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Spatial distribution and fixed-precision sampling plans for the ladybird *Harmonia axyridis* in sweet corn

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Abstract. To improve the utilization of the multicolored Asian lady beetle, *Harmonia axyridis* (Pallas), as a natural enemy in integrated pest management (IPM) programs for sweet corn, *Zea mays* L., fixed-precision enumerative sequential sampling plans for this coccinellid were developed and validated. Data were collected from sweet corn plots during 2000 to 2004, with means ranging from 0.02 to 11.83 *H. axyridis* (larvae and adults combined) per plant. Taylor's power law parameters from the regression of log variance versus log mean suggested an aggregated distribution for larvae and the combined count of larvae and adults in sweet corn. For IPM purposes, a plan was developed for *H. axyridis* larvae and adults to provide a desired precision level of 0.25 (SEM/mean), resulting in an average sample number of 65 whole plants. However, for the purposes of ecological or applied research (with desired precision = 0.10), an average sample number of 205 plants was necessary.

Key words: biological control, Coccinellidae, integrated pest management, resampling software, sweet corn

Introduction

In Minnesota, USA, sweet corn, *Zea mays* L., is grown on 57,466 ha (processing: 53,419 ha; fresh market: 4047 ha) with a total value of $106,750,000 (Hutchison and O'Rourke, 2003). Key pests in this system are the European corn borer, *Ostrinia nubilalis* (Hübner) and corn earworm, *Helicoverpa zea* (Boddie). Management of these lepidopterans has depended on intensive use of insecticides (Bartels and Hutchison, 1995; Hutchison et al., 2004). An additional pest, of infrequent economic importance, is the corn leaf aphid, *Rhopalosiphum maidis* Fitch (Foster and Flood, 1995). With numerous pesticide options being banned by the USA Environmental Protection Agency and increased social and environmental concerns with pesticide use, alternative management tactics are needed for sweet corn.

A favorable alternative to pesticide use is the promotion of existing natural enemies to increase natural pest suppression (i.e. conservation biological control) (Obrycki and Kring, 1998). The guild of generalist predators in Minnesota sweet corn has traditionally consisted of Coccinellidae [e.g. *Coleomegilla maculata* (De Geer), *Hippodamia convergens* (Guerin), *H. tredecimpunctata* (Say), and *Adalia bipunctata* (L.)], Chrysopidae, Anthocoridae, and Nabidae (Bolin et al., 1996; Schellhorn and Andow, 2005). An exotic coccinellid of east-Asian origin, the multicolored Asian lady beetle, *Harmonia axyridis* (Pallas), has recently invaded this system. *Harmonia axyridis* was first recorded in Minnesota in 1994 (Koch and Hutchison, 2003), and its biology and impacts were reviewed by Koch (2003). Since its initial detection, *H. axyridis* has rapidly become the most abundant generalist predator in Minnesota sweet corn (Galvan et al., 2005a). Recent research confirmed that *H. axyridis* will prey on *O. nubilalis* and *R. maidis* (Hoogendorn and Heimpel, 2002; Musser and Shelton, 2003a). Because of the potential for *H. axyridis* to prey on pests in sweet corn, researchers are trying to incorporate this coccinellid, along with other natural enemies, into an integrated pest management (IPM) program for this system (Musser and Shelton, 2003b; Galvan et al., 2005a, b).

Sampling plans are created to provide guidelines for estimating populations over a wide range of densities with an acceptable level of precision (Nyrop and Binns, 1991). A large body of literature exists on sampling plans for pest species; however, relatively little work has been conducted on the sampling of natural enemies (Binns and Nyrop, 1992). Flint and Dreistadt (1998) stated that “monitoring of natural enemies is a key component of IPM and essential for getting a maximum understanding of, and benefit from, biological control”. For Coccinellidae in particular, a paucity of literature exists on the development (Iperti et al., 1988; Elliott et al., 1997; Ren et al., 2000; Musser et al., 2004) and validation (Elliott et al., 1997) of sampling plans to provide density estimates with known levels of precision.

A resampling simulation approach was developed for validating sampling plans (Hutchison et al., 1988; Naranjo and Hutchison, 1997). The use of resampling for validation of sampling plans has been effective for pests in a diversity of systems, including forage crops (Hutchison et al., 1988), row crops (Hodgson et al., 2004), vegetable crops (Burkness and Hutchison 1998; O’Rourke et al. 1998), and stored grains (Subramanyam et al., 1997). We are unaware of this resampling approach being used to validate a sampling plan for a natural enemy.

