The Great Lakes Entomologist

Volume 22
Number 3 - Fall 1989 *Number 3 - Fall 1989*

Article 3

October 1989

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SAMPLING TECHNIQUE FOR LARVAE OF THE ALFALFA SNOUT BEETLE, *OTIORHYNCHUS LIGUSTICI* (COLEOPTERA: CURCULIONIDAE)

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ABSTRACT

This paper presents a sampling procedure for estimating larval populations of the alfalfa snout beetle, *Otiorhynchus ligustici*. The method is based on counts of the larvae taken in 16 × 16 cm quadrats of soil during early fall when the grubs are in their final two instars and feeding just below the crowns of the plant. Analysis of sampling variability showed that 200 quadrats per field are necessary to obtain adequate precision for intensive population studies but that 50 quadrats are sufficient for survey work. The pattern of counts was overdispersed but conformed to the negative binomial distribution.

The alfalfa snout beetle (ASB), *Ortiorhynchus ligustici* (L.), is a relatively new pest of forage crops in the lower Great Lakes region of Canada. It was first discovered on the eastern Ontario mainland in 1986 (Loan et al. 1986) and is currently restricted to a 12 km^2 area in south Grenville County (Harcourt and Guppy 1987).

The life cycle of the snout beetle in Ontario requires 2 years (Guppy and Harcourt 1989) and there are even- and odd-year broods, Brood A and Brood B, respectively, based on the year of adult activity. The adults emerge in early spring and lay their eggs in the soil near the alfalfa crowns. These hatch in early June and the young grubs feed on the root hairs and fine lateral roots, gradually moving deeper into the soil during the summer months. The third to fifth instars feed at soil depths of 20 to 30 cm but the final two instars move upward in early fall to feed on the tap roots at levels of 5 to 15 cm. In November, the fully-fed larvae retreat to a depth of 20 to 30 cm and hibernate until June of the following year when they pupate. The resulting adults remain dormant for ca. 9 months.

Owing to its cryptic habits and the small size of the early instars, a census of ASB populations during spring and summer is costly and labor-intensive (Mellors et al. 1982). On the other hand, sampling in early fall, when the grubs are almost fully grown and are feeding just below the crowns of the plant, would appear to be economically feasible, not only for surveys, but for more intensive studies aimed at detecting population change from generation to generation. This paper describes methods for estimating numbers of such larvae, and reports on the analysis of data leading to the development of a reliable sampling system.

SAMPLING METHODS

The study was carried out during 1987 and 1988 in 2- to 4-year old stands of alfalfa on five dairy farms within the new population epicentre (Loan et al. 1986). In each of the two

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years, numbers of large larvae (instars 6 and 7) were estimated on 14 occasions. The sampling was carried out in late September and October when the grubs were feeding in the tap roots just below the alfalfa crowns. Prior to sampling, an area measuring 30 × 30 m was marked off within the fields and divided into four blocks of equal size. These were further subdivided into four equal plots. Then, four sample units were selected at random from within each plot, making a total of 64.

The sample unit was a 16 × 16 cm quadrat of soil, 15 cm deep, containing one or more crowns of alfalfa. It was selected as the smallest entity that could be removed by shovel conveniently. In preliminary studies, this unit was compared for efficiency to a quadrat double this size; relative efficiency, defined as the reciprocal of the product of variance per m^2 and cost in human-minutes (hm), was 94% greater for the smaller unit. The quadrats were delineated by a metal frame and the soil was removed systematically to unearth the grubs. A total of 4.5 hm was required to select and process a single quadrat.

Numbers of larvae were recorded in each quadrat. The totals for individual sample units ranged from 0 to 58 and means per population sample (of 64 units), from 0.27 to 12.16.

STATISTICAL ANALYSIS

Detection of the spatial pattern. Using the MLP program of Ross (1987), the Poisson (variance (s^2) = mean (m)) and negative binomial ($s^2 = m + m^2/k$) distributions were fitted to the 28 sets of data and the goodness-of-fit was tested by χ^2. When Poisson distributions were fitted to the observed distributions, discrepancies between observed and expected values were significant in all 28 sets. However, the frequencies of all counts closely approximated the negative binomial series, and none of the deviations between observed and expected values was significant. Individual k values ranged from 0.16 to 2.33, implying a good deal of overdispersion.

