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**POPULATION ASSESSMENT DURING THE ADULT STAGE  
OF THE ALFALFA BLOTCH LEAFMINER, *AGROMYZA FRONTELLA*  
(DIPTERA: AGROMYZIDAE)<sup>1</sup>**

D. G. Harcourt<sup>2</sup>

ABSTRACT

This paper presents a sampling procedure for estimating adult populations of the alfalfa blotch leafminer, *Agromyza frontella* (Rondani). The method is based on counts of the flies as they emerge from the soil following adult metamorphosis, taken in a series of funnel traps. Analysis of sampling variability showed that 80 traps per field will give adequate precision for life table studies in alfalfa. The pattern of counts was overdispersed but conformed to the negative binomial distribution.

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This paper is the third in a series dealing with sampling procedures for natural populations of the alfalfa blotch leafminer, *Agromyza frontella* (Rondani) in fields of alfalfa. Earlier papers considered population assessment of the egg and larval stages which occur on the plant (Harcourt and Binns 1980a), and the prepupal and pupal stages which are soil-borne (Harcourt and Binns 1980b). This article describes a method for estimating numbers of adults as they emerge from the soil, and the analysis of data leading to development of a reliable sampling plan for this stage. The technique is so designed that the data obtained can be integrated directly into life tables.

The life history of the leafminer has been described by Guppy (1981). In eastern Ontario there are typically three generations a year. Briefly, the adults emerge from hibernation in early spring and deposit their eggs within the alfalfa leaflets. The larvae feed between the epidermal tissues, developing a characteristic blotch-type mine during the course of three instars. The mature larvae emerge from the mined leaflets and drop to the ground where they pupate just beneath the soil surface. The insect overwinters as a partially developed pupa that completes its development in early spring. Peaks of adult emergence occur in late May, early July, and early August.

EXPERIMENTAL PLOTS

The data were collected during 1980–1981 in conjunction with life table studies of the pest at two locations in eastern Ontario, the Central Experimental Farm, Ottawa, and the College of Agricultural Technology, Kemptville. The fields, each of which comprised a 0.4 ha (1 ac) stand of pure Vernal alfalfa, were situated in flat terrain and contained an average of 440 stems per m<sup>2</sup> during the course of the study. Soil types ranged from clay loam to sandy loam.

SAMPLING METHODS

The adults were trapped as they emerged from the soil in each generation. Following harvest of the crop, an area measuring 30 × 30 m (100 × 100 ft) was marked off within each field and divided into four blocks of equal size. These were further subdivided into four equal plots and four traps were placed at random within each plot, making a total of 64.

The traps comprised 22-cm diameter plastic funnels<sup>3</sup> fitted with 24-oz screw-cap styrene jars as shown in Figure 1. These were inverted over the crop stubble and secured by a nail

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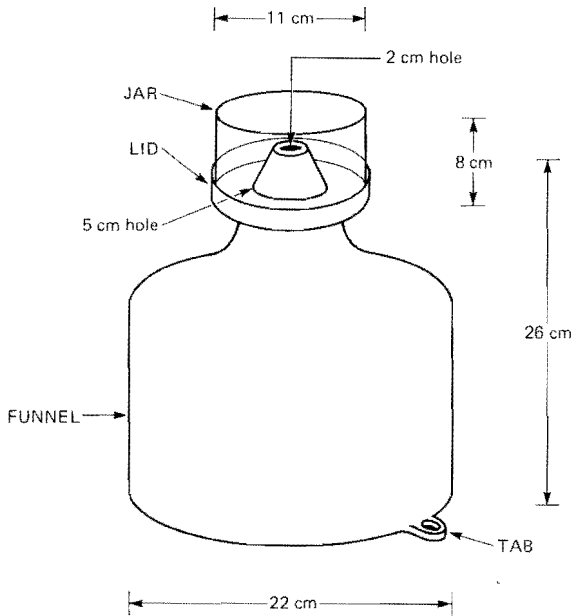


Fig. 1. Emergence traps used for *A. frontella* adults. The flies are trapped in ethylene glycol contained in the lid of the jar. The union between lid and funnel is sealed with plastic glue.

driven through the funnel tab. A thin layer of ethylene glycol was poured into the lid of each jar to trap and preserve the flies as they emerged. The basal area covered by the trap was  $0.034 \text{ m}^2$  ( $0.375 \text{ ft}^2$ ).

