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(R4)

~~McGOMB~~  
~~MESO~~  
~~PETTINGER~~  
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~~GREGG~~  
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*Twardus*  
*Wistle 197*  
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PROTECTION OF DOUGLAS-FIR FOLIAGE FROM WESTERN  
SPRUCE BUDWORM (LEPIDOPTERA: TORTRICIDAE) DAMAGE BY EARLY  
APPLICATIONS OF ACEPHATE (ORTHENE 75S)<sup>1</sup>

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**Abstract**

*Can. Ent.* 110: 1127-1132 (1978)

Acephate (Orthene 75S) was applied to Douglas-fir (*Pseudotsuga menziesii* Mirbl. [Franco]) in spring 1976 to determine its efficacy for protecting current year's foliage from western spruce budworm (*Choristoneura occidentalis* Freeman). Results showed budworm mortality for both treatments exceeded the checks by at least 91% at all sample periods. A significant degree of foliage protection was also achieved as measured by mean twig length and mean number of missing needles per twig. Samples of new growth were analyzed for residues of acephate and methamidophos (an active metabolite of acephate) by gas-liquid chromatography. Results showed that high levels of insecticide persisted for about 10 days, then dropped over a 5-day period to lower levels for the rest of the study, about 35 days.

**Introduction**

Acephate is an effective insecticide against two species of spruce budworm, *Choristoneura occidentalis* Freeman and *C. fumiferana* (Clemens) (Armstrong and Nigam 1975; Hopewell and Nigam 1974). The usual recommendation has been to apply insecticides to fourth and fifth stadia larvae that are feeding externally on needles (Carolin and Honing 1972). In this method, acephate is used primarily as a contact insecticide. Acephate has also been reported to have systemic activity (Lyon 1973; Werner 1974). Brewer and O'Neal (1977) demonstrated "some type of systemic action" when acephate was applied to second and third stadia spruce budworm larvae which were feeding in the old growth needles or new buds.

In spring 1976, we made a small-scale ground application of acephate on second and third stadia spruce budworm larvae to test its potential as a systemic insecticide and its efficacy in protecting current year's new growth. When buds on Douglas-fir (*Pseudotsuga menziesii* Mirbl. [Franco]) begin to develop, second and third stage larvae of western spruce budworm (*Choristoneura occidentalis* Freeman) enter the new buds and begin feeding. Prior to bud development most larvae are mining into old growth needles. Photosynthate necessary for bud development comes from old growth needles next to the buds (Crafts and Crisp 1971: 168-266). Because systemic insecticides penetrate the needles and move with plant sugars to developing buds, foliar application to the old growth should result in sufficient toxicant accumulation in the buds to kill larvae feeding there, and also to kill insects feeding directly on the old growth needles.

The tests took place in the Sugarloaf Mountain area of the Arapaho-Roosevelt National Forest, near Boulder, Colorado, where infestations of spruce budworm had occurred for many years (Johnson and Minnemeyer 1975).

<sup>1</sup>This paper reports research involving insecticides. It does not report recommendations for their use nor does it imply that any uses described here have been registered. All use of insecticides must be registered by the appropriate State and/or Federal Agencies before they can be recommended.

### Materials and Methods

**Insecticide application.** Spraying commenced when the majority of the buds were starting to swell. At this time, most of the insects were mined into the buds or old growth needles. Acephate was mixed just before spraying. Treatment 1 consisted of 4.8 g a.i./l water. Treatment 2 consisted of two applications 10 days apart, the first at 4.8 g a.i./l water and the second at 2.4 g a.i./l water. A Bean hydraulic sprayer (Model 1010a)<sup>2</sup> equipped with an FMC 29 adjustable nozzle and a D-9 orifice was used to apply the formulations at 14 kg/cm<sup>2</sup>. Nine 0.04 to 0.08 ha plots containing 10 or more 6- to 11-m tall Douglas-firs were established, and assigned to each treatment. All plots were accessible to a pickup-mounted sprayer and were separated by a minimum of 100 m. Plots were screened from one another by intervening vegetation or changes in aspect. Each tree was sprayed until thoroughly wet.

**Insecticide efficacy.** Five trees in each plot were selected for determination of insecticide efficacy. Trees chosen had sufficient foliage to ensure sampling throughout the study with no adverse effects on the trees. Only the upper two-thirds of the crown on each sample tree was studied. This area of the crown was divided into eight sampling sites. Prior to each sample period, three sites per crown were randomly selected for sampling.

