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ABSTRACT

A sequential sampling system for classifying infestation levels of overwintering larvae of the spruce budworm, *Choristoneura fumiferana*, (Clemens), is described. Sequential tables for critical densities of larvae on balsam fir and red spruce are presented for 70%, 80%, and 90% confidence intervals. The system is based on data collected in Maine and uses a maximum of 6 branches, 3 from fir and 3 from spruce. Simulations illustrating error rates and effort saved using the system are discussed.

INTRODUCTION

Population surveys of spruce budworm, *Choristoneura fumiferana* (Clemens), have been widely used for planning annual management programs and for forecasting damage in infested stands in eastern North America. Although several stages of the insect may be sampled, surveys of overwintering, second instar larvae (L_2) are used extensively, "to refine and check results of egg-mass surveys and to provide an up-dated forecast of infestation and defoliation," (3). Host-tree branch samples are soaked in hot NaOH to remove larvae from hibernacula. The larvae are then separated from the aqueous mixture with hexane and filtered (9, 8). Extracted larvae on the filter paper are counted under a dissecting microscope.

L_2 sampling involves taking one branch from the mid or upper-crown of each sample tree, and numbers of trees sampled per site are few, 3 to 10, depending on the State or Province. Intensive population sampling (e.g. for life-table studies) requires many more trees (11). The low precision of single L_2 estimates is balanced by sampling 100-700 sites in each jurisdiction (3). However, even with few branches processed per site, a sequential plan could reduce the numbers of branches further.

Sequential sampling differs from conventional sampling in that, with the latter, a decision is made after examining an entire sample, e.g. 20 branches. With a sequential system, a decision is made after each component (branch) of a sample is examined. The decision might be to cease examining components and classify the sample, e.g. as heavy, or to examine another component because there is insufficient information to make a classification at that point. Sample size in a sequential system is, therefore, variable but usually averages less than in a fixed-sample system while providing equivalent information. Sequential systems are most efficient where the question asked is whether the sample exceeds a certain critical density, e.g. 10 insects per branch, or is less than an economic threshold, e.g. two insects per pheromone trap, rather than more specific questions, e.g. is the population intensity between 5 and 7 per leaf.

METHODS

We based the plan on the L_2 sampling system now used in Maine. Three 1-1.5 m branches (1 per tree) are sampled per host species and per site. Stands usually contain both balsam fir (*Abies balsamea* (L.) Mill.) and red spruce (*Picea rubens* Sarg.) so that the typical sample for a site is 6 branches, 3 fir and 3 spruce. Because the system already has the arbitrary limit of 6 branches, we were not interested in reaching any specific level of precision, but only in maintaining most of the precision inherent in the 6-branch sampling system.

Larval densities are expressed here as numbers per 10 m^2 of branch surface, as used in Quebec and Newfoundland (3). We also provide tables based on insects/branch, the measure used in the Maritime provinces. Densities expressed as larvae/100 ft^2, used in the U.S., are nearly identical with numbers of larvae/10 m^2, and the same sequential tables could be used or small corrections made (1 larva/10 m^2 = 0.93 larvae/100 ft^2).

Most jurisdictions use four infestation-intensities of budworm larvae: light, medium, heavy, and extreme. Critical densities, or bounds, for these classes are:

Infestation Level

	Light	Medium	Heavy	Extreme
larvae/10 m^2 (Maine) (14)	<188	188-538	538-1184	>1184
larvae/10 m^2 (Que., Nfld.) (3)	<100	100-300	300-650	>650
larvae/branch (Maritimes) (3)	<6	7-20	20-40	>41

Critical densities vary, e.g. between Maine and Quebec, because of subjective differences in spray policies. The per-branch values of the Maritimes are comparable to the Quebec values, assuming an average surface area of branches of about 1.5 m^2. Sequential plans calculated from Maine data should be applicable in other areas, given a 6-branch collection system, and the mean-variance relationships of counts are assumed to be the same.

Basic data for calculating sequential plans were derived from L_2 collections made at 28 sites throughout the budworm-infested region of Maine in the winter of 1982-83. The sites exhibited broad ranges of budworm-infestation level and past spray history, but stands with dead or moribund trees were avoided. One 1-1.5 m branch from each of 20 balsam-fir and 20 red-spruce trees at each site was collected and processed. Average population densities ranged from 148 to 3493 per 10 m^2 on fir and 95 to 4168 per 10 m^2 on spruce among the 28 plots. On a per branch basis, the range was 5 to 155 on fir and 4 to 194 on spruce.