Sweet corn IPM could benefit from the development and validation of sampling plans for key predators, such as *H. axyridis*. Precise sampling could be used to estimate *H. axyridis* abundance as part of comprehensive action thresholds for sweet corn IPM. In addition, such sampling could also be used in ecological studies to improve our understanding of the biology of this invasive coccinellid. Therefore, the objective of this study was to characterize the spatial distribution and develop and validate fixed-precision enumerative sequential sampling plans for *H. axyridis* in sweet corn.

Materials and methods

Sampling for *H. axyridis* adults and larvae on sweet corn was conducted during the growing seasons of 2000–2004 at the University of Minnesota Outreach Research and Education Park, Rosemount, Minnesota, USA. Sweet corn varieties used in all years were non-transgenic. In 2000, 'Code 40' and 'Code 39' sweet corn (General Mills, Green Giant, Le Sueur, Minnesota) were each sown separately into 6.1× 30.5 m plots on 1 May. A second replicate of both of these varieties was sown on 5 June. For both sowing dates, seed was sown 5.08 cm deep with 76.2 cm rows. In 2001, 'Code 40', was sown on 9 May into a 15.2×15.2 m plot. Seed was sown 3.8 cm deep with 76.2 cm row spacing. In 2002, 'Code 40' was sown on 15 May into a 4 × 5 array of two-row plots, with plots being 7.6 m long. Adjacent plots were separated by a fallow row. Seed was sown 4.5 cm deep with 76.2 cm row spacing. In 2003, 'Code 40' was sown on 8 May into a single 30.5×45.7 m plot. Seed was sown 4.5 cm deep with 76.2 cm row spacing. Additionally, in 2003 and 2004, untreated control plots from the field study described in Galvan et al. (2005a) were used for sampling. In all years, standard sweet corn production practices were followed (Foster et al., 2003).

Plants were sampled using nondestructive, visual whole-plant inspection. Based on the balance between precision and cost (i.e. time), Musser et al. (2004) determined that this sampling method was superior to destructive sampling or sticky cards for monitoring larval and adult *H. axyridis* in sweet corn. The sample unit size for all samples was a single plant, and sample number (i.e. sample size) ranged from 30 to 90 plants. In 2003 and 2004, when samples came from the untreated control plots of a randomized complete block design, the sample was split equally among the replicates. In each plot, samples were taken approximately every week from first silk to harvest. When sampling a plot, plants were selected randomly from the interior of the plot while avoiding the edge rows. Larval and adult *H. axyridis* were identified using a diagnostic guide (Schellhorn, 2003) and stage specific descriptions provided by Koch (2003). However, first instars were omitted from these counts due to difficulties distinguishing first instars among coccinellid species in the field. Stage specific counts of *H. axyridis* were recorded per plant. A total of 42 data sets were collected with mean densities ranging from 0.02 to 11.83 *H. axyridis* larvae and adults per plant.

Based on the sample counts, inferences for the spatial patterns of adults and larvae of *H. axyridis* separately and combined were made using Taylor's power law:

$$s^2 = a\,m^b \tag{1}$$

where s^2 is the sample variance, m is the sample mean, and b and a are the slope and antilog of the intercept, respectively, of the regression of the log variance versus log mean (Taylor, 1961). This variance-to-mean relationship describes the spatial distribution of a population, with $b<1$, $b=1$ and $b>1$ indicating uniform, random and aggregated dispersion, respectively. After randomly selecting and withholding data sets for sampling plan validation (explained below), 24, 30 and 31 data sets were used for estimation of the Taylor's power law parameters for larvae, adults and both stages combined, respectively.

The protocol given by Naranjo and Hutchison (1997) was followed for development and validation of the sampling plans. Using resampling simulation software (Resampling for Validation of Sampling Plans [Naranjo and Hutchison, 1997]), the resulting a and b values from Taylor's power law were used to develop Green's (1970) enumerative sampling plan with precision levels of 0.10 and 0.25 for ecological and pest management purposes, respectively, as suggested by Southwood (1978). Under Green's plan, the sampling stop line is calculated as:

$$T_n \geq \left(a\,n^{1-b}/D^2\right)^{1/(2-b)} \tag{2}$$

where T_n is the cumulative number of individuals sampled, n is the total number of sample units, D is the precision (SEM/mean), and a and b are Taylor's power law parameters. From the data sets collected, 9, 9 and 11 sets for larvae, adults and the two stages combined, respectively, were randomly selected and withheld from the estimation of the Taylor's power law parameters, and were used as independent validation of the sampling plans. These validation data sets were selected to reflect a broad range of population densities. Simulations were used to randomly sample with replacement from each of the validation data sets until the stop lines were crossed, which generally indicates that the desired precision has been achieved. After 500 iterations of sampling from each data set, the mean density, sample number and precision were obtained. The averages across validation data sets of the resulting mean sample number for each of the precision levels are the recommended sample numbers for estimating *H. axyridis* density.

