

# Development and Validation of a Fixed-Precision Sampling Plan for Estimating Striped Cucumber Beetle (Coleoptera: Chrysomelidae) Density in Cucurbits

ERIC C. BURKNESS AND WILLIAM D. HUTCHISON

Department of Entomology, University of Minnesota, 1980 Folwell Avenue, St. Paul, MN 55108-6125

Environ. Entomol. 27(2): 178-183 (1998)

**ABSTRACT** The striped cucumber beetle, *Acalymma vittatum* (F.), has been identified as one of the most damaging insect pests of vegetables in Minnesota. In an effort to develop practical methods for estimating adult beetle density, beetles were sampled in cucurbit fields throughout central and southern Minnesota during 1994-1995. *A. vittatum* samples were collected in several cucurbit crops including cucumber, pumpkin, and squash. A sample unit consisted of 7 consecutive plants within a row. Beetle counts were recorded for each of the 7 plants in a sample unit and the sample size consisted of a total of 48 sample units in each field. Counts from individual plants in a sample unit were combined to evaluate progressively larger sample units of 1, 2, . . . 7 consecutive plants. An enumerative fixed-precision sampling plan was developed based on Green's method and the Taylor power law. Performance of the plan was validated using bootstrap (resampling) simulations and 9 independent data sets. Final analysis indicated that a sample unit of 2 consecutive plants provided the highest relative net precision (RNP) for estimating *A. vittatum* adult density. On average, over a range of densities, thirty 2-plant sample units per field were necessary to maintain the desired precision ( $SEM/\bar{x}$ ) level of 0.25. This plan should aid further research on *A. vittatum* population dynamics, the yield-density relationship, or the correlation between density and incidence of bacterial wilt. This plan also can be used in tandem with recently developed economic thresholds for pest management decision making.

**KEY WORDS** *Acalymma vittatum*, cucurbits, enumerative sampling, relative net precision, bootstrap simulation, resampling software

THE STRIPED CUCUMBER BEETLE, *Acalymma vittatum* (F.), is a major pest of cucurbitaceous crops in the north-central United States. A recent survey of Minnesota vegetable growers indicated that *A. vittatum* was the number 2 insect pest of vegetables in the state (Subramanyam et al. 1993). In the absence of insecticide control, potential annual losses from  $\approx 1,400$  ha of cucurbits were estimated to exceed \$2.6 million in Minnesota (Noetzel et al. 1985). Because of the damage potential of *A. vittatum*, via direct defoliation or vectoring of bacterial wilt, and historical insecticide use (Noetzel et al. 1985), it was necessary to develop an integrated pest management (IPM) program in Minnesota. Specifically, we initiated research to develop practical sampling plans and economic thresholds to aid growers in making informed pest management decisions (Burkness 1996).

Density estimation, or enumerative sampling, is often used for research purposes but also has use for development and implementation of sequential sampling plans for IPM (Hutchison 1994). As with other sequential sampling methods, this approach for enumerative sampling uses a flexible sample size, often reducing sample size up to 50%. The primary use of enumerative sequential sampling plans is to estimate population density. Density estimates then can be

used in conjunction with economic injury levels (EIL), or economic thresholds, to determine pest status. Estimates of population density using sequential sampling can provide a predetermined, fixed level of precision at minimum cost (Hutchison 1994), and requires that all individuals be recorded on each plant examined.

The purpose of this article was to develop and validate a sequential sampling plan based on Green's (1970) method, using bootstrap resampling simulations, for use in research as well as IPM applications.

## Materials and Methods

**Data Sets.** In 1994 and 1995, in central and southern Minnesota (Anoka, Dakota, Dodge, Mower, Steele, and Wright counties), 20 fields of either cucumber, *Cucumis sativus* (L.); pumpkin, *Cucurbita moschata* (Duchesne) Poir.; or squash, *Cucurbita pepo* (L.) were sampled for adult *A. vittatum* populations. In total, 36 data sets were collected over 19 dates. Fields ranged in size from 0.4 to 40 ha.