In preparing the sequential sampling plan, we followed the method of Iwao (5) because it requires no assumption that population counts follow a uniform aggregation pattern over all population densities. Such an assumption is required in older plans, based on Wald (15), but that assumption is not valid in the case of spruce budworm (11). The Iwao method has been applied recently to several other insects (1, 2, 12, 13).

The method is based on the relationship between the mean, m, and the mean crowding parameter, $\overset{*}{m}$, of Lloyd (7), taking the form, $\overset{*}{m} = \alpha + \beta m$ (5). Where this relationship exists, upper and lower limits of the confidence intervals of critical mean densities can be calculated by:

$$\text{upper limit: } T' = q\,mo + t\sqrt{q\,[(\alpha + 1)mo + (\beta\text{-}1)mo^2]}$$

$$\text{lower limit: } T'' = q\,mo - t\sqrt{q\,[(\alpha + 1)mo + (\beta\text{-}1)mo^2]}$$

where q is the sample size (number of branches used, 1-6 sequentially in this case); mo is the critical mean population density (which separates high or intolerable population densities from low or tolerable ones); t is the critical value for Student's t-distribution for n-1 degrees of freedom (n = maximum number of branches per site) and the desired confidence level, e.g. 2.02 for 90%, 1.48 for 80%, etc. when $n = 6$; and α and β are the intercept and slope from the relationship, $\overset{*}{m} = \alpha + \beta\, m$ (6, 4).

In practice, if after counting 1 to 5 branches, the cumulative number of insects exceeds T', one can cease counting and assume, the degree of confidence corresponding to t, that m exceeds mo. Similarly, if cumulative counts are less than T'', one ceases counting and assumes that m is less than mo. If cumulative counts remain between T' and T'', one counts all six branches and arrives at the population class estimate based on the total sample.

As noted earlier, critical population densities in terms of whether or not to spray vary among and are flexible within jurisdictions, with lower population thresholds used in years or regions when tree condition is poor. For that reason, we selected a range of critical densities in this work. We calculated the tables so that one can conclude that population densities are greater than 538, 753, 969, or 1184 larvae/10 m^2 or less than 538, 323, 188, or 108 larvae/10 m^2. These values are the densities used to separate infestation levels and make spray decisions in Maine plus some intermediate values.

A sequential table was prepared for combined samples of fir and spruce with infestations expressed as larvae/10 m^2 of branch surface and for t-values corresponding to 70%, 80%, and 90% confidence. In addition, we re-worked all of the steps using insects/branch rather than insects/10 m^2.

Finally, error rates and effort saved by the system were evaluated by subsampling within the data sets for the 28 sites. Of the 40 branch counts for fir and spruce per site, 6 counts were selected randomly by computer for 100 trials for each site. Numbers of branch counts needed to reach a decision were recorded along with numbers of times that infestation levels of the site were incorrectly classified. This evaluation was done with the 70% confidence interval table only. It can be assumed that, at higher confidence levels, more branches would need to be processed to reach decisions and that incorrect decisions would be fewer.

RESULTS

The original plot data used in the sampling plans are listed as Appendix 1a and 1b.

A regression of $\overset{*}{m}$ on m for balsam-fir only at the 28 sample sites, with counts expressed as insects/10 m^2 was:

$$\overset{*}{m} = 139.8 + 1.26m \; (R^2 = 0.95,\ t = 35.4,\ p < 0.001)$$

The same relationship for red-spruce was:

$$\overset{*}{m} = 195.2 + 1.24\, m \; (R^2 = 0.95,\ t = 22.4,\ p < 0.001)$$

Comparisons of slopes and elevations of these two regressions by ANOVA showed no significant differences (F = 0.098 and 0.199 for slopes and intercepts respectively). Therefore, data for fir and spruce were pooled, producing the relationship:

$$\overset{*}{m} = 161.1 + 1.25\ m\ (R^2 = 0.96,\ t = 35.5,\ p < 0.001)$$

Using these values for α and β (161.1 and 1.25 respectively), Table 1 was calculated with three levels of confidence. One point of caution in use of Table 1 can be illustrated using the 70% confidence-interval section. If the larval count on the first branch was 1200, one could assume only that the larval population exceeded 538/10 m². It is incorrect to assume that the population lies between 538 and 753/10 m². To make that assumption requires that the larval count both exceed the upper confidence limit of *mo* =

Table 1

Sequential sampling decision limits for overwintering larval populations of spruce budworm on balsam fir and/or red spruce for three confidence probabilities; populations expressed as larvae/10 m² of branch foliage surface.