In preliminary studies during 1979, the funnel traps were compared for efficiency to larger pyramid traps ( $0.83 \text{ m}^2$ ) previously used at Ottawa for emergence of the cereal leaf beetle, *Oulema melanopus* (L.), and its parasites (for description, see Gage and Haynes, 1975). Data for 17 sampling dates on which 32 traps of each size were compared showed that relative efficiency, defined as the reciprocal of variance per  $\text{m}^2$ , was 2.5 times greater for the smaller trap than for the larger.

The flies were counted at weekly intervals during peak periods of emergence. At sampling, the jars were removed and the flies separated from the liquid in the lids by means of a small art brush. An average of 1.5 min was required to count and remove the contents of each trap.

In the two years, numbers of emerging adults were recorded on a total of 35 occasions. The totals for individual traps ranged from 0 to 131 and means per population sample (of 64 traps), from 1.0 to 31.5.

#### STATISTICAL ANALYSIS

**Detection of the spatial pattern.** Using the Fortran program of Gates & Ethridge (1972), the Poisson ( $s^2 = m$ ) and negative binomial ( $s^2 = m + m^2/k$ ) distributions were fitted to the 35 sets of data and tested by  $\chi^2$ . When Poisson distributions were fitted to the observed distributions, discrepancies between observed and expected values were significant in 27 cases. However, the frequencies of all counts closely approximated the negative binomial

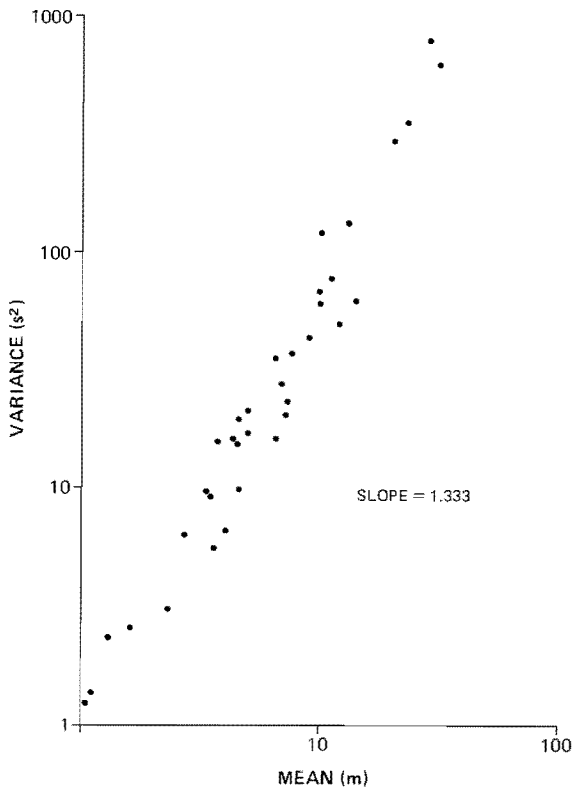


Fig. 2. Variance-mean relationships for 35 counts of *A. frontella* adults taken in emergence traps. Each point plotted is based on a sample of 64 traps.

Table 1. Results of analysis of variance<sup>a</sup> for counts of emerging adults of the alfalfa blotch leafminer, 14 June 1980.

Source of variation	df	Observed mean square	F
Blocks	3	0.066	0.316
Plots within blocks	12	0.209	1.215
Traps within plots	48	0.172	

<sup>a</sup>Transformed scale.

series, and deviations between observed and expected values were significant in only one case. Individual  $k$  values ranged from 1.01 to 7.56. The common value of  $k$  as determined by the regression method of Bliss (1958) was 1.42.

Variance-mean relationships for the 35 counts are illustrated in Figure 2. The overdispersed nature of the data is clearly shown by the plotted values, which depart noticeably from the 45° line of Poisson expectation to attain a slope of 1.33.

Table 2. Estimates of the numbers of traps required for two confidence probabilities and a 15% level of precision in sampling for adults of the alfalfa blotch leafminer.

