A SAMPLING TECHNIQUE FOR OVERWINTERING SPRUCE BUDWORM AND ITS APPLICABILITY TO POPULATION SURVEYS

by

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INFORMATION REPORT M-X-25

CANADIAN FORESTRY SERVICE
Department of Fisheries and Forestry

APRIL 1971

INTRODUCTION

The spruce budworm, Choristoneura fumiferana Clem., is a major forest pest in eastern Canada and the present widespread infestations have been a continuous threat to pulpwood forests since the mid-1940's. Many man-hours are spent in monitoring the intensity and distribution of these infestations to obtain both ecologic data on the pest and to determine the need to protect particular forests from further damage. Thus the development of more efficient systems for monitoring populations and predictive tools is a continuing objective of budworm research programs. One such system and its application are discussed in this report.

The budworm overwinters as a second-instar larva in a hibernaculum spun under bark scales and lichen of the host tree. Miller and McDougall (1968) observed that the hibernaculum could be destroyed and the larva washed from the foliage with a sodium hydroxide solution. A note on this sampling procedure was published (op. cit.) but subsequent modifications in technique warrant the following resumé. Equipment comprises a 9-quart container; a foliage 'basket' made of hardware cloth, ¼-inch mesh, and constructed to fit inside the container; a coarse sieve with a 0.8-mm mesh; a fine sieve with a 0.2-mm mesh; and a 4000 ml separating funnel.

Step 1. Clip foliage sample, usually a whole branch, into wire basket and immerse in a hot (50°C) 2% solution of NaOH.

2. Stir foliage once per hour and remove from solution after 8 hours.

3. Pour the NaOH solution through the two sieves. Needles are retained in the coarse sieve, the larvae and fine bark debris in the fine sieve.

4. Wash the twigs and needles in the foliage basket with water and pour effluent through the sieves.

5. Wash the larvae and debris from the fine sieve into the separating funnel with water. The total 'wash' from a mid-crown balsam fir branch will usually consist of 100 ml of debris in 600 ml of water.

6. Add 10 drops of methylene blue (4% solution) to stain the plant debris.

7. Add a sufficient amount of benzene, or similar type of light oil, to give an ⅛ inch layer on top of the aqueous solution.

8. Shake the water-benzene mixture vigorously to obtain a thorough mixing, then allow to settle. About 70 to 90% of the plant debris settles to the bottom of the funnel and 99% of the larvae collect at the oil-water interface. Too much debris hinders the separation process.

9. Draw off the plant debris and discard. Draw the oil-water fraction and vacuum filter. Use 95% alcohol to remove all larvae and debris from the separating funnel. The solids from the oil-water fraction for one branch can be collected on three to five, 6-in. filter papers. These can be examined in a few minutes under a microscope.

Various NaOH solutions were tested -- 2.5% cold, 2% hot, and 1% hot. Figure 1 shows that the hot solutions are much more effective than the cold and that about 90% of the larvae are extracted after 3 hours.

RELATIVE EFFICIENCY OF TECHNIQUE

Two types of large-scale surveys are used currently in eastern Canada to assess budworm abundance:

1. The Forest Insect and Disease Survey beat large larvae into collecting trays from standing trees to assess annual budworm density at permanent sampling stations. This is referred to as the 'beating' method.
2. Protection agencies conduct egg-mass surveys in one year to predict potential tree damage in the next year and the need for protective treatments.

Both types of surveys provide the required information and the introduction of another system would not be warranted unless it is more efficient or if a particular research program required population fixes on as many life stages of the pest as possible. We believe that washing over-wintering larvae from foliage has certain advantages over current survey techniques and these advantages are discussed below.

Sampling cost: The cost, on a time scale, of beating large larvae from standing trees is very low because no time is spent examining the foliage. Egg sampling is expensive because of the time spent examining individual needles for egg masses. The cost of washing larvae from the foliage is higher than the beating method but only one-third that of counting egg masses.

