

Pesticides  
aerial application  
Dy Budworm

2669 (55)

## Study Plan

### Small Scale Field Tests of Matacil and Orthene Aerially Applied to Western Spruce Budworm Populations

1975

by

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## I. INTRODUCTION

### A. Selection of Chemicals

In June 1974, the Dow Chemical Company terminated production of mexacarbate (Zectran<sup>(R)</sup>), the most effective insecticide currently registered with the Environmental Protection Agency (EPA) for the control of the spruce budworm species (Choristoneura spp.) complex in the United States. This development and the general increase in Choristoneura populations in North America make field tests of potentially effective insecticides to find a replacement for Zectran imperative.

The Insecticide Evaluation Project (IEP) (PSW-2203, USFS) has selected two insecticides that we feel have the best potential for effectively controlling spruce budworm (SBW) populations in North America. They are aminocarb (Matacil) and Orthene. Our selections are based on several of the following criteria:

1. Potential high field effectiveness in SBW population suppression due to high toxicity and/or high residual biological effectiveness.
2. Potential high effectiveness in the protection of tree foliage.
3. Safety to non-target organisms.
4. Amount of agricultural and/or forest experience with the material in the USA and Canada.
5. Prospects of obtaining early registration.

The general descriptions of the two candidate insecticides, their contact and feeding toxicities, and safety data are presented elsewhere.<sup>1/</sup> These

<sup>1/</sup> IEP Staff 1974. A proposal for laboratory and field tests of four insecticides on spruce budworm populations in 1975. An unpublished manuscript on file at PSW-2203. 16 p. 10/2/74

chemicals are not as toxic as Zectran to spruce budworm in laboratory tests. However, they do have good potential for high field effectiveness against spruce budworm in the forests. The candidate insecticides have a comparable or higher safety factor than Zectran which is the environmentally safe alternative to DDT in spruce budworm control. Field use of Matacil and Orthene against forest and agricultural insect pests have not shown any serious adverse effects against mammals, birds, fish, and other nontarget organisms. Additional safety testing may be needed for registration purposes.

This study plan describes proposed field tests for the two candidate insecticides against populations of the western spruce budworm (Choristoneura occidentalis Free.) in central Washington. However, the test objectives, experimental design and study procedures are suitable for tests against spruce budworm populations anywhere in North America.

### B. Application Strategies

Both candidate insecticides will kill spruce budworm larvae by contact activity and by ingestion. The field effectiveness of contact insecticides is greatest when the application is timed to the stage when the target insect is most exposed to the spray. With the budworm, this is during the 4-5th larval instars. Most of the defoliation damage caused by the insect during its life cycle occurs during the last two instars. They consume a substantial amount of the current year's foliage which helps shield them from aerially applied contact insecticides. They are full grown and hence much larger than earlier instars. Consequently, they are more exposed and therefore more vulnerable to a contact insecticide than at any other time during their life cycle.

Contact insecticides applied during the exposed late larval stage may cause very high mortality in the target insect populations. This would have greater effect on generation survival and population densities of the next generation than high mortality in the early instars. Practically all spraying operations against spruce budworm populations in the United States are conducted during the late larval stages. This application strategy would provide very little protection to the current year's foliage. Canadian experience with the contact insecticide DDT shows that the overall effect of a late DDT application (against the large larval instars) would be the reduction of the insect population with very little foliage protection (Randall 1968).

Orthene exhibits systemic activity. Orthene is a phloem mobile systemic insecticide with high potential for the control of a variety of forest insects--particularly those difficult to reach with a contact insecticide.

Spruce budworm larvae are difficult to control in late May-early June while they are in the 2nd instar and predominantly in the needle-mining or bud-mining stage. They are not exposed at this stage and therefore not vulnerable to a contact insecticide, but are vulnerable to systemic insecticides. Systemic insecticides should be applied when 50% of the bud caps have fallen away. The applications should coincide with budworm emergence from winter hibernacula. Upon emergence the 2nd instar larvae commence mining old needles, new buds, and staminate flowers. At budbreak they move to the elongating shoots to feed on the current year's foliage.

