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Guide to Testing Insecticides on Coniferous Forest Defoliators

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INTRODUCTION

Coniferous forests of the Western United States are periodically infested by epidemic populations of defoliating insects. Effective and safe use of insecticides, an integral part of most pest management programs, is one method of control.

Inherent in the insecticide application process, however, is an extremely high degree of variation. Chemicals used may be ephemeral or persistent; one species of insect may be susceptible to a particular chemical while another may not; there may be differences in susceptibility or tolerance to a specific chemical insecticide among populations of an insect species; the pest's vulnerability and susceptibility may vary at different developmental stages; weather conditions in the test areas may differ, together with forest type and stand structure; dosage rate, type and volume of formulations vary with insecticide; spray nozzle systems and aircraft types are many; accurate deposit analysis depends on an understanding of the physics of droplet spectra and impingement, and these differ with insecticide formulations and test conditions.

Human variation plays its role as well. Entomologists have varying degrees of experience in planning and supervising research, pilot and control projects, and in analyzing and interpreting the data.

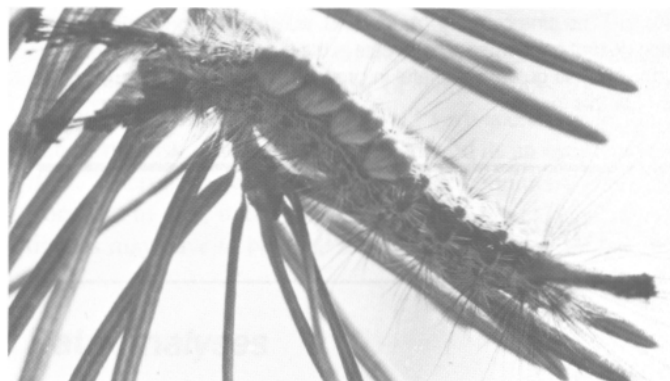
Many sources of variation can be better controlled by careful planning. In developing plans to test insecticides, the manager must rely on the best available information. Some information on biological evaluations about insect outbreaks and control operations has been published, but much of it exists only in unpublished reports and, therefore, is not easily accessible. We have sought to bring together in this report much of the information necessary for planning.

This report provides a guide to testing insecticides applied to coniferous forest defoliators. It outlines techniques for designing, installing, conducting, and evaluating various types of projects, and describes the sampling considerations, methods, and analytical methodology necessary to do the job.

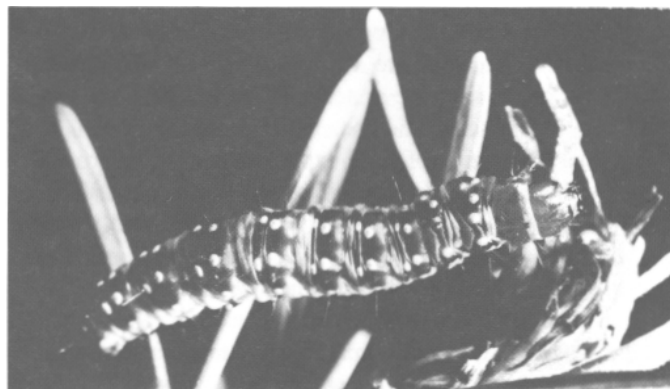
This guide is based both on our experiences in the forests of Washington, Oregon, Idaho, and Montana, with populations of western spruce budworm (*Choristoneura occidentalis* Freeman)

and Douglas-fir tussock moth (*Orgyia pseudotsugata* McDunnough), and in the Northeastern United States with populations of the spruce budworm (*Choristoneura fumiferana* [Clements] Freeman). Although the guide is based primarily on studies of budworm populations, much of the information reported can be applied to other defoliating insects such as the Douglas-fir tussock moth, hemlock looper (*Lambdina fiscellaria lugubrosa* Hulst.), the pine butterfly (*Neophasia menapia* Felder and Felder), and the larch casebearer (*Coleophora laricella* Hubner) (figs. 1, 2).

No guide can substitute for work habits and attitudes necessary for a thorough, careful collection of data. Care and thoroughness are particularly vital at the interface between biological phenomena that are observed and data that represent them.

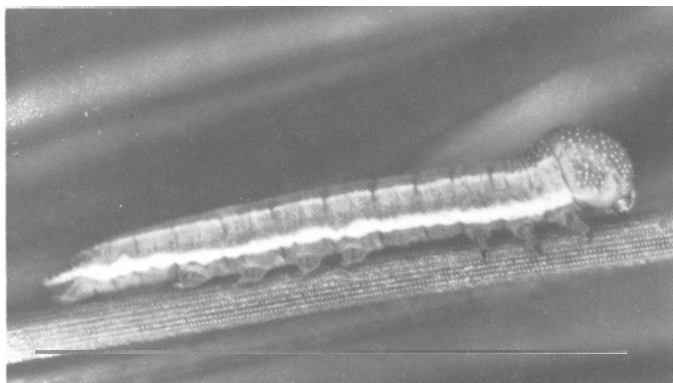


A



B

Figure 1—Douglas-fir tussock moth (A) and western spruce budworm (B) are the most important defoliating pests of Douglas-fir and true fir forests in the United States. The larvae of both species are similar in their feeding habits and distribution in the host trees. Their populations can be sampled with similar methods.



A



B

Figure 2—The pine butterfly (A, larva; B, adult) is the most serious defoliating pest of ponderosa pine forests in the United States. The methods described in this guide are useful in projects to suppress epidemic populations of this insect.

EXPERIMENTAL DESIGN

Objectives

The Forest Service, U.S. Department of Agriculture, uses a progression of tests to evaluate and register various insecticide treatments for use in resource protection. These are field experiments, pilot control tests, and operational control projects.

The primary objective of field experiments is to determine minimum effective dosage of one or several treatments against a specific target pest. Treatments may involve different insecticides, different dosage rates or formulations of the same material, or different application equipment. Ensuring adequate replication within the confines of operational constraints, such as manpower and cost, usually requires that treatment plots be small.

Pilot control tests evaluate the most promising treatment identified by field tests under operational conditions. Usually, only one dosage is evaluated and the plots are large enough to simulate operational conditions. Data from field experiments and pilot control tests are used to register highly promising insecticides

with the U.S. Environmental Protection Agency. Operational control projects use registered pesticides in a safe manner to achieve resource protection. Resource management objectives are defined in operational control programs and benefit-cost effects of alternative control activities on these objectives are evaluated. These programs may attempt to treat an entire infestation and are carried out on large areas, broken up into spray blocks of thousands of acres. Because their objectives differ, the design and plan for each will differ. Nevertheless, many considerations and activities are similar among them.

In planning a field experiment, a pilot control test, or an operational control project, the first step is to state the objectives clearly, concisely, and as specifically as possible. The objectives may be to estimate treatment effects or specifications to be met, or to test hypotheses. Some field tests of insecticides may be planned to determine the lowest effective dose to achieve a certain percent control or residual population for each treatment and to compare treatments. Usually, experiments with such general purposes are evaluated by analysis of variance (ANOVA). If overall treatment effects are indicated as significant by the F test, tests for differences between pairs of means are the next focus of interest. In other tests and control projects, more specific purposes may be to estimate percent reduction or initial as against residual population size.

To arrive at an effective design for either hypothesis testing or estimation, we must consider variability and cost. Because the criteria for judging effectiveness for the two goals are different, the design parameters—such as number of plots, number of trees, will likely be different. In some instances, both hypothesis testing and estimation are important in a single field test. The test should then be designed using both criteria. The set of design parameters that both meet design criteria for one goal and exceed the criteria for the other goal should be used.

Hypothesis Testing

In choosing the framework for testing insecticides, we need to consider the power of the tests, that is, the probability of detecting treatment effects when they exist. Power is the criterion by which confidence in the experimental design can be measured.

If we designate the hypotheses

H_0 : no differences exist between treatments, or between treatments and control, or

H_1 : one or more treatments differ significantly from the control or from each other,

then the two kinds of error that can be made in hypothesis testing are Type I or Type II, in which:

Type I error is to decide there is a treatment effect when, in fact, there is none; or to reject a true hypothesis (accept H_1/H_0)

Type II error is to decide there is no treatment effect when, in fact, there is one; or accept a false hypothesis (accept H_0/H_1).

Results of possible decisions in hypothesis testing are as follows:

		Correct Decision	
		H_0	H_1
Actual Decision	H_0	No error $P = 1 - \alpha$	Type II $P = \beta$
	H_1	Type I $P = \alpha$	No error $p = 1 - \beta$

The size of the Type I error is referred to as α and the size of the Type II error as β . The power of the test is $1 - \beta$, or the probability of rejecting a false hypothesis. In the field experiment, $1 - \beta$ is equivalent to the probability of detecting a treatment effect when one exists.

Suppose, for example, we are interested in detecting a difference of 10 percent in number of larvae killed between two doses of an insecticide, and that we erroneously decide that there is not this much difference when, in fact, there is a difference of more than 10 percent. Then a Type II error has been made. If the probability that a Type II error will occur is greater than or equal to 20 percent, given the design of our test procedure, the power of this test is less than 80 percent. Keeping the power above a given level ($1 - \beta$) requires keeping the Type II error below β . We always want to maximize the power and minimize the probability of making a Type II error, given an initial choice of acceptable size for the Type I error, α .

Variance, cost, and experimental design influence power. For a given sample size, the larger the variance within or between experimental units, or both, the lower the power. Consider, for example, an experiment in which n trees on a plot are sprayed with an insecticide and n trees on another plot serve as controls. If the number of insects, after spray, varies considerably from tree to tree (which is usually the situation after treatment), it is more difficult to detect if the treatment has an effect. The variance is larger than it would be if all trees within a plot had the same postspray count (low variance).

The relation of cost to power concerns sample size, because increased sample size reduces variance, leading to increased power. A problem then confronting the investigator is choosing the maximum possible sample size for a cost that is to be held at a given level.

Factors in experimental design that influence power include number of levels per chemical, the spread of differences between levels, number of replications, and number of sampling and subsampling units. Power can be increased by decreasing the number of treatments and by selecting treatments for comparison with expected large differences in treatment effects. For a given number of observations, power increases as number of observations per treatment increases.

Costs

The cost of sampling is, of course, a primary constraint on sample size, and must be considered when the experiment is designed. A cost function can be constructed to take into account both fixed and variable costs. Fixed costs, such as rental of aircraft, do not fluctuate particularly with sample size, but costs for

plot selection, layout, and sampling depend on worker hours. Cost of personnel will vary with number of units and subunits sampled, and with other factors peculiar to the sampling situation, such as roughness of terrain, weather conditions, and accessibility and condition of roads.

A typical cost function for this type of experiment would include items such as the following:

1. Aircraft cost
2. Experimental design and plot selection
3. Travel time between worker's residence and study areas and between plots
4. Plot layout, flagging and corner marking, and removal of markers
5. Selection, marking and mapping of trees, travel between trees on plot
6. Setting out and picking up spray cards associated with study trees
7. Reading of spray cards in the laboratory
8. Field collection of samples (branches), number of branches per tree, travel between trees on plot
9. Prespray examination of samples in the laboratory
10. Postspray examination of samples in the laboratory.

The cost for item 1 depends on carrying capacity and type of aircraft, distance between landing strip and experimental plots, number of plots and number of acres per plot, distance between plots, and topographic features of plots. The cost of items 2, 3, and 4 depend upon number of worker-hours per plot, and items 5 and 6 upon number of worker-hours per tree. Item 7 may be a fixed cost per spray card (and therefore per tree), while items 8, 9, and 10 depend on number of worker-hours for handling branches. Costs per worker-hour will vary according to the nature of the task, but can usually be entered as an estimated constant. If terrain, accessibility, and other conditions vary much from plot to plot, initial plot selection and insect population estimates may have to be done separately by plot.

Data Analyses

To account for treatment differences, given natural differences and sampling error, we can define the variables of interest to be the ratio of insects to foliage after treatment to that before treatment. An estimate of this variable and the components of the variances of the estimates can be computed on the basis of ratios of the plot means. Usually, the experiment will be designed to test differences between dose levels, or between treatments and control, for a number of insecticides. The model for analysis of the experiment will then be evaluated by a one-way ANOVA with plot ratios as observations and insecticides at various levels with controls as treatments.

Variability in sampling within such an experimental framework is often so high, however, that the power of tests of differences within the bounds of feasible sampling configurations is not high enough. It may have been decided ahead of time, for example, that in testing a hypothesis of no difference between treatment and control no more than 21 trees on each plot with two branches per tree can be sampled

because of cost and other considerations. Yet, the variance estimate for such a sampling configuration may be such that the probability of detecting a desired 10 percent difference between treatment and control would be only 50 percent.

We will first develop and illustrate the design procedure for one-way ANOVA with randomized blocks and then provide an alternative framework which, when appropriate, can somewhat alleviate the need for large sample size.

The process of design consists of selecting a set of design parameters that maximize the power of the F test of the hypothesis $H_0: B_1 = B_2 = \dots B_r$ against the alternative hypothesis $H_1: B_1 = \beta_1$ $i = 1, \dots, r$. The B_i are the treatment effects, in our example, the true mortality under treatment i . The parameters we can set are:

r = number of treatments
 nb = number of blocks (replications)
 nt = number of trees per plot
 nbr = number of branches per tree

To maximize power we need to calculate the noncentrality parameter Φ of the noncentral F distribution:

$$\Phi = \frac{\sqrt{\sum_{i=1}^r \beta_i^2}}{\sigma \sqrt{r/nb}}$$

One of the variables, nb , in Φ is a member of our set of design parameters that will be varied in searching for a maximum. Of the other three variables, the σ must be estimated from data on hand, the β_i 's set to reflect our alternative hypothesis, and the r is given when we set our objectives.

The usual error mean square from the one-way ANOVA with randomized blocks is used to estimate σ^2 :

$$\hat{\sigma}^2 = \sum_{i=1}^{nb} \sum_{j=1}^r \frac{(y_{ij} - y_i - y_j + y_{..})^2}{(r-1)(nb-1)}$$

in which

y_{ij} = after/before ratio of number of insects for block i , treatment j (the number of insects is totaled over all branches and trees on each plot)
 y_i = mean for block; y_j = mean for treatment; and $y_{..}$ = overall mean.

This shows $\hat{\sigma}^2$ as a function of nb and r , but not explicitly as a function of nt and nbr . To let the number of trees and branches vary in our search for maximum power, therefore, we must break σ^2 into its components; the variability between plots, σ_e^2 , and the sampling variance in estimating each plot mean, σ_s^2 . Specifically, experimental error = $\sigma^2 = (nt)(nbr)\sigma_e^2 + \sigma_s^2$. The sampling variance in estimating each plot mean is estimated (Kendall and Stuart 1963, p. 247) by:

$$\hat{\sigma}_s^2 = \left(\frac{E(X2)}{E(X1)} \right)^2 \left\{ \frac{\text{Var}(X2)}{E^2(X2)} + \frac{\text{Var}(X1)}{E^2(X1)} - \frac{2\text{Cov}(X1, X2)}{E(X1)E(X2)} \right\} = \text{Var} \left(\frac{X2}{X1} \right)$$

in which

$X1$ = number of insects per plot before treatment

$X2$ = number of insects per plot after treatment.

We can use sample estimates for each component. The formulas for estimating two-stage sampling variance are from Cochran (1963, p. 277). For both $X1$ and $X2$ we have:

$$\begin{aligned} \hat{\text{Var}}(X) &= \frac{s_1^2}{nt} + \frac{s_2^2}{(nt)(nbr)} \\ s_1^2 &= \frac{\sum_{j=1}^r s_{1j}^2}{r(nt-1)}, s_2^2 = \frac{\sum_{j=1}^r s_{2j}^2}{r(nt)(nbr-1)} \\ s_{1j}^2 &= \frac{\sum_{k=1}^{nt} (x_{k.} - x_{..})^2}{nt-1}, s_{2j}^2 = \frac{\sum_{k=1}^{nt} \sum_{m=1}^{nbr} (x_{km} - x_{k.})^2}{nt(nbr-1)} \end{aligned}$$

in which

x_{km} = insect count on branch m , tree k , plot j
 $x_{k.}$ = mean count for tree k , plot j
 $x_{..}$ = mean count on plot j

$E(X)$ = grand mean over all branches, trees, plots

$$= \frac{\sum_{i=1}^r \sum_{j=1}^{nb} \sum_{k=1}^{nt} \sum_{m=1}^{nbr} x_{ijkm}}{r(nb)(nt)(nbr)}, \text{ in which } x_{ijkm} \text{ is the count on branch } m, \text{ tree } k, \text{ block } j, \text{ treatment } i.$$

An unbiased estimate of

$$\text{COV}(X_1, X_2) = \sum_{i=j}^r \sum_{j=1}^{nb} \left(\frac{\sum_{k=1}^{nt} x_{1ijk} x_{2ijk} - (nt x_{1ij.} x_{2ij.})}{r(nb)(nt)(nt-1)} \right)$$

in which x_{1ijk} and x_{2ijk} are sample means for the k th tree, block j , treatment i

and

$x_{1ij.}$, $x_{2ij.}$ are plot means for treatment i , block j .

Substituting in σ_s^2 , we get $\hat{\sigma}_s^2$ with nt and nbr equal to the number of trees and number of branches in the data set used. Then, with $\hat{\sigma}^2$ = error mean square (EMS) from ANOVA

$$\text{and } \hat{\sigma}_s^2, \text{ we get } \hat{\sigma}_e^2 \text{ from } \hat{\sigma}_e^2 = \hat{\sigma}_e^2 = \frac{\hat{\sigma}^2 - \hat{\sigma}_s^2}{(nt)(nbr)}.$$

We now have estimates of everything we need to get an estimate of $\hat{\sigma}^2$ for any combination of nt and nbr . We use s_1^2

and s_2^2 to estimate $\text{Var}(X1)$ and $\text{Var}(X2)$; these are combined with $E(X1)$, $E(X2)$ and $\text{Cov}(X1, X2)$ to obtain an estimate of σ_s^2 we can call $\hat{\sigma}_s^2$ for a particular combination of nt and nbr . Then

$$(nt)(nbr) \hat{\sigma}_e^2 + \hat{\sigma}_s^2 = \hat{\sigma}^2$$

an estimate of between-plot variation for the chosen values of nt and nbr .

