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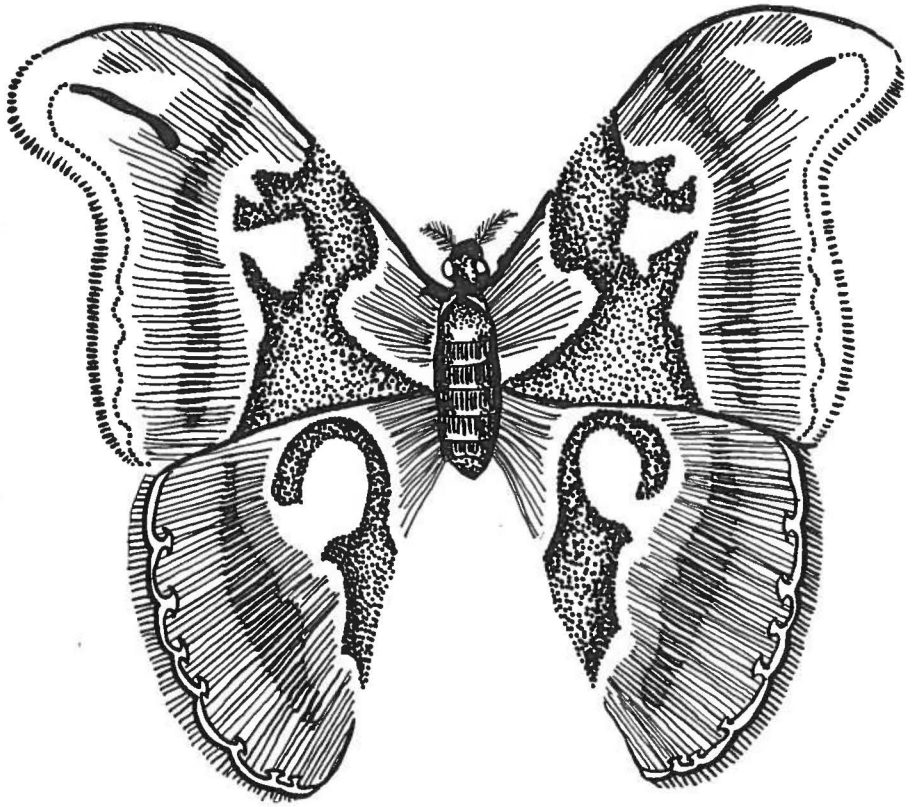

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