These phenological events coincide with the physiological stage of the tree when the maximum quantity of photosynthate is being translocated from the photosynthate source (the old needles adjacent to and on the terminals) to the photosynthate sinks (the developing shoots and leaves of the current year). Orthene has the ability to move with the assimilate stream from the mature needles to the elongating shoots and new needles.

Systemic insecticides applied in the early spring may give greater foliage protection than contact insecticides applied later. This is suggested by some data showing that an early spray strategy directed against 2nd instar budworm protected more foliage than a late spray strategy directed at 6th instar budworm larvae (Randall 1968). However, the late spray strategy killed a much greater percent of the budworm population than did the early spray strategy. The field tests described in this study plan are designed to test early and late application strategies with Orthene.

## II. OBJECTIVES

The primary objective is to determine and compare the field efficacy of Orthene and Matacil following spray application in terms of insect mortality/survival and foliage protection obtained with equal spray coverage.

Meeting this objective will provide the following information: 1. An evaluation of the systemic properties of Orthene and the effectiveness of these properties against the 2nd instar budworm larvae; 2. an appraisal of the effectiveness of the contact activity of the two candidate insecticides against the 4th-5th instar larvae of the budworm. Orthene is most effective when ingested, whereas, Matacil is highly toxic by contact; 3. an appraisal of early versus late applications of Orthene in terms of foliage protection and budworm survival; and 4. determine the effects of the various treatments on the primary parasites, Apanteles and Glypta, and other parasites of spruce budworm.

## III. EXPERIMENTAL DESIGN

The five treatments (including untreated checks) will consist of two different application strategies for each of the two insecticides and a check plot. Each treatment will be replicated three times, the checks twice. The statistical design will either be a simple random design or a randomized block design, depending on the layout of the study areas. Each insecticide treatment will be aerially applied by helicopter to 20-acre study plots in western budworm infested forest stands. The untreated checks will help monitor the natural progression of larval mortality and defoliation of infested trees. A fifteen tree<sup>2/</sup> cluster in each study plot will be selected for spruce budworm (SBW) population sampling. The study trees will be relatively open-grown and suitable for SBW population sampling by 35-foot extendable pole pruners.

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<sup>2/</sup> The selection of number of trees per treatment plot is based on a telephone conversation with V. J. Carolin. It should be emphasized that numbers and cluster(s) configuration is subject to change pending review by Carolin, John Hazard and/or Floyd Johnson, PNW, Portland, Oregon.

#### IV. STUDY AREAS AND PROCEDURES

##### A. Selection of Study Areas

The area tentatively selected for these field tests is near Winthrop, Washington on the Okanogan, N. F.

##### B. Plot Selection

The selection of treatment plots will depend on the availability of suitable sample trees. Ideally the fifteen tree cluster should be in a circle covering not more than 3-5 acres.

Individual plots will be selected by the following criteria: 1. Easy access by road; 2. contain a medium to high population of western budworm over an area of at least 40 acres;<sup>3/</sup> The minimum population density needed for testing is 15-29 larvae/100 buds;<sup>3/</sup> 3. contain enough sample trees within the 20 acres, i.e., 30 feet tall, open grown and not overtopped by larger trees; 4. be isolated from other plots either by geographical distance or topographical features to prevent any cross contamination with other study plots.

##### C. Marking Spray Plots and Swath Spacing

All plots shall be marked to assure uniform spray coverage in the manner by Maksymiuk *et al.* (1968). The corners of each spray plot will be marked by placing a brilliant light-reflecting plastic panel on the top of a dominant tree. These markers will be placed on the treetop with the aid of slingshot, crossbow, or line-throwing gun. Similarly, both ends of the plot will be marked at 200-foot intervals by placing alternate yellow and white flagging at the top of dominant trees for swath spacing. The aircraft pilot will line up on two similar colored flags to obtain the 100-foot spacing intervals.