We now need only to select values of β 's to calculate power for a range of design parameters. Reasonable values of differences between the β_i 's under alternate hypotheses in this problem can be taken as between 0 and 1. If, in an experiment with two levels of treatment, we wish to reliably detect differences when the pattern of treatment mortalities is at least as different from equal as $\beta_1 = 0.8$, $\beta_2 = 0.9$, then the β_i are calculated as follows:

$$\begin{aligned} \beta_2 &= \beta_1 + 0.1 \\ \text{with } \sum_{i=1}^2 \beta_i &= 0 \\ \beta_1 + (\beta_1 + 0.1) &= 0 \\ \beta_1 &= 0.05 \\ \beta_2 &= -0.05 \end{aligned}$$

We now have everything we need to calculate power. For example, if $r = 2$, $nb = 8$, $\hat{\sigma} = 0.10$, $\alpha = .05$, then

$$\Phi = \frac{\sqrt{\sum \beta_i^2}}{\sigma \sqrt{r/nb}} = \frac{\sqrt{0.05^2 + 0.05^2}}{0.10 \sqrt{2/8}} = 1.41$$

The degrees of freedom are 1 and 7, the same as those for the F test for treatment, namely $r - 1$ and $(nb - 1)(r - 1)$. From charts by Pearson and Hartley (1951), we find for $df_1 = 1$, $df_2 = 7$, and $(\Phi = 1.41)$, the power is 0.41.

When the cost function has been defined in terms of r , nb , nt , and nbr , power and cost can be computed for enough values of these parameters to construct isopower and isocost curves.

To illustrate, some of the results of designing new field tests for Orthene against larch casebearer are based on variance and cost data from an earlier test (Hard and others 1979).¹ A question often asked is whether $\frac{1}{2}$ lb of Orthene is as effective as 1 lb of Orthene. The null hypothesis is that $\frac{1}{2}$ lb yields the same result as 1 lb, that is, $\beta_1 = \beta_2$. To justify the expense of the field test, we would like the probability of rejecting the null hypothesis if $\frac{1}{2}$ lb gives at least 0.1 less mortality than 1 lb ($\beta_1 - \beta_2 \geq 0.1$). The β_i 's for the alternate hypothesis can be calculated from $\beta_1 - \beta_2 = 0.1$, $\beta_1 + \beta_2 = 0$. From this we obtain $\beta_1 = 0.05$ and $\beta_2 = -0.05$. We then calculate power and cost for a number of combinations of nb and nt , with nbr set to 2, 4, 6, and 8.

¹ This report neither recommends the pesticide uses reported, nor implies that they have been registered by the appropriate governmental agencies.

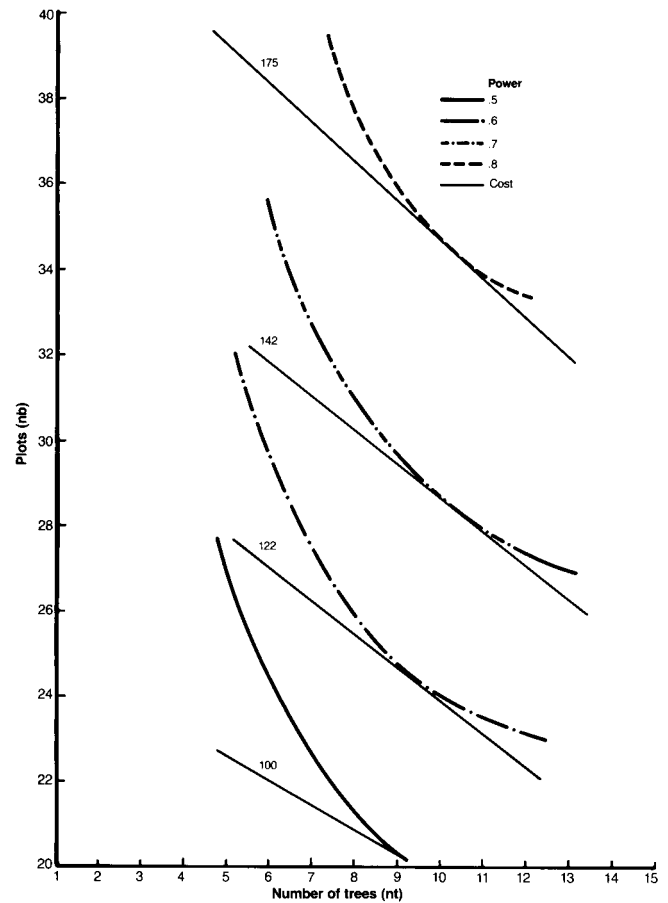


Figure 3—Isopower and isocost curves for various integer values of number of trees per plot or block (nt). The combination of number of blocks or plots (nb) and nt that provide a given level of power for the least cost is found at the point where a cost curve is tangent to the power curve. Cost is expressed as hundreds of dollars.

For a given power, cost is always lowest for two branches per tree ($nbr = 2$). We then calculate power and cost for all combinations of nb from 20 to 40, and of nt from 1 to 15, with nbr set to 2. Isopower and isocost curves are then fit to power and cost values at the integer values of nb and nt (fig. 3). The combination of nb and nt that provides a given level of power for the least cost is found at the point where a cost curve is tangent to the power curve. The point of tangency may not fall on integral values of total number of plots, nt , or both. For power = 0.8, the minimum cost is slightly above \$17,500 and could be obtained with 17 replications (34 plots), 11 trees per plot and 2 branches per tree, or 36 plots, 9 trees per plot. For power of at least 0.7, we can choose either $nb = 30$, $nt = 9$, or $nb = 28$, $nt = 11$. In each situation, the power will be above 0.7, and the cost will be about \$14,200. We could, of course, look at the actual cost of each of the combinations and choose the combination with minimum cost. Because we are designing a new study with estimated variances and average costs from previous studies, however, this refinement is not necessary. Either combination available is a reasonable choice if at least 0.7 power is desired. We believe it is unreasonable to plan an experiment to answer an important question with power less than 0.8.

As design parameters, therefore, we choose either 34 plots, 11 trees and 2 branches, or 36 plots, 9 trees and 2 branches. But in many instances an experiment of this size is not feasible. Achieving proper time of applications on all plots may be impossible; or, it may be impossible to find enough infested plots in a compact enough area so that the variance used in calculating power still applies; or it may be too expensive.

What can we do in these situations to be reasonably sure of conducting a successful experiment? If we reword our question from "Is ½ lb Orthene as effective as 1 lb Orthene?" to "Are ½ lb and 1 lb each effective?" we may then use a more powerful test. But we must first define effectiveness in quantitative terms. We may believe arbitrarily, for example, that a treatment is effective if the mortality is at least 95 percent, or a treatment is effective if fewer than 5 budworms per 100 buds remain after treatment (McGowan and others 1967, Stipe and others 1977). We may now use a one-sample Student's t-test:

$$t_{n-1} = \frac{(\bar{r} - \mu_r)\sqrt{n}}{s_r}$$

in which

r_i = ratio of before and after counts on the i^{th} plot

$$\bar{r} = \sum_{i=1}^n r_i$$

$$s_r^2 = \sum_{i=1}^n \frac{(r_i - \bar{r})^2}{n-1}$$

μ_r = criteria for effectiveness

To design an experiment we again look at the power. With the same number of trees, variance, and alternate hypothesis as above, we calculate the noncentrality parameter of the t distribution as:

$$\delta = \frac{(\mu_r - \mu_a)\sqrt{n}}{\sigma} = \frac{(0.9 - 0.8)\sqrt{n}}{0.10}$$

in which μ_a = the mortality under the alternate hypothesis.

With the power curves (Owen 1962) for three values of n we get the following:

n:	δ	Power
6	2.5	0.72
7	2.7	0.78
8	2.9	0.85

To achieve 0.8 power for each of these individual tests of effectiveness, therefore, we need only 8 plots per treatment instead of 17 needed using an analysis of variance design. The variable cost of the experiment is only \$8,341, compared with \$17,726 for the analysis-of-variance design.

Other Methods

Previous experimental designs for pilot control tests and operational control projects have stressed the reduction of variance in the allocation of resources to obtain good estimates of population densities and mortalities (Mounts 1976). Carolin and Coulter (1972) described a procedure that allocates sampling effort optimally among plots, subplots (clusters), and trees with a multistage computer program of Hazard and Stewart (1974). Analyses are based on variance in numbers of larvae on four twig lots and relative costs attributed to plots, subplots (clusters), and trees.

Some statistical and practical considerations in sampling favor fewer trees per cluster and more clusters per area. A cluster represents a single sample point no matter how many trees are in the cluster. The more trees and branches sampled, the more information obtained about the insect population at that sample point or cluster, which usually covers less than 1 acre (0.405 ha). Beyond a certain point, which depends upon the variability of the data, additional trees in a cluster do not contribute substantially to precision of population estimates. Cluster means are usually more variable than measurements between trees within clusters. In this case, more clusters with fewer trees in each one give a more precise estimate of the population mean. When precision of the estimate is the only criterion for choice of sampling design, single tree clusters are, for the usual case, the most efficient. The advantages of cluster sampling schemes can then be considered in terms of cost and practicality.

Cost per sample unit is the major cost of sampling, and traveling between trees and plots is the major expense incurred. Costs of sampling each tree are essentially similar. Because distances between trees in a plot or cluster are small it may be more economical to choose more than one tree per plot or cluster and thereby reduce the number of plots or clusters in an area. Instead of 60 one tree plots on which to observe treatment effects in a spray area, for example, it may be more cost-efficient to observe treatment effects with 20 three tree plots (clusters). Also, it is easier to locate 20 three tree plots (clusters) on the more accessible sites in a study area than it is to locate 60 plots. The cost-effectiveness of various combinations of clusters and trees is a major part of a computer program by Hazard and Stewart (1974) that allocates sampling effort.

SPRAY AREAS

Boundaries

Our working assumption is that a western spruce budworm outbreak is damaging trees in several National Forests in

Montana. After 3 to 5 years of heavy defoliation, widespread tree mortality will result in one forest if heavy defoliation continues for an additional season. A summer aerial reconnaissance shows large areas of severe defoliation caused by budworm feeding in spring and early summer. A fall budworm egg mass survey indicates continued high larval populations for the next spring. In early fall, research entomologists, with the cooperation of the forest pest management staff and the forest supervisor, decide to conduct field experiments in the infested forests to determine the effectiveness of several promising new insecticides; or alternatively, they decide, in cooperation with the forest supervisor and pest control specialists, to spray those areas where widespread tree mortality is expected and those forests stands to be protected for timber, esthetic high-use areas or winter game range.

A control project (field experiment, or pilot control test) is set up. Responsibilities are delegated. You are the project entomologist. Much of the success of the project depends upon what you do in the next few months.

First, boundaries of the proposed spray area must be determined. Aerial detection and damage reconnaissance surveys are the best sources of infestation damage for determining proposed spray boundaries. Aerial surveys of forest defoliating insects such as the spruce budworm or Douglas-fir tussock moth begin in midsummer after pupation and moth flight are completed. The survey lasts for 3 to 4 weeks and evaluates the current season's damage. Survey procedures, equipment standards and personnel training have been described (Wear and Buckhorn 1955). The procedures allow large areas of visible defoliation to be surveyed quickly and cheaply, and to be accurately mapped. Survey maps broadly delineate proposed spray units in forest areas. Degree of defoliation damage is thought to indicate population levels of the defoliating insect.

Aerial survey maps have limitations, however. They record defoliation damage caused by previous populations but furnish no information for predicting the coming year's defoliations. A biological evaluation to estimate the damage possible from the next generation (that is, the generation that will be treated), therefore, is necessary. This evaluation permits an investigator to determine need and extent of direct control and to draw boundaries around the proposed spray units more precisely.

Reconnaissance

Once boundaries of proposed spray areas are broadly determined, aerial photos and up-to-date maps of the areas are obtained. Aerial reconnaissance is necessary to acquaint the investigator with the topography, forest types, and host and nonhost areas. Infestations occurring in canyons and "hot spots"—most heavily defoliated forest areas—can be identified. Visibly infested areas are outlined on photos with ridge tops, roads, and nonhost areas as boundaries. As much of the infested area as possible should be included within the

proposed spray units. Land ownership patterns are determined; state cooperation and clearance from private owners are obtained, if needed. Road systems in proposed spray areas must be known, and quickly identifiable from the air and on the ground. New roads are added to photos and maps after aerial reconnaissance. Natural openings such as meadows, ridgetops, clearcuts, and other areas that are accessible to tank trucks and have potential as heliports are recorded. All roads should be driven, portions of the area that are inaccessible by road should be walked. Infestation, tree conditions, and forest types should be delineated on the maps during reconnaissance.

Spray Blocks

After boundaries of proposed spray areas are determined and the areas reconnoitered, plots are selected for field tests, or the proposed spray area is divided into blocks for pilot tests or operational control programs. A spray block—an operational unit within the proposed spray area—contains similar forest conditions, pest population levels, and developmental stages within the range of variation present in the spray area. Spray block boundaries are selected to coincide with easily recognizable features such as ridges, streams, meadows, or roads.

Spray blocks are usually larger than several thousand acres. In the 1958 Spruce Budworm Control Project in Oregon, spray blocks varied from 106 acres (42.9 ha) to almost 33,000 acres (13,360.3 ha), averaging 7400 acres (2996.0 ha). In the 1974 Cooperative Douglas-fir Tussock Moth Control Project, spray blocks averaged about 1677 acres (679.0 ha). The project consisted of 203 spray blocks organized into seven control units headquartered in nearby towns, from which the various activities on the spray blocks were administered.

Experimental Plots

Plots are usually much smaller than spray blocks, varying in size from approximately 20 acres (8.10 ha) to more than 1000 acres (404.86 ha). To replicate treatments under uniform conditions, plots are located within areas of similar insect population level, developmental stage, and forest habitat type. A spray block may be used as a treatment unit or it may be blocked to contain all of the replicated experimental plots for one study.

Field experiments should provide estimates of the performance of an insecticide that could be expected in an operational control project. Experimental plots, therefore, need to be large enough to include environmental and spray application conditions similar to those in spray blocks of operational control programs. Sufficient area depth is necessary to allow for spray turbulence, drift, and settling within treated areas. Edge effects—which are pronounced in small plots—should be minimized. Small plots require careful spray ap-

plication because application errors can be intensified by greater edge effects. Slight downslope winds (0 to 5 mph [0 to 8 km/h]) in early mornings when spray is normally applied, for example, can cause more than one-third of a 40-acre (16.20 ha) plot to be missed if the pilot does not offset enough uphill to allow for proper downslope air movement.

Field Laboratory

Once spray blocks, experimental plots, or both are chosen, the project director selects the largest town within a reasonable commuting distance to spray areas. The town preferably contains Forest Service facilities and is large enough to provide an adequate local labor supply and provide comfortable living facilities for project personnel.

A building should be located in or near the town for use as a field laboratory. The main functions of the laboratory are to:

- Serve as project headquarters and radio base
- Provide a place for processing field samples and diagnostic work
- Provide suitable conditions for cold storage and laboratory rearing of larvae to determine incidence of parasitism, and laboratory bioassay of specimens collected in the field
- Provide accommodations for examining preliminary data
- Provide for storage of equipment and supplies

SAMPLING

Life Stages

Appropriate life stages—During reconnaissance of the proposed spray area, the insect population is monitored. If the population drops to a low level, particularly before the appearance of the instar to be sprayed, it may be necessary to delete that spray unit in a control program. A field experiment may be canceled because of an inadequate target population. Density of budworm egg masses must be determined in fall and the survival of the overwintering population of small larvae assessed in early spring. Densities of the number of egg masses and hibernating larval populations are directly related to extent of tree defoliation the next spring (Terrell and Fellin 1960).

Evaluating an infestation requires adequate sampling techniques to determine population levels at certain life stages. The various life stages considered to be appropriate sampling periods for the spruce budworm have been thoroughly discussed (Morris 1955). Information can be obtained from examining all periods of the budworm's life cycle: egg masses,

Table 1—Head capsule width and general characteristics of spruce budworm larvae, Western United States

Instar	Average width of head capsule	Characteristics
1	—	Head capsule brown. Prothoracic shield light brown. Body light green.
2	0.35	Head capsule dark brown. Prothoracic shield brown to dark brown and entire body orange brown to yellow orange. Body length above 4 mm.
3	0.49	Head capsule dark brown to black. Prothoracic shield dark brown to black; rear margin slightly undulate in center. Body orange brown with setal areas visible as dots. Body length 5 to 7 mm.
4	0.76	Head capsule black. Prothoracic shield black with slight medial notch on rear margin. Body orange brown; setal areas small and pale; anal shield ivory with brown pattern. Body length 6 to 10 mm.
5	1.26	Head capsule yellow brown with black triangular markings at base. Prothoracic shield black; rear medial notch halfway through shield. Body pale olive brown above, pale yellow brown below with lateral orange stripes. Setal areas a conspicuous ivory color, not raised. Anal shield tan. Body length 10 to 16 mm.
6	2.02	Head capsule chestnut brown to tan. Prothoracic shield divided in the middle and collar-like; same color as head capsule. Body olive brown to dark brown above, orange brown to yellow brown below. Setal areas large, raised, ivory color. Anal shield tan. Body length 16 to 30 mm.

Source: Lyon and others (1972)

hibernating larvae, feeding larvae, pupae, and adult moths. The larval stages of the spruce budworm can be identified from known characteristics of the insect (*table 1*) (Carolín and Stevens 1979, 1981). Population surveys to assess the need for direct control and to evaluate control action have mainly focused on egg masses, hibernating larvae, large larvae, and pupae.

Generally, the small sizes of egg masses and difficulty in finding enough of them when examining samples of foliage make surveys of budworm egg masses time-consuming, expensive, and subject to substantial human errors that must be checked constantly. The egg stage, however, is a period of relative population stability and offers the advantages of a reasonably long period in which to accomplish the work. Also, past and present budworm population levels can be compared directly by distinguishing and counting samples of the current year's and older egg masses obtained from the egg mass surveys of the current year.

A population survey of diapausing (hibernating) larvae also offers the advantage of a long period in which to do the work. This type of survey is difficult, however, and presents special

problems. Hibernating larvae are surveyed during early spring, a time when forests may not be easily accessible. The work requires some method of selecting sample trees, felling the trees, transporting samples from the forests to the rearing rooms, and placing them in tightly sealed boxes to force larvae to break diapause. All subsequent sampling must be done on a different set of trees with all the attendant problems of intertree variation in the sample estimates.

Most direct control programs of the spruce budworm are aimed at the feeding larvae (3d to 6th instars) life stage. These larvae defoliate trees in spring. Large larvae are highly active and special precautions must be taken to prevent their loss during sampling. The large larval stage (5th and 6th instars) is also a period of high budworm mortality and rapid change in population densities. It is a difficult phase in which to sample large areas in intensive life table studies.

Pupal and prepupal stages of early summer, however, are periods of stability and, therefore, are favorable to sampling of large areas. Pupae can be easily found on foliage samples for rapid and accurate sampling. From live and empty pupa cases data on pupal density, pupal parasites, sex ratio, and moth abundance can be readily obtained. These data are particularly valuable for more completely evaluating direct control treatments than just the data comparing pretreatment and posttreatment population densities of large larvae.