Plants from the edge of the field as well as the center portions of the field were sampled. Fields were sampled using a stratified design to ensure adequate coverage of each field. Fields were stratified into 4 quad-

rants. The 1st plant of a sample unit was selected by walking in a random direction within a quadrant, then stopping arbitrarily after  $\approx 15$  s. The sample unit then began with the 1st plant directly facing the sampler. Each subsequent sample unit was located following the same procedure, walking in any direction, in most cases not more than 20–30 steps and stopping arbitrarily. Twelve sample units were collected per quadrant for a total of 48 sample units in a field (with the exception of 2 occasions where data for 24 sample units were recorded).

Each sample unit consisted of 7 consecutive plants within a row. Data were recorded for each of the 7 consecutive plants and were later divided into separate sample units of 1, 2, . . . 7 consecutive plants for analysis. Sample size and sample unit were selected to ensure that, for most beetle densities, the data sets would be large enough to include subsequent estimates of the optimum sample size and sample unit. To provide a realistic time interval for sampling and to minimize possible error involved with sampling larger plants, beetles were counted from plants that were at the cotyledon to 4th true-leaf growth stage. Each plant within a sample unit was checked completely, as well as the soil surface in a 30-cm radius around the plant.

**Sample Unit Analysis.** Individual sample units were defined by combining data for consecutive plants sampled (e.g., plants 1 and 2 were combined for a sample unit of 2; plants 1, 2, and 3 for a sample unit of 3, up to a sample unit of 7 consecutive plants) and then placing each sample unit on a per-plant basis. Sample unit analysis was done by calculating relative net precision (RNP) (e.g., Pedigo et al. 1972, Bechinski and Pedigo 1982).

RNP gives equal consideration to precision ( $SEM/\bar{x}$ ) and time as variables. The higher the precision (or lower relative variation) and lower the cost, the higher the RNP, which indicates a more efficient plan. RNP is calculated by:

$$(1/RV * C_s) * 100, \quad [1]$$

where  $RV$  is relative variation ( $= [SEM/\bar{x}] * 100$  [Southwood 1978]) and  $C_s$  is the total cost, in time, to collect a given number of sample units, usually measured in person-hours.

Conventional RNP calculations were done on all 36 fields using a fixed sample size of 48 samples per field. Precision was calculated based on the means and variances of all 48 samples. Cost (time) estimates of sampling were 15 s per plant and on average 15 s to walk to each new sample site.

RNP calculations were also done using actual mean estimates of sample size and precision from Green's plan, following resampling validation with Resampling Validation for Sample Plans (RVSP) software (Naranjo and Hutchison 1997). Naranjo and Hutchison (1997) provide a detailed explanation of RVSP, and its advantages for development and validation of sequential sampling plans for arthropods. For resampling analysis, 23–27 data sets (depending on sample unit) were selected to represent a range of *A. vittatum* densities. Nine data sets were excluded from the orig-

inal 36 to be used eventually as independent data for final resampling validation of optimum sample size. The spatial pattern for each sample unit was assessed using the Taylor power law,  $s^2 = am^b$ , which describes the variance-to-mean relationship (Taylor 1961). Resampling analysis uses the  $a$  and  $b$  parameters from Taylor's power law in Green's (1970) plan, to generate actual estimates of precision and sample size based on independent data sets. An initial precision level of 0.25 was selected as acceptable for pest management purposes (Southwood 1978). The sampling stop line for Green's (1970) plan is calculated by:

$$T_n \geq (an^{1-b}/D^2)^{1/(2-b)}, \quad [2]$$

where  $T_n$  is the cumulative number of individuals sampled,  $n$  is the total number of samples,  $D$  is the precision ( $SEM/\bar{x}$ ), and  $a$  and  $b$  are Taylor's power law parameters. All resampling analyses for sample unit, using Green's plan, were based on 500 sampling runs (bouts) of each data set and sample unit. Resampling also was done with replacement because of low population densities. RNP, precision, and sample size obtained from RVSP were used to make final comparisons among the different sample units.