##### D. Selection of Sample Trees

Plot locations are specifically chosen so that they will contain suitable sample trees. Individual sample trees will be selected using the following criteria:

1. The sample (study) trees will be Douglas-fir and grand fir, if possible in equal numbers.
2. Be small enough to be sampled with a 35-foot extendable pole pruner but contain enough foliage for the samples needed through the whole study.
3. Show some of signs of defoliation from the previous year indicating that it contains a high SBW population.
4. Relatively open-grown and not overtopped by adjacent trees.
5. Within 20 feet of the base of the sample tree there must be an opening for the placement of spray cards and plates to obtain data on spray coverage of the plot and study trees.

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<sup>3/</sup> This minimum population density was suggested by V. M. Carolin, PNW, Portland, Oregon.

The position of the treatment plots and limited acreage involved should increase the possibility of applying an even and consistent dosage of insecticide.

#### E. Monitoring budworm development and tree phenology

The early treatment strategy (ETS) of Orthene should be timed according to bud development. Within each ETS plot five buds per sample tree will be marked (flagged) to monitor bud development. Buds selected should be as high up the crown as the sampler can reach and distributed around the entire circumference of the crown. Spray application for the ETS will commence 3 days after 40% of the bud caps have fallen away.

The three remaining treatments will coincide with larval development. This necessitates selecting five additional trees per plot to be used exclusively for this purpose. Two mid-crown branches from these five trees per plot will be sampled and all the larvae on each branch collected in alcohol for instar determination. It is exceedingly important that the larval development tree be interspersed within the population sampling cluster. When 50% of the larvae reach the fifth instar, spraying should begin no later than 24-48 hours after the pre-spray sample. Development sampling should begin as soon as the plots are established and should continue on a periodic basis until the population approaches the fifth stadium. At this time, development samples should be taken at least every other day.

#### F. SBW Population Sampling

Spruce budworm larval population densities will be estimated on each tree, cluster and treatment just prior to spraying (24-48 hours) and at 7 and 14 days following treatment application. A pupal sample will also be taken in mid-July.

The sampling procedure used is basically that described by Carolin and Coulter (1972) and consists of counting the number of larvae and buds and measuring the foliage area of two 15-inch branches removed from mid-crown of the sample trees at pre-spray and four branches at post-spray. The population will be expressed as the number of SBW larvae per 1000 buds and 1000 square inches of foliage.

Two, 15-inch twigs will be cut from the approximate mid-crown with a 35-foot extendable pole pruner with a 18-20" cloth basket fastened just below the cutting head and individually lowered to the ground and placed in plastic bags. Bags must be marked with plot number, tree number and date, and stored out of direct sunlight.

During the pre-spray sampling, 5 unopened buds/sample tree will be tagged. These buds will be collected during the post-spray sampling (7 days after spray application). See section K Residue Analysis for collection procedures.

All samples will be returned to a field laboratory where workers will examine each branch and record on a data sheet (see appendix 1) the following information:

1. Date and treatment
2. Plot number
3. Tree number
4. Branch number
5. Branch length
6. Branch width
7. Total number of buds or shoots
8. Total number budworm

A procedural description and accompanying data forms for estimating bud defoliation will be added to this study plan at a later date but before population sampling begins.

Approximately 200 SBW larvae will be collected from each plot during each sampling period and placed into rearing for parasite emergence. The collections will be reared on artificial diet in petri dishes. Several SBW larvae (no more than 10) will be placed in each petri dish in such a manner as not to injure them. The date, study plot number or cluster number will be recorded on each dish. The petri dishes will be held at the field laboratory and processed to determine percent parasitism including identity of the parasite complex.

The parasite emergence data will indicate the possible effects the various treatments may have on the three hymenopterous parasites which have attacked second instar larvae the previous fall. These parasites issue from their SBW hosts and form cocoons when the SBW larvae are 5-6th instars. We may also see treatment effects on tachinids which attack 5-6th instar budworm and emerge during the budworm pupa stage. Petri dishes and media will be supplied by PSW-2203.