The life stages have been examined to determine their usefulness in predicting subsequent defoliation damage for western budworm infestations in the central and southern Rocky Mountains (McKnight and others 1970), for western budworm in the northern Rocky Mountains (Terrell and Fellin 1960), and for budworm infestations in eastern Oregon and Washington (Carolyn and Coulter 1972). In every instance the egg stage was found particularly helpful to making control decisions, and was used for predictive purposes as an index of subsequent defoliation. It is expensive, however, and could be supplemented by analysis of weather from selected weather stations (Hard and others 1980) and a reduced egg mass survey.

In eastern Oregon, old egg masses to represent the previous year's new egg masses can be used to predict budworm population trends (Buffam and Carolyn 1966). The infestation trend in the northern Rockies, however, could not be predicted by a single sampling of foliage and recording of old and new egg masses (Terrell 1961).

Sampling universe: trees—In forest insect sampling, it has become common practice to refer to the forest stand or habitat in which the "population of interest" occurs as the "sampling universe" (Morris 1955). It is necessary in any sampling problem, therefore, to define the sampling universe carefully, and thereby avoid the danger of applying conclusions to a broader or more narrow universe than that sampled. The terms sampling universe and target universe have specific meanings in statistics. The *target universe*, or population, is the one to which we would like our inferences to apply: it contains all of the elements of concern, that is, all of the defoliating insects in the stand. The *sampling universe* is that portion of the target universe available for us to sample. The two universes will

differ because of practical reasons such as accessibility, ability to count elements, and others. When we speak of the sampling universe in field tests of insecticides, we are usually referring to insects on foliage of open-grown host trees 30 to 50 ft (9 to 15 m) tall that are amenable to sampling with a 35 ft (10.6 m) extendable pole pruner. Tree foliage is the primary habitat for larvae of all forest defoliating insects. It is the habitat for most of the life stages of the spruce budworm and the Douglas-fir tussock moth, except for a portion of the overwintering budworm larvae populations hibernating on tree boles and limbs.

Host tree species of spruce budworm are found in various major forest types, age classes, and stand conditions depending upon the action of past influences on the forest. Substantial evidence indicates that budworm density, survival, and larval development rates vary with tree species, forest types, and stand conditions (Morris 1963, Williams and others 1971). It is a mistake, therefore, to choose sample trees from just one host tree or one forest type, or both, for assessing budworm population trends and status, and also for assessing results of various insecticide and other control treatments. The spruce budworm feeds on five species of conifers (Bean and Waters 1961). Balsam fir (*Abies balsamea* [L.] Mill.) is the preferred host. Its counterpart, the western spruce budworm, has at least 14 acceptable hosts (Carolyn and Honing 1972), with these as the preferred hosts: Douglas-fir (*Pseudotsuga menziesii* var. *glauca* [Beissn.] Franco), Engelmann spruce (*Picea engelmanni* [Parry]), grand fir (*Abies grandis* [Dougl.] Lindl.), and subalpine fir (*Abies lasiocarpa* [Hook] Nutt.). In comparison with the spruce budworm on balsam fir, the western spruce budworm on Douglas-fir and grand fir deposit larger egg masses, hibernate farther inside the crown, and show greater diversity in age distribution of feeding larvae (Carolyn and Coulter 1972).

Examining the effects of chemical and other kinds of control on budworm or other defoliating insects under a variety of forest conditions requires description of each of the conditions. These may be stand density, species composition, number of canopy layers, aspects, and others. Once the conditions are described, any forest stand characterized by a specific set of conditions will be part of a reasonably homogeneous forest universe for sampling. If the objective of the study requires estimates of budworm population change on an area basis throughout a forest type, however, stratified random sampling within the heterogeneous forest universe is necessary. Which ever the interest is—to examine budworms under a variety of forest conditions or to examine budworms on an area basis—the basic sampling universe is trees. It is necessary, therefore, to know between-tree and within-tree variability of budworm population densities.

More population variability exists between trees, even those growing in homogeneous forest conditions, than exists within a tree. Frequently, intertree variance for a given plot is higher than interplot variances within a homogeneous forest. Also, a correlation exists between successive samples from the same tree (Morris 1955). Taking consecutive samples from the same trees, or remeasuring the same trees when the estimates of

interest trend—change between points in time—has a statistical advantage. Also, precision can be gained through re-measuring the same trees if the correlation between successive samples of insects is high enough. More precise estimates of budworm population densities can be obtained, therefore, by taking egg mass samples and larval and pupal samples from the same trees. An individual tree can withstand only so much twig removal, however, and if a large number of samples are desired, it may be necessary to take them from nearby trees.

Sample units—Although trees are the major source of variation in estimates of budworm population densities, to examine all foliage on a tree as a sample unit is neither practical nor statistically desirable (Morris 1955). Instead, some portion of the foliage within a tree must be considered. In a study to sample spruce budworm populations on balsam fir in New Brunswick, branch surface (the foliated length and width of the whole branch) was more suitable as a sample unit than were twigs or shoots (Morris 1955). The whole branch has these attributes: (a) an equal chance of selection; (b) stability; (c) small enough to be handled to allow collection of enough units to provide an adequate estimate of variance; (d) lends itself to estimating population on a per acre basis; and (e) can be collected without serious loss or disturbance of the insect population.

Most sampling and population dynamics work in Western United States has been done on Douglas-fir. This species is more abundant on a variety of exposures and sites and has a greater ability to endure budworm feeding over a period of years than other host species. It also is valued for economic reasons (Carolin and Coulter 1959). But restriction of sampling to Douglas-fir has hindered population dynamics work in the West because budworm survival and population densities are related to tree species (Williams and others 1971).

Branches of Douglas-fir, however, are not as symmetrical and are much thicker, heavier, and longer than those of true firs. It is practically impossible, therefore, to obtain enough whole branch samples with a pole pruner to adequately estimate budworm populations within and between trees. Entomologists in the Western United States, therefore, have used whole branches and 15-inch (45 cm) twigs to estimate egg mass densities, and 15-inch twigs (cut from the apical portion of the branch) to estimate western spruce budworm and Douglas-fir tussock moth larval populations in Douglas-fir and true firs (Campbell and others 1982, Carolin and Coulter 1972, Mason 1970, Srivastava and Campbell 1982). The twigs are small enough to provide flexibility of sampling design and are easily collected. The proportion of budworm population on the terminal 15-inch portion of the branch, however, may not be as constant as if the whole branch were used. Larvae will move from the exposed periphery of the branch in a negative response to high temperatures and also will move inward or drop down as twigs are defoliated. The apical portion of the branch defoliates first because most of the current year's foliage (the primary food) is located there.

Variation in estimates of larval populations was studied on Douglas-fir whole branches and 15-inch twigs to determine relative efficiency of the two units (Carolin and Coulter 1959).

Two whole branches were removed from the middle of the lower crown half from each of 10 trees and four 15-inch twigs were removed by pole pruner from the lower crown half from each of 25 trees. The 15-inch twig samples were considerably more efficient, and took 2.5 to 3 times less work to obtain an accurate assessment than did whole branches.

It is difficult, however, to cut an exact 15-inch branch or twig in the upper crown of a tree with a fully extended 30 to 35 ft (9 to 10.7 m) pole pruner. The length of cut branches or twigs usually varies from 10 to 25 inches (25 to 64 cm). The problem is solved by measuring length and width of all sample branches and dividing the product by 2, and by counting the number of new shoots or buds and expressing the population in terms of food supply; that is, number of insects per 1000 inches² of foliage (or per square meter of foliage), or number of insects per 100 buds, or both. These procedures put data on a continuous variable basis rather than on a discrete variable basis.

Egg Masses

Decisions to control insect populations are based partly on their known ability to damage trees, current health of trees, target pest insect population trends, and forecasts of additional effects on the trees. It is imperative, therefore, to have some indication of what the population density of the pest would be the next spring. Weather analysis of the previous season (Hard and others 1980), supplemented by an estimate of density of egg masses produced by the previous generation in critical areas, has predictive value for the early larval density of the next generation. For budworm and tussock moth, egg masses are usually sampled in late summer or early fall to forecast population trends. Timing of sampling at any locality is aimed at an approximate 90 percent egg hatch to ensure the deposition of most eggs before sampling (Carolin and Coulter 1972).

Sampling Procedures—Sample trees similar in appearance to those used to estimate larval populations are selected at various locations within spray areas. The following procedure applies to each sample tree:

1. Use 30-foot extension pole pruners with baskets attached, to cut off the entire foliated portion of branches. Cut one foliated, branch from the upper crown, two from the middle crown, and one from the lower crown of each tree to conform to the distribution of egg masses and foliage for most trees (Carolin and Coulter 1959).

2. After cutting, place each branch sample into a polyethylene bag, a tightly sealed paper bag, or a cotton bag. Properly identify the sample by a card that lists area, plot number, and tree number. A large burlap sack conveniently holds 10 to 15 bags that enclose the samples. Place the bags in the shade during sampling to prevent an oven or sauna effect that can occur if eggs or larvae are stored in paper or polyethylene bags exposed to direct sunlight. Heat and humidity buildups are less of a problem when cotton bags are used because the cotton fabric allows limited air circulation.

3. The samples should be held in a walk-in cooler at 45° to 50 °F (7° to 10 °C) until processed.

To process samples, the foliated length and width of each branch sample are measured and recorded and needles with egg masses are removed for examination.

Foliage measurements—Length and width of the foliated branch is measured and area of foliage calculated by multiplying the two measurements and dividing the product by 2. The foliated branchlets are cut from the main branch with hand clippers, and are cut into twigs of convenient handling sizes.

Overlapping grids are drawn on a large cloth with the number of square feet or square meters for each combination of grid dimensions. Branchlets are laid on the grid cloth in a layer spread evenly with no open spaces. Usually only one-quarter to one-half of the cloth is covered. The dimensions of the covered portion of the cloth are recorded in square feet or meters.

To further process the samples, needles of each twig are closely examined for budworm egg masses. All needles with egg masses and other insect material are removed from twigs with tweezers by technicians and placed in a petri dish containing a card that identifies the sample. The contents of each petri dish are examined by an entomologist. Budworm egg masses of each sample are removed and placed in a pill box labeled for sample identification.

Egg masses can generally be separated into "new"—those laid the current year—and "old"—those laid in past years (fig. 3). A combination of characteristics are helpful for separating spruce budworm egg masses (table 2). The separation of new from old egg masses, on the basis of these characteristics is not foolproof, however. Generally, most separations will be correct. A certain number of new egg masses, however, may be called "old" or old called "new." Counts of old and new egg masses are compared to provide trend information on population levels for 2 years. If the total number of new egg masses is substantially less than the total number of old egg masses, chemical control may not be necessary. In addition, the total number of new egg masses may have predictive value on the subsequent defoliation of Douglas-fir in many western coniferous forests (Bullard and Young 1980).

Parasites

Spruce budworm egg masses are parasitized almost entirely by one species of chalcid: *Trichogramma minitum* (Riley). Once this tiny wasp invades a budworm egg mass, it usually parasitizes every egg in the mass. The parasitized egg masses are easily recognized by their black color. If a high proportion of new egg masses are parasitized, a control project may not be necessary. Estimates of the small larval budworm population density in diapause on the trees can be used to determine if control is necessary.

Cocoons of budworm parasites, particularly *Apanteles fumiferanae* (Vier.) and *Glypta fumiferanae* (Vier.), are

Table 2—Characteristics for distinguishing between new and old spruce budworm egg masses

Characteristics	Egg masses	
	New	Old
Color of larval head capsule on unhatched egg	Brown to black	Yellow to light brown
Larval exit holes in eggs	Circular exit holes	Gaping exit holes
Brightness of egg mass	Shiny and clear	Opaque
Dirt particles	None	Tiny, black specks
Stains	None	Present
Color and condition of eggs containing <i>Trichogramma</i> ¹	Black, no exit holes	Brown, exit holes
Color of budworm moth's wing scales on top of eggs	Dark	Bleached
Color of specks of yolk	Orange yellow	Bleached
Egg mass adherence to old needles	Sticks well	Falls off easily
Shrinkage of needles	Shrink	Do not shrink

¹ A hymenopteran parasite of budworm eggs.

usually found associated with spruce budworm egg masses on needles. Adults of these species attack 1st and 2d instar budworms. The parasite larvae overwinter in diapausing budworm larvae and subsequently emerge from late instar budworm larvae, killing them in the process. Cocoons found on foliage in fall during sampling of egg masses are mainly those of parasites that emerged during early summer of the current year. Because of weathering, few parasite cocoons remain attached to foliage beyond 1 year. Cocoons per 1000 inches² of foliage provide an estimate of parasite density during early summer. These parasites presumably are available to attack 1st and 2d instars of the larval population that hatch from the new egg masses laid that summer.

Overwintering Populations

When field tests and suppression programs are planned, a survey is desirable in the spring to determine if budworm population losses from fall dispersal and overwintering mortality have made direct control unnecessary, reduced the suitability of the area for field tests, or both. The survey is particularly needed if winter was severe. If intense storms occurred earlier than usual, high overwintering mortality in the budworm population could result.

The significance of sampling overwintering western budworm populations is supported by data that show that the density of larvae hibernating on the boles and branches to be a reliable index of the numbers of feeding larvae (Carolin 1950, Carolin and Coulter 1972). A small sampling study of larvae hibernating at midcrown boles and limb sections on nine Douglas-fir trees showed that limb sections were the best sample units for predicting size of western spruce budworm larval populations feeding in the spring on the same trees (Carolin and Coulter 1972). About 79 percent of the larvae at midcrown were found on the limb components. Four regression equations predicting the number

of feeding larvae and mined needles on the basis of limb section data are included in the report. Carolin and Coulter (1972) caution that their results apply specifically to the Blue Mountains of Oregon and those stands in which Douglas-fir trees develop roughened bark on limbs and boles at a relatively early age. Other larger sampling studies are needed to determine the predictive value of estimates of hibernating larvae for feeding larvae on Douglas-fir and other hosts in the geographic range of the western spruce budworm.

The distribution of hibernating larvae on Douglas-fir in Colorado was studied by McKnight (1969). He concluded that the lower crown is a suitable index for estimating hibernating larvae of the western budworm on Douglas-fir in the Rocky Mountains. Whole branch samples can be cut from the lower portion of the tree crown without felling the tree or preventing its use and data recorded for later correlation with numbers of feeding larvae and defoliation. Unfortunately, no sampling design was provided on the basis of the study. Although it may not be necessary to quantify the overwintering population on a per unit basis—simply to determine if enough population survived the winter—some sampling design is necessary to correlate the overwintering population with feeding population and defoliation.

Bolt sections cut from the midcrown of 5- to 8-inch-diameter Douglas-fir trees were used to estimate overwintering western budworm populations in the Northern Rocky Mountain Region (Terrell 1959). Such bolt sections or billets are cut about 3 ft (1 m) below the junction of smooth and rough bark. They are 15 to 17 inches (38 to 43 cm) long and have a bark surface of 1.35 to 2.50 ft² (0.41 to 0.76 m²) that can be measured easily. Only one billet is taken per tree. Five bolt sections per sampling point gave less variable results at each point than did the use of fewer sections. Although Terrell (1959) did not study the distribution of hibernating western budworm larvae on Douglas-fir in the Northern Rocky Mountain Region, he compared larval emergence from overwintering sites on limb and bolt samples. An average of 2.9 larvae per square foot emerged from limb samples and 58.0 larvae per square foot emerged from the bolt sections of the same trees.

The number of hibernating larvae on Douglas-fir was directly related to the roughness of bark, large bark scales, and lichens (Carolin and Coulter 1972, McKnight 1969). The bark of grand fir and subalpine fir is much smoother than that of Douglas-fir of comparable age and size, and studies are needed to determine the distribution of hibernating larvae on limbs and boles of these true firs and Douglas-fir throughout the mountainous West. The studies are necessary to develop sampling methods that are biologically and statistically sound to estimate hibernating population of the western spruce budworm on its major tree hosts. The study procedures described by Terrell (1959) and McKnight (1969) or the one described in this guide could be the basis for obtaining the needed information on the distribution of hibernating larvae on the various tree hosts of the western spruce budworm.

The best time for sampling is when the probability of late winter storms is low. For many locations within the geographic range of the western spruce budworm, this period occurs between late March and early April. Fall sampling and storing of infested material until spring (before forcing larval emergence) results in a substantially lower (fourfold to fivefold) estimate of overwintering populations (Terrell 1959).

Selection of sample trees will usually be restricted to the most accessible areas because of weather conditions. Nevertheless, the sampling party may need snow machines, snowshoes, skis or all three to obtain the samples. Because of high intertree variance for budworm populations, we believe it best to sample as many points as possible in each drainage or other geographical stratum that will be treated. We also believe it is necessary to include at least two host tree species of the budworm whenever more than one is present within the proposed spray area. The trees should be the same type as those normally selected as sample trees to estimate pre- and postspray larval population densities: about 25 to 50 ft (7 to 15 m) tall and not overtopped or heavily shaded by adjacent trees.

Several sample trees are felled at each sampling point and 4 whole branches taken randomly from each crown third—a total of 12 whole branches per tree. Branches are tied in a bundle with cord and a tag is attached to identify the tree, the sampling point, and the area. The bolt of the tree is cut into 2-ft (0.6 m) sections. Every other 2-ft bolt within the crown, and to 6 ft (2 m) below the crown, should be brought in from the field. If the bolt within the crown is 20 ft (6.1 m) long, then five 2-ft bolts will be taken from within the crown and two 2-ft bolts from the portion of the bolt immediately below the crown, giving a total of seven 2-ft bolts for that sample tree. Each bolt is marked to identify tree and area, and its former position in the tree. The bolt from the topmost position in the crown will be number 1 and the bolt from the bottom position will be number 7.

Branches and bolts from the sample trees are brought into the laboratory or a rearing room and sealed in cardboard containers. Small emerging 2d instar budworm larvae have a strong tendency to seek light and could easily escape if the box is not sealed tightly. A large glass vial inserted into each cardboard container will provide a means to entrap emerging larvae for counting because they are photopositive. Each 2-ft (0.6 m) bolt should be placed into a separate cardboard container. Four branches from the lower crown third are placed in one container, those branches from the middle crown third in another container, and so on. Samples from each tree with a 20-ft (6.1 m) crown, therefore, are contained in 10 cardboard boxes (seven bolt samples, three sets of branch samples).

Second instar budworms usually break diapause and begin to emerge in about 3 to 4 days in a well-heated room with an average temperature of 75 ° to 80 °F (24 ° to 27 °C). They can be easily collected and counted through use of the glass vials inserted and tightly sealed into the cardboard containers. After a week of emerging it should be obvious

if sufficient budworm larvae survived the winter to warrant a control operation. A sample of budworms (3d and 4th instars) taken the next spring on trees on the same plot that provided the hibernating larvae samples can provide the data necessary to determine if overwintering survival on the various tree components is related to total population in the spring.