A 38-cm branch was removed from each sampling site using pole pruners. The branches were individually bagged and taken to the laboratory for analysis. The number of living and dead insects in each bag was tabulated. Larval density data was collected at prespray and 3, 7, 14, 21, 28, and 35 days postspray. It was necessary to remove infested buds and needles to determine the number of living insects. The needles were spread out on a small light table. Live larvae were readily visible through the tissue, whereas dead larvae appeared dark and shriveled. All infested buds were dissected to determine the number of living and dead insects. This procedure was continued until the population was no longer feeding inside the needles and the buds had opened. The branches were measured and the area of foliage calculated. Population densities were expressed as number of living larvae per 1000 cm<sup>2</sup> foliage. The branch data was pooled to form the data base for that tree.

**Foliage protection evaluation.** On postspray days 21, 28, and 35, a random selection of five of the most distal new growth shoots from each sampling site was made for each treatment. These shoots were used to evaluate the amount of foliage protection achieved in the study. The shoots were removed and the length of new growth and the number of missing needles on each shoot was tabulated. The number of missing needles on each shoot was tabulated by counting needle scars and stubs.

**Residue sampling.** Residues from new growth (buds and shoots) from eight sample trees per treatment were analyzed. These sample trees were divided among three plots – two of three trees each, and a third of two trees. Untreated check plots were used to check for chemicals which interfere with residue analysis. Some of the untreated check foliage was fortified by the addition of a known concentration of insecticides and analyzed to determine percentage recovery.

Treatment 1 samples were taken for residue analysis on the day of spray application and 7, 14, and 35 days postspray. Treatment 2 samples were taken on the day of the first spray, then 4 and 9 days postspray, and on the day of the second spray, 4, 7, 15, 28, and 41 days after the second spray day. One sample from a check tree was taken on each day of sampling.

Each sample consisted of a small branch from each of the eight sections of the sample tree. Buds and shoots from each section were pooled to give a 10 g sample. Each sample was bagged, frozen with dry ice, and stored until processed for analysis.

<sup>2</sup>Trade names are mentioned solely for information. No endorsement by the U.S. Department of Agriculture is implied.

Table I. Mean number of western spruce budworm larvae per 1000 cm<sup>2</sup> foliage treated with acephate, in Colorado, 1976

Treatments	Pre-spray day	Postspray day					
		3	7	14	21	28	35
Treatment 1*	22.2	1.2	0.3	0.7	0.3	0.2	0.2
Treatment 2†	19.7	0.9	1.3	0.03	0.1	0.1	0.6
Check	31.6	—	40.2	36.0	22.1	15.5	10.3

\*†Treatment 1 = 4.8g a.i./ℓ water and treatment 2 = 4.8g a.i./ℓ water plus an additional 2.4g a.i./ℓ water 10 days later, respectively.

**Sample preparation and analysis.** All plant material was weighed and placed in a Titeseal vial containing 18 g of anhydrous sodium sulfate for every 5 g of tissue. Twenty milliliters of ethyl acetate (Mallinckrodt AR) were added to the vial for every 5 g of tissue, and the contents homogenized, using a Brinkman polytron (Model PT). The homogenate was centrifuged for 5 min at 1000 rpm at  $-10^{\circ}\text{C}$ . The supernatant was decanted into a disposable screw top vial containing 1 g of activated Nuchar C190-N per 20 ml of supernatant. The charcoal and supernatant were thoroughly mixed by shaking on a vortex shaker. The mixture was again centrifuged. A 4-ml aliquot was removed from each 20 ml of supernatant and evaporated to dryness under a stream of dry nitrogen. One milliliter of methyl isobutyl ketone (Mallinckrodt AR) was added to dissolve the residue. A Varian aerograph gas-chromatograph (Model 2700) equipped with an alkali flame ionization detector was used to measure the residue.

### Results and Discussion

**Efficacy results.** Population density estimates were analyzed for significant differences by Tukey's test (Bancroft 1968). Treatment population means were significantly different from check population means ( $P = 5\%$ ) at each time interval, but not significantly different from each other (Table I). Insecticide efficacy was determined by the ratio of two ratios (Simmons and Chen 1975) at postspray days 8, 14, and 35 (Table II). Mortality at all sample periods exceeded the check by at least 91%.