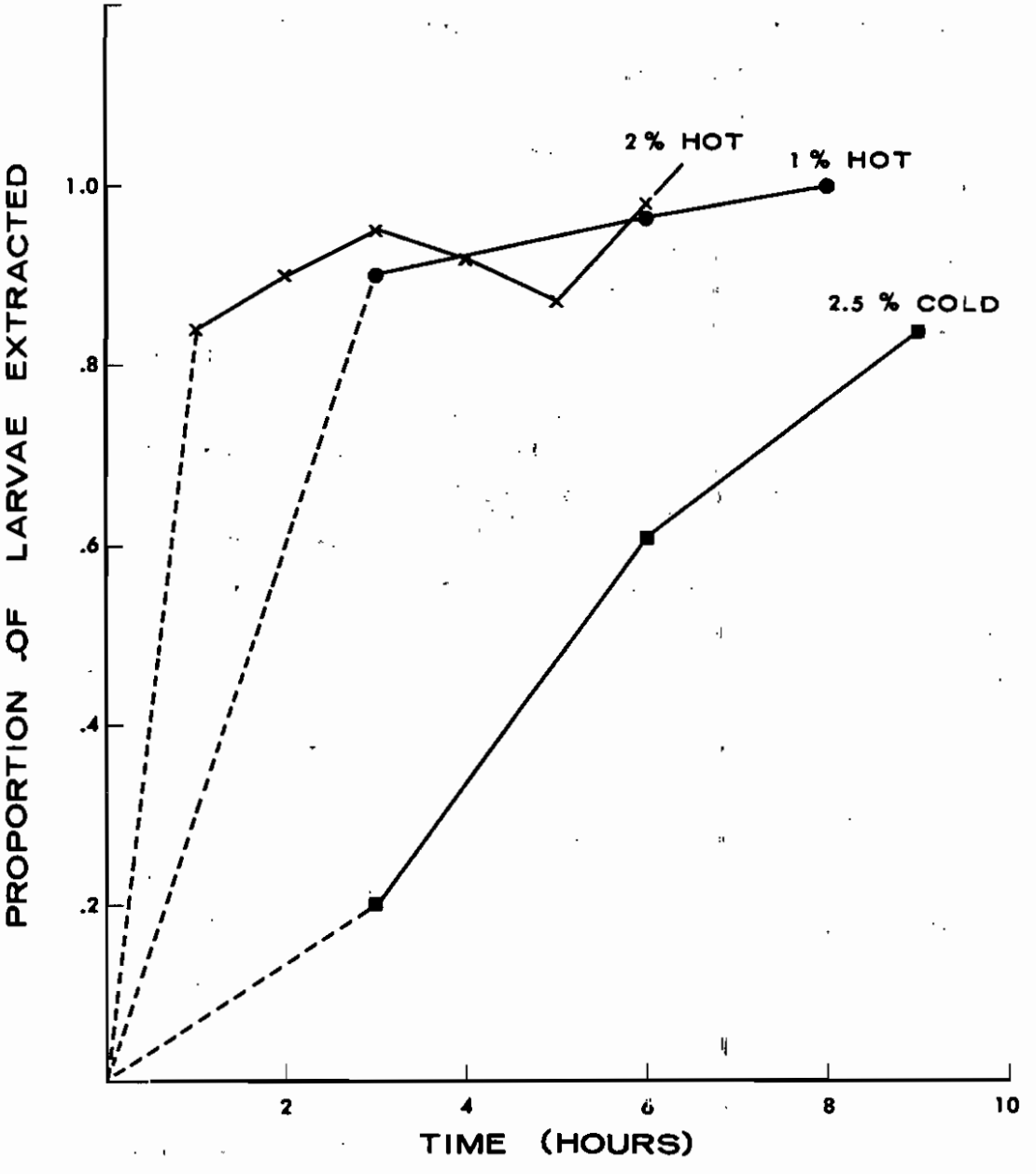

Figure 1. Proportion of larvae extracted by different treatments (NaOH concentration, temperature, and time).

Population stability: The longer a particular stage of a pest population remains numerically stable, the more readily an unbiased density fix can be obtained from a large number of scattered locations. Overwintering populations remain stable for about 7 months; egg populations for about 1 month. However, large larval populations are very unstable. Dynamic seasonal changes occur over a short period and thus the correct 'timing' to avoid a biased sample is extremely critical in the beating method.