**Sample Size Analysis.** Following the protocol given by Naranjo and Hutchison (1997), 9 data sets were held out from the original 36 for use in validating the sampling plan. The 9 data sets were not used for estimation of Taylor's  $a$  and  $b$ , and reflect *A. vittatum* adult densities likely to be encountered in midwestern cucurbit fields (Brust et al. 1996, Burkness 1996), ranging from 0.1 to 2.5 beetles per plant. The simulation selected samples randomly, with replacement, from a given data set until the stop line was exceeded. A fixed-precision level of 0.25 and the  $a$  and  $b$  parameters (1.33 and 1.31, respectively) for the optimum sample unit of 2 consecutive plants were used in the simulation. The simulation was run using the 9 independent data sets. Mean actual density, sample size, and precision were obtained based on 500 sampling bouts for each data set. Mean actual precision was then examined to determine if it was above or below the desired precision level of 0.25. If the mean actual precision level was above or below the desired precision level, the precision level was adjusted from 0.25 to achieve a final mean actual precision of 0.25. Subsequent mean actual sample size (ASN) was then selected as the optimum sample size for the range of beetle densities tested.

## Results and Discussion

**Sample Unit Analysis.** Taylor's power law regression showed a positive correlation between variance and mean for all sample units, with  $r^2$  values  $\geq 0.88$  (Table 1). The  $b$  value for all sample units was found to be significantly  $>1$  ( $P = 0.05$ ) using a  $t$ -test comparison (Table 1). Values for  $b > 1.0$  suggest an aggregated spatial distribution. Radin and Drummond (1994) observed similar results in their study of *A. vittatum* on cucumber and squash, where  $b = 1.986$ . Aggregated distribution patterns seem appropriate for

Table 1. Taylor power law (variance-mean relationship) statistics for *A. vittatum* sampling data collected in cucurbits, southern Minnesota, 1994-1995

Sample unit, no. plants	Regression statistics ( $\pm$ SEM)				Density range <sup>b</sup>
	<i>a</i>	<i>b</i> <sup>a</sup>	<i>r</i> <sup>2</sup>	<i>n</i>	
1	2.621 $\pm$ 0.22	1.386 $\pm$ 0.11	0.88	23	0.04-2.65
2	1.335 $\pm$ 0.21	1.307 $\pm$ 0.08	0.92	25	0.01-2.13
3	0.979 $\pm$ 0.18	1.293 $\pm$ 0.07	0.94	26	0.01-2.08
4	0.800 $\pm$ 0.18	1.286 $\pm$ 0.06	0.95	27	0.01-2.13
5	0.658 $\pm$ 0.19	1.271 $\pm$ 0.06	0.95	27	0.01-2.13
6	0.585 $\pm$ 0.20	1.266 $\pm$ 0.06	0.94	27	0.01-2.36
7	0.566 $\pm$ 0.21	1.266 $\pm$ 0.07	0.93	27	0.01-2.39

<sup>a</sup> All *b* values were significantly  $>1$  ( $P = 0.05$ ) using *t*-tests. Values for *t* were as follows: sample unit 1 = 3.55, *df* = 22; sample unit 2 = 3.73, *df* = 24; sample unit 3 = 4.33, *df* = 25; sample unit 4 = 4.92, *df* = 26; sample unit 5 = 4.66, *df* = 26; sample unit 6 = 4.35, *df* = 26; sample unit 7 = 4.03, *df* = 26.

<sup>b</sup> Density converted to mean number of beetles per plant, regardless of sample unit size.

*A. vittatum*, given their intense mating behavior (i.e., attempting to mate with both males and females) (Gould 1944).

Several studies have compared different sampling methods by estimating RNP (Bechinski and Pedigo 1982, Pedigo et al. 1972). RNP allows one to determine the best balance between cost (time) and precision. For *A. vittatum*, we used RNP to evaluate sample units ranging from 1-7 consecutive plants. RNP analysis based on a fixed sample size of 48 (e.g., Pedigo et al. 1972, Bechinski and Pedigo 1982) indicated that a sample unit of 1 plant was optimum (Table 2). These results reflect a rapid increase in cost (time) as sample unit size increases, compared with a more gradual increase in precision. As Table 2 shows, a sample unit of 1 plant provided an average precision level of 0.28, which is slightly lower than desired, and sample units 2-7 all had precision levels  $\leq 0.20$ .