Distribution of the overwintering population on the trees should be apparent after larval emergence data is analyzed. Analysis of variance (nested) may indicate the distribution and variance of various densities of hibernating larvae among sections within the tree, by tree species, sampling point, and area. Significant differences in population distribution are represented by different value coefficients for tree species, crown size, and other variables in equations that describe the distribution of the hibernating population. Thereafter, those portions of the tree containing the most constant and greatest proportion of large or dense overwintering populations are brought in from the field the next time samples need to be taken. Alternatively, a weighted tree sampling procedure could be designed with the variance component technique.

Sampling hibernating larvae to prevent unnecessary spraying requires considerable labor for often questionable results (Carolin and Coulter 1972). Most sampling efforts have used inadequate sampling designs, and methods of forcing the emergence of hibernating larvae from diapause can result in large errors if the rearing containers are not tightly sealed. Other techniques for sampling hibernating spruce budworm larvae based on washing larvae from their hibernacula have been described (Miller and McDougall 1968, Miller and others 1971). So far we have not seen any published results of these techniques for western spruce budworm, nor do we know their predictive value for estimating populations of feeding larvae.

LARVAL DEVELOPMENT

Spray Applications

Timing of insecticide application and, in a large measure, the success of the control operation, depends upon the correct assessment of spruce budworm larval development rates. Timing is even more crucial when nonpersistent insecticides are used. Most insecticide treatments against the budworm have been applied against 3d to 6th instars. Budworms in these large larval stages cause practically all defoliation and are vulnerable to both stomach and contact insecticides. Insecticides applied against larval instars (3d to 6th) are usually timed so that all larvae have emerged from diapause but none

have pupated. If the insecticide is applied while a significant proportion of the larval population is in diapause or is feeding inside tightly closed buds and needles of host trees whose buds have not broken, these larvae may escape any contact with the spray, particularly if the insecticide is nonpersistent.

Insecticides are not as effective against fully developed 6th instar budworm and pupae as they are against young 6th instar and other instars (Robertson and others 1976). If the insecticide is applied after pupation is widespread, a higher percentage of the generation can survive to adults and lay eggs. Consequently, population density of the subsequent generation may be high enough to result in substantial tree mortality and growth loss in forest stands sprayed by the insecticide.

Timing is not as crucial with a persistent insecticide, such as DDT, which has been reported to be available to the target organism for at least 10 days after application (Hurtig and Rayner 1953). Aerial spraying began in most DDT projects at the peak of the 4th instar. Persistence of the DDT on foliage caused it to remain available to the last larvae breaking diapause. Relatively nonpersistent insecticides—pyrethrins, mexacarbate, naled, or other—are applied against the peak of the 5th instar but before noticeable pupation (Williams and others 1978a, Williams and Walton 1968). Spraying was postponed to the 5th instar to increase the probability of contacting all of the larval population—particularly those that break diapause last. Occasionally, however, adequate spraying of nonpersistent insecticides require two applications: an early application timed to affect 3d and 4th instars to achieve early foliage protection; and a late application timed to the peak of the 5th instar to achieve maximum population reduction and protect the remaining foliage (*fig. 4*).

It is necessary that the criteria for timing the insecticide application be stated clearly in the project work plan; for example, "Spraying will begin within 48 h after 25 (or 50 or 75) percent of the larvae reach 5th instar." Forty-eight hours is enough time to allow for prespray population sampling and other project preparations to be completed, and it is not enough time for substantial numbers of rapidly developing larvae to reach the fully developed 6th instar or pupal stages.

Other insects, such as the Douglas-fir tussock moth, may have extended hatch periods and require different criteria for timing treatment application. To ensure maximum population availability to insecticide exposure before substantial defoliation occurs, applications of relatively persistent insecticides against Douglas-fir tussock moth are timed to begin 4 to 5 days after 70 percent of the tussock moth egg masses begin to hatch. Sometimes two applications 5 to 10 days apart may be needed with nonpersistent insecticides (Williams and others 1978a).

Larval Development Rates

Rates of budworm larval development depend upon several environmental variables of which weather and food quality are two of the most significant. Hot, dry weather while larvae are large instars (3d to 6th) favors high survival and rapid

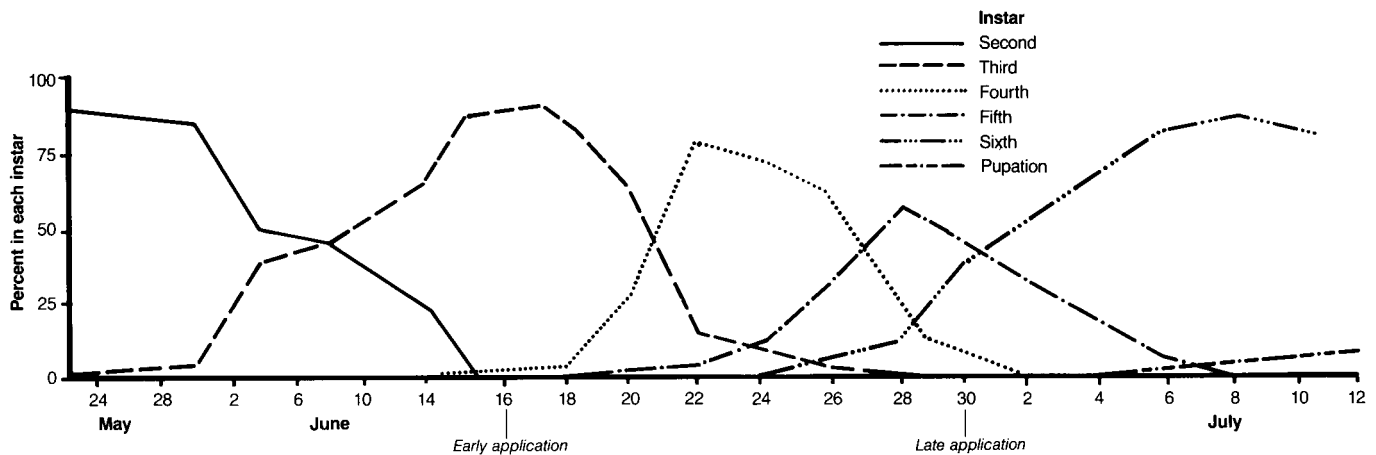


Figure 4—Idealized western budworm larval development following the emergence of the 2d instars from hibernation. An early application of a non persistent pesticide would be made when most of the larvae are in

the 3d instars. A late application would be timed for the peak of the 5th instars.

development. Increased infestation in an area of northern Idaho and Montana was directly related to hot, dry weather of the previous summer (Hard and others 1980). Larval development rate and survival, however, decrease during cold, rainy weather (Shephard 1959, Morris 1963). Larval development rate is influenced also by the food quality available when diapause is broken. Food quality varies by host tree species and may also depend on host phenology. An extreme example of this situation is provided by comparing survival of spruce budworm on balsam fir with survival on black spruce, *Picea mariana* (Mill) B.S.P.

In the eastern provinces of Canada and in the Northeastern United States, spruce budworm larvae feeding on balsam fir buds develop faster than those feeding on black spruce. This is because of bud phenology and food quality differences between the host tree species (Blais 1957, Swaine and Craighead 1924). Balsam fir buds open approximately when 2d instars break diapause, a time when an ample supply of high quality foliage is available. Black spruce buds, however, open several weeks after larvae break diapause. Larvae on black spruce are forced to mine the hard, less nutritious old needles until buds of the current year open. New black spruce foliage hardens more quickly than foliage of other host tree species and larvae feed on them with difficulty.

In Montana, development rates of Western spruce budworm on true fir—subalpine fir (*Abies lasiocarpa* [Hook.] Nutt.) and grand fir (*A. grandis* [Dougl.] Lindl.)—appear to be analogous to those of spruce budworm in the East on balsam fir. True fir buds open about the same time western spruce budworm larvae break diapause. New succulent foliage of true firs appears to be highly nutritious to larvae. Buds on Douglas-fir trees present in the same stand, however, break about 5 to 7 days after those of the true firs (Williams 1968). Variation in time of bud break among Douglas-fir trees at a particular location is large—sometimes 7 to 14 days—compared with the variability shown by true firs—less than 5 days.

On Douglas-fir, budworm larvae are forced to mine hard, less nutritious old needles until the current year's buds break. Once Douglas-fir buds break, however, larvae feeding on the succulent new foliage develop rapidly but do not completely overtake the development of those feeding on grand or sub-alpine firs. The phenological differences in bud break among host species appear to be responsible for the apparent differences in larval development rate.

Engelmann spruce (*Picea engelmannii* Parry ex Engelm.), and western larch (*Larix occidentalis* [Nutt.]), are also hosts to budworm in the West. Larch buds break earlier than those of any of the host tree species. Buds on Engelmann spruce break after buds on true fir, but before buds on Douglas-fir. Budworm development rates and survival are poor on larch but survival rates on Engelmann spruce are comparable to those on Douglas-fir.

Larval development rates may differ among host tree species at similar locations. It is necessary to keep a record of these rates on the several hosts present in the spray units. In the past, budworm development in Western United States was checked only on Douglas-fir because that species was the most widespread and most commercially valuable. Consequently, many larvae developing on true firs in the spray units were pupae by the spray date. If true firs comprised a significant portion of forest stands in the spray units, a substantial portion of the budworm population was less vulnerable to the insecticide treatment or escaped it altogether. The number of budworm moths available for egg laying in the treated stand, therefore, could be large. Except for some highly localized situations, however, Douglas-fir is overwhelmingly dominant in host western spruce budworm forests. Applications of insecticides timed to budworm development on that species should normally suffice.

If a nonpersistent insecticide is used in the control treatment, it may be necessary to spray twice those areas that have a high complement of true firs and Douglas-fir. The first application should be timed to treat budworm rapidly devel-

oping on the exposed elongating shoots of true firs, recognizing that many budworms on Douglas-fir will be feeding in the still tightly closed buds, or partially closed buds, capped buds, or mining old needles and, therefore, shielded from contact with the insecticide. The second application should be timed to treat budworm developing on Douglas-fir. Peak of the 5th instar could be the target in both situations.

Collection Points

Because the spruce budworm's larval developmental rate depends partly on weather and host tree species, it is necessary to collect larvae from enough locations to cover the range of temperature conditions and host species present in the control area. In a hypothetical V-shaped drainage, for example, larval development sampling or collection points should be established on (a) valley bottoms, (b) midslopes (southern-western exposures), (c) midslopes (northern-eastern exposures), (d) ridgetops (southern-western exposures), and (e) ridgetops (northern eastern exposures).

Significant numbers of grand fir, subalpine fir, or both, are present in moist valleys of some high-elevation areas of Montana, Idaho, and eastern Oregon. Nevertheless, Douglas-fir is the common host tree species throughout forested areas. In this situation, two tree species should be sampled at the sampling point: grand fir (or whichever true fir is predominant), and Douglas-fir. Budworm larvae from each species would be collected and kept separate. Douglas-fir would be the only host species sampled at elevations or slopes where few trees of other host species are present. When recording data, it may be useful to include the locations used to determine larval developmental rates within the sampling framework. The framework is designed according to statistical needs of treatment objectives to estimate budworm larval population densities on foliage. The sampling framework could also provide information on mortality and larval developmental stages.

Sampling need not be done from a single tree of a particular host species but, preferably, from several adjacent trees of the same species or other host species if they are present in substantial numbers.

Two size classes of trees have been used for developmental rate samples:

- Trees 30 to 40 ft (9 to 12 m) tall, with full crown covering at least one-half the length of the bole and not overtopped by larger trees. Larvae are collected from foliage in the top one-half of the tree with a 30 ft (9 m) extendable pole pruner.
- Trees 10 to 15 ft (3 to 4.5 m) tall, with full crown covering almost all the bole, open grown (not overtopped by larger trees). Foliage containing larvae are collected with hand clippers and a net.

The primary criterion is that the tree crowns are open and generally exposed to full sunlight during most of the day. We prefer the shorter trees for sampling ease, and because work can be done more quickly. Developmental samples are processed the same day they are collected. The sampling



Figure 5—The development rate of spruce budworm larvae in the proposed treatment area must be closely monitored to estimate the date when most of the larvae will be exposed and vulnerable to insecticide treatments.

(collection) points are marked with ribbons and tags and their locations plotted on the project map.

Sampling Procedure

Sampling collection points are established no later than 1 week after substantial numbers of budworms break diapause. Early development of insects can be watched every 3 to 4 days. If development is rapid, however, insects should be watched more frequently—perhaps every 2 days. It may be possible to estimate probable spraying dates on the basis of current early developmental rates and information of previous years.

Samples should be collected daily starting at approximately 2 weeks after the budworms break diapause and at least 10 days before estimated spray date. Enough buds and developing shoots with signs of current larval feeding are clipped at each sampling point to fill a 1-gal (4.5 l) ice cream carton. Enough foliage is collected to allow examination of 50 to 100 larvae per sampling point or location. Many buds will show signs of larval feeding but are later abandoned by the larvae. If larvae are collected directly from foliage and buds are not dissected, the field developmental rate data will be heavily biased toward larger, easily seen late instars.

Samples can be collected in ice cream cartons and each carton labeled by location, tree species, and area. Cartons from all sampling locations are brought into the laboratory for processing by early afternoon of the day samples are collected.

Buds and developing shoots from each carton are examined thoroughly and dissected. All larvae are removed and placed into a vial or jar containing 70 percent alcohol. The vial is properly labeled to identify the sampling point, tree species, and area. The work must be done carefully. Second and third instars are tiny and easily missed. If substantial numbers of early instar larvae are missed, the results of sampling will not represent actual field conditions and will lead to erroneous conclusions about field developmental rates. This work could be used as training exercises for workers hired to examine foliage from pre- and postspray sampling periods of the proposed field experiment or control job.

Larvae are removed from the vials and examined by an entomologist. The entomologist identifies various instars with the aid of a microscope and counts the number of budworm larvae in each instar. The project entomologist graphs the data that illustrates larval developmental rate at each sampling point (*fig. 5*). All work should be completed before the project entomologist concludes the work day. During hot weather it is necessary to plot instar development daily to determine precise timing for effective application.

THIRD TO SIXTH INSTARS

Two main procedures for ascertaining effects of various insecticide treatments are hypothesis testing and population estimates. Differences in density estimates of budworm and other target pest populations are compared before and after treatment; mortality estimates are compared between treated and untreated populations (see *Experimental Design*). Estimates of mortality and pretreatment and posttreatment population densities must be as statistically precise as a given level of financing can afford. Statistical precision for pretreatment and posttreatment population estimates is aided by remeasuring populations on the same tree.

Sample Trees

Species—Several budworm host tree species are usually present throughout the insect's geographic range. Because the population densities, survival and larval development rates vary with tree species, it is a mistake to choose sample trees for estimating population densities from just one host tree

species in proposed spray areas. Choosing sample trees from the several host trees present in the spray area is particularly important in operational projects. Because of the desire to limit variables, however, restricting sampling to the dominant host tree species may be appropriate in some field experiments. However, the recommended procedure to follow where several budworm hosts are present is the following:

- Divide the proposed spray area delineated on the map into its constituent forest types, for example: Engelmann spruce—subalpine fir, grand-fir—Douglas-fir, Douglas-fir—ponderosa pine, nonhost types.
- Determine acreage for each forest type and use some form of random sampling-tree selection within the stratified host types.

Size—Whenever possible, select trees most representative of the forest canopy for sample trees. In most instances, trees in the dominant position of the forest canopy are too large to adequately sample with a 30-ft extension pole pruner. Of necessity, therefore, the choice is limited to those trees that can be reached with the pole pruner. Sample trees should have open crowns, that is, crowns not overtopped or sheltered by taller adjacent trees. Pre- and postspray population estimates on the basis of sampling small, overtopped trees are sometimes in error because of budworm larvae dropping onto the study trees from taller adjacent trees between sampling periods. It is also desirable to use open-grown trees to obtain an optimal number of buds during sampling. Portions of the crown that are shaded (usually the lower one-third) do not produce many buds. Developing vegetative buds are the natural feeding sites for larvae. Population density of western budworm larvae on open-grown Douglas-fir trees 70 to 80 ft tall do not differ significantly from that of open-grown trees 30 to 40 ft tall (Campbell and others 1982).

Because the primary purpose of our population sampling is to obtain data on effects of insecticide treatments on target pest populations, study trees selected should be those on which target pest populations are most exposed to aerial applications of insecticides. Situations where treatment effects are confounded by variables associated with tree height, stand density, overhead canopy, and other factors that serve to shield populations on study trees from aerially applied insecticides should be minimized.

We are not interested in estimating population densities on representative forest trees in various host forest types. It is on representative forest trees where random sampling and independence of observations are of paramount importance.

Although we use sampling formulas appropriate for random sampling and data distribution described by a normal universe (bell-shaped data distribution) to compute the sampling errors for our estimates, some form of systematic sampling to estimate spruce budworm populations in the forests must be used, because it is too expensive to randomly sample trees in a forest due to limited accessibility and high travel costs. Some system of incorporating random sampling, stratification, and sometimes systematic sampling, is usually developed.

Unless the sample is very small, there is a slight risk in making statements about differences between estimates of sample universe characteristics and true universe characteristics on data from a nonnormal universe (Cochran 1963). The derived universe (consisting of a large number of sample estimates of a particular universe characteristic obtained from a large number of samples) must be distributed normally. If the parent universe deviates even widely from normal, the derived universe may still be near enough to normal to allow the use of the sampling error formulas. This is more true in larger samples.

Plots—After the proposed spray area is stratified into its component host and nonhost types, a number of sample trees are allocated to each host type according to that host type's dominance in the spray area. Actual selection of tree plots is based on accessibility to road systems, helicopter landing sites, navigable streams, and forest trails. Selection of plots dependent upon accessibility introduces another consideration into the sampling effort. We have no evidence, however, that the budworm population or any insect populations are distributed in the forest according to avenues of human accessibility.

Plot locations are randomly selected as soon as possible within the various host-type strata. Number of plots depends upon the sampling effort needed and number of samples deemed adequate to estimate a certain size population within a specified sampling error or precision.

Some forest pest managers believe that area coverage is best achieved by systematic sampling. They select trees systematically with a grid or line transect system over the area. Plots consist of single trees equidistant from each other. This kind of distribution is difficult to obtain in natural forests, particularly in the mountainous West. It could be obtained in plantations, in flat areas of the South, and in upper mid-West areas at a high cost of sampling.

We recommend using the road system and other avenues of accessibility as much as possible to cover the area. Supplement this coverage by several compass lines locating tree plots at specified intervals within the budworm host type.