Mechanical extraction: Washing overwintering larvae from foliage is amenable to mechanization. No system has yet been devised to remove egg masses from foliage but DeBoo and Copeman (1969) developed a procedure to dislodge large larvae mechanically which is more efficient than examining individual feeding sites visually.

Inter-tree variation in egg and larval counts: In budworm population studies, the tree is the basic sampling universe and inter-tree variation is of particular concern when attempting to define mean density within a forest type. Morris (1955, figs. 9 and 10) has shown that the inter-tree variance of egg counts is higher than the variance of either large larval or pupal counts. In a pilot sample, we found that the variance of egg counts is also higher than the variance of overwintering larval counts (Table 1).

Table 1. Inter-tree variance in population counts of eggs and overwintering larvae[a]

	Density per mid-crown branch				
Stage	5	10	15	20	30
Egg masses	10	36	80	114	300
Overwintering larvae	10	30	56	92	190

a. Variance based on one branch from each of three trees.

Thus more sample units (trees) are required to establish mean density of the egg stage than that of other life stages with a set degree of precision. Egg-mass sampling is, therefore, costly in terms of the number of sample units to be examined as well as the time required to examine each unit (as noted above).

Sampling precision: Precision is another factor that must be considered in selecting a survey technique, particularly if the counts of one developmental

stage of the pest are used to predict the abundance of a later stage. Budworm egg-mass counts in August are used to predict the abundance and potential for damage caused by large larvae in the next year. A pilot sample of egg-counts (August), second-instar counts (September - March), and fourth-instar counts (late May) were obtained at each of a number of locations, to answer the question are egg-mass counts a more reliable predictor than second-instar counts? Regression analyses in which the fourth-instar counts were treated as a function of (1) egg counts and (2) second-instar counts showed that the latter explained 45% of the variation in the fourth-instar density but the egg sample only accounted for 22%. Neither figure is impressive for predictive purposes partly because only three mid-crown branches were examined per location and the same trees were not sampled each time. However, the greater precision of second-instar counts as a predictive tool warrants further testing and we strongly suspect that a second-instar survey is the better method to define and predict budworm population levels.

APPLICABILITY OF TECHNIQUE

If a decision is made to use the washing technique as a survey tool, then information is required on the type of collection unit, size of sample unit, etc. We have obtained some pilot data on these points as outlined in the following sections.

Location of hibernacula: One problem in sampling an overwintering population is that some larvae spin hibernacula on the stem and thus a branch sample may not provide a 'total' collection unit. Terrell (1959) compared stem and branch samples from Douglas-fir and found 2.9 larvae on the branch as opposed to 58 larvae on the stem on a per unit area of surface basis. However, Miller (1958) estimated that the 'stem' population of second-instar larvae constituted only 1% of the total tree population in open, mature balsam fir stands and 4% in dense, middle-aged stands. Red spruce is another common host of budworm in New Brunswick and to check stem versus branch populations on this species nine bolts, each 2 feet long, were removed from each of four trees and four mid-crown branches were selected from each of the same trees. Branch and stem samples were washed in NaOH and totals of 376 larvae were counted on the stem and 2,200 on the branch units. Conversion of these data show that the stem population was about 2% of the total tree population and thus we conclude that branches are an adequate collection unit for second-instar population surveys of both fir and red spruce.

Selection of the sample unit: There are gradients in the growth characteristics of branches within the crown, and the exposure of branches within the canopy of conifer stands. The budworm responds to, and is affected by, these gradients and they must be recognized when selecting branches as sampling units. For survey purposes, it has been found that a mid-crown branch from balsam fir provides a representative sample for egg and third-instar larval surveys. To determine the within-crown distribution of second-instar larvae, single branches were selected from the top, middle, and basal third of the crown of each of 10 balsam fir and 10 red spruce and washed in NaOH solution. The mean counts per level on the two species were similar (Table 2).