RNP estimates based on the use of the sequential sampling plan, with flexible sample size (via RVSP validation), indicated a sample unit of 2 consecutive plants provided the best balance between cost (time) and precision (Table 3). Resampling analysis using Green's plan for *A. vittatum* showed that sample units of 2 and 3 consecutive plants provided very similar estimates of RNP (Table 3), but a sample unit of 2 consecutive plants required  $\approx 10$  fewer plants to be sampled. Therefore, a sample unit of 2 consecutive plants was considered optimum for density estimation using Green's sequential sampling plan. By contrast,

RNP analysis using a fixed sample size ( $n = 48$ ), where a 1-plant unit was optimum (Table 2), revealed how the conventional analysis can lead to either too few or too many plants being examined, resulting in increased cost. Estimates of optimum sample unit, obtained from the simulations run with RVSP, should provide more realistic estimates of RNP because it is based on a variable sample size, indicative of sequential sampling plans.

**Sample Size Analysis.** Resampling analysis for Green's plan, using a sample unit of 2 consecutive plants and a desired precision of 0.25, resulted in an actual average precision of 0.23 and an actual average sample number (ASN) of 37 2-plant samples per field (Table 4). This was more conservative than generally required for IPM application (e.g., Southwood 1978, Hutchison 1994). Therefore, additional RVSP simulations were run with lower levels of precision. A fixed precision level of 0.28 was subsequently found to provide an actual average precision level of 0.25, with an ASN of 30 per field (see overall mean, Table 4). This resulted in the desired average precision for IPM purposes, with 7 fewer samples per field. The over-estimation of sample size, resulting in a higher than necessary precision level, was discussed by Hutchison et al. (1988) in a comparison of Kuno's and Green's fixed-precision sampling plans. Using a resampling approach, Hutchison et al. (1988) found that the fixed-precision level often could be relaxed (e.g.,  $>0.25$ ) to

Table 2. Relative net precision (RNP) and mean precision calculations using a fixed sample size of  $n = 48$  for *A. vittatum*, southern Minnesota, 1994-1995

Sample unit, no. plants	Total plants	Mean precision	Time (h) <sup>a</sup>	Mean RNP <sup>b</sup>
1	48	0.28	0.40	10.27
2	96	0.20	0.60	9.18
3	144	0.17	0.80	7.90
4	192	0.16	1.00	6.76
5	240	0.15	1.20	6.21
6	288	0.14	1.40	5.71
7	336	0.13	1.60	5.28

<sup>a</sup> Time calculated by:  $((ASN * 15 s) + (total plants * 15 s)) / 60 s / 60 min =$  sample time (h).

<sup>b</sup> RNP (relative net precision) =  $(1/RV(C)) 100$  (e.g., Pedigo et al. 1972), based on a conventional fixed sample size ( $n = 48$ ).

**Table 3.** Sample unit analysis for *A. vittatum* enumerative sequential sampling plan; mean precision, relative net precision (RNP), and average sample number (ASN) based on resampling analysis of Green's fixed-precision sequential sampling plan,  $D = 0.25$ ; southern Minnesota, 1994-1995

Sample unit (number of plants)	Actual mean				
	Sample no. (ASN)	Total plants	Precision (D)	Time (h) <sup>a</sup>	RNP <sup>b</sup>
1	60.6	60.6	0.25	0.51	10.47
2	35.0	70.0	0.24	0.44	12.60
3	26.6	79.8	0.24	0.44	12.61
4	22.9	91.6	0.23	0.48	11.65
5	19.7	98.5	0.22	0.49	11.36
6	17.8	106.8	0.22	0.52	10.93
7	17.6	123.2	0.22	0.59	10.13

RVSP analysis for data sets where mean *A. vittatum* densities ranged from 0.1 to 2.5 beetles per plant.

<sup>a</sup> Time calculated by:  $((ASN * 15 s) + (total\ plants * 15 s)) / 60 s / 60 min = sample\ time\ (h)$ .

