AN ADDITIONAL NOTE ON SAMPLING OVERWINTERING SPRUCE BUDWORM LARVAE

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INTRODUCTION

In a recent report (Miller et al. 1971), we outlined a method of extracting and counting overwintering larvae of the spruce budworm (Choristoneura fumiferana (Clem.)) from sample branches at minimum cost. The essence of the technique was to wash the branches in a warm sodium hydroxide solution that dissolved the hibernaculum webbing and freed the larvae. Since publication, we have field tested the technique by paired counts of overwintering second-instar larvae and feeding third-instar larvae in selected plots. In most instances, the counts were very similar indicating little or no spring dispersal loss. This was unexpected because earlier investigations (Miller 1958) revealed that spring dispersal losses up to 71% were not uncommon. In this report, we discuss these conflicting results and reevaluate the washing technique.

METHODS AND RESULTS

Endemic populations of the spruce budworm were monitored in northwestern New Brunswick where the budworm remained at a low density in the 1960's after the 1949-1959 outbreak. Washing overwintering larvae (L2) from 20 to 100 branches per plot and counting feeding larvae (L3) in the same plot in the spring provided an opportunity to compare the two sets of counts (Fig. 1). We expected Figure 1 to show a spring dispersal loss with most of the points falling below the 45° line. However, it did not and population gains were observed in many instances. Similar results were obtained in heavily infested plots in central and southern New Brunswick (Table 1). Thus comparison of L2 and L3 counts in both endemic and epidemic areas suggested that the L2 counts were too low and the washing technique inadequate.

A third comparison, involving egg counts, also suggested that the washing technique was inadequate. Egg-mass and L2 counts obtained from the extensive survey that is carried out annually in New Brunswick to define tree hazard were plotted (Fig. 2) and an analysis of the regression showed an L2 survival (L2/eggs) of 34% in low level populations decreasing to 10% in dense populations. These survival rates are low