#### G. Spray Formulations

Orthene - The field formulation containing Orthene will be a dilution of 1.0 lb of Orthene in one gallon of water with 3.8 gm Rhodamine B extra base (0.1%) per gallon for droplet assessment. The rate of application will be one gallon of formulation per acre containing 1 lb of Orthene. Two applications will be made, depending on the timing of tree development and insect development. One at 40% bud cap fall plus 3 days and the other when 50% of the budworm larvae are in the 5th instar.

Matacil - The field formulation containing Matacil will be a dilution of 0.15 lb and 0.3 lb in one gallon of diesel oil with a 4% solution of Rhodamine B in oleic acid at a rate of 1 quart/10 gallons of formulation or 15 quarts of liquid dye to 150 gallons total of formulated Matacil. There will be two applications with different dosages: 1. one gallon of formulation per acre containing 0.15 Matacil and 2. one gallon of formulation per acre containing 0.30 lb Matacil.

#### H. Spray Application

The afternoon before spraying is to begin the aircraft with an observer will visit each of the plots to familiarize the pilot with their location and show him the prospective method in which each plot is to be treated. Emergency landing sites, landmarks, dangerous topographical features, etc. will be pointed out to the pilot on these orientation flights. Spraying will begin as early in the morning as light conditions will allow. Continuous radio communication will be maintained between the pilot and the aerial observer and the ground crew at all times.

Spraying will be postponed or prohibited under the following conditions:

1. Wind velocity exceeds 6 mph.
2. Temperature exceeds 65° F.
3. Snow, water, or ice covers the foliage.
4. Rain is predicted to fall within 6 hours.
5. Fog covers part of the area to be treated, or the flight path of the helicopter between heliport and the test site.
6. Air turbulence is so great as to effect the spray pattern and deposition.

A pint sample will be taken from each treatment formulation at the time of mixing. These samples will be shipped to PSW-2203 for a biological and chemical assay.

#### I. Meterology

Meterological measurement of air temperature, humidity, and wind at ground level will be made by personnel from the R-6 Pilot Project of fenitrothion.

#### J. Spray Deposit Assessment

The deposit sampling units will consist of two white Kromekote cards and two aluminum plates placed in "cardholders" at ground level. This set of four deposit samplers will be placed around each tree in the nearest opening. Cards and plates will be put in place immediately before spraying and must be collected no more than one hour later.

Both the cards and the plates will be put out the morning of spray. Immediately after the spraying of a plot the plates must be picked up. This must not be longer than 1 hour after spraying, preferably within 1/2 hour. In picking the plates up the collector should lay the contaminated portion of the two plates from each tree face to face. Two small pieces of masking tape used to keep the plates together as well as to record the appropriate data (tree #, plot, date, etc.). All aluminum plates will be shipped as soon as possible to Dr. Richard Roberts, U. S. Forest Service, P. O. Box 245, Berkeley, California 94701. A note should be attached to the shipping box to have the airport notify Dr. Roberts or Dr. Pieper upon arrival, at

415-486-3681 or 415-486-3373.

The cards should be left out for 1-2 hours to allow them to dry. This will allow for simple shortage after collection since the materials actually penetrate the Kromekote cards the droplets can't be rubbed off.<sup>4/</sup>

PNW-2208 will analyze the cards with an automatic spot counter and provide data on VMD and number of drops per cm<sup>2</sup>. PSW-2203 will analyze the aluminum plates using dye sensitive fluorometric techniques and provide data on gal/acre deposited.

#### K. Residue Analysis

Foliage Handling and Procedures - A small amount of residue analysis will be performed for the early treatment with Orthene. Analysis will be conducted on developing buds in order to assess the systemic movement of Orthene from old mature needles to developing new buds. The buds will be selected and tagged for analysis the day before spray. These will be expanding, but closed buds with their caps still in place and the bud scales completely covering the entire portion of new growth. Five buds will be tagged on each of the 15 tree/plot and on each plot including the check plot. This gives a total of 300 tagged buds for the three Orthene treated plots and one check plot.