Table 2. Counts of second-instar larvae on balsam fir and red spruce

Species and sample	Crown level		
	Top	Middle	Basal
Balsam fir			
Per branch	45	75	75
Per unit area of foliage	15	15	13
Red spruce			
Per branch	64	88	70
Per unit area of foliage	15	15	9

The lower density of the basal branches (statistically significant on spruce) was not unexpected because egg counts and third-instar counts show a similar gradient. However, the data show that a branch selected from the mid-crown would not be an unduly biased sample unit for a survey of overwintering larvae.

Sample size: Sample size, or the number of foliage units to be examined to determine population density with precision, is the function of a number of variables such as the size of the collection unit, population density, distribution of the population within and between trees, and whether the objectives of the sampling program are intensive or extensive in nature. Considerable pilot sampling is needed to obtain these data but a first approximation of sample size for an overwintering budworm population is shown in Table 3 where it is assumed that:

(a) one mid-crown branch is drawn per tree;

(b) the infestation level ranges from 10 to 60 larvae per branch; and

(c) the required precision (standard error) is either 10 or 20% of the mean. A standard error of 10% of the mean is the usual arbitrary limit set for intensive population studies.

Table 3. Sample size for population counts of overwintering spruce budworm on balsam fir

Expected mean density per branch	Observed variance	Number of trees required[a] for standard error of --	
		10% of mean	20% of mean
10	30	30	8
30	190	21	5
60	660	18	5

a. One mid-crown branch per tree.

Population Density in Relation to Host: In sampling mixed conifer stands, some consideration must be given to the potential differences in second-instar density on different host species. The following are preliminary observations on fir, red spruce, and white spruce. In a 48-year-old, severely infested stand containing 60% balsam fir and 40% red spruce, counts of second-instar larvae from washing were not significantly different between species. In contrast, five times as many larvae were found on balsam fir as on white spruce in a 'young to middle-aged' stand (80% fir and 10% spruce with a developing infestation. These data are illustrated in Figure 2 where the second-instar counts per mid-crown branch on fir (Lines A and B) and on spruce (Line C) are plotted over tree height. Figure 2 also shows that three times as many larvae were washed from 'flowering' fir (Line A) as non-flowering fir (Line B). The term 'flowering' refers to scars of staminate flower. Although the non-flowering trees, ranging from 20 to 30 feet in height, were subdominant to the 30- to 45-foot flowering trees, the three-fold difference in budworm density is impressive. These results diverge from earlier findings by Miller (1958) in which he was unable to show that the overwintering larvae were concentrated in the flower scars on a branch as opposed to the twigs of the same branch. The precise role