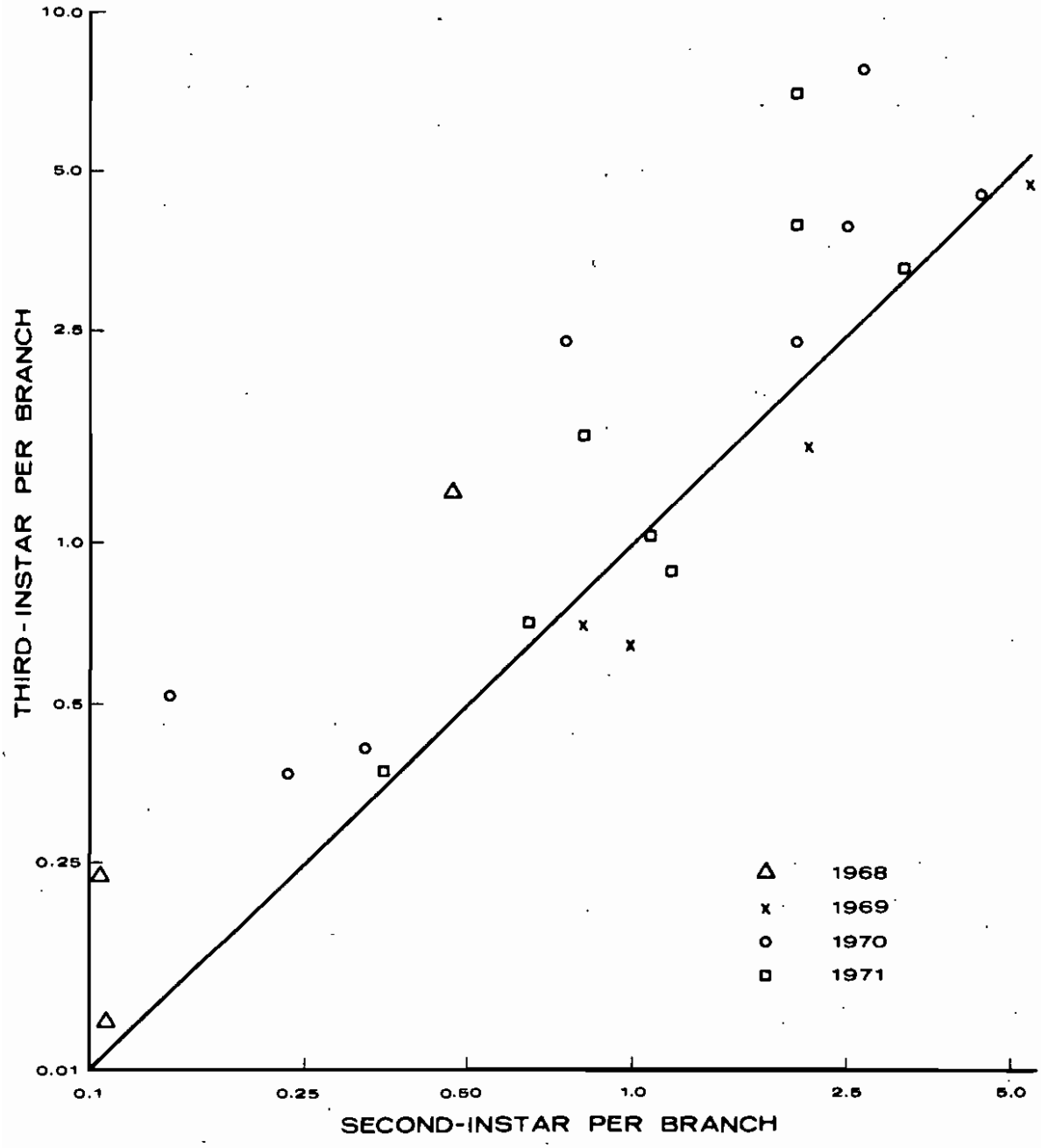

Fig. 1. Third-instar counts as a function of second-instar counts in the same plot.

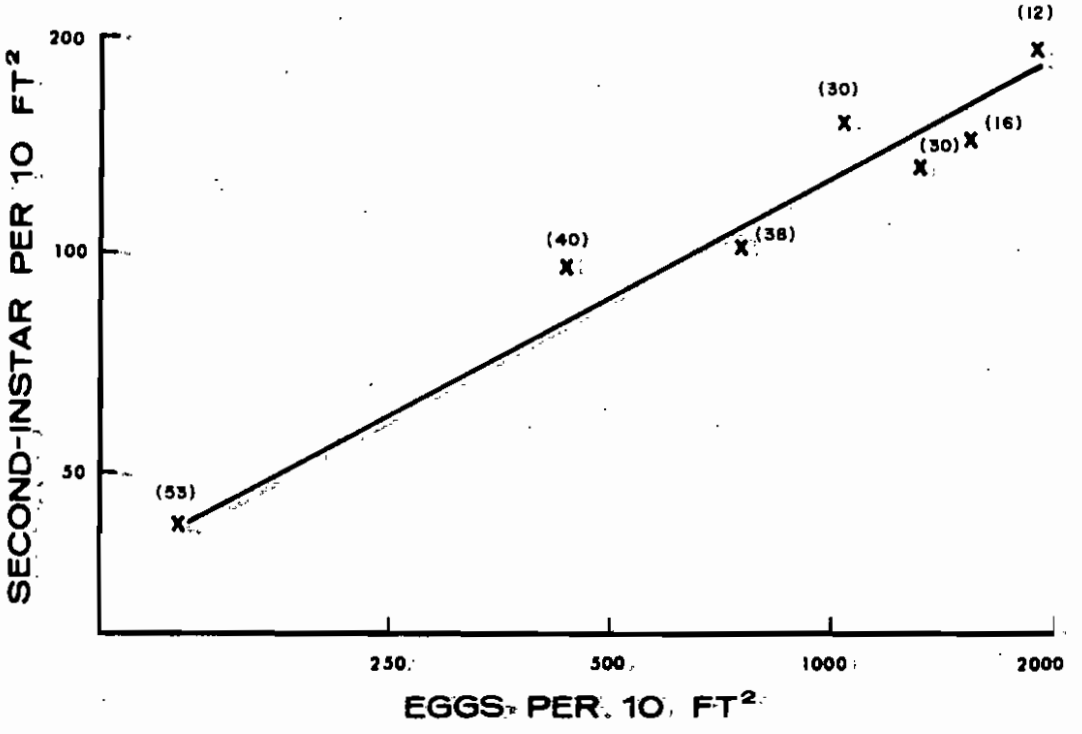

Fig. 2. Mean second-instar counts as a function of egg counts. Number of observations in 4 years shown in parentheses