These tagged buds will be collected seven days post-spray. Only the new growth should be collected leaving the old bud cap and bud scale. Care should be taken not to contaminate the new growth with Orthene by handling the surrounding foliage before collecting each sample. If possible, one person should hold the branch while another person collects the new growth or clips the bud into a plastic sandwich "baggy". In this manner no contaminated foliage is handled by the person collecting the sample. If a tagged bud has not opened, collect the entire bud. Each bud should be placed individually in a baggy and the five buds from one tree placed in a paper sack. The tree and plot number should be clearly marked on each baggy and paper sack with water insoluble ink or with soft pencil. Immediately after collecting and bagging the buds, they should be placed in cold (dry ice) storage and shipped air freight to:

Dr. Carl Crisp	(415) 486-3100 (lab)
U.S. Forest Service	(415) 486-3373 (office)
P.O. Box 245	(415) 254-1915 (home)
Berkeley, California 94701	

A note should be attached to the shipping box to have the airport notify Dr. Crisp upon arrival. If possible, please ship the samples to the Oakland airport. Please label the box "Biochemical Residue Samples".

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<sup>4/</sup> Information provided by George Markin, PNW 2208.

The chemical residue procedures will be adapted from Leary (1974).

#### L. Assessment of Foliage Retention

Defoliation assessments will be done in two ways on trees and branches.

A whole tree visual classification of crown defoliation will be done for every sample tree. The crown will be divided into three areas; upper, middle, lower. Old and new foliage will be classified separately and recorded on separate data sheets. It is important that this classification take place before spray application begins and again in August after pupation.

#### New Foliage Intensity Codes

<u>Class</u>	<u>Defoliation</u>	<u>Intensity Code</u>
None	No visible damage	0
Light	Less than 10%	1
Moderate	11-50% damage	2
Heavy	51-90% damage	3
Very Heavy	91-100% damage	4

#### Old Foliage Intensity Codes

<u>Class</u>	<u>Defoliation</u>	<u>Years Back</u>	<u>Intensity Code</u>
None	None visible	0	0
Light	Defoliation barely visible	1	1
	Defoliation barely visible	2	3
	Defoliation easily visible	1	2
Moderate	Defoliation easily visible	2	4
	Defoliation of old needles	1	6
Heavy	Severe to complete defoliation of old needles	2	5

Using the same 15-inch sample branch collected during the pre- and post-spray larval sampling period, 25 apical buds will be examined on each branch making a total of 50 pre-spray and 100 post-spray per tree. An optical examination will be made of the degree of feeding on each bud or new shoot, and this data recorded as the percent defoliation to the nearest 10 percent. The amount of foliage saved will be determined by comparing the pre- and post-spray samples.

#### V. DATA SUMMARY AND ANALYSIS

The field effectiveness of the various treatments and control strategies in suppressing SBW population and protecting tree foliage can be evaluated by the following sets of data:

### A. Population Data

Estimates of pre- and post-spray SBW population densities for each tree and cluster in the spray plots for each sampling period.<sup>5/</sup> This data gives the mortality rate per plot. The mortality estimates will be determined by ratio estimation, e.g. the survival rate is given by:

$$r_i = (X_{2i}/Y_{2i})/(X_{1i}/Y_{1i})$$

where  $X_1$  and  $X_{2i}$  denote pre- and post-spray insect counts and  $Y_{1i}$  and  $Y_{2i}$  denote pre- and post-spray measurements of branch surface for the  $i$ th tree. The SBW population data will be used to:

1. Compare mortality rates and post-spray or residual larval population densities over time among the treatments.
2. Compare pupal population densities among the treatments.

These comparisons will be tested by Analysis of Variance for each sampling period using SBW population densities, mortalities as the (Y) variables. The least significant difference (LSD) techniques of multiple comparisons will be used for comparisons among means. If sufficient ranges exist in the pre-spray population density data, analysis of co-variance will be used. With pre-spray SBW population density as the co-variate, the effectiveness of the insecticide treatments on a range of population densities will be investigated.

To measure the current years foliage saved, covariance analysis will be used to test significant differences of slope and intercept between untreated and treated plots where the independent variable is the pre-spray population and the dependent variable is the percent defoliation.