Branch No.	Population less than:				Population more than:			
mo =	108	188	323	538	538	753	969	1184
70% Confidence Probability (n − 1 = 5, t = 1.16)								
1	--	--	--	75	1001	1348	1695	2038
2	--	51	187	421	1731	2383	2964	3576
3	44	166	406	812	2416	3291	4164	5031
4	100	292	642	1226	3078	4203	5328	6444
5	169	426	889	1654	3726	5097	6468	7829
80% Confidence Probability (n − 1 = 5, t = 1.48)								
1	--	--	--	--	1129	1513	1895	2274
2	--	--	60	240	1912	2581	3248	3909
3	--	56	251	590	2637	3575	4511	5439
4	9	165	463	970	3334	4532	5728	6915
5	67	284	688	1368	4012	5465	6916	8356
90% Confidence Probability (n − 1 = 5, t = 2.02)								
1	--	--	--	--	1345	1790	2233	2671
2	--	--	--	--	2217	2973	3725	4471
3	--	--	--	217	3011	4055	5096	6128
4	--	--	161	539	3765	5086	6404	7710
5	--	45	350	886	4494	6084	7671	9245

538 and be less than the lower limit of *mo* = 753. We have not calculated the latter because upper and lower limits of adjacent values of *mo* overlap at low numbers of branch counts.

The regression relationships of $\overset{*}{m}$ on m with insect densities expressed as insects/branch for fir and spruce, respectively, were:

$\overset{*}{m} = 2.48 + 1.29\ m\ (R^2 = 0.98,\ t = 42.6,\ p < 0.001)$ and
$\overset{*}{m} = 5.58 + 1.21\ m\ (R^2 = 0.96,\ t = 30.7,\ p < 0.001)$

The slopes and intercepts of the two regressions were not different ($F = 0.13$ and 2.35 respectively), and a pooled regression for spruce and fir was:

$$\overset{*}{m} = 4.3 + 1.24\ m\ (R^2 = 0.98,\ t = 49.1,\ p < 0.001)$$

Table 2 was calculated using these values for α and β. The critical population densities listed at the top of Table 2 represent the same population

Table 2

Sequential-sampling-decision limits for overwintering larval populations of spruce budworm on balsam fir and/or red spruce for three confidence probabilities; populations expressed as larvae/branch.

	70% Confidence Probability (n − 1 = 5, t = 1.16)							
Branch No.	*Population less than:*				*Population more than:*			
mo =	2	5	10	18	18	26	35	43
1	--	--	--	3	33	46	60	73
2	--	1	6	14	58	80	106	128
3	--	4	12	28	80	113	149	181
4	0	7	20	41	103	144	191	232
5	1	10	27	56	124	175	232	282
	80% Confidence Probability (n − 1 = 5, t = 1.48)							
1	--	--	--	--	37	52	67	81
2	--	--	2	9	63	88	116	140
3	--	--	8	20	88	122	161	195
4	--	3	14	33	111	155	205	249
5	--	6	21	46	133	187	247	301
	90% Confidence Probability (n − 1 = 5, t = 2.02)							
1	--	--	--	--	45	61	79	95
2	--	--	--	--	74	101	132	160
3	--	--	--	8	100	139	182	220
4	--	--	5	19	125	174	228	277
5	--	--	10	31	149	208	274	332

densities expressed as insects/10 m^2 in Table 1. They were calculated using a regression of the original data expressed as insects/10 m^2 on the same data expressed as insects/branch for fir and spruce pooled:

$$\text{ins/br} = -2.19 + 0.038 \text{ ins/10 m}^2 \; (R^2 = 0.88,\ t = 19.8,\ p < 0.001)$$

For those who have not previously used sequential tables, we have included a hypothetical example as Appendix 2.

The effort saved in using this sequential system at high population densities (Figure 1) was estimated by selecting 6 branch counts at random 100 times from each of the 28 data-sets (28 sites, spruce and fir combined), and determining the numbers of branches used in each case to reach a decision. The percent of branches that needed to be counted in the 100 trials for each site was related to the population mean (Figure 1). Since 1 branch of each 6 collected must be counted, a minimum of 16.6% of branches must be used, and the maximum effort that can be saved is 83.4%. Little effort was saved when the observed mean population density, m, was close to the critical population level, mo, but effort saved approached the maximum at m = 3000 − 4500/10 m^2 for mo levels of 538 - 1154. An analysis of effort saved at low population densities was not done because of the few collection sites with low populations.