<sup>b</sup> RNP (relative net precision) =  $(1/RV(C)) 100$  (e.g. Pedigo et al. 1972), but based on actual ASN for each sample unit (RVSP results), versus a conventional fixed sample size.

achieve the desired actual average precision (e.g., 0.25).

Sample size requirements for achieving a desired precision level of 0.25 declined rapidly as mean density increased (Fig. 1), and the maximum mean sample size for densities of 0.25 beetles per plant was  $\approx 75$ . Once mean density increased above 0.25 beetles per plant, mean sample size declined to  $< 35$ . At low mean densities ( $< 0.25$  beetles per plant), actual mean precision exceeded the desired level of 0.25. At higher mean densities ( $> 0.25$  beetles per plant), the actual mean precision was better than desired or exceeded the desired precision by  $< 0.05$  (Fig. 2). The desired precision level of 0.25 fell within the maximum and minimum boundary and corresponded closely with the actual mean precision level (Fig. 2). In general, the

modified fixed-precision sequential sampling plan, with precision set at 0.28, performed well for density estimation over a range of *A. vittatum* population densities.

Until recently, sampling plans for *A. vittatum* have not been developed. Radin and Drummond (1994) developed a sampling plan and determined the sample size necessary to achieve a desired level of precision at a given density. However, their plan did not consider evaluation of optimum sample unit, and was based only on 10 data sets collected for 1 growing season from the same plot. In contrast, the results presented here were based on 36 data sets collected from 6 counties in Minnesota over 2 growing seasons. Therefore, this plan should provide a reliable estimate of *A. vittatum* density on cucurbits, particularly for

**Table 4.** RVSP simulation results used to validate Green's fixed-precision sequential sampling plan for *A. vittatum* using desired fixed-precision levels of 0.25 and 0.28, for a sample unit of 2 consecutive plants

Field	Observed mean density	Average statistics for 500 sequential sampling simulations						
		Density	Precision (D)			Sample no. (ASN)		
		Mean	Mean	Max	Min	Mean	Max	Min
Desired D = 0.25								
1	0.14	0.15	0.33	0.41	0.23	87	162	45
2	0.21	0.22	0.19	0.25	0.14	64	105	42
3	0.44	0.46	0.24	0.37	0.15	39	55	22
4	0.45	0.45	0.17	0.24	0.11	39	53	28
5	0.56	0.60	0.23	0.34	0.14	32	51	19
6	0.93	0.97	0.26	0.35	0.18	24	38	13
7	1.14	1.15	0.17	0.24	0.09	20	31	15
8	1.78	1.85	0.22	0.35	0.10	15	23	10
9	2.44	2.55	0.23	0.37	0.13	12	19	10
Overall mean	0.90	0.93	0.23	0.32	0.14	36.9	59.7	22.7
Desired D = 0.28								
1	0.14	0.15	0.36	0.51	0.24	71	150	29
2	0.21	0.22	0.21	0.28	0.14	52	80	34
3	0.44	0.46	0.26	0.44	0.14	31	47	17
4	0.45	0.46	0.19	0.25	0.13	31	46	20
5	0.56	0.61	0.25	0.39	0.13	26	42	14
6	0.93	0.97	0.29	0.40	0.18	19	35	11
7	1.14	1.16	0.19	0.27	0.10	16	24	11
8	1.78	1.82	0.24	0.40	0.08	12	21	10
9	2.44	2.50	0.26	0.45	0.13	11	16	10
Overall mean	0.90	0.93	0.25	0.38	0.14	29.9	51.2	17.3

Regression parameters for the Taylor's power law for a sample unit of 2 consecutive plants;  $a = 1.335$  and  $b = 1.307$  (see Table 3).