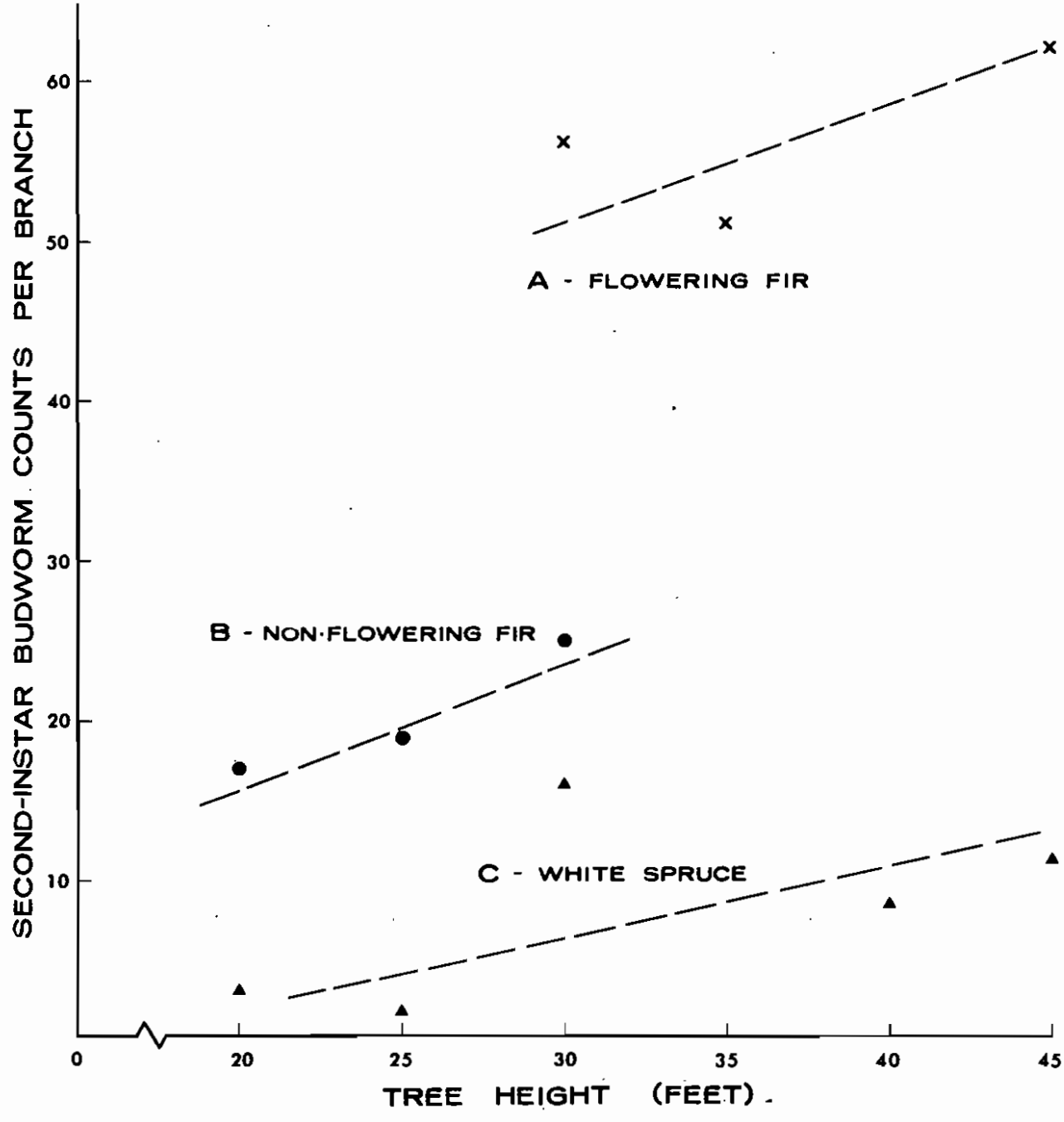

Figure 2. Density of overwintering budworm on three specified hosts in a middle-aged fir-spruce stand.

of old flower scars in budworm dynamics was therefore questioned, but it is now apparent that more data are required to define second-instar densities in conifer stands and the role of the flowering-fir component. If flowering fir is the major component in the stand, then it may determine the number of overwintering budworm and thus greatly affect the susceptibility of the stand to a population explosion. In any event, Figure 2 shows that, in a general survey of overwintering populations in predominately balsam fir stands, the potential differences between the flowering and non-flowering components must be recognized.

Predicting Damage: Protection agencies currently use the egg-mass survey to predict the expected degree of current defoliation in the next year (Fig. 3). Thus egg density becomes one input value in the decision to apply a control treatment. The relationship between overwintering larvae per branch and expected defoliation is also shown in Figure 3. The correlation of larval density and defoliation is somewhat less than the value for eggs but wholly adequate to predict that a particular pest density would cause low, moderate, or severe defoliation.

DISCUSSION

Washing overwintering spruce budworm larvae from foliage is an efficient method of obtaining a population fix. We are convinced that the technique could partly replace the current 'beating' survey and supplement the egg-mass survey. The term 'partly replace' is used because the beating method would doubtless be retained to obtain data on parasites or to sample those areas where budworm appear unexpectedly. Similarly, protection agencies would continue to conduct egg-mass surveys because egg counts are the first population fix obtainable in a particular generation and the need for a control treatment is decided 8 months in advance. This is essential in large-scale programs and is a strong argument for an egg survey. However, overwintering larvae can also be counted early and give 5 to 7 months preparation time. Thus we believe that the egg survey data could be supplemented with counts of overwintering larvae to give a more precise and less costly definition of budworm abundance.