Error rates using the sequential plan were estimated by simulation in similar fashion to effort saved. There are two aspects to this error, 1) classifying a population level in a higher category than it really is, and 2) classifying a high population as low. As an example of the former case, we can use the regression line (Figure 2) for critical population level, mo = 538 since this line has the greatest R^2. Populations with mean infestation levels of 400-525 were incorrectly classified to exceed 538 insects/10 m^2 10 - 20% of the time. At lower populations, error rates decreased below 10%, reaching zero at populations of about 130/10m^2.

With the latter case, classifying a high population at a lower population level, there were too few collection sites of low population to establish regression lines. The results are presented, however, as Table 3 showing only one case where the error rate exceeded 10%.

DISCUSSION

The error rates in using Iwao's sequential decision system require more discussion in view of a recent evaluation of the total errors involved (10). These authors have shown that actual errors inherent in the procedure may be greater than the nominal error established and assumed in the calculation of decision limits, and they describe three sources of error.

One of these is the sequential error, the error involved when reaching a decision with less than the maximum number of sample components (branches), and in the present case is the error illustrated in Figure 2 and Table 3. For

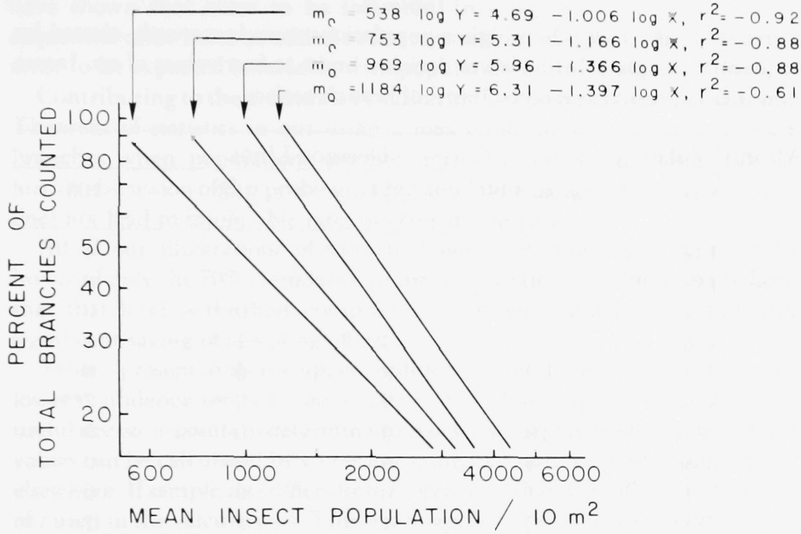

Figure 1. The percent of branches from a total sample of 6 branches that must be searched to classify spruce budworm populations as exceeding a population threshold (*mo*), at increasing mean population densities.

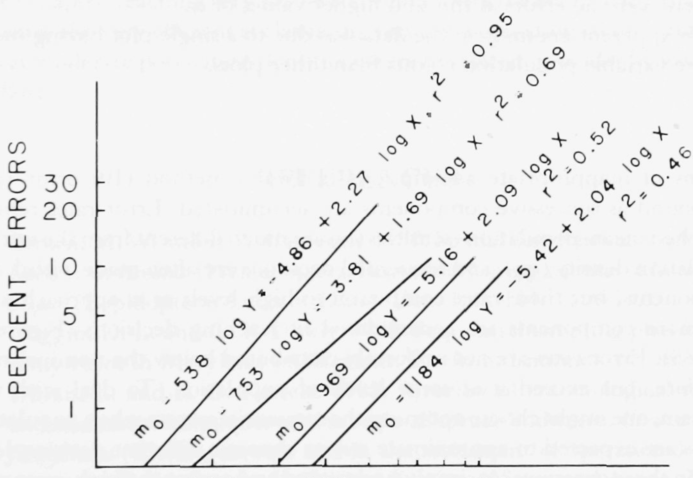

Figure 2. The percent of trials wherein lower population densities of spruce budworm were incorrectly classified as exceeding a population threshold (*mo*).

Table 3

Percent of 100 trials when higher populations were incorrectly classed by sequential sampling below lower decision limits at four values of *mo*. Insect densities expressed as insects/10 m^2 of branch surface.