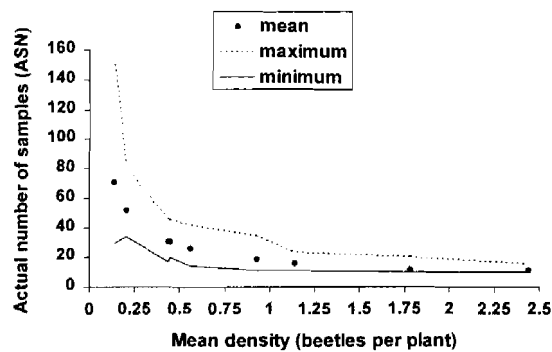


Fig. 1. Summary of resampling (RVSP) validation analysis showing actual average sample number (ASN) for Green's sequential sampling plan over a range of *A. vittatum* adult densities (independent data sets), where the sample unit is 2 consecutive plants; Green's plan parameters were:  $D = 0.28$ ,  $a = 1.335$ ,  $b = 1.307$  (actual mean  $D = 0.25$ ).

beetle densities commonly observed in the Midwest (Burkness 1996, Brust et al. 1996). RVSP simulations to validate Green's fixed-precision sampling plan showed that an average of thirty 2-plant sample units provided a final actual mean precision level of 0.25.

Resampling analyses, using RVSP or similar software, also have been used successfully for the validation of sequential sampling plans for the pea aphid, *Acyrtosiphon pisum* (Harris) (Hutchison et al. 1988); flower thrips, *Frankliniella* spp. (Cho et al. 1995); sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (Naranjo and Flint 1995); leaf-curling plum aphid, *Brachycaudus helichrysi* (Kaltenbach) (Badenhausser 1996); and the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Subramanyam et al. 1997). In each of these studies, data were analyzed using bootstrap simulations with Green's (1970) plan and the Taylor

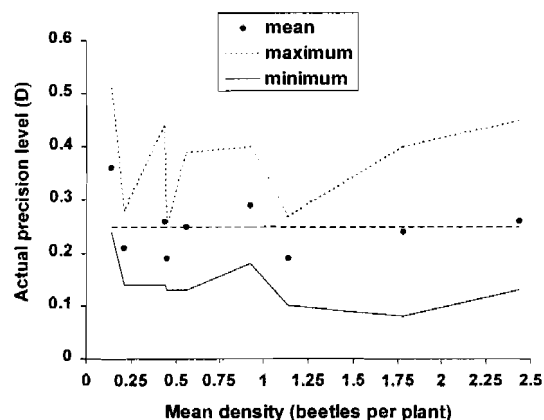


Fig. 2. Summary of resampling (RVSP) validation analysis showing actual precision level ( $D$ ) for Green's sequential sampling plan over a range of *A. vittatum* adult densities (independent data sets), where the sample unit is 2 consecutive plants; Green's plan parameters were:  $D = 0.28$ ,  $a = 1.335$ ,  $b = 1.307$ . Dashed horizontal line shows the desired precision level of 0.25.

power law. Although the variance-mean relationship must fit the Taylor power law to provide a valid sampling program, and independent data sets are necessary for validation, the resampling approach is intuitively attractive because it does not rely on theoretical sampling distributions characteristic of Monte Carlo analyses (e.g., Nyrop and Binns 1991).

In summary, this sampling plan should allow for efficient estimation of *A. vittatum* density for both research and IPM applications. For research, this plan should be useful for estimating *A. vittatum* population density over time and relating population levels of *A. vittatum* to defoliation injury, or the incidence of bacterial wilt. For IPM, the plan can be used in tandem with recently developed economic injury levels of 0.1–6.5 beetles per plant for processing cucumbers (Burkness 1996), or action thresholds of 0.5–1.0 beetles per plant for muskmelon (Brust et al. 1996).

#### Acknowledgments

We thank Sandy Bird, Pat Bolin, Laurie Cooper, Brady Lenzen, Andy Miller, and Patrick O'Rourke for assistance in the field and with data analysis. We thank Steve Naranjo (Western Cotton Research Laboratory, USDA-ARS, Phoenix, AZ) and Roger Moon (Department of Entomology, University of Minnesota) for their suggestions and comments in reviewing a draft of the article. This work was supported by North Central Region Pesticide Impact Assessment Program Project No. 583 (Ohio State University Research Fund No. 675841) "Loss of Furadan 15G for Cucumber Beetles on Cucurbits: Biological, Environmental and Economic Impact of Alternatives," and the University of Minnesota Agricultural Experiment Station. This article is No. 971170109 of the Minnesota Agricultural Experiment Station.

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*Received for publication 11 April 1997; accepted 3 October 1997.*

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