Table 1. Comparison of second-instar (L2) counts to third-instar (L3) counts within epidemic populations, 1969 and 1970

Year	Mean counts per branch	
	L2	L3[a]
1969	26	22
1970	18	18

a. Converted from counts per 18-inch branch tip using the equation: Larvae/18 inch tip = 0.51 x larvae/whole branch.

and again suggested an apparent failure of the washing technique. We therefore decided to test a number of possible explanations.

(1) *That washing foliage with sodium hydroxide solution fails to remove all larvae from the foliage.* We washed the same branch samples repeatedly and found that the first washing did, in fact, remove over 95% of the "extractable" larvae from branches. In a follow-up test, paired samples were collected from four red spruce trees. One sample was washed in the prescribed manner; the other was exposed to room temperature and the living larvae forced from hibernacula. The comparative counts were 63 washed to 67 living larvae, again indicating that the wash was extracting most of the larvae.

(2) *That a gradual erosion of larvae takes place in the overwintering period.* Mid-crown samples from 10 trees were taken on 13 January, 11 and 29 February, and 27 March. The washing counts per branch were 88, 64, 60, and 99, respectively, showing no erosion loss during the winter.

(3) *That second-instar larvae are lost with the needles and bits of bark that drop when the branch samples are stored for long periods and allowed to dry.* Four samples were collected in mid-January and allowed to dry for 0, 2, 4, and 7 weeks. Collecting and washing the debris showed that 12% of the population could be "lost" in the dropped needles and bark bits (Table 2).

Table 2. "Loss" of larvae with drying period

Weeks of drying	Mean L2 per branch[a]	Mean L2 in debris	Loss, %
0	112	0.0	0
2	90	5.2	6
4	74	10.4	12
7	109	15.0	12

a. Sample of 5 branches.

(4) *That second-instar larvae spin hibernacula on both host and non-host trees while emerging larvae only establish on host trees.* If this were the case, the L2 counts on fir could appear low in relation to L3 counts, particularly in mixedwood stands with a scattered fir-spruce component. However, most of our test locations had a fir-spruce component greater than 90% which would negate this assumption.

(5) *That either a density gradient or different spatial distributions of L2 and L3 populations within the crown could account for our deceptively low L2 counts.* To check this assumption, a total of 25 balsam fir trees were sampled by crown quarters (A = top quarter; B; C; and D = bottom quarter) in three sampling periods (see Appendix for results).

(a) In January, four branches were taken from each quarter of 100 trees (40 branches) and washed separately to give counts of hibernating larvae on each branch (L2H counts).

(b) In late April, four branches were taken from each quarter of five trees. The four branches were wrapped together in paper towelling and the emerging second-instar larvae were recorded as they crawled over the paper (L2E counts).

(c) In mid-June, one branch was taken from each quarter of 10 trees. The new shoots were examined and the number of third- and fourth-instar larvae recorded (L3 counts).

Figure 3 shows the mean number of overwintering larvae per branch (L2H and L2E) and the mean number of L3 larvae within each crown quarter. Note the marked difference in the spatial distribution of the L2H (dotted line) and L3 (solid line) populations. There were few