The logarithmic relationship between variance and mean (Taylor 1961) for the 28 counts is illustrated in Fig. 1. A regression analysis showed that the slopes and intercepts for the two years (i.e. broods) were not significantly different; hence, a relationship with one slope and one intercept was used for the entire dataset. The overdispersed nature of the data is again clearly shown by the plotted values, which depart noticeably from the 45° line of Poisson expectation to attain a slope of 1.41 ($s^2 = 3.28\ m^{1.41}$). Therefore the transformation required to obtain homogeneous variances is $x^{0.3}$ ($0.3 = 1 - 1.41/2$).

Analysis of variance. The statistical methods used in this study follow those of Harcourt and Binns (1980). The counts were analysed using a nested analysis of variance (among blocks, among plots in blocks, and among quadrats in plots) and the data were stabilized by the transformation $x^{0.3}$, where x is equal to the observed count. The analysis of variance is illustrated in Table 1, using one of the 28 sets of data.

Analysis of the 28 larval counts showed that variation between blocks and plots was significant in 13 and four cases, respectively. This implied that considerable heterogeneity occurred throughout the field. For this reason, it was deemed advisable to adopt a single stage sampling procedure and to develop a sampling plan that provides for a number of sample units well spread over the field.

Sampling precision. Table 2 lists the estimates of population density together with their standard errors in the untransformed scale. Mostly, the latter were within 20% of the mean. They exceeded it on nine occasions.

OPTIMUM ALLOCATION OF SAMPLING RESOURCES

To avoid interpretive problems associated with presenting sampling recommendations in a transformed scale, raw data were used to investigate the sample size for predetermined confidence limits. The inter-quadrat percent coefficients of variation (CV) were derived as 100s/m. These ranged from 73 to 250 (Table 2).

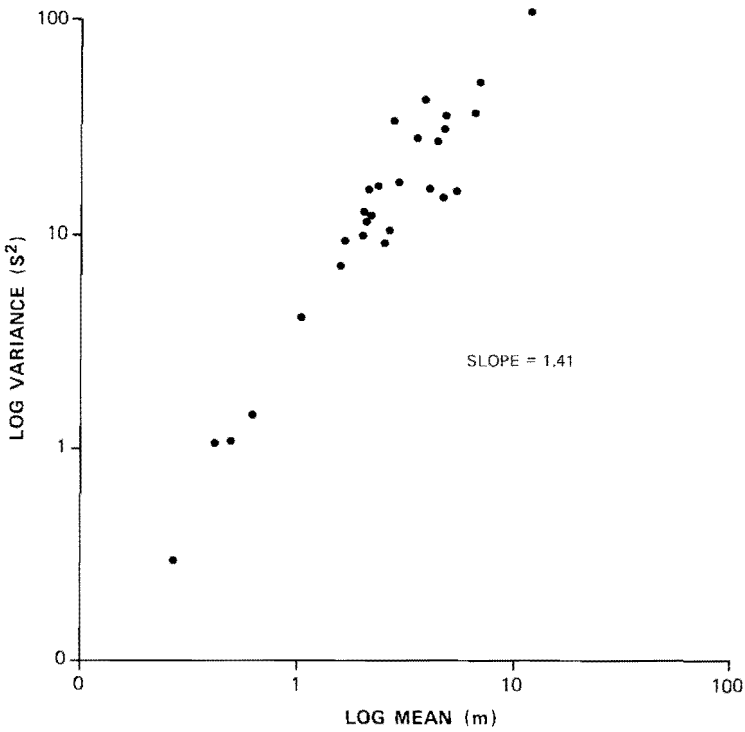

Figure 1. Variance-mean relationship for 28 counts of *O. ligustici* larvae from quadrats of soil. Each data point is based on a sample of 64 quadrats.

Table 1.—Results of analysis of variance[a] for counts of large larvae of the alfalfa snout beetle, 24 September 1987.

Source of variation	df	Observed mean square	F
Blocks	3	2.677	6.168
Plots within blocks	12	0.434	0.814
Quatrats within plots	48	0.533	

[a]Transformed scale, $y = x^{0.3}$

The number of sample units corresponding to a confidence interval of width p% of the mean (m) is given by

$$N_s = (CV/p)^2 \quad (1)$$

where CV is the percent coefficient of variation and p is the level of precision (Snedecor and Cochran 1967).