### B. Spray Deposit Data

The spray deposit data will provide the following information for each tree and cluster of trees used in the SBW population sampling:

1. The approximate gallons per acre (GPA) of spray formulation reaching the ground in the open spaces beside the study trees.
2. The densities of the spray droplets reaching the ground in the open beside the study trees.
3. The VMD of the spray droplets reaching the ground in the open beside the study tree

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<sup>5/</sup> These estimates will be obtained by use of a computer program designed for these analyses by Wilson and Williams, 1972.

These spray deposit data will describe a range of deposited dosage for the study tree sites in each sampling cluster and treatment. The field effectiveness of each treatment can be partly evaluated by determining the relationships between SBW larval mortalities (survivorship) and deposit for each insecticide application.

The relationship between deposit and mortality will be investigated by regression analysis (Davis et al. 1956; Maksymiuk et al. 1971). In all cases larval mortality will be the dependent variable (Y) and the spray deposit will be the independent variable (X). For the purpose of such analysis, percent mortality will be converted to probits, and deposit values at each sample tree site converted to logarithms. Correlations will be made using various deposit (X) variables: 1. volume of spray in the open, 2. drop density sampled in the open, 3. produce of 1 and 2. Figure 1 is an example of such a graph, when one of the deposit variables (X) is plotted against insect mortality (Y) (Maksymiuk et al. 1971).

## VI. BUDGET

Insecticides - Orthene and Matacil will be provided without cost by the respective chemical companies.

Total Costs by Fiscal Year<sup>6/</sup>

	FIDM(R-6) FY 75	FIDR(PSW)	FIDM(R-6) FY 76	FIDR(PSW)
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### Applications:

Helicopter - 240 acres (12 hrs @ 165/hr)		2000.		
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Personnel: @ \$163. wk. including overtime

15 Forestry Aides for 10 wks	14670.		9780.	
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### Vehicles

6 pickups 25,000 mi. at .16	2400.		1600.	
2 sedans 10,000 mi. at .22	1320.		880.	

### Per Diem

Permanent Personnel	1750.	1725.	875.	550.
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	FIDM(R-6) FY 75	FIDR(PSW)	FIDM(R-6) FY 76	FIDR(PSW)
<u>Equipment and Other Services</u>				
Organizational & Planning	500.	2000.	1000.	1000.
Communication	200.		200.	
Electronic Spotscanner				720.
Dye		300.		
Supplies (includes alum. plates & analyses)		1500.		1000.
Residue analyses				1000.
Computer time				100.
Other misc. supplies	1000.	1000	500.	
Preparation of Reports			1000.	
	<hr/>	<hr/>	<hr/>	<hr/>
Sub totals	21840.	8525.	15835.	4370.
Totals FY 1975	30,365.			
FY 1976				20,205.
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Grand Total	\$ 50,570.			

6/ Permanent salaries contributed. Costs for each item are listed under Column headings FIDM or FIDR according to which office will spend the funds; it does not indicate the source of funding.

## Literature Cited

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## 1975 Spruce Budworm Insecticide Test Data Format (IEP)

Orthene	Matacil	Check
1. 1E	7. .15L	13. 1L
2. 2E	8. .15L	14. 2L
3. 3E	9. .15L	15. 3L
4. 1L	10. .30L	
5. 2L	11. .30L	
6. 3L	12. .30L	

E, and L denote Early and Late treatments; .15 lb/acre and .30 lb/acre

Date \_\_\_\_\_ Insecticide \_\_\_\_\_ Block \_\_\_\_\_

Field  
Cols.

Data Cards

Field Description

	1	2	3	4
1-2				
3-4				
5-6				
7-10				
11-12				
13-14				
15				
16				
17-20				
21-24				
25-28				
29-32				
33-36				
37-40				
41-44				
45-48				
49-52				

Card ID: 01 or 02 for pre-post-spray respectively.

Area Code: See above.

Cluster (Plot) No: 1-6.

Tree Number

Tree Species, DF, TF

Crown Level: M

Branch Number, 1-4

Branch Length, inches

Branch Width, inches

Number of Shoots

Total No. of SBW

Other.