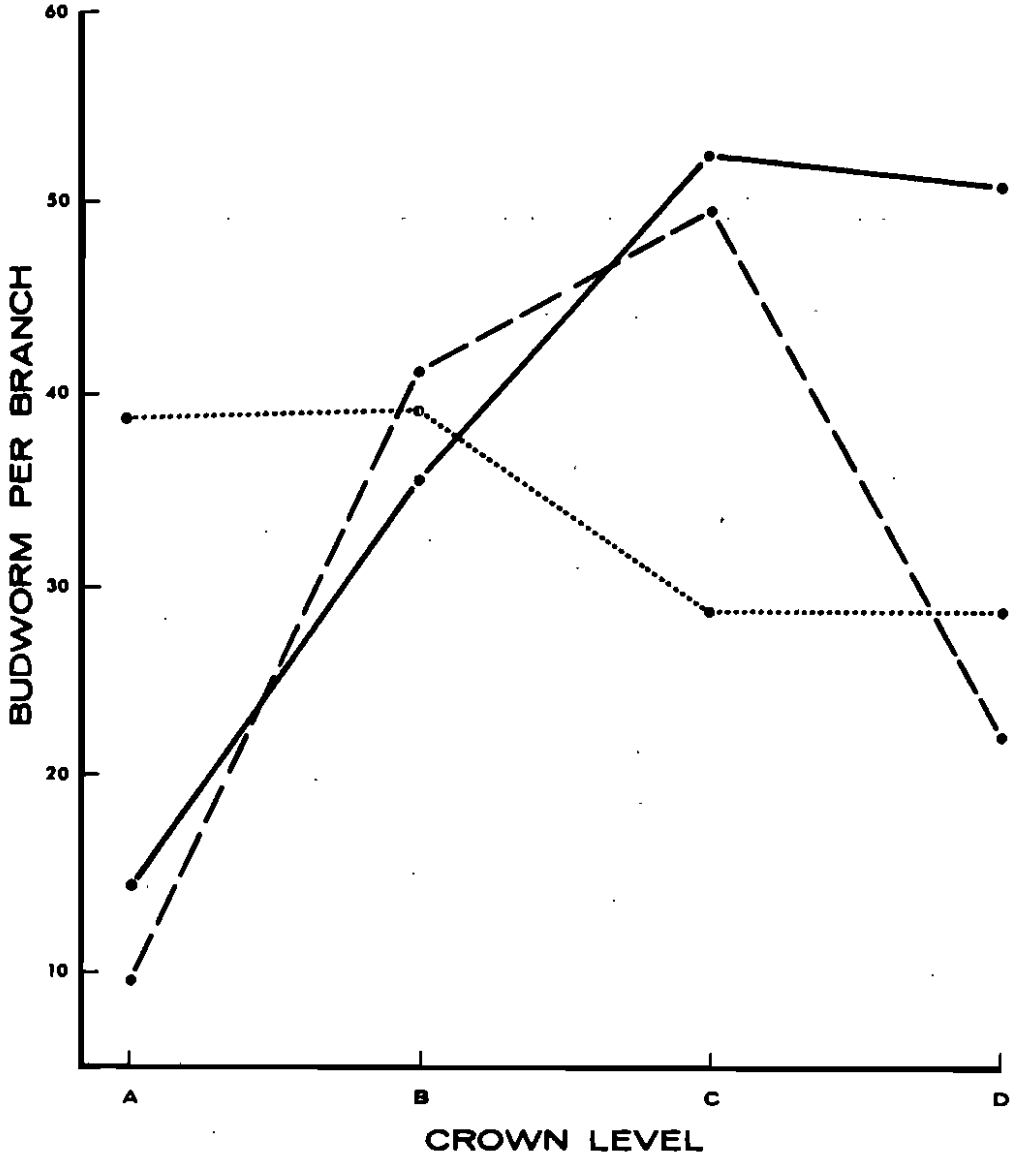

Fig. 3. Mean second-instar larvae per branch in hibernacula (L2H, solid line) and emerging in the spring (L2E, broken line) with mean third-instar larvae (L3, dotted line). Data from same plot.

overwintering larvae on the small A and B level branches while the feeding third-instar larvae preferred these strata: possibly because of the density and development of the new shoots. Consequently, if sample branches were taken from the B level, the counts would show very little difference in L2H and L3 densities (Table 3). If samples were taken from the C level, however, the counts would show a marked difference (55.6 to 28.7) which might be inadvertently attributed to a spring dispersal loss of 26.9 larvae or 48% of the population. Figure 4 shows the same L2H and L3 counts corrected for branch size. The number of L2H per 10 ft^2 of foliage is fairly consistent throughout the crown while L3 per 10 ft^2 shows a sharp gradient.