Sampling Procedure

Prespray sampling of the insect population begins about 48 hours before the spray date and is completed by evening of the day before application begins. The sampling procedure is basically that described by Carolin and Coulter (1972), and Campbell and others (1982). It consists of counting the number of larvae and buds and measuring the foliage area of 15-inch (45 cm) twigs or branches removed from the midcrown of sample trees. Branches are cut with a 30-ft extendable pole pruner from midcrown of the sample tree for prespray larval sampling (*fig. 6*).

Postspray sampling begins after the insecticide activity has completely ended. This will vary with insecticides, formulations, weather, and surfaces. In the past, postspray sampling

began 4 to 5 days after the relatively nonpersistent chemical mexacarbate was applied and 10 days after a more persistent chemical such as DDT was applied. Several postspray samples may be desirable.

Two or more postspray samples of 5th and 6th larval instars provide data for mortality curves and their slopes for treatments and areas. In addition to at least two postspray sampling periods during the 5th and 6th instar stage, it may be desirable to sample the pupal stage and egg masses laid by the treated generations of insects to discern ultimate treatment effects.

Branch samples taken in late summer according to the following procedure provide information on spruce budworm pupae, adults, and new egg masses—all at the same time:

- Some statistically determined number of 15-inch branches are cut from crowns of sample trees for the postspray larval sampling. Data from previous work shows that average budworm population densities occur at midcrown in most trees (Williams and Walton 1968). That crown portion is adequate for prespray sampling. It may be inadequate, however, for providing a large number of postspray samples. It may become necessary, therefore, to use other portions of the crowns to obtain all the postspray samples. Sampling crews must avoid taking lower crown branches that contain no buds.

- Each 15-inch twig is cut and allowed to fall into an 18- to 20-inch (45 to 50 cm) cloth basket attached to the pruner just below the cutting head. The basket catches larvae from branches that drop during cutting. The pole pruner is carefully lowered to avoid hitting other tree branches and to prevent the cut twig from falling out of the cloth basket.

- Samples and larvae are carefully placed into a large polyethylene bag, tough paper sack, or cloth bag along with a card stating study area and date, plot number, tree number and species, and branch number.

- The opening of the sample bag should be closed by twisting it tightly, doubling the twisted end, and retaining it in that position by strong rubber bands. This procedure reduces the opportunities for larval escape.

Sample bags are placed into large burlap sacks (old feed sacks are excellent) and placed in the shade. Usually 10 to 15 bags can be placed in a sack depending on the size of the branches. Samples from the same tree are kept together. This makes it easier to assign specific trees to an insect checker so that the same person examines both pre- and postspray branches. The procedure helps minimize variation in insect counts and branch measurements because of individual checkers.

If a laboratory is used to process branch samples, the samples are taken to the field laboratory and placed overnight in a walk-in cooler. Temperature of the cooler, between 40 ° and 45 °F (4 ° and 7 °C), is enough to reduce activity of the larvae without killing them.

Processing Branch Samples

The branch samples can be processed in the field for Douglas-fir tussock moth larvae (*fig. 6*) or in the laboratory for



A
Figure 6—The sampling of Douglas-fir tussock moth and spruce budworm larval populations consists of removing twigs or branchlets from host trees by an extendable pole pruner (A). These twigs are either ex-



C
 amined for insects and measured at the sampling site (B) or are placed in bags and transported to the field laboratory (C).

udworm larvae (*fig. 7*) to obtain insect and bud counts and branch measurements. Difficulties of obtaining accurate insect counts in the field and laboratory were discussed by Morris (1955). A processing procedure has been developed for use in field laboratories and is accurate enough to replace expensive foliage examination commonly used in the past (DeBoo and others 1973, Martineau and Benoit 1973). This procedure uses a screen table inserted into a galvanized drum held at an angle on a folding wooden stand. The apparatus consists essentially of these five parts:

- A perforated cap (for a 16-oz screw-top widemouthed glass jar) welded near the bottom end of the galvanized drum to fit a 2-inch hole, and a handle fixed near the point of balance of the drum on the opposite side
- A removable rectangular iron screen tray (23.2 by 18 inches, mesh 0.5 inch) framed with a welded steel rod
- A 16-oz collecting jar
- A paint brush (1½ inches wide)
- A folding wooden stand built so as to keep the drum at the required angle and height when in operation and fitting inside the drum during transportation.

Insect larvae are separated from the foliage in three steps: (a) beating the branch sample vigorously against the screen table and side of the drum (30 strokes in all), (b) brushing

down the screen and the inside of the drum to direct larvae into the jar, and (c) removing the jar for examination of contents.



Figure 7—Technicians in the field laboratory carefully examine foliage samples for larvae. The buds or new shoots are counted and the foliated areas of the samples are measured.

Foliage samples taken before the peak of 3d instar should be placed in nylon mesh bags and returned to the field laboratory. -Here the budworm larvae can be allowed to develop to 3d instar on foliage at room temperature before branch samples are processed in galvanized drums. All other branch samples for pupae and egg masses can be processed at the field laboratory.

Budworm and other insect larvae dislodged from each branch sample are counted and total number for each branch and tree recorded on data forms. Length and width of the foliated portion are measured to the nearest one-half inch. Total number of buds or shoots of the current year are counted, examined for feeding injury or defoliation, and placed into different damage classes. Damage Class I = 1 to 25 percent, II = 26 to 50 percent, III = 51 to 75 percent, and IV = 76 to 100 percent defoliation. Changes in actual shoot counts and percentage of total counts in each category over time help to assess defoliation damage.

For each branch the following information should be recorded:

1. Date and treatment
2. Study plot number
3. Tree number and species
4. Branch number
5. Total number of the target insect



Figure 8—Larvae removed from the foliage samples are counted, and then placed in petri dishes for examination by the supervisory entomologist. A number of these larvae may be fed an artificial diet and reared for possible emergence of parasitoids.



Figure 9—The effects of various suppression programs by insecticides on the parasitoids of forest defoliating insects must be monitored to obtain data necessary for the development of pest management strategies. Such strategies may use a combination of chemicals and biological control agents.

6. Branch length
7. Branch width
8. Total number of buds or shoots
9. Number of undamaged shoots
10. Number of shoots in Damage Class I (1-25 pct defoliation).
11. Number of shoots in Damage Class II (26-50 pct defoliation)
- 12.- Number of shoots in Damage Class III (51-75 pct defoliation)
13. Number of shoots in Damage Class IV (76-100 pct defoliation).

About 100 budworm larvae are collected from each tree cluster or plot during each sampling period and placed into rearing for parasite emergence. The larvae are reared in petri dishes (*fig. 8*). Several budworm larvae (no more than 10) are placed in each petri dish—in a manner not to injure them—and are fed an artificial medium (Lyon and others 1972). Date, study plot or cluster number, or both, are recorded on each dish. Petri dishes are held at the field laboratory and processed to determine percent parasitism, including identity of the parasite complex. Parasite emergence data will indicate the possible effects the various treatments may have had on hymenopterous parasites that parasitized 2d instars the previous fall. These parasites emerge from 5th and 6th instars and form cocoons. Treatment may also affect tachinids that attack 5th and 6th instar budworms and emerge from budworm pupae (*fig. 9*). Processing the parasite samples can be done after the peak work periods for the field tests have passed.

We recommend the following procedure for processing pre- and postspray branch samples at the laboratory:

1. Samples are removed from the cooler and sorted according to tree. Tree numbers are assigned to technicians counting the insects so that each person will examine foliage from the same tree for pre- and postspray population counts.

2. The 15-inch sample branches are removed from the polyethylene or paper bags. All insects are removed from the branches and bags and placed into petri dishes by technicians supervised by an entomologist (*fig. 8*). It is necessary to have enough technicians (8 or 10) so samples can be processed rapidly. With rapid processing, storage of samples is minimal and the probability of significant budworm larval mortality or escape during processing is reduced.

3. After insects are placed in petri dishes, those examining the foliage measure the longest length and width of each sample branch and count the number of current year's buds (including, of course, developing foliage shoots). Total number of buds or shoots of the current year are examined for feeding damage and placed into one of the four damage categories previously described. Data for bud counts, branch measurements, and damage classification are recorded on the card taken from each bag identifying the sample.

4. The identification card is affixed to the petri dish (dishes) containing the insects removed from the branch samples. The dishes are given to the supervising entomologist;

5. Budworm larvae are separated from other insects and counted. Insects found associated with the budworm are identified and counted. Two identification keys have been useful in separating larvae of associates of the western spruce budworm on new foliage of Douglas-fir and true firs. One (Carolin and Stevens 1979) separates small larvae in opening buds and new shoots, and the other (Carolin and Stevens 1981) separates advanced instars on expanded foliage of Douglas-fir and true firs.

6. Tree and branch numbers, bud counts, sample branch measurements, damage counts, and budworm and associate insect counts are recorded on an appropriate data form, and larvae are placed in petri dishes containing food medium for parasite rearing (*fig. 8*).

INSECTICIDE APPLICATION

The task that perhaps influences success or failure of field tests and control programs most is field application of the insecticides. Efficacy and safety of insecticide formulations developed in the laboratory ultimately are determined in the field. Field effectiveness of formulations against target insects are influenced by many more variables than those affecting the organisms in laboratory bioassay. Efficacy is determined not only by properties of the chemical formulation and dosage applied, but also by a complex system of other variables that

affect coverage of the treated areas and deposit on target organisms. These include types of aircraft, application equipment and techniques, temperature, rain, sunlight, wind turbulence above and within the forest canopy, spray physics, and forest stand density and structure. Insecticides must be applied in a manner to maximize coverage of the target area and enhance the probability of impingement of the insecticide droplet on the target insects.

Equipment

Equipment designed to meet job specifications and properly calibrated is required. If this equipment is commercially available, owners or lessors must be sought out and encouraged to bid on the advertized or contemplated control operations or field tests.

The aircraft (airplane or helicopter) must be powerful enough to carry a full load of spray from the airport (heliport) to the spray blocks, spend sufficient time for orientation, apply treatment, and return with an adequate reserve of fuel. The aircraft must be capable of carrying the full load with a safe margin of fuel at all elevations and must have the desired spray system attached with adequate power to run it.

The spray boom should be made of a strong, lightweight material and be large enough to allow easy flow of the insecticide formulation under pressure. Nozzles should be designed to produce a restricted range of spray droplet sizes without leakage.

The aircraft and spray system must be calibrated before they are accepted and used on the tests or operations. Calibration of hydraulic pump systems generally consists of setting up the entire spray system on the aircraft, and spraying the insecticide-dye mixture and running it while on the ground (*fig. 10*). At this time all nozzles should be checked to see if (a) they are new, (b) they are applying the spray uniformly at the desired pressure, and (c) they (or other parts of the



Figure 10—The spray equipment and system attached to the aircraft must be inspected to see that it is in proper working order and calibrated to deliver the spray formulation at the desired volume rate and droplet size over the target area.

spray system) are not leaking or drooling. Small fixed-wing aircraft with spray systems operated by a wind-driven pump must be flown for calibration.

Once the spray system is determined to be satisfactory for operational conditions, the aircraft should be required to make at least three passes over a line of Kromekote or other deposit cards to check spray pattern at sunrise or just after sunset during a period of little or no wind. Cards are placed 10 ft apart in a line 200 ft long at 90 degrees perpendicular to the direction of the prevailing wind. The aircraft is required to fly upwind over the center of the line at 50 ft above the ground while spraying the insecticide dye mixture. Cards are then checked to determine overall swath width of the aircraft and spray system and the working swath of the aircraft. The working swath is defined as that part of the swath at which the deposit is more than over 20 percent of the volume applied (usually 0.2 gal/acre [1.9 l/ha]). To compute the 0.2 gal per acre, the cards from the calibration test are compared to standard cards with drops of the same size and similar colored dye. The cross-swath check also can be used to determine if the swath pattern is uniform. Areas of very low deposit at swath center indicate nonfunctioning or misoriented nozzles on the aircraft. If such skips are found, the aircraft contractor is required to change or rearrange the nozzles so that a uniform swath is obtained.

A method for determining optimum swath widths and spray application rates to obtain a uniform spray coverage of 20 to 25 drops per square centimeter at the top of the forest canopy was described by Dumbauld and others (1980). A computerized atmospheric dispersion and canopy-penetration model is used to demonstrate the method under various meteorological conditions for five different types of spray aircraft.

Formulation

Usually, some mixing of the formulation is necessary before it is loaded into spray tanks. A small quantity of dye (such as Rhodamine B Extra base) or tracer is added to aid in assessing deposit. Some formulations must be diluted with

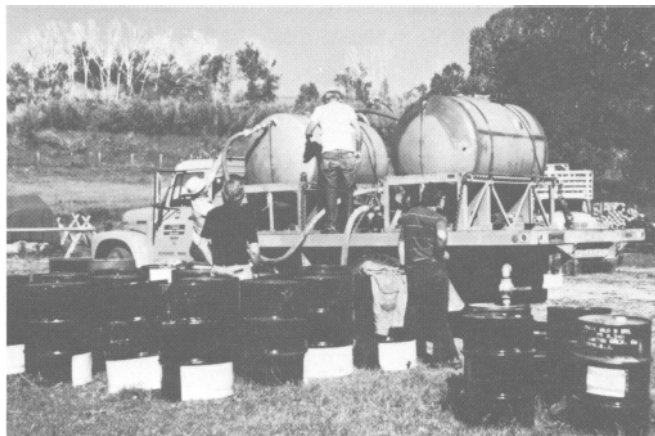


Figure 11—The spray formulation must be properly mixed and free of dirt and other contaminants that would plug the spray system of the aircraft.

a carrier material. Also, some dilutions are not stable mixtures and will settle out if allowed to stand for more than a few hours. Once settled, some mixtures may not resuspend. It is necessary, therefore, to know exactly how much will be needed for each day's work and mix that amount just before treatment. If help is desirable, a representative of the insecticide manufacturer is usually available and can be contacted for consultation.

Mixing of the formulation preferably should be done in a 500-gal or smaller mixing tank (*fig. 11*). A suggested sequence of adding and mixing of ingredients is as follows:

1. Determine exact amounts of formulation that will be used each day.
2. Check the mixing tank and hoses to see that they are clean and contain no sediment or water.
3. Place the diluent (usually no. 2 fuel oil or water) into the mixing tank.
4. Add the dye or tracers to the mixture in the mixing tank.
5. Mix thoroughly for 10 minutes (this mixture can be formulated and stored the day before).
6. Add the insecticide formulation from the manufacturer to the mixture in the tank.
7. Mix thoroughly for 30 minutes.
8. Mix again for 15 minutes each hour while loading the formulation into the aircraft.
9. Set aside some tank samples for bioassay and laboratory analyses.
10. Do not attempt to save and reuse any formulation not used the day it was mixed unless it is a solution that does not separate over time. Place any extra formulation in the drum for disposal.

Spray Application

Boundaries of the treatment area are identified with markers that are readily visible to pilots. Corners may be marked with fluorescent streamers located on the top of the dominant tree nearest to each plot corner. Streamers can be positioned in the top of a tree by shooting a line over the tree with a cross-bow (*figs. 12, 13*) or line guns and pulling the streamer into the tree's crown (U.S. Department of Agriculture, Forest Service 1974). The corner markers must be conspicuous from the air to assist spray pilots with locating plots and to provide reference points to help in gauging spray width (*fig. 12*). Proper location of highly visible markers increases the accuracy, efficiency, and safety of aerial application. Several methods for placing colored markers in and above the treetops so that they are easily visible from the air to spray pilots have been described (Maksymiuk 1975).

Before applying the insecticide, the pilot must be oriented to the spray area boundaries, the working swath widths of the aircraft, and dangerous topographic features (*fig. 14*). The pilot and the aerial observer must plan flight lines for applying the insecticide. Because most spraying is done in early morning, flight lines are oriented so that the pilot can avoid facing



A



B

Figure 12—Fluorescent streamers located on the tops of dominant trees (A) or in the open (B) are highly visible to spray pilots and help mark plot corners and boundaries. Proper location of these streamers increase the accuracy, efficiency, and safety of aerial application of insecticides.

the sun. If plots to be treated are small, 20 acres (8 ha) or less, it may be possible for an aerial observer to guide the pilot on spray runs. This guidance is best achieved if the observer is in another aircraft flying above the spray aircraft. In this position the aerial observer can see how evenly the spray coverage is applied, can pick out uneven swathing and missed spots, and can guide the pilot to obtain desired coverage. Each spray swath should be marked on a black-and-white aerial photo. The application rate in acres per minute and the number of acres treated can be calculated by knowing



A



B

Figure 13—Fluorescent streamers can be positioned in the tops of trees by using crossbows (A) or signal guns (B) to shoot lines with attached streamers over the trees.



Figure 14—Project supervisor must orient pilots and aerial observers to the spray area boundaries and dangerous terrain features of the area. Flight lines must be planned to insure proper spacing of the working swath widths of the spray aircrafts.

the working swath width, swath length, and speed of the aircraft (Barry 1978).

While applying the insecticide, the aircraft is required to fly at a height of 50 ft above treetops at a designated speed and swath width.

Spraying should be prohibited when any of the following conditions exist:

- Wind velocity exceeds 6 mph, and an upslope wind pattern develops
- Temperature exceeds 70 °F (20 °C)
- Snow, water, or ice covers the foliage
- Rain is predicted to fall within 6 hours
- Fog covers part of the area to be treated or the flight path of the aircraft between the airport (heliport) and the test site.

Meteorological conditions should be monitored in each spray area before and during spray operations. A meteorological station should be set up at least 1 week before spraying to determine wind patterns over the spray area during the

day. Temperature, relative humidity, wind velocity and direction, cloud conditions, and presence or absence of an inversion layer should be measured in the vicinity of the spray site (figs. 15, 16, 17). An inversion layer can be detected by means of a wire sound weather balloon, inflated with helium, which can be used to record temperature at 10-ft intervals

above ground level to an elevation of 150 ft (fig. 17). Inversion layers form an impenetrable barrier for small spray droplets. The layers begin to break up at sunrise, and mild turbulence results, which aids the distribution of the small droplets within the target area. Meteorological data are collected at the start, midpoint, and end of spraying (Barry and others 1978).