Tukey's test ( $P = 5\%$ ) was used to test the significance of mean twig length and mean number of missing needles per twig, between treatments at each time interval (21, 28, and 35 days postspray day). The differences between treatment 1 and treatment 2 at all time intervals for both the mean twig length and mean number of missing needles per twig were not significant (Table III). This indicates the additional application of acephate in treatment 2 did not contribute significantly to the overall foliage protection.

The mean number of missing needles per twig was significantly greater for both treatment 1 and treatment 2 than for the checks at all time intervals. Mean twig lengths

Table II. Estimated<sup>1</sup> population reduction in western spruce budworm populations treated with acephate in Colorado, 1976

Comparison	Postspray day		
	7	14	35
Treatment 1* with check	99	97	98
Treatment 2† with check	95	99	91

<sup>1</sup>Estimate based on the ratio of two ratios (Simmons and Chen 1975).

\*†See footnote Table I.

Table III. Mean ( $\bar{x}$ ) and standard deviation (S.D.) of twig lengths (cm) and number of missing needles per twig of Douglas-fir treated with acephate for the suppression of western spruce budworm in Colorado, 1976

Variable	Post-spray day	Check ( $\bar{x}$ + S.D.)	Treatment 1* ( $\bar{x}$ + S.D.)	Treatment 2† ( $\bar{x}$ + S.D.)
Twig length	21	14.7±4.56	23.4±3.77	24.8±3.34
"	28	11.2±4.67	27.3±4.55	22.5±4.93
"	35	11.5±5.77	29.5±4.73	28.7±4.23
Missing needles per twig	21	29.6±13.0	0.58±0.7	0.75±0.7
"	28	31.0± 9.74	1.01±1.96	0.89±1.56
"	35	30.3±10.8	0.91±1.27	0.99±2.68

\*†See footnote Table I.

recorded in treatments 1 and 2 were significantly greater than the checks only on postspray day 35.

Surviving larvae remaining on branch samples were collected and reared on artificial diet to determine the degree of parasitism encountered. No significant differences between total parasitism existed between plots. However, the parasite guild of the check plot was comprised of more genera than treatment plots. Parasites found in the check plots were: (1) *Apanteles* spp., (2) *Glypta* spp., (3) various dipterous parasite species, and (4) hyperparasites in the genera *Mesochorus*, *Hypoteromalus*, and *Amblymerus*. *Apanteles* and *Glypta* were also found in both treated plots; however, no Diptera or hyperparasites were found.

**Residue analysis results.** To test our recovery methods we fortified some check foliage with known concentrations of both acephate and methamidophos. Methamidophos (O,S-dimethylphosphoramidothioate) is an active metabolite of acephate. The overall recovery for acephate was 86% ( $S\bar{x} = \pm 2.92$ ), replicated 3 times over three different concentrations; that for methamidophos was 81% ( $S\bar{x} = \pm 2.41$ ), replicated 3 times over two different concentrations. The GLC analysis for acephate also detects any methamidophos present in the sample. The amount of methamidophos was rarely over 2 ppm ( $\bar{x} = 1.08$  ppm) and did not change appreciably with time; therefore, it was added to the acephate concentration to give the total amount of insecticide recovered in ppm (Fig. 1).

Tukey's test ( $P = 5\%$ ) was also used to assess the significance between differences in residues (in ppm) found in the treatments at three time intervals during the experiment. Again, results showed that the differences between treatment 1 and treatment 2 at each time interval were not significant. Differences between treated and untreated were significant only through postspray day 7. By postspray day 14, differences between residues in each treatment were not significant. Treatment 2 residues increased sharply as expected after the second spray day, but to only 40% as much as after the first spray day. No appreciable residues were found in the checks.

The  $LC_{50}$  for western spruce budworm for sixth stage larvae is 7.7 ppm by feeding.<sup>3</sup> The  $LC_{50}$  value for second and third stage larvae is probably much lower. A lethal concentration existed in the treated buds and new growth for at least 12 days after treatment (Fig. 1). Because of the theoretically lower  $LC_{50}$  value for second and third stage larvae, it is possible that we achieved foliage protection for as long as 35 days postspray.