Table 3. Comparative counts by crown level of larvae in hibernacula (L2H) and third-instar larvae (L3)

Sample	Counts per branch	
	L2H	L3
B	38.1	39.4
C	55.6	28.7
4-level	39.8	33.8

To summarize, intensive sampling of L2H and L3 populations by crown levels reveals two different spatial distributions and these distributions are such that samples taken from one crown level must be interpreted with care. This in no way detracts from the usefulness of the washing technique to measure second-instar abundance. Rather it means if the investigator wishes to obtain the additional data of *change in abundance* from L2 to L3, sample branches should be taken from all crown levels to account for the distribution of larvae within the crown. For example, our four-level sampling showed a population change from 118 L2H per 10 ft^2 to 79 L3 per 10 ft^2, or a dispersal loss of 33%. We consider this to be the most accurate estimate of population change.

The washing counts (L2H) are essentially identical within levels A, B, and C to the counts of emerging larvae (L2E) (Fig. 3) and lead us to believe that the significant difference in the D level is the result of branch selection and not a true population difference.

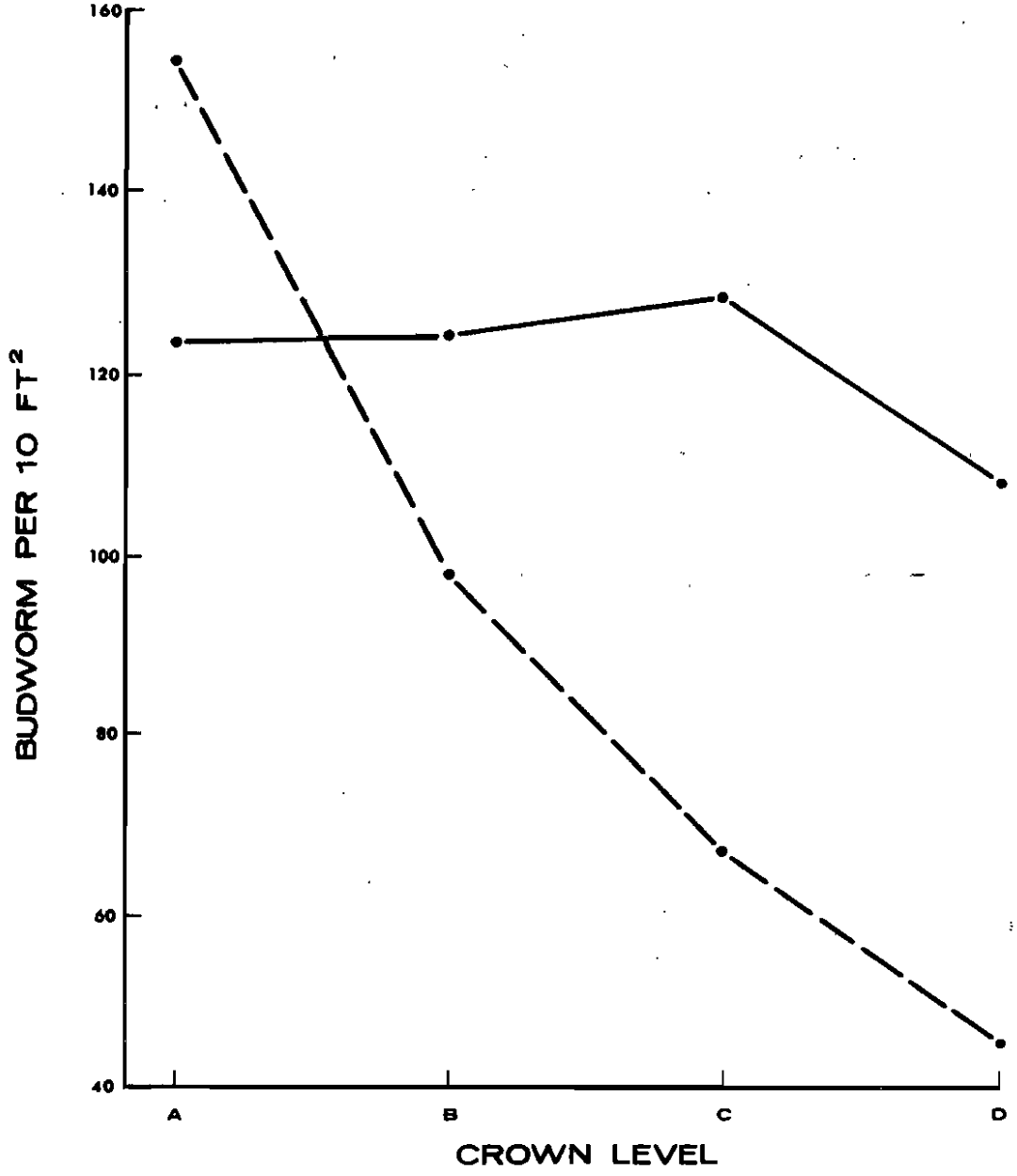

Fig. 4. Mean second-instar (L2H, solid line) and third-instar (L3, broken line) larvae per 10 ft^2 of foliage by crown level.

Within this level, it is suspected that the L2H samples were taken from the upper D level whereas the L2E samples were from the base of the crown. We conclude from Fig. 3 (L2H and L2E samples) that the washing technique extracted most of the larvae from the foliage.

We were confident in the conclusion that a very small proportion of the L2 population overwinters on the trunk and therefore the whole branch is an adequate collection unit.

SUMMARY