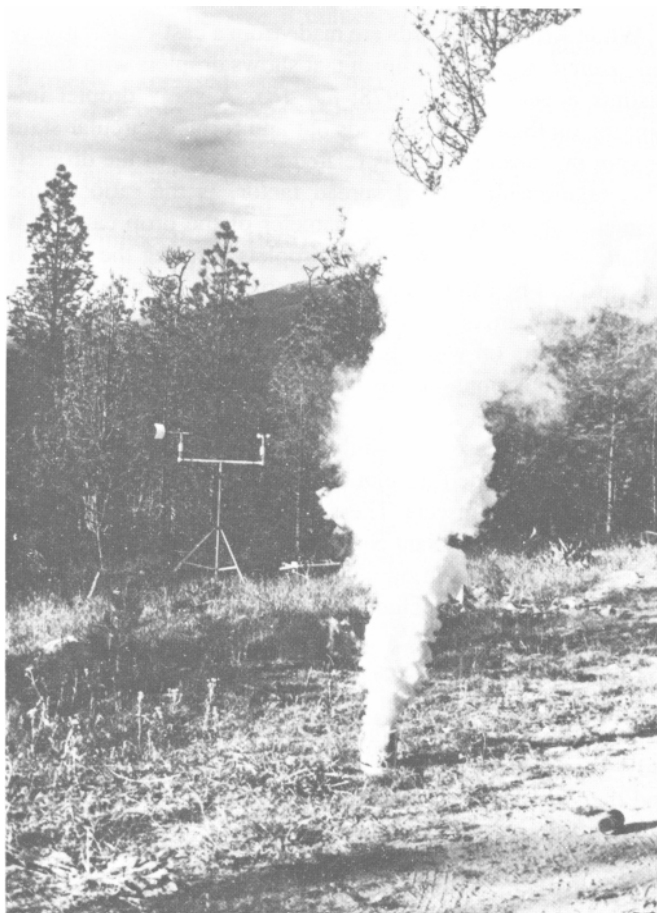


Figure 15—Smoke released in the spray plot can indicate wind speed and direction within and above the forest canopy. This knowledge allows the spray pilot to compensate for wind drift during his spray runs.

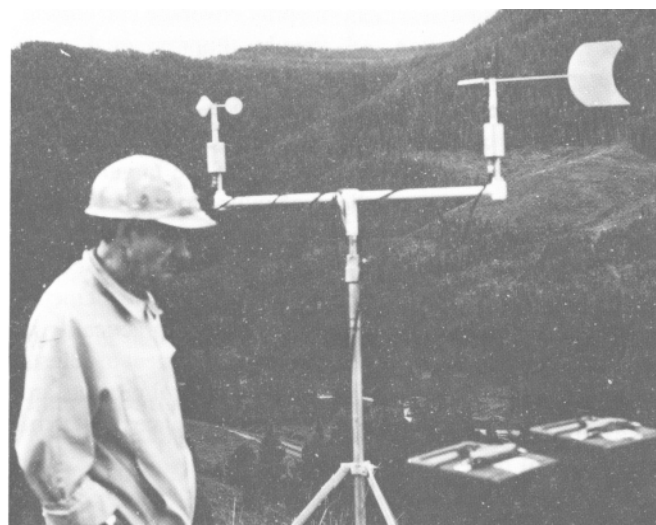


Figure 16—The various meteorological parameters of the spray area should be monitored before as well as during the actual spray application. Wind patterns over the spray area should be known for different periods of the day.

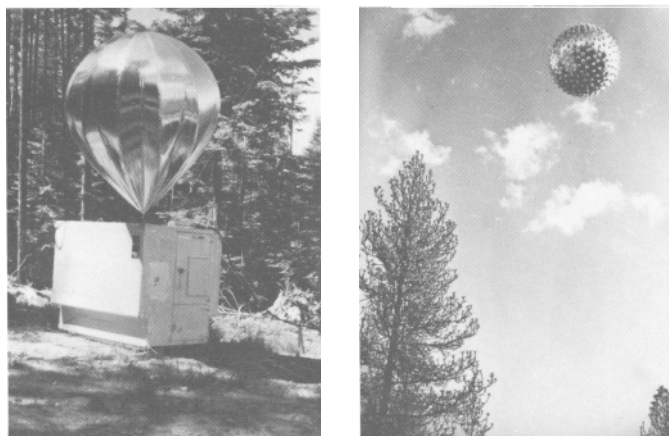


Figure 17—Inversion layers over the spray areas can be detected and measured with a wire sound weather balloon inflated with helium. It can take vertical temperature profiles at 10-foot intervals from ground level to 150 feet.

SPRAY COVERAGE

Barry and others (1978) have described the equipment and materials, and field and laboratory procedures for assessing insecticidal sprays released over forests. Conversion and computation tables, spread factor equations for some formulations, and sample forms are found in their reference source and training guide.

An assessment of spray coverage helps to determine the quality of the application to the target area. It indicates whether the spray equipment was working properly and if the spray was applied evenly to the target area. It shows gaps in the coverage and indicates areas that were missed. It can also show spray contamination in nontarget areas if those areas are sampled. Rapid assessment of spray coverage can correct deficiencies in application and, thereby, improve quality.

Assessment of spray deposit provides information on the physical characteristics of the spray such as droplet size, droplet density, and spray mass. These characteristics are sometimes related directly to mortality of the target insect, and inversely, to various indices of injury to the host. The best way to evaluate spray effectiveness on defoliating insects is to directly examine spray deposits on the target insect or its substrate—the tree foliage. Usually these assessment methods are tedious and time-consuming, and their practicality is limited to research. Spray deposit in aerial treatment of forests in experiments, and pilot and operational control projects are usually assessed with paper cards, aluminum plates, or both.

Spray Deposit Cards

Oil-sensitive cards have been used in control projects to help evaluate the spray coverage over the treated area. Be-

cause the solvent or insecticide carrier usually has an oil base (for example, cycle oil), spray droplets 40 μ and larger register on oil-sensitive cards. The cards are placed around the sample trees and at specified intervals along lines laid out perpendicular to the airplane swath widths. The quantity of spray on a card can be roughly estimated by visually matching the card with standard cards carrying known amounts of deposit (Davis 1954).

White Kromekote cards are made from a cast-coated highly calendered stock with a finish that shows droplets with sharp, distinct edges (Markin 1978; *fig. 18*). A spray droplet impinging on these cards spreads out and forms a circular stain or spot the size of which is related to the size of the droplet. This relationship, called spread factor, is the ratio of the diameter of the stain to the diameter of the drop causing it (Waite 1978a). The size of the droplets forming the spot on the card can be calculated by measuring the diameters of the spots on the card and knowing the spread factor of the spray. Spread factors on white Kromekote cards differ among pesticidal formulations but many have been calculated (Waite 1978a).

Dyes that increase the visibility of spray droplets are usually added to insecticide formulations to help register droplets on cards, foliage and insects. These include fluorescent dyes such as water soluble Brilliant Sulpho Flavine FFA (BSF), Rhodamine B extra S, and oil soluble Rhodamine B extra base. Nonfluorescent dyes include water soluble Nigrosine and oil soluble Sudan Deep Black. Although analytical methods that use soluble fluorescent dyes are more sensitive than those that use soluble nonfluorescent dyes, fluorescent dyes generally fade rapidly in intense sunlight (Himel and others 1965). Also, only fluorescent dyes have been used on trees and insects, and they are absorbed in an irregular, unpredictable manner by both foliage and insects.

Spectral counts of droplet stains on Kromekote cards are used to determine the deposit characteristics of spray dissemination systems on the spray aircraft and also the quality of

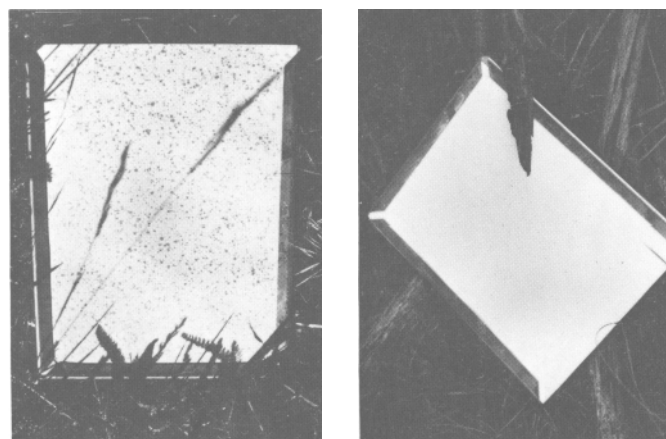


Figure 18—Spray deposit cards can indicate the spray coverage, the spray droplet spectrum, and the volume of insecticide deposited on the target area. Without this information it would be difficult to evaluate the quality of the spray application job.

the aerial application of the insecticide. Procedures for making the spectral counts have been described (Dumbauld 1980; Dumbauld and Rafferty 1977, 1978; Dumbauld and others 1980; Maksymiuk 1978).

Spray droplet size registered on cards aids in determining spray atomization (Maksymiuk 1964, 1978). The degree of spray atomization can significantly assist the effectiveness of aerial spraying because it influences spray coverage through distribution, evaporation, and drift. The degree of atomization formerly expressed as mass median diameter, is now expressed as volume median diameter (VMD). This is the drop diameter that approximately divides the spray volume into two equal parts—50 percent of the volume is in droplet sizes above the VMD and 50 percent in droplets below it (Maksymiuk 1964, 1978). The VMD is determined from samples of the drop size spectrum visible on the cards. Consequently, any inferences from sampling apply only to the droplet spectrum above 40 μ in diameter.

Density of the spray droplets on the cards indicates the quality of insecticide coverage and, therefore, if a forest has been treated satisfactorily. The amount of active ingredient deposited is related directly to the number and size of the drops being deposited. The number and size range of spray droplets that impact on spray cards and plates are highly variable and are largely influenced by the volume and size range of the droplets in the material that is applied, by the location of the cards, the size of the forest openings, density of the forest canopy, wind speed and direction, and the time of day. Nevertheless, pest control specialists have generally assumed that mean droplet density, or the amount of deposit in gallons per acre, estimated from spray cards placed near sample trees and in open areas in the forest is a reliable index of insect mortality. Several studies have reported good correlation between spray droplet densities and volumes of deposit on the cards, and insect mortality in the area (Flavell and others 1977, Hard and others 1979, Johnson 1963, Young 1978). In other studies, little or no correlation was found between insect mortality and spray deposit (Buffam 1965, Maksymiuk 1963b).

One study showed that the portion of the spray droplet spectrum below 50 μ resulted in 97 percent mortality of the budworm populations treated with mexacarbate (Himel and Moore 1967). Because few droplets below 40 μ are visible on spray deposit cards (Thornton and Davis 1956), the most effective portion of the droplet spectrum could not be detected. High correlations between number of spray droplets and budworm mortality were obtained when the unseen portion of the droplet spectrum (drops 40 μ) accompanied the visible portion of the droplet spectrum down to the target. At those times when the correlation was low, small unseen droplets evidently did not accompany the larger visible droplets to the target, but fell elsewhere.

Despite difficulties in recording the presence of the small droplets and their reliability in predicting insect mortality, spray cards are useful in indicating areas that may have been missed or that were inadequately covered by the spray. The cards are easy to use and are the most practical sampling

surface for experimental, pilot, or operational control projects.

Fluorescent Particles

Fluorescent particles aid field experiments because deposit can be estimated by counting particles on insect larvae and foliage with an ultraviolet light source and a stereomicroscope. Counting particles on larvae and foliage, however, is time-consuming and, therefore, impractical in pilot and operational projects. Insoluble fluorescent particles were used experimentally in western Montana to determine size and number of aerial spray droplets impinging on spruce budworm larvae (Himel and Moore 1967). A sample of 1113 larvae affected by the spray was microscopically examined with ultraviolet light. Fluorescent particles on each larva were counted to determine the size of the largest droplet. Because the number of the micron-sized fluorescent particles and volume of insecticide spray liquid were known, the number of fluorescent particles in each spray droplet was a direct measure of droplet size (Himel and others 1965).

Ninety-three percent of the spruce budworm larvae had no drops larger than 50 μ in diameter (Himel and Moore 1967). Distribution of droplet size was examined on a subsample of 346 larvae. Ninety-seven percent of the total droplets on these larvae were below 46 μ . Because 95 percent of the total volume of most conventional sprays used in spruce budworm control consists of droplets larger than 50 μ in diameter, only 5 percent of the insecticide applied to the forests was effective in killing spruce budworm larvae from direct aerial contact (Himel and Moore 1967). These conclusions have been supported by other studies (Barry and others 1977).

Fluorescent particles offer improvement over spray deposit cards. Larvae and foliage from various plants in the treated area can be illuminated by ultraviolet light, examined under a microscope, and the number of fluorescent particles counted to determine spray atomization in insecticide coverage. Droplets smaller than 21 μ , however, have a high probability of having no fluorescent particles and, therefore, are not visible (Himel and Moore 1967).

Foliage

Because tree foliage intercepts much of the spray and is the actual habitat of target insects, it should provide a better index of the amount of insecticide actually available to them than cards and plates. Although deposit data from foliage is more expensive to obtain, it may be more closely related to insect mortality than data from cards and plates. Foliage samples are being used increasingly in research to assess spray coverage and deposit (Maksymiuk and others 1975, Barry and others 1977, Williams and others 1978a, Barry 1978). Data on the amount of spray deposit, spray droplet sizes, densities, and quality of coverage can now be obtained from foliage. The amount of spray deposit is estimated by spec-

trofluorometric analysis. Spray deposit sizes and density data are obtained by examining foliage under a dissecting microscope equipped with a reticle (Barry and Ekblad 1978).

Several criteria must be met when assessing foliage (Barry 1978):

- Dye concentrations must provide suitable contrast.
- The droplet spread factor on target foliage must be determined.
- The sensitivity and detection threshold of the magnifying instrument used must be known.
- Droplets must dry in a reasonable time without excessive running.

Because foliage assessment methods are time-consuming and costly, various investigators have sought relationships between insect mortality and spray deposit densities on foliage and on Kromekote cards placed on the ground (Barry and others 1977, Maksymiuk 1963a, Maksymiuk and others 1975). To date, we have no evidence that foliage assessment methods provide better data on spray coverage of the target area or are better predictors of insect mortality than Kromekote cards and aluminum plates.

Volumetric Methods

Many investigators have not been satisfied with the quality of information obtained from spray cards or with the limitations of tracer materials such as fluorescent particles. Greater research effort has been expended on volumetric methods to estimate the number of gallons per acre present on the target area. Currently, much of this information is being obtained from tree foliage and aluminum plates, which are washed and the resulting solutions analyzed with a fluorometer (Yates and Akesson 1963). The amount of dye in the wash solution is compared with the amount originally added to the spray and, given the area of the plates, the volume of spray deposited, in gallons per acre, can be calculated.

Large variations in spray deposit and larval counts have been found between trees because of (a) screening effects of adjacent trees on spray deposited on foliage and on plates and cards placed on the ground near sample-trees (Maksymiuk 1963a) and (b) natural variation of larval distribution within and between trees compounded by sampling error. Consequently, when amount of deposit and percent larval mortality were analyzed on a tree-by-tree basis, correlation between spray deposit and larval mortality was low (Maksymiuk and others 1975).

High correlations were obtained between amount of deposit on tree foliage and larval mortality when sample trees were grouped in various categories or classes on the basis of mean spray deposit recovered from trees (Maksymiuk and others 1975) or when sampling of larvae and spray deposit on cards were based on tree clusters (Flavell and others 1977).

Recently, high correlations between western spruce budworm and Douglas-fir tussock moth larval mortality and volume deposits, estimated from spray cards and aluminum plates placed beneath sample trees, were obtained on a tree-by-tree

basis. Volume deposit estimates for three data sets were used to estimate dosage to which the insects were actually exposed (Williams and Robertson 1983). The estimated dosage variable provides an interface for use with the logic of a laboratory efficacy model that calculates expected mortality (Force and others 1982). For the three data sets examined, prediction success (as measured by a χ^2 goodness-of-fit test) ranged from 73 to 95 percent (Williams and Robertson 1983). And mean mortality predicted or calculated by the model was within 5 percent of that actually observed in all situations.

Spray Deposit Samples

The spray deposit assessment methods used in the 1972-1974 Douglas-fir—tussock moth insecticide program were foliage, aluminum plate, and spray card sampling. The basic units at each study tree used for insect population and spray deposit sampling were four 15-inch branches taken from mid-crown. Two aluminum plates (6 by 6 inches) and one white Kromekote card were also used to sample spray deposit. Plates and white cards were placed in metal or plastic holders on the top of wire supports and positioned near the ground but above the general level of ground vegetation in the nearest open area adjacent to each sample tree. Additional cards and plates were randomly distributed in the open spaces.

Kromekote cards and aluminum plates have been used as an index of spray coverage on the sample trees and target area by providing data on spray droplet density (droplets/cm²) and amount of insecticide (gallons per acre, GPA) at ground level. Care must be taken to keep the cards dry because on moist cards the spray spots are not as sharp or distinct and they spread differently. Moist cards tend to warp and, as a result, the reading of spray spots is further confounded (Barry and Markin 1978).

Cards and plates are placed in the field just before spraying. They are prenumbered to correspond with the sample trees, with the number written on the underside. A technique for defining optimum sample size procedures for spray deposit assessment using cards has been developed by Wong (1980). Cards and plates should be left out for at least 45 min after the spray is applied to allow the small spray droplets to settle and dry (Barry and Markin 1978). During the collection, each aluminum plate pair is placed face-to-face (deposit sides together), stored in a slotted box for transportation, and sealed against any light. Kromekote cards can be stored in the same box.

The foliage samples (15-inch or 45-cm twigs) removed from trees for density estimates of pretreatment insect population can be used for the pretreatment foliage assessment samples. One hundred needles should be removed at random from each of the four foliage samples, placed into opaque paper bags marked with the appropriate tree and foliage number, and placed in a larger single paper bag. Foliage samples removed during the first posttreatment density counts, however, cannot be used to assess spray coverage, because they are not taken immediately after spraying and the dye will

have faded. A set of four foliage samples, therefore, should be removed from the sample trees within 3 hours after spraying. These samples should be collected from the four cardinal directions at midcrown and placed in individual paper bags and, with the prespray samples, sent to the laboratory for spray deposit assessment.

Some monitoring of spray drift is recommended. Kromekote cards and aluminum plates can be placed along some of the block edges and in the check areas. In the 1973 Cooperative Tests of Chemical Insecticides for Control of the Douglas-fir Tussock Moth, possible spray drift into the check plots was monitored by placing 10 Kromekote cards and 10 pairs of plates in the 10-tree cluster closest to the nearest treated plots and in a line parallel to the plot border.

Aluminum plates, Kromekote cards, and foliage samples may be processed by the following procedure: spray deposit is washed from each pair of aluminum plates with 10 ml of 95 percent ethanol. The deposit, in terms of concentration (g/ml) of the fluorescent tracers, is measured with a Model 430 Turner Spectrofluorometer and corrected for background fluorescence from prespray samples and percent recovery. Percent recovery is determined by applying known volumes of the formulations to aluminum plates and the deposit then determined by the method described earlier. The ratio of known to measured deposit gives percent recovery.