Mean Population	Decision Limit			
(*m*)	*mo* = 538	323	188	108
134				4
152				0
221			0	0
330		3	1	0
334		8	0	0
528		9	7**	7**
581	18	0	0	0
665	4	0	0	0
751	1	0	0	0
775	3	1	1	1
823	0	*	*	*
826	1			
840	1			
1042	*			

*There were no errors at this and higher values of *m*.

**This apparent anomaly in the data was due to a single plot having much more variable population counts than other plots.

reasons of inappropriate assumptions in Iwao's method (10) error rates compound as successive components are accumulated. Error rates remain low when mean-population-densities (*m*) are much different from the critical population density (*mo*), and sequential decisions are often made with 1 or 2 components, but these rates compound to high levels as *m* approaches *mo* and more components are accumulated in reaching decisions (Figure 2, Table 3). Error rates are not uniformly distributed below the nominal rate, therefore, but exceed it at some levels of population. To deal with this problem, one might choose not to use the sequential system when population means are expected to approximate *mo*, or choose a different decision level (*mo*) on those occasions, or simply understand and accept the high error rate. The higher confidence probabilities sections of Tables 1 and 2 may also be used in these cases, but this results in less effort saved.

A second source of error is the terminal error, that involved in decisions based on counting all components. We have not addressed terminal error in this paper, but several years of use of L_2 sampling with fixed-size samples have shown that error to be tolerable. In using the sequential system, sequential error must be added to the terminal error. The amount of added error to be expected is variable with population mean (Figure 3, Table 2).

Contributing to these errors is violation of the criteria of the Central Limit Theorem of statistics in our using a maximum sample size as small as 6 branches when populations are not normally distributed. (See (10) for further discussion of this problem.) Our simulations suggest that this violation does not lead to intolerable rates of error in this case.

All of our illustrations of simulated sampling (Figures 1, 2, Table 3) involved only the 70% confidence probability section of Table 1. We believe that that level is the best compromise between tolerable error rate and significant saving of sampling effort.

Table 1 presents only the upper confidence limits for $mo = 538 - 1184$ and lower confidence limits for $mo = 108 - 538$. These appear to be the most useful decision-points in determinations of what to spray in our region. Other values can be calculated by a user assuming that our values of α and β apply elsewhere. If sample size differs from 6 branches, that will influence the value of t used in the calculations. This might apply to pure stands of fir or spruce where fewer than 6 trees might be sampled.

This sequential sampling system has been used in Maine since 1983. At that time heavy and extreme population levels of budworm were common in Maine, and these could be quickly classified as such with the sequential system, often with one or two branches. Since 1983, budworm populations have collapsed reaching very low levels over much of the State. The sequential system is also very efficient at classifying very low population counts properly. It is at moderate population densities where the system provides little saving of effort.

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Appendix 1a

Table of mean, standard deviation, and mean crowding calculations for overwintering larval counts on 28 plots, expressed as insects per 100 ft^2 of branch surface.

Plot No.	Fir			Spruce		
	Mean	Standard Deviation	Mean Crowding	Mean	Standard Deviation	Mean Crowding
1	885.6	570.3	1251.8	1505.3	805.3	1935.1
2	1333.7	615.1	1616.4	1759.2	888.7	2207.1
3	1829.6	913.3	2284.5	3872.9	1712.7	4629.3
4	1114.3	683.4	1532.4	2697.9	1066.7	3118.7
5	1412.8	537.9	1616.7	2388.2	1728.9	3638.9
6	1777.0	1163.0	2537.2	1929.5	927.3	2374.2
7	1570.6	959.9	2156.3	2907.5	2164.2	4517.4
8	936.8	609.4	1332.3	1000.9	697.8	1486.4
9	824.8	383.4	1002.0	1224.7	549.9	1470.7
10	2339.9	1476.9	3271.0	2097.7	952.6	2529.3
11	736.2	562.6	1165.2	797.9	603.9	1254.0
12	1909.4	1062.5	2499.6	2504.8	1241.6	3119.2
13	846.4	582.4	1246.2	681.4	579.3	1172.9
14	593.3	475.9	974.2	888.5	728.8	1485.3
15	564.4	297.9	720.7	670.6	573.6	1160.3
16	188.8	124.5	269.9	221.4	195.5	393.0
17	388.2	468.6	952.8	249.7	230.1	460.7
18	340.3	295.8	596.4	272.7	174.6	383.6
19	238.4	234.0	467.1	656.5	568.1	1147.1
20	138.6	105.6	218.0	110.5	112.5	214.1
21	140.6	118.1	238.8	140.9	87.8	194.6
22	889.3	569.8	1253.4	1373.9	690.6	1720.1
23	903.9	670.9	1400.8	1450.8	926.9	2041.9
24	661.6	476.4	1003.7	850.3	622.0	1304.4
25	639.3	310.9	789.6	756.9	441.9	1013.9
26	1005.6	685.8	1472.2	1799.3	1520.7	3083.5
27	473.1	391.5	795.5	606.1	413.8	887.6
28	3245.9	1781.3	4222.5	2282.4	1132.7	2843.5