Results and discussion

In our sampling for this study, *H. axyridis* was the most abundant coccinellid, comprising 77.4% of the Coccinellidae observed across all samples (R.L.K. unpublished data); therefore justifying our focus on this single species. For counts of *H. axyridis* larvae, adults, and the two stages combined, log variance increased with increasing log mean (Table 1). The b values from Taylor's power law were significantly greater than one ($p < 0.05$) for larvae and the stages combined, and indicated an aggregated distribution for this species in sweet corn (Table 1). However, the adults showed a random distribution ($b \approx 1$) (Table 1). In comparison, b values for several coccinellid species in wheat (m^2-quadrat sample units), *Triticum aestivum* L., ranged from 0.96 to 1.21 (Elliott et al., 1997).

Park and Obrycki (2004) also found an aggregated distribution for lady beetles, including *H. axyridis* larvae and adults, during peak populations in corn, but a random distribution before and after the

Table 1. Taylor's power law ($s^2 = a\,m^b$) parameters for counts of *Harmonia axyridis* larvae and adults on sweet corn

Stage	a (95% CI)	b (95% CI)	n	r^2
Larvae	1.60 (1.40, 1.82)	1.15 (1.09, 1.21)	24	0.99
Adults	1.16 (0.95, 1.43)	1.03 (0.95, 1.11)	30	0.96
Combined	1.52 (1.35, 1.72)	1.18 (1.09, 1.25)	31	0.97

s^2 is the sample variance, m is the sample mean, b and a are the slope and antilog of the intercept, respectively, of the regression of the log variance versus log mean.

population peak. In addition, *H. axyridis* showed an aggregated distribution on tobera trees, *Pittosporum tobira* (Thunb.) Ait. (Johki et al., 1988). Furthermore, Ren et al. (2000) considered *H. axyridis* larvae to be aggregated on tobacco, *Nicotiana tabacum* L., with $b = 1.019$. The aggregated distribution of this species, along with that of other Coccinellidae, may be due to the aggregated distribution of their prey (Dixon, 2000). Kawai (1976) found that the aggregated distribution for *H. axyridis* larvae on corn can result from a 'trapping effect,' in which larvae, while randomly searching for *R. maidis*, spend relatively more time in areas with high prey density; therefore, over time, resulting in aggregations in those areas. In our study, the random distribution for adults may have been due to the greater mobility of adults compared to larvae. Further work is needed to verify the spatial distribution of this predator in large, commercial sweet corn fields.

To our knowledge, this is the first use of resampling to validate a sampling plan for a natural enemy. Within each precision level for each developmental stage, the sample number required to achieve the desired precision decreased rapidly with increasing mean density of *H. axyridis* per plant (e.g. Figure 1A, C). Across the range of densities of *H. axyridis* per plant, a greater average sample number (ASN) was needed to attain the desired precision at the 0.10 level (ASN = 235, 390 and 205 whole plants for larvae, adults and the combined count, respectively) than the 0.25 level (ASN = 46, 90 and 65 whole plants for larvae, adults and the combined count, respectively) (Table 2). For example, in order to achieve the desired precision level with an average sample number less than 100, the mean density of *H. axyridis* adults and larvae would need to be ~2.0 *H. axyridis* per plant at the 0.10 precision level compared to ~0.25 per plant at the 0.25 precision level (Figure 1A, C). The desired precision levels of 0.10 and 0.25 were between or below their respective maximum and minimum boundaries at all *H. axyridis* densities (e.g. Figure 1B, D). Average precision varied slightly above and below the desired precision levels (e.g. Figure 1B, D). Because of the similarity (overlap of 95% confidence intervals) of the Taylor's power law parameters between *H. axyridis* adults and larvae, the same sampling plan can be used to estimate the density of these two life stages. Therefore, to obtain estimates of *H. axyridis* larval and adult densities in sweet corn with 0.10 or 0.25 precision, we recommend visually inspecting 205 or 65 whole plants, respectively (Table 2). This sample number will provide the desired precision averaged over many