Using this equation, values for N_s were obtained for two levels of precision corresponding to the 28 counts (Table 2). To obtain an overall sample requirement, the

Table 2.—Estimates of the number of quadrats required for two levels of precision in sampling for larvae of the alfalfa snout beetle.

Sample	Mean number per quadrat	CV	Precision level 10%	20%
		1987 (Brood B)		
1	2.63 ± 0.457	139	194	49
2	2.97 ± 0.519	140	196	49
3	2.67 ± 0.402	120	144	36
4	2.16 ± 0.416	154	237	60
5	2.16 ± 0.442	164	269	68
6	1.05 ± 0.253	193	372	93
7	2.31 ± 0.508	176	310	78
8	12.16 ± 1.318	87	76	19
9	2.19 ± 0.423	154	237	60
10	6.56 ± 0.764	93	87	22
11	2.14 ± 0.388	145	210	53
12	0.50 ± 0.148	201	404	101
13	0.27 ± 0.067	199	396	99
14	5.28 ± 0.502	76	58	15
		1988 (Brood A)		
1	0.41 ± 0.128	250	625	157
2	1.69 ± 0.383	181	328	82
3	3.97 ± 0.815	164	269	68
4	7.05 ± 0.876	99	98	25
5	0.47 ± 0.126	214	458	115
6	1.56 ± 0.338	173	299	75
7	4.75 ± 0.747	126	159	40
8	3.70 ± 0.665	144	208	52
9	2.16 ± 0.496	184	339	85
10	2.55 ± 0.375	118	139	35
11	4.80 ± 0.696	116	135	34
12	3.86 ± 0.502	104	108	27
13	4.44 ± 0.405	73	53	14
14	4.59 ± 0.662	115	132	33

CVs in Table 2 were averaged to determine the precision corresponding to certain given values of N_s (average CV = 146). Precisions corresponding to sample sizes of 50, 100, 150, and 200 quadrats are given in Table 3. These results indicate that an acceptable level of precision (10%) for intensive population studies would require 200 quadrats per field. At the same time, they indicate that an adequate level of precision (21%) for survey work, or for studies that require only a measure of gross change in population density (Southwood 1978, Horn 1988), would require just 50 quadrats per field.

It should be noted, however, that the actual sampling requirement will vary with population density. Using equation (1) along with the variance–mean relationship, $s^2 = 3.28\ m^{1.41}$, the equation for N_s becomes

$$N_s = 32800/(m^{0.59}\ p^2) \qquad (2)$$

(eg. Harcourt et al. 1983). The values for N_s based on equation (2) agree well with those obtained directly from Table 2. Thus equation (2) can be used to plot N_s against m for any value of p.

Table 3.—Levels of precision and sampling time corresponding to certain values of N_s for larvae of the alfalfa snout beetle.

N_s	Percent precision (p)[a]	Time, in hm[b]
200	10	900
150	12	675
100	15	450
50	21	225

[a] $p = CV/\sqrt{N_s}$, where CV = 146
[b] 4.5 hm per quadrat

DISCUSSION

In determining the sample size for target insect stages, it is important to evaluate the costs of sampling. In these terms, a total of 900 hm would be needed to select and process 200 quadrats (Table 3). Thus a team of two persons would require a 7.5 h day to take a sample for intensive studies of ASB larval populations. However, the same team would require less than 2 h to take a 50-quadrat sample for survey purposes.

In the foregoing statistical appraisal, untransformed data were used to set the sample size for specified precision. As a rule, we have found that sampling plans based on log-transformed data tend to suggest smaller sample sizes; therefore, in situations where such a transformation is appropriate, a plan based on non-transformed data should ensure adequate precision. It is likely that the same conclusion holds for a transformation like $x^{0.3}$.

ACKNOWLEDGMENTS

The authors are grateful to J. S. Walker and V. J. Margetts of the Plant Research Centre for technical assistance and to F. Meloche for fitting the spatial distributions. This article is contribution number 1193 of the Plant Research Centre and number C087 of the Engineering and Statistical Research Centre.

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