Appendix 1b

Table of mean, standard deviation, and mean crowding calculations for overwintering larval counts on 28 plots, expressed as insects per branch.

	Fir			Spruce		
Plot No.	Mean	Standard Deviation	Mean Crowding	Mean	Standard Deviation	Mean Crowding
1	36.5	27.2	55.7	51.3	29.3	67.1
2	62.2	32.6	78.3	85.8	43.2	106.5
3	92.1	55.0	123.9	151.6	67.9	181.0
4	53.5	27.9	67.1	110.0	36.2	120.9
5	73.9	30.7	85.7	111.3	80.1	167.9
6	90.8	60.0	129.5	79.7	36.7	95.6
7	77.4	42.5	99.8	116.2	67.3	154.2
8	44.0	24.8	56.9	39.9	27.9	58.5
9	42.9	21.3	52.5	54.1	29.0	68.6
10	66.0	39.7	88.9	46.6	22.1	56.0
11	18.3	11.9	24.9	19.0	18.5	36.1
12	53.7	30.9	70.5	64.4	35.0	82.5
13	24.3	16.6	34.7	16.0	15.2	29.5
14	16.4	12.7	25.2	21.2	18.8	36.9
15	14.6	7.7	17.7	13.3	9.0	18.5
16	7.6	4.7	9.5	8.1	6.7	12.6
17	17.9	20.8	41.0	9.3	8.3	15.6
18	13.4	11.3	21.9	9.7	6.4	12.9
19	14.4	11.2	22.2	23.5	22.1	43.3
20	5.6	4.6	8.4	3.6	3.2	5.4
21	5.1	4.1	7.3	4.6	2.7	5.2
22	34.5	27.8	48.6	59.8	27.3	71.3
23	28.3	23.1	46.1	52.7	34.2	72.9
24	29.4	19.1	40.8	34.3	25.6	52.5
25	17.6	6.7	19.1	22.9	13.4	29.7
26	34.3	21.4	46.6	39.6	24.9	54.4
27	19.4	14.9	26.9	23.9	16.3	33.9
28	155.4	86.9	203.0	105.4	49.5	127.6

Appendix 2

Use of the sequential sampling table.

1. One has made a judgement that, in the absence of other considerations, one will spray areas having budworm populations of 753 larvae/10m^2 or more in the coming year and will exclude from treatment areas with less than 323/larvae 10m^2.
2. For each collection of six branches from a site, the first branch is extracted and counted.
3. For the first site, the count on the first branch is 1565 larvae/10m^2. Consulting Table 1, this exceeds the table value of 1348 (70% confidence probability, *mo* = 753), and one can cease further analysis of that collection with the decision that it exceeds the spray threshold.
4. For a second site, the count on the first branch is 936. Since this does not exceed 1348, a second branch must be counted. The second count is 1213, which when summed with the first count, totals 2149. A third branch produces a count of 1562 which brings the accumulated total to 3711. Since this total exceeds the table value for three branches of 3291, one discontinues counts for that collection and includes the site in a spray plan.
5. For a third site, the counts on the first two branches are 52 and 46, totalling 98. Since this is less than the table value for two branches, *mo* = 323, of 187, one discontinues work on the collection and excludes the site from the spray plan.
6. On a fourth collection, the counts for six branches are 743, 530, 826, 415, 622, and 930. Cumulative totals are 743, 1273, 2099, 2514, 3136, and 4066. Since the cumulative totals have never exceeded the table values for *mo* = 753, it has been necessary to count the entire collection of six branches. The mean count for the six branches is 4066/6 = 678. The decision must now be made whether this is sufficiently close to the threshold of 753 to include in the spray plan or not. Consideration would probably be given to the results of other samples taken in the immediate vicinity.