Investigations during a budworm outbreak (Miller 1958) suggested that the population "loss" during the spring dispersal of second-instar budworm larvae could be as high as 71%. Thus, when more recent comparisons of second- and third-instar budworm abundance revealed little or no dispersal loss, it was assumed that the sampling technique of washing second-instar larvae from the foliage with sodium hydroxide solution was at fault. However, an intensive test showed that the washing technique does extract most of the larvae from the foliage and counts on mid-crown branches give an accurate estimate of budworm abundance. The test also showed that, if the investigator wishes to measure population change (dispersal loss) from second- to third-instar larvae, sample branches would have to be taken from all crown levels rather than just one.

REFERENCES

Miller, C. A. 1958. The measurement of spruce budworm populations and mortality during the first and second larval instars. Can. J. Zoo. 36: 409-422.

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Table 4. Overwintering larvae per branch (L2H) by crown level on 10 trees

Level	Branch	Tree									
		1	2	3	4	5	6	7	8	9	10
A	1	15	8	12	15	17	15	24	10	8	19
	2	26	5	10	9	15	13	20	8	3	18
	3	17	19	22	11	10	25	32	7	2	22
	4	25	34	19	12	13	14	15	10	3	14
B	1	27	62	33	27	26	35	32	18	10	22
	2	22	84	54	26	43	22	27	13	11	40
	3	33	114	57	31	34	26	58	17	14	34
	4	40	126	79	49	23	36	42	17	10	48
C	1	27	63	49	53	62	33	50	35	43	77
	2	42	59	68	57	73	76	65	52	30	62
	3	39	71	30	41	96	40	91	44	15	53
	4	44	70	46	43	55	25	66	27	24	83
D	1	27	55	45	33	62	46	74	42	32	70
	2	65	27	34	47	53	35	32	65	22	84
	3	46	31	41	32	49	50	48	67	51	62
	4	44	42	62	69	70	65	35	89	44	75

Table 5. Emerging larvae (L2E) by crown level on five trees

Level	Tree 1	2	3	4	5
A	29[a]	37	82	37	9
B	129	170	182	272	88
C	145	201	239	207	193
D	65	69	64	118	126

a. Values are totals on four branches from each crown level.

Table 6. Third-instar larvae (L3) per branch by crown level on 10 trees

Level	Tree 1	2	3	4	5	6	7	8	9	10
A	24	29	18	26	46	60	13	58	65	46
B	43	24	48	40	54	58	31	19	48	29
C	16	29	6	15	40	34	39	42	60	6
D	26	22	9	52	10	14	0[a]	49	71	31

a. Few new shoots.