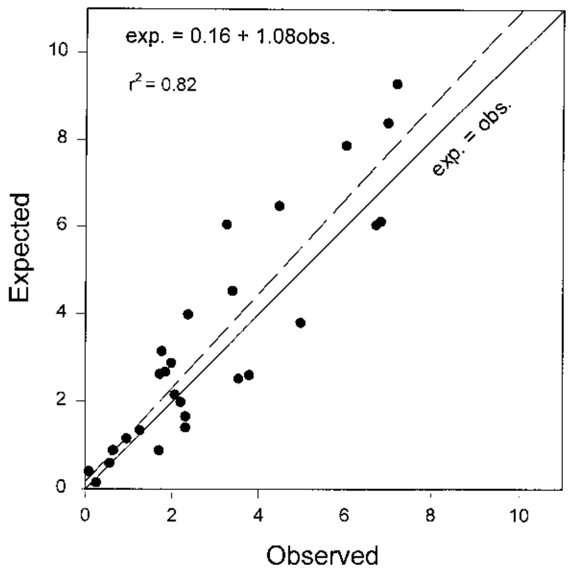

Figure 4. Expected versus observed number of adult coccinellids per 1-min count for 27 samples each consisting of 18 1-min counts.

of $\bar{x}$ from Equation (5). If Equation (5) is acceptable for estimating $\bar{y}$, the estimates of $\bar{x}$ predicted from Equation (5) should be consistent with the values of $\bar{x}$ observed for the series of samples. In this case, neither the intercept nor the slope of a regression of predicted versus observed $\bar{x}$ should differ significantly from zero or one, respectively, and the coefficient of determination of the regression should be large (close to 1.0). Results of this regression are illustrated in Figure 4. Neither the intercept (0.16, SE $= 0.37$) nor the slope (1.08, SE $= 0.10$) of the regression differed significantly from zero or one, respectively. The coefficient of determination was relatively large ($r^2 = 0.82$). This result supports the analysis based on the statistical properties of the sampling procedure, and supports the contention that the sequential sampling procedure is acceptable for sampling adult coccinellids in wheat fields to obtain estimates of absolute density with precision levels close to those specified.

Discussion

The desirability of evaluating sequential sampling procedures prior to their implementation has been discussed (e.g., Hutchison et al., 1988; Kuno, 1969; Nyrop & Simmons 1984). For example, Hutchison et al. (1988) found that actual precision achieved with a fixed-precision sequential sampling procedure developed for the pea aphid, *Acyrthosiphon pisum* (Harris), using Green's (1970) method differed markedly from specified values. In our study, comparison with independent field data yielded no significant discrepancy between observed and predicted precision. We demonstrated by application of reasoning based on the statistical properties of the sampling procedure and comparison with field data that the procedure yields estimates with average precision similar to that specified over a broad range of adult coccinellid densities.

The sampling procedure was acceptable for sampling adult coccinellids in South Dakota wheat fields even though it was developed from data collected in Texas and Oklahoma. Coccinellid communities occurring in wheat in Texas and Oklahoma differ somewhat in species composition from those in South Dakota. For example, *Hippodamia tredecimpunctata tibialis* (Say) is a common coccinellid in wheat fields in South Dakota (Elliott & Kieckhefer, 1990) but does not occur in Oklahoma or Texas. Likewise, *H. sinuata* is common in Texas but absent from South Dakota wheat fields. The sampling procedure may be robust with respect to minor differences in species composition of coccinellid communities in different geographic regions. This may be primarily the result of similarity in variance-mean relationships of various species, as summarized by Taylor's power law, which suggests that species have similar spatial distribution patterns, at least at the spatial scale of our sampling protocol.

Attempts have been made to incorporate natural enemies into sampling procedures for aphid pests of wheat in the Great Plains. For example, Texas guidelines for managing greenbugs in wheat advise farmers to delay control measures in fields where greenbug populations are near economic thresholds when coccinellids are present until it can be determined whether greenbug populations will be controlled by the natural enemies. This approach is advised when there are one or more coccinellids per 0.3 m of row (≈12 or more coccinellids per m^2) (Patrick & Boring 1990). The guidelines are relatively crude because methods are not provided for assessing the reliability of estimates of coccinellid density, and to our knowledge, there are no experimental data that specify the relationship between coccinellid and greenbug density and the resulting level of greenbug population suppression. Results of this study should contribute toward improved IPM decision-making for greenbugs and other cereal aphids in wheat. Even though the number of minutes required to obtain density estimates for a precision level of $c = 0.10$ are excessive, 8 or fewer minutes are required on average to estimate coccinellid density in wheat fields when $c = 0.25$ and density is greater than 2.3 adult coccinellids per m^2 (2 adult coccinellids per minute of counting). Thus, sampling coccinellids by timed counts may be an appropriate method for use in an IPM program. However, if the procedure were to be used with $c = 0.25$, an excessive number of samples would need to be taken to estimate density in fields with very low coccinellid densities. It might be advisable to truncate sampling after a specified number of minutes regardless of whether the stop-line has been crossed. In this case, estimation would be less precise than specified when the procedure is used to sample low density populations. However, sparse coccinellid populations (< 2 coccinellids per m^2) are unlikely to exert significant biological control over cereal aphids in wheat, so that estimates with low precision may be adequate for assessing the potential for biological control, or lack of it, under such conditions.

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