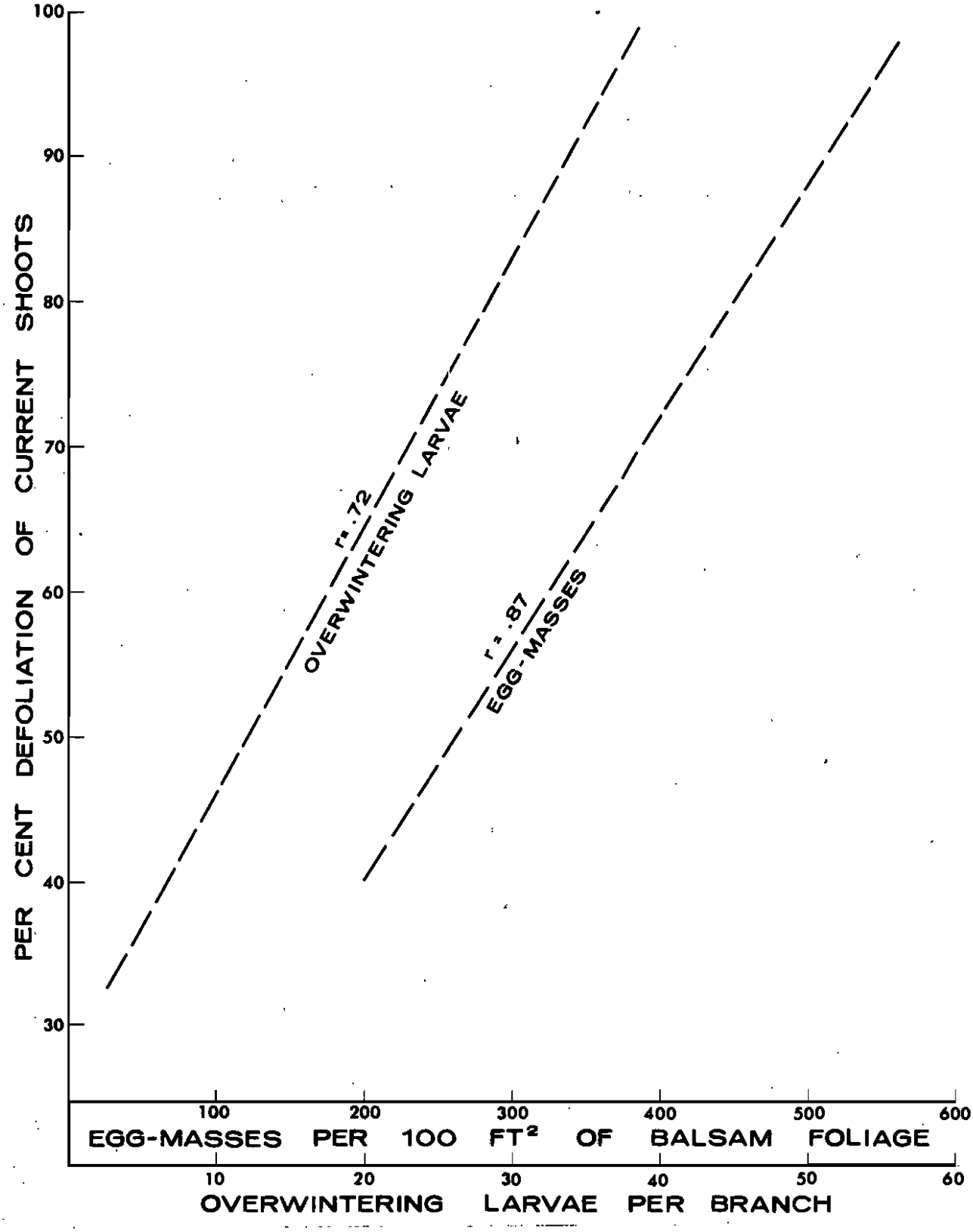

Figure 3. The relationship between expected defoliation of current balsam fir shoots and the density of eggs and overwintering larvae.

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