Concentrations are converted to deposit in GPA, per sampling station. Actual tracer concentrations of a sample of formulation are collected when loading the aircraft. Amount of tracer removed from the plates and area of the plates can be determined with this formula (Maksymiuk 1964):

$$\text{GPA} = \frac{(\text{g.dye} \times 10^{-7})}{\text{ml}} \times \frac{(\text{ml wash}) 94.356 \times 10^4 \text{ ft}^2 / \text{acre}}{(\text{g.dye} / \text{gal}) (0.25 \text{ ft}^2 / \text{plate})}$$

Spray drops per square centimeter deposited on the white Kromekote cards can be determined in various ways. Droplets containing fluorescent particles or stains can be counted on the cards. For nonwhite cards, however, a dissecting microscope with an ultraviolet light is needed to illuminate the fluorescent deposit. An image analyzer computer, Quantimet, also may be used for rapid sizing and counting of spray deposit stains on white cards (Waite 1978b). Spray droplets can be sized for atomization analysis. Spray atomization can be determined by the drop-size spectra (D-max) method (Maksymiuk 1964).

An Automatic Spot Counting and Sizing (ASCAS) computer program analyzes the spot count data obtained from spray deposit cards (Young and others 1977). It computes various droplet diameters of the spray cloud in terms of mass media diameter and volume media diameter, spray droplet density, and of volume (ounces and gallons) of spray deposited per acre. The program is on line at the computer center of the University of California at Davis, California, and the U.S. Department of Agriculture's Fort Collins Computer Center in Colorado.

The procedure for assessing spray deposit from foliage samples begins with random selection of 100 needles from

each foliage sample collected within 3 hours of treatment. The needles are dried, weighed, and spray deposit removed by washing with 10 ml of 30 percent ethanol. Concentration of fluorescent tracer (g/ml) is measured with a spectrofluorometer. Concentrations are then corrected for background fluorescence, based on the average of the prespray samples and percent recovery. Amount of insecticide on the foliage, in terms of micrograms of insecticide per 100-needle sample, can be estimated if the amount of tracer removed from the needles and the insecticide concentrations from the formulation are known.

MONITORING EFFECTS ON NONTARGET INSECTS

In field and pilot control tests with experimental insecticides it is necessary to monitor effects of chemicals on populations of nontarget organisms for possible registration requirements of the Environmental Protection Agency. Also, it may be necessary to obtain similar data in control programs when registered materials are used at dosages near the upper limit of the material's environmental safety range. Normally, these monitoring studies are the responsibility of various Federal and State wildlife agencies. Also, the Forest Service has assessed the effect of the experimental insecticide on nontarget insects as part of the field tests through cooperators at universities and its own field crews (Shea and others 1982).

These monitoring efforts have used drop cloths to sample arboreal insects and some parasites, malaise traps to sample certain groups of flying insects, and drift nets and surber samples for aquatic insects in small streams. Occasionally, subsamples of the large larval instars are dissected to determine parasitism.

Drop Cloths—Collections of insects from drop boxes or drop cloths placed beneath selected study trees can be examined for the presence of arboreal insects and for parasites and predators associated with the target insect pest. Drop boxes can be particularly valuable in monitoring "knock-down" (insects which are stunned by the insecticide, but recover later) and mortality of arboreal insects.

Malaise Traps—Malaise traps can be constructed and set up in the insecticide treatment plots. Collections made over time from these traps can help determine the qualitative and quantitative changes in insect biomass of those groups normally attracted to such traps. The traps can provide substantial information on the Hymenoptera, Lepidoptera, and Diptera affected by the treatments.

Parasites—A subsample of budworm larvae or other target insect larvae collected during pretreatment and posttreatment

sampling periods can be reared on artificial diet for parasite emergence. Rearing is described in the section on Sampling. Another small sample of larvae from each population sampling should be dissected to determine the incidence of parasitism. Dissecting larvae for parasites is tedious and time-consuming and the procedure cannot be used to process large numbers of larvae as easily as rearing. Dissection is a more accurate procedure in determining parasitism, however, because it can account for all internal parasites, whether or not they emerge. Periodic dissection of small subsamples of larvae can be used to ascertain the accuracy of the larger, more comprehensive rearing program.

The effect of various treatments on internal parasites can be assessed by comparing the incidence of parasitism (expressed as parasites of a particular species per 100 larvae) among the treated populations pretreatment and posttreatment (Williams and others 1969). Incidence of parasitism should also be determined for budworm larvae from the check plots.

Drift Nets—Drift nets can be used to monitor treatment effects on aquatic insects in small streams. Samples should be taken periodically for at least 24 to 48 hours before treatment at various locations along streams running through treatment plots. These samples should indicate the normal diurnal drift pattern and diversity of the aquatic insect fauna in the stream. Periodic posttreatment sampling at the same locations should begin 1 to 2 hours after treatment and continue for at least several days.

Insect populations should be monitored periodically during the season and perhaps several times the following year. If there are ill effects from the insecticides, additional sampling will determine the rate at which nontarget populations recolonize in treatment areas and the rates at which their population sizes increase.

FOLIAGE RETENTION

The major purpose of an operational control project is to minimize injury to the forest resource. One way to assess the effectiveness of the control project is to determine how much defoliation was prevented. How much protection did the treatment provide? Methods to assess foliage retention are generally subjective and, if quantitative, are not very accurate. Despite inaccuracies, however, some assessment of treatment protection should be recorded and analyzed.

Trees and Branches

Currently, foliage retention on trees and branches can be assessed in at least two ways. The first is to visually classify

crown defoliation and crown damage on the whole tree (Williams 1967). Crown defoliation and damage are first classified when sample trees are selected in late May before the budworm or target defoliator begins feeding. Crown damage is classified again in August or September during the egg mass sampling period, after the budworm or target defoliator has completed its development. Results are examined to empirically determine variation in time by tree and treatment area. The second method is to compare branch samples. Each new (current year's) shoot on the 15- to 18-inch (45 cm) sample branch collected during each larval sampling period and the current year's shoot on the whole branch collected during the egg mass sampling period is counted and placed in one of the, following categories: light damage (0-25 pct defoliation), moderate damage (26-50 pct defoliation), heavy damage (51-75 pct defoliation), severe damage (76-100 pct defoliation) (Williams and others 1971, Grimbale and Young 1977). Counts placed into these categories can be tested by covariance analyses to determine significance for each sampling period and area.

Aerial Photography

Although false-color aerial photography is not used to assess defoliation damage in coniferous forests, it has been shown to be a valuable tool for assessing effects of aerial sprays on defoliating pests in hardwood forests. In hardwood forests, false-color aerial photography can provide estimates of foliage saved in spray plots as compared with surrounding untreated areas (Ciesla and others 1971). Aerial photography of each spray plot can be obtained during early June, before defoliation occurs, and again in late July when larvae have completed feeding and defoliation is most conspicuous. Aerial photos should be examined in stereo to compare defoliation in areas protected by spray with that in adjacent forests. Maps showing areas of foliage protection, degrees of defoliation and zones of spray drift in relation to spray plots can be prepared in accordance with procedures described by Ciesla and others (1971).

DATA ANALYSIS AND SUMMARY

Statistical inference and graphic interpretation are relied on to evaluate the effectiveness of the spray applications on target insects, protection of the resources, or both. We recommend those methods described in the section on Experimental Design. We also recommend assessing relationships between insect mortality and various spray deposit parameters such as volume deposits and deposit densities (Hard and others 1978, 1979; Williams and others 1978b; Williams and Robertson 1983; Young 1978). Field effectiveness of various treatments

Table 3—Significance¹ of numbers and mean percent mortality of tussock moth larvae on treated plots of Douglas-fir, British Columbia

Dimilin treatment (oz a.i./gal)	Days postspray					
	0	7	14	21	28	35
	Mean number of larvae/1000 inches ¹					
2.0	314	165	66	30	7	1
1.0	193	132	106	52	34	15
0.5	276	194	126	65	37	22
Untreated	155	130	86	89	72	60
	Mean percent mortality					
2.0		49.9	79.4	91.4	97.9	99.7
1.0		31.3	46.2	73.9	82.1	92.2
0.5		36.3	61.0	75.8	86.1	89.7
Untreated		18.6	33.2	17.5	15.8	10.0

Source: Hard and others (1978)

¹Means not connected by a bar are significantly different at the 95 percent level.

and control strategies and protection of tree foliage can be evaluated from data on populations, spray deposit, and foliage retention.

Insect Populations

Population data obtained in the sampling procedures described previously can be used in hypothesis testing and also to provide estimates of pre- and postspray population densities and mortality, by tree, cluster, and treatment area for each sampling period. These estimates can be easily obtained with minimum time and expense with a computer program designed for such analyses (Wilson and Williams 1972). The data format for this program is provided in the *appendix*. The analyses are based on the ratio of insect counts to branch surface area or bud counts. For example, the survival rate (mortality estimate) is given by:

$$r_i = ({}^X2i/{}^Y2i)/({}^X1i/{}^Y1i)$$

in which X1i and X2i denote pre- and postspray insect counts and Y1i and Y2i denote pre- and postspray measurements of branch surface (or bud counts) for the i th tree.

The budworm population data can be used to

- Compare mortality rates and postspray or residual larval population densities among the treatments over time
- Compare pupal population densities among the treatments
- Compare number of egg masses laid by survivors in treated and untreated populations, among treatments and with those laid by the previous generation.

These comparisons can be tested by ANOVA for each sampling period with budworm population densities and mortalities as the (Y) variables (*tables 3 and 4*). Multiple comparison techniques can be used to find the means responsible for the differences. If sufficient ranges exist in the prespray population density, analysis of covariance should be used. With prespray budworm population density as the covariate,

Table 4—Probability of detecting a significant difference in mortality among Dimilin-treated plots, with treatments replicated three times¹

Difference in mortality to be detected (pct)		Probability at days postspray				
Range	Deviation from mean	7	14	21	28	35
10.0	5.0, 0, -5.0	< 20	< 20	33	50	89
15.0	7.5, 0, -7.5	21	22	66	83	> 99
20.0	10.0, 0, -10.0	37	40	89	97	> 99
35.0	17.5, 0, -17.5	83	84	> 99	> 99	> 99

Source: Hard and others (1978)

¹Outlined area shows that experimental power is high if the threshold for adequate power is arbitrarily set at 80 percent of detecting a significant difference.

the effectiveness of the insecticide treatments on a range of population densities can be investigated.

Spray Deposits

Spray deposit data provide the following information for each individual and cluster of trees used in sampling the target insect populations:

- Approximate gallons per acre (GPA) of spray formulation reaching the ground in open spaces beside the study trees
- Densities of the spray droplets reaching the ground in the open beside the study trees
- Volume median or mean diameter (VMD) of the spray droplets reaching the ground in the open beside the study trees

The spray deposit data describe a range of deposited dosage for the study tree sites in each sampling cluster, study plot, and treatment. The insect population data and spray deposit data should be summarized in tabular form (*tables 5 and 6*) (Williams and others 1978b, Young 1978). Field effectiveness of each treatment can be partly evaluated by determining relationships between target insect mortalities (survivorship) and deposit for each insecticide application.

Any possible relationship between deposit and mortality, or residual population levels, or both, should be investigated by regression analysis (Davis and others 1956; Hard and others 1978; Maksymiuk and others 1971, 1975; Young 1978). In all situations, larval mortality or residual population levels are the dependent variable (Y) and the spray deposit is the independent variable (X). For the purpose of such analyses, percent mortality may be converted to probits and deposit values at each sample tree site converted to logarithms (*fig. 19*) (Maksymiuk and others 1975). The graphic relations between the 10-day mortality and spray deposit data (*table 6*) are displayed in *figures 20 and 21* (Young 1978). Additional graphic relations of insect mortality and survival, with dosage rate expressed as ounces of actual insecticide applied per acre

Table 5—*Insect population density and mortality and spray deposit estimates in insecticide tests against the Douglas fir tussock moth in Washington and Oregon, 1972*¹

Plot number and location	Treatment dosage	Population density		Mortality	Spray deposit on ...		
		Prespray	Postspray		Foliage	Aluminum plate ³	Kromekote card ³
	<i>Lb/acre</i>	<i>Insects/1000 inches² of branch area</i>		<i>Percent</i>	<i>µg a.i./1100 needles²</i>	<i>Gal/acre</i>	<i>Droplets/cm²</i>
Pyrethrins							
Washington:							
I (Oroville) ⁴	0.05	51.84(22.91)	20.71(40.9)	60.04(35.90)	5.42(9.79)	0.08(.06)	11.08(7.66)
2 (Oroville)	Control	37.10(10.76)	33.28(32.20)	10.30(46.70)	—	—	—
I (Oroville) ⁵	0.05	20.72(12.58)	11.17(19.00)	46.06(18.85)	—	0.06(.05)	—
2 (Oroville)	Control	33.52(9.80)	18.64(22.50)	44.40(23.50)	—	—	—
1 (Oroville) ⁶	0.10	51.84(22.91)	11.17(19.00)	78.45(10.31)	—	—	—
2 (Oroville)	Control	37.10(10.76)	18.64(22.50)	49.76(41.43)	—	—	—
Mexacarbate							
Washington:							
3 (Riverside)	0.15	18.57(61.29)	10.50(13.09)	87.13(17.69)	7.88(4.33)	0.49(.16)	74.43(22.40)
4 (Riverside)	Control	7.60(3.57)	3.67(6.24)	51.71(11.83)	—	—	—
Oregon							
5 (Elk)	0.15	13.49(3.84)	1.98(4.05)	85.29(9.89)	—	0.69(.27)	64.41(25.07)
6 (Ridge)	0.30	45.26(7.25)	17.34(14.48)	61.69(14.40)	—	0.12(.12)	7.71(4.97)
7 (Farmhouse)	0.15	19.22(1.05)	9.27(.77)	51.85(2.84)	—	.32(.16) ⁷	29.35(18.34)
8 (Elk)	Control	8.35(1.67)	3.20(2.14)	61.68(10.77)	—	—	—

Source: Williams and others (1978a)

¹ Values in parentheses are standard errors of the mean.² Amount of dye recovered.³ Placed in openings in forest canopy nearest study trees.⁴ First application.⁵ Second application. Spray deposits sampled on aluminum plates only.⁶ Two applications compare the prespray population density of the first application with the prespray populations density of the second application. No spray deposits.⁷ Based on treatment of 20 trees only.Table 6—*Prespray and postspray insects per 100 buds, with corresponding spruce budworm mortality for one spray block with 15 single trees sampled within a block*

Trees	Prespray	Postspray		Mortality		Spray deposit	
		3 days	10 days	3 days	10 days		
		<i>Insects/100 buds</i>		<i>Percent</i>		<i>Gal/acre</i>	<i>Droplets/cm²</i>
1	6.304	1.301	2.964	0.794	0.530	0.03	7
2	7.087	3.033	2.049	0.572	0.711	0.05	6
3	5.732	1.691	2.239	0.705	0.609	0.06	12
4	5.299	0.702	0.463	0.868	0.913	0.11	12
5	3.759	0.159	0.000	0.958	1.000	0.16	22
6	4.050	0.532	0.986	0.869	0.757	0.05	13
7	5.398	0.426	0.000	0.921	1.000	0.10	21
8	6.558	1.196	0.849	0.818	0.871	0.09	11
9	11.628	3.764	2.711	0.676	0.767	0.07	16
10	11.449	4.745	5.734	0.586	0.499	0.01	4
11	17.886	13.747	8.513	0.231	0.524	0(.01)	1
12	5.702	3.052	3.781	0.465	0.337	0.02	7
13	6.634	1.875	1.760	0.717	0.734	0.03	5
14	5.065	4.351	3.384	0.141	0.332	0.01	4
15	8.244	4.661	5.128	0.435	0.378	0.02	4

Source: Young (1978)

are displayed in figures 22 and 23, respectively (Hard and others 1978).

Damage

Amount of defoliation of the new foliage can be classified for each sample branch during each of the larval and pupal sampling periods. These classifications can be examined by covariance analyses to test the amount of defoliation damage resulting from target pest populations within and among the treated and untreated plots. Covariance analyses allow the statistician to handle problems resulting from the statistically unacceptable practice of preselection of check areas and the continued use of Abbott's formula (Abbott 1925).

Check Areas

Check areas are commonly set aside from control projects to estimate natural larval mortality. Spray induced mortality is distinguished from mortality from natural causes by Abbott's formula ($100(x-y)/x = \% \text{ control}$, in which $x =$ percent survival in check population and $y =$ percent survival in treated population) (Abbott 1925). The result is larval mortality creditable to spraying based on the premise that natural mortality within the two areas occur at similar rates. This is a questionable assumption.

Use of check areas and Abbott's formula are justified in agricultural experiments on annual crops, where environmental conditions between treated and untreated blocks are similar and somewhat more controllable. The check areas, therefore, are representative of the treated areas. But forests present entirely different environmental conditions.

First, representative check areas are difficult to find, particularly in the mountainous West, because of variable topography, local weather conditions, site qualities, and forest stand structures. It is difficult, therefore, to find sufficient similarities between areas so that natural mortalities can be compared with treatment-induced mortalities. Second, workers in control projects tend to spray all areas with high larval populations and, as a result, it is difficult to set such areas aside as checks. Frequently, areas with population densities lower than those to be treated are used as check areas. But this does not provide a comparable situation because natural mortality rates usually correlate with larval densities. Third, if an area with high population densities and environmental conditions similar to areas to be treated is set aside, it is usually so close to the spray blocks that a high probability of contamination by the small, but highly effective, spray droplets exists.

Although there are many difficulties in obtaining comparability among target pest populations in different forest areas, check areas and control populations are necessities in experimental designs to assess treatment effects on target pest populations. Check areas with their resident target pest populations serving as controls are the only areas and pop-

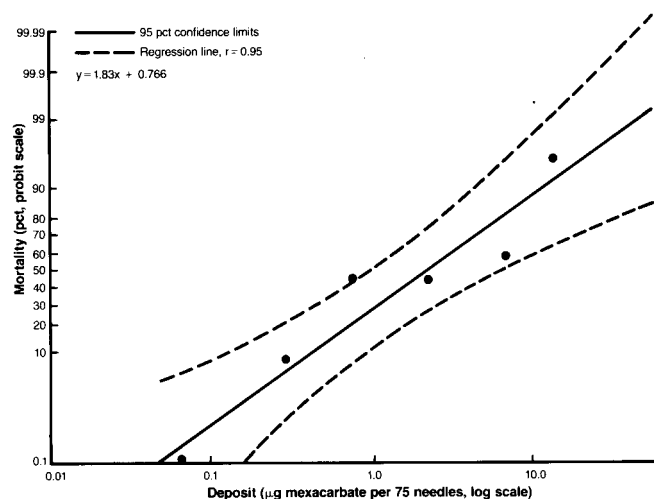


Figure 19—Relationship between average mortality of the spruce bud-worm in probits and mean deposit in logs for the treated plots. The least squares equation is $Y = 1.831 X + 0.766$, in which Y is the mortality in probits and X is the log (100Xdeposit). The factor of 100 was used to convert the deposit values to a convenient scale for computations (Maksymiuk and others 1975).