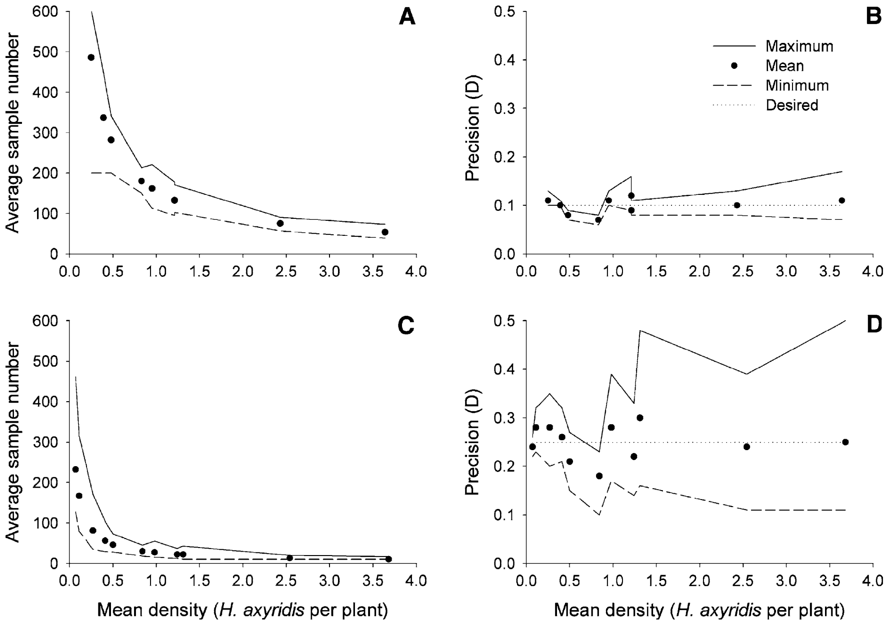

Figure 1. Summary of resampling validation analysis for the combined count of *Harmonia axyridis* larvae and adults on sweet corn showing average sample number (graphs A and C) and average precision level (graphs B and D) for Green's (1970) sequential sampling plan over a range of *H. axyridis* densities. Precision levels for the sampling plan were 0.10 (graphs A and B) and 0.25 (graphs C and D). Taylor's power law parameters were: $a = 1.52$ and $b = 1.18$.

sampling bouts across the range of observed *H. axyridis* densities. In other words, for a specific sampling bout, the average precision might be better (i.e. lower precision value) than desired if densities are high, or worse (i.e. higher precision value) than desired if densities are low.

This work provides a research tool for monitoring populations of a natural enemy in sweet corn. For example, these sampling plans could be used in monitoring for adverse non-target effects of transgenic sweet corn hybrids. Visual whole-plant sampling has been used to monitor natural enemy populations in plots of transgenic and non-transgenic corn (e.g. Pilcher et al., 1997; Wold et al., 2001). However, in these studies, the authors do not address the precision of their estimates for making comparisons among treatments. The sampling plans presented here could be used to more precisely monitor populations of *H. axyridis*, as a surrogate species, for potential adverse effects of

Table 2. Resampling simulation results for validation of Green's (1970) fixed-precision sampling plans using desired fixed-precision levels of 0.10 and 0.25 for counts of larvae and adults of *Harmonia axyridis* on sweet corn

Data set	Observed mean density	Average statistics for 500 sequential sampling iterations [a]						
		Density	Precision (D)			Average sample No. (ASN)		
		Mean	Mean	Min	Max	Mean	Min	Max
			Desired $D = 0.10$					
Larvae	1.20	1.21	0.10	0.08	0.12	235	140	294
Adults	0.46	0.47	0.10	0.09	0.12	390	181	513
Combined	1.25	1.27	0.10	0.08	0.12	205	129	261
			Desired $D = 0.25$					
Larvae	1.08	1.12	0.25	0.16	0.36	46	23	87
Adults	0.38	0.41	0.25	0.19	0.33	90	37	166
Combined	1.04	1.09	0.25	0.16	0.35	65	35	122

[a] Data sets were resampled with replacement using Resampling for Validation of Sampling Plans simulation software (Naranjo and Hutchison 1997).

the transgenic crop. In doing so, more valid conclusions could be drawn about the differences in insect populations among treatments.

Furthermore, these sampling plans could be used to estimate *H. axyridis* densities as part of a decision-making program, in which the ratio of pest to natural enemy densities are used in conjunction with the relationship between pest density and plant damage to make management decisions (e.g. Nyrop and van der Werf, 1994). To obtain a sample number of 65 whole plants for a precision level of 0.25 required a reasonable amount of cost (~1.95 person hours); however, if a precision level of 0.10 is necessary, the cost (~6.15 person hours) to inspect 205 whole plants may be prohibitively high in some circumstances. Nyrop and van der Werf (1994) and Musser et al. (2004) discuss the benefits of classification sampling for natural enemies, which could be incorporated into a monitoring program with less cost than enumerative sampling. However, for enumerative or classification sampling plans to be implemented to incorporate biological control, the in-field predation rates of *H. axyridis* on various pests must be quantified to determine what predator/prey ratio is necessary to suppress pest populations (e.g. Nyrop and van der Werf, 1994). With such information, management decisions could be better made to incorporate natural enemy induced mortality on pest population growth, which should lessen the use of insecticides.

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