Sample	Mean number per trap	CV	Confidence probability	
			.80 <sup>a</sup>	.90 <sup>b</sup>
<u>1980</u>				
1	9.9 ± 1.2	83	50	83
2	7.0 ± 0.7	64	30	49
3	4.5 ± 0.5	70	36	59
4	14.2 ± 1.1	55	22	36
5	4.0 ± 0.4	64	30	49
6	1.7 ± 0.2	94	65	106
7	12.1 ± 1.0	58	25	40
8	23.0 ± 2.7	82	49	81
9	4.5 ± 0.6	90	59	97
10	3.4 ± 0.4	89	58	95
11	28.8 ± 3.6	96	67	111
12	31.5 ± 3.1	78	44	73
13	20.0 ± 2.1	84	52	85
14	6.5 ± 0.7	93	63	104
15	4.5 ± 0.6	99	72	118
16	4.4 ± 0.5	90	59	97
17	3.6 ± 0.3	65	31	51
18	7.3 ± 0.6	66	32	52
19	2.3 ± 0.2	74	40	66
20	1.1 ± 0.1	109	87	143
<u>1981</u>				
21	13.0 ± 1.4	88	57	93
22	5.1 ± 0.6	88	57	93
23	10.1 ± 1.0	76	42	69
24	3.4 ± 0.4	91	60	100
25	9.2 ± 0.8	71	37	61
26	2.8 ± 0.3	89	58	95
27	9.7 ± 1.4	113	93	154
28	11.0 ± 1.1	80	47	77
29	7.1 ± 0.7	100	73	120
30	6.7 ± 0.5	60	26	43
31	3.7 ± 0.5	106	82	135
32	7.7 ± 0.8	79	46	75
33	5.1 ± 0.5	81	48	79
34	1.3 ± 0.2	114	95	156
35	1.0 ± 0.1	107	84	138

<sup>a</sup><sub>Z</sub> = 1.2816.

<sup>b</sup><sub>Z</sub> = 1.6449.

**Analysis of variance.** The statistical methods used in this study follow those of Harcourt and Binns (1980ab). The data were analysed using a nested analysis of variance (among blocks, among plots in blocks, and among traps in plots). In these analyses the data were stabilized by the transformation  $\log(x + 1)$ , where  $x$  is equal to the observed count. The analysis of variance is illustrated (Table 1) using one of the sets of data.

Analysis of the 35 counts showed that variation between blocks and plots was significant in just two and seven cases, respectively. Although this implied that variation between traps

Table 3. Levels of precision corresponding to certain values of  $N_s$  and  $1 - \alpha$  for adults of the alfalfa blotch leafminer.

$N_s$	Confidence probability ( $1 - \alpha$ )	Percent precision (p) <sup>a</sup>
80	0.8	12
	0.9	15
70	0.8	13
	0.9	17
60	0.8	14
	0.9	18
50	0.8	15
	0.9	20

$$^a p = z CV \sqrt{N_s}$$

comprised most of the sampling variance, it also indicated that some heterogeneity occurred throughout the field. For this reason, it was deemed adequate to adopt a single stage sampling procedure. Hence, a sampling plan for estimating numbers of emerging adults should provide for a number of traps well spread out over the field.

**Sampling precision.** Table 2 lists the estimates of population density together with their standard errors in the untransformed scale. Invariably, the latter were within 15% of the mean.

#### 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-trap coefficients of variation (CV) were derived as 100s/m. These ranged from 55 to 114 (Table 2).

Following Snedecor and Cochran (1967), the number of traps corresponding to a confidence interval of width p% of the mean (m) at a probability level of 100 ( $1 - \alpha$ )% was calculated as

$$N_s = (z_{\alpha} CV/p)^2$$

where CV is the percent coefficient of variation, p is the level of precision, and  $z_{\alpha}$  is the  $1 - \alpha/2$  significant value for the standard normal distribution.

Using this equation, values for  $N_s$  were obtained for confidence probabilities of 0.8 and 0.9, and a 15% level of precision (Table 2). The data were then averaged to determine the precision corresponding to certain given values of  $N_s$ ,  $1 - \alpha$ , and a CV of 84; the latter was chosen by taking its rounded arithmetic mean over dates. Precisions corresponding to sample sizes of 50, 60, 70, and 80 traps, with confidence probabilities of 0.8 and 0.9 are given in Table 3. From these results, it is evident that an acceptable confidence probability (0.8) and level of precision (12%) for life table studies can be obtained with 80 traps per field. To obtain a greater or lesser degree of precision, numbers of traps should be increased or decreased accordingly.

#### DISCUSSION

In determining the sample size for target insect stages, it is important to evaluate the costs of sampling. In these terms, only 120 min (=2h) would be required to count and remove the contents of 80 traps. However, where resources are limited or less intensive data are required (as in surveys), a lower level of precision could be adopted; Table 3 shows that just 50

traps would yield a population estimate that provides a confidence probability of 0.8 and a level of precision of 15%.

In the foregoing statistical appraisal, untransformed data were used to set the sample size for specified precision. As a rule, sampling plans based on log-transformed data tend to underestimate the sampling requirement; therefore, in situations where log-transformed data are appropriate, a plan based on non-transformed data should ensure adequate precision.

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