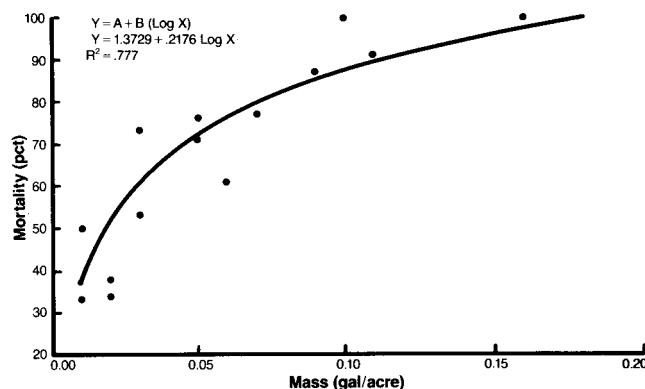


Figure 20—Regression curve of insect mortality over mass recovery in one spray block with 15 single trees (Young 1978).

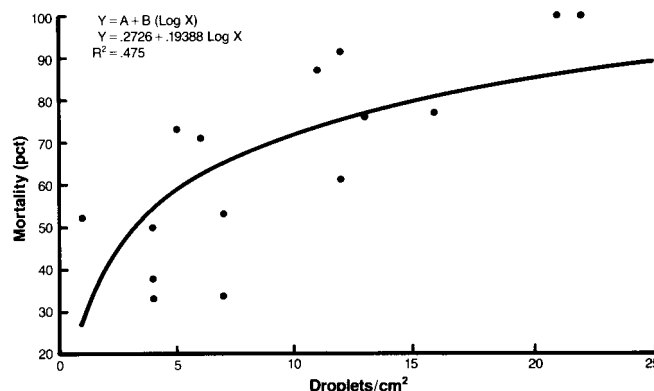


Figure 21—Regression curve of insect mortality over droplets/cm² in one spray block with 15 single trees (Young 1978).

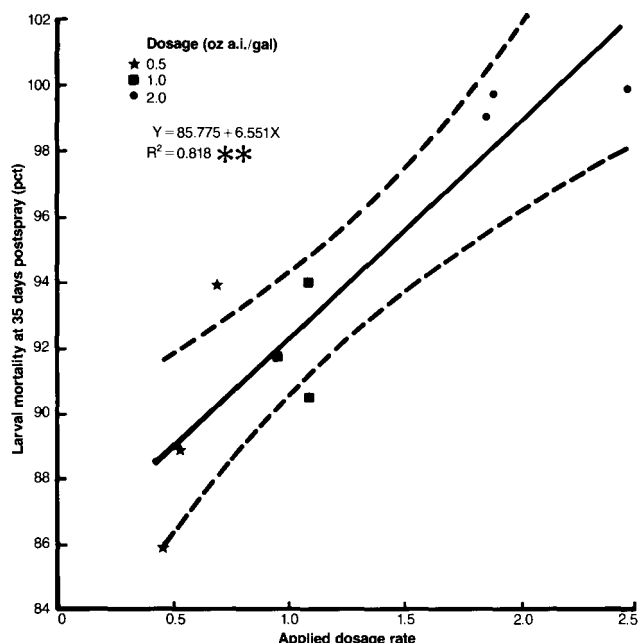


Figure 22—Douglas-fir tussock moth larval mortality, in mean percent per plot at 35 days postspray, is highest in plots treated with 2 ounces of Dimilin per gallon of spray. Applied dosage rate is calculated as a.i. applied per acre = (spray flow in gal) (oz a.i. per gal formulation)/plot area in acres. Dotted lines show 95 percent confidence limits; ** = significant at 1 percent level (Hard and others 1978).

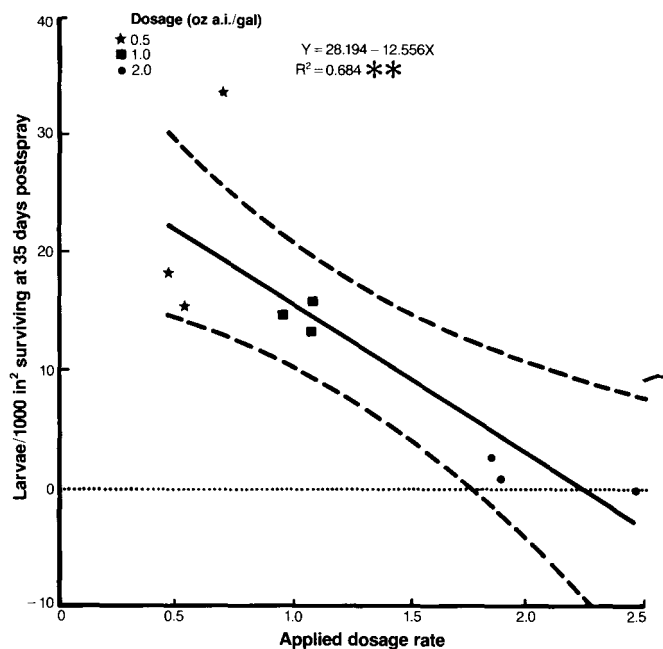


Figure 23—Mean number of surviving Douglas-fir tussock moth larvae/1000 in² per plot at 35 days postspray is lowest in plots treated with 2 ounces of Dimilin per gallon of spray. Applied dosage rate is calculated as in figure 22. Dotted lines show 95 percent confidence limits; ** = significant at 1 percent level (Hard and others 1978).

ulations in the experiment without a treatment component. High variability in populations within or between locations in a test lowers the power of the test. The lower the power the more difficult it is to determine differences among treatment effects. The investigator must design the test to increase the power of the test within the limitations of cost. Power can be increased by increasing the number of observations per treatment, by decreasing the number of treatments, and by selecting treatments for comparison with expected large differences in treatment effects. In field tests of insecticides the largest differences are expected to occur between treatment(s) and check or controls (no treatments) (see *Experimental Design*).

Check areas are also worthwhile to determine effects of insecticides on internal parasites of the budworm. Parasitized larvae treated with mexacarbate or DDT survive proportionately much better than unparasitized budworm larvae (MacDonald 1959; Williams and others 1969, 1979). This difference is strikingly illustrated when apparent parasitism of prespray and postspray budworm populations of treated and untreated areas are compared. Check areas are useful to ascertain the apparent effects of insecticides on birds, small mammals, and terrestrial insect populations—particularly those insecticides with high mammalian toxicities.

Sequential Sampling

Sometimes not enough money or workers are available to estimate reliably budworm population densities to determine whether some control action needs to be done. Under these circumstances, sequential sampling may be used profitably. The sequential plan has no fixed sample size and permits classification of pre- and postcontrol population levels within some specified limit of accuracy. Prespray sampling may be done to decide whether control is justified or not. A postcontrol sample is done to determine the effectiveness of a spray project. Of course, the surviving population level is the primary concern in spruce budworm control. Several sequential sampling plans have been developed for the spruce budworm (Cole 1960, McKnight 1970, Morris 1954, Waters 1955). Computer programs are available to develop sequential plans when certain information is available (Talerico and Chapman 1970).

For a sequential plan, sufficient information must be available on:

- The relationship between number of insects per sample unit (15-inch or 45-cm branch) and subsequent defoliation of that unit. This information is needed to set class limits or degrees of infestation for the precontrol sampling.
- Frequency distribution of the prespray insect population (usually negative binomial).
- Frequency distribution of the postspray insect population (a Poisson distribution) (Cole 1960).
- Calculation of the constant k by one of several methods (Cole 1960, Mason 1970).
- Determining what constitutes satisfactory or unsatisfactory control, and the class limits.

Table 7—Sequential table for field use in prespray sampling of spruce budworm larval populations

Twigs examined	Cumulative number of budworm larvae			
	Class I against Class II		Class II against Class III	
5	5	19	7	48
10	17	32	34	75
15	30	44	62	10
20	42	56	89	13
25	54	68	116	15
30	67	81	144	18
35	79	93	171	21
40	91	105	198	24
45	103	118	226	26
50	116	130	253	29

Source: Cole (1960)

The sequential plan developed from population-damage studies in southern Idaho (Cole 1960) illustrates the usefulness of this sampling method in budworm control projects (tables 7, 8, figs. 24, 25). A 95 percent reduction of a Class III (heavy) infestation was used as the dividing line between satisfactory and unsatisfactory control. The sequential plan was used in 1965 to classify the mexacarbate and naled insecticide tests in the Bitterroot National Forest, in Montana, as satisfactory or unsatisfactory (Williams and Walton 1968). This National Forest is not far from where the original research was done and, therefore, the plan should apply.

A major problem with sequential sampling in insect surveys is that it assumes that observations are randomly selected. Unfortunately, we seldom know the underlying distributions of our biological subjects, and neither insects nor trees are randomly distributed in the field. When defining a sampling rule for collection of units, therefore, a random order of the

Table 8—Sequential table for field use in postspray sampling of spruce budworm larval populations

Twigs examined	Cumulative number of budworm larvae	
	Satisfactory control	Unsatisfactory control
15	—	12
20	2	14
25	4	17
30	6	19
35	8	21
40	11	23
45	13	25
50	15	27
55	17	29
60	19	31
65	21	34
70	24	36
75	26	38
80	28	40
85	30	42
90	32	44
95	34	46
100	36	48

Source: Cole (1960)

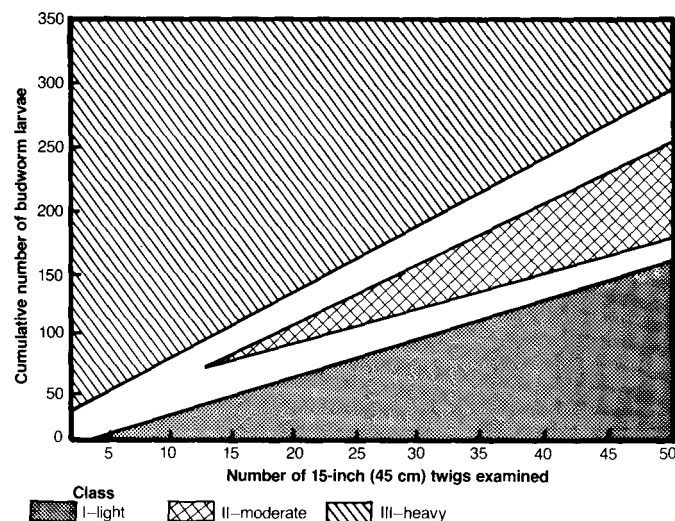


Figure 24—Sequential graph for prespray sampling of spruce budworm populations (Cole 1960).

sample cannot be ensured. Random order also assumes that all items in the population can be sampled. In fact, it is highly probable that if samples are not selected until one visits the field, all sampling will occur in a limited part of the total area.

All of the sequential plans referred to previously assumed a constant value of the negative binomial parameter K. Recent studies on the western spruce budworm, however, have shown that K values varied systematically with budworm density for the 4th larval instar, residual pupae, and egg masses (Campbell and Srivastava 1982). New sequential sampling plans have been developed for classifying insect populations with unstable K values, such as the western spruce

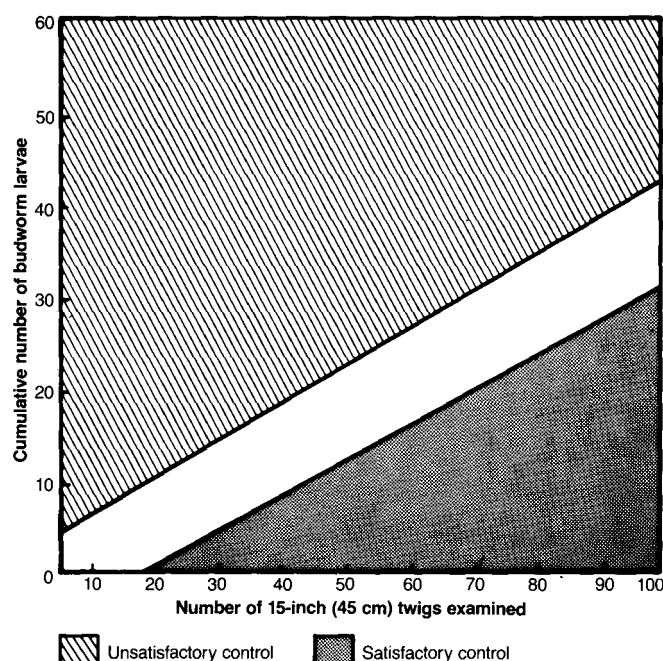


Figure 25—Sequential graph for postspray sampling of spruce budworm populations (Cole 1960).

budworm (Srivastava and Campbell 1982). These sequential sampling plans can classify population levels of 4th instar budworm larvae and egg masses (*tables 9, 10*).

The sequential sampling plan is most reliable in small homogeneous areas (about 5-ha plots) and is used by removing a 45-cm terminal tip from the midcrown of each tree sequentially. A minimum of 1 tree is needed to classify 4th instar larval populations and at least 14 trees are needed to classify egg mass populations.

Srivastava and Campbell (1982) also developed another sampling scheme called "Sequential Count Plans" that can both classify population levels and provide estimates of population density at a prespecified precision level at a lower cost than is needed for fixed-size plot sampling schemes. These sequential count plans provide estimates of budworm densities for 4th instar larvae, egg masses, and residual pupae. For a 20 percent precision level, a minimum of 4 trees must be sampled to obtain larval estimates (*table 11*), a minimum of 9 trees at the pupal stage (*table 12*), and a minimum of 17 trees at the egg mass stage (*table 13*). Density is estimated as the total number of insects at the stopping point per number of trees sampled. This count is converted to density per square meter by dividing the number by 0.082.

Table 9—Sequential sampling scheme at 95 percent confidence level for larval population (4th instar) on midcrown 45-cm terminal tips

Trees	Cumulative number of budworm larvae			
	Light	Moderate	Moderate	Heavy ¹
1	2	3	9	10
2	4	6	17	20
3	6	9	25	28
4	9	13	33	37
5	12	16	40	46
10	25	35	77	88
15	39	53	114	130
20	53	72	150	171
25	67	91	187	212
100	285	383	723	825

Source: Srivastava and Campbell (1982)

¹ Infestation limits defined by Carolin and Coulter (1972).

Table 10—Sequential sampling scheme, at 95 percent confidence level, for egg-mass populations on midcrown 45-cm terminal tips

Trees	Cumulative number of egg masses			
	Light	Moderate	Moderate	Heavy ¹
10	3	5	9	10
14	4	6	13	15
15	5	7	14	16
20	6	10	19	21
25	8	12	24	26
50	16	24	48	52
60	19	29	58	63
70	23	34	67	74
80	26	39	77	84
90	29	43	87	95
100	32	48	97	105

Source: Srivastava and Campbell (1982)

¹ Infestation limits defined by Carolin and Coulter (1972).

Table 11—Sequential count plans for estimating 4th instar budworm larvae on 45-cm terminal tips (midcrown)¹

Trees (n)	Cumulative number of budworm larvae		
	D _o = 0.15 ²	D _o = 0.20	D _o = 0.25
3	—	—	15
4	—	71	8
7	162	10	5
10	26	8	4
15	16	7	4
20	13	6	4
25	12	6	4
30	11	6	4
50	10	6	4
75	10	6	3

Source: Srivastava and Campbell (1982)

¹ Sampling terminated when cumulative number of larvae $\geq T_n$ at n trees.

² Fixed level of precision.

Table 12—Sequential count plans for estimating residual pupae on 45-cm terminal tips (lower crown)

Trees (n)	Cumulative number of pupae and pupa exuviae (T _n)		
	D _o = 0.15 ¹	D _o = 0.20	D _o = 0.25
6	—	—	30
9	—	45	3
10	—	12	2
15	—	4	2
16	81	4	2
20	12	3	2
25	7	3	2
30	6	3	2
40	5	2	1
50	4		1

Source: Srivastava and Campbell (1982)

¹ Fixed level of precision.

Table 13—Sequential count plans for estimating egg masses on 45-cm terminal tips (midcrown)

Trees (n)	Cumulative number of egg masses (T _n)		
	D _o = 0.15 ¹	D _o = 0.20	D _o = 0.25
11	—	—	47
14	—	—	6
17	—	111	4
20	—	14	3
30	114	5	2
40	14	4	2
50	9	3	2
65	7	3	2

Source: Srivastava and Campbell (1982)

¹ Fixed level of precision.

APPENDIX A—Sample Data Collection Sheet

Area code 1= Chamberlain; 2 = Belmont; 3 = Check

Field Columns	Data Cards								Field description								Field Description
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
1-2																	Card I.D.: 01 or 02 for pre or post-spray
3-4																	Area code: see above
5-6																	Plot-number 1-3
7-10																	Tree number
11-12																	Tree species (DF, TF)
13																	Crown level
14-16																	Branch number
17-20																	Branch length
21-24																	Branch width
25-28																	Number of shoots
29-32																	Number defoliated
33-40																	
41-44																	Total number of WSBW

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The Forest Service, U.S. Department of Agriculture, is responsible for Federal leadership in forestry. It carries out this role through four main activities:

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- Cooperation with State and local governments, forest industries, and private landowners to help protect and manage non-Federal forest and associated range and watershed lands.
- Participation with other agencies in human resource and community assistance programs to improve living conditions in rural areas.
- Research on all aspects of forestry, rangeland management, and forest resources utilization.

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- Represents the research branch of the Forest Service in California, Hawaii, and the western Pacific.
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Williams, Carroll B., Jr.; Sharpnack, David A.; Maxwell, Liz; Shea, Patrick J.; McGregor, Mark D. **Guide to testing insecticides on coniferous forest defoliators.** Gen. Tech. Rep. PSW-85. Berkeley, CA: Pacific Southwest Forest and Range Experiment Station, Forest Service, U.S. Department of Agriculture; 1985. 38 p.

This report provides a guide to techniques for designing field tests of candidate insecticides, and for carrying out pilot tests and control projects. It describes experimental designs for testing hypotheses, and for sampling trees to estimate insect population densities and percent reduction after treatments. Directions for applying insecticides by aircraft and for evaluating the effectiveness of such applications on target and nontarget insects are explained. The guide can help practitioners improve decisionmaking, efficiency, and safety in the use of insecticides to control forest pests. Although it is based primarily on budworm populations, the information reported is applicable to other coniferous forest defoliators.

Retrieval Terms: insecticide tests, forest defoliators, western spruce budworm, Douglas-fir tussock moth, experimental designs, sampling