<sup>3</sup>Personal communication from J. L. Robertson, Forest Service, U.S. Dep. Agric., Berkeley, Calif., 1976.

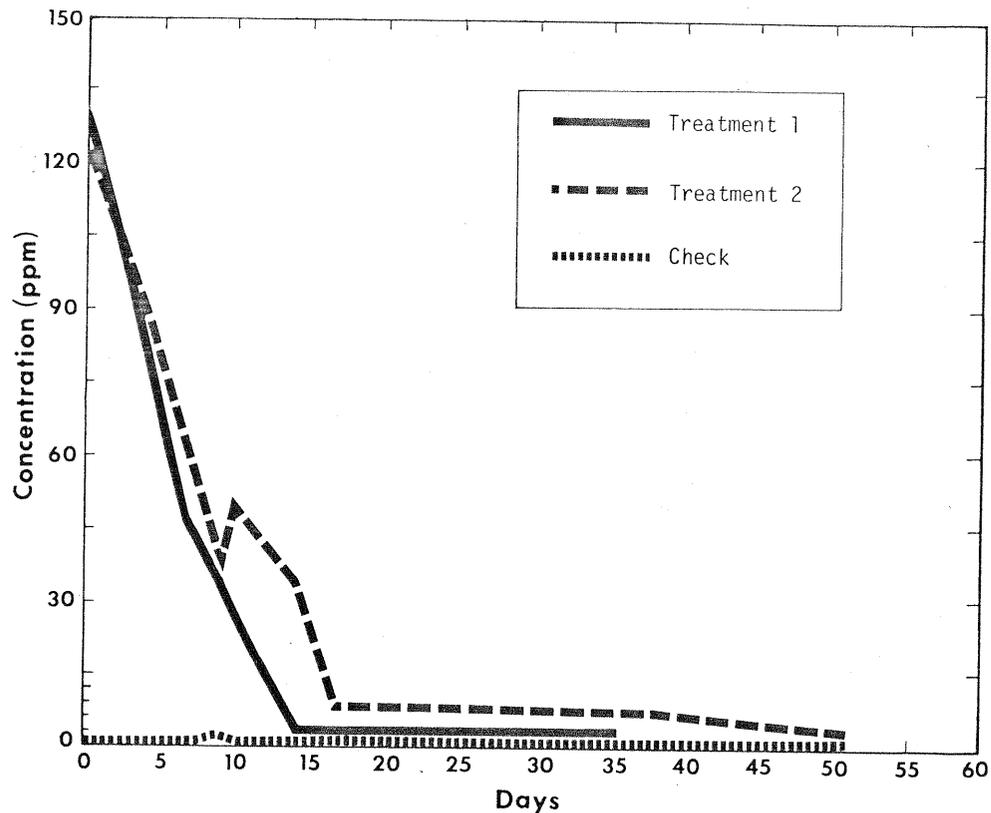


FIG. 1. Mean residue levels of methamidophos and acephate from new buds and shoots of Douglas-fir sprayed by ground applications for the suppression of western spruce budworm. Treatment 1 consisted of 4.8 g a.i./ $\ell$  water; treatment 2 consisted of 4.8 g a.i./ $\ell$  water plus an additional 2.4 g a.i./ $\ell$  water 10 days later.

### Conclusion

The results of this study confirm the observations of Brewer and O'Neal (1977). Like them, we achieved high budworm mortality and current year's foliage protection. Although we cannot rule out some degree of contact action as causing the mortality, the data suggest a possible systemic mode of action because most of the killed insects had mined into old-growth needles or buds. Residue analysis further suggests the systemic action of acephate because it seems unlikely that the high residue levels (Fig. 1) found in the buds can be explained solely on the basis of penetration. The success of these small-scale pilot tests suggests the need for further testing on a larger scale. The feasibility of this approach for protecting large forest areas as well as for small numbers of trees or single trees in urban areas needs to be determined. Because of its possible systemic action and its lower toxicity, acephate should be a more acceptable chemical, in terms of environmental safety, than many insecticides now used.

### Acknowledgments

We thank Anna Tang for her assistance in preparing samples for residue analysis; Dr. Philip S. Magee, Chevron Chemical Co., for providing analytical standards of methamidophos and acephate insecticides; and Dr. J. L. Robertson for providing unpublished feeding toxicity data for western spruce budworm and acephate.

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(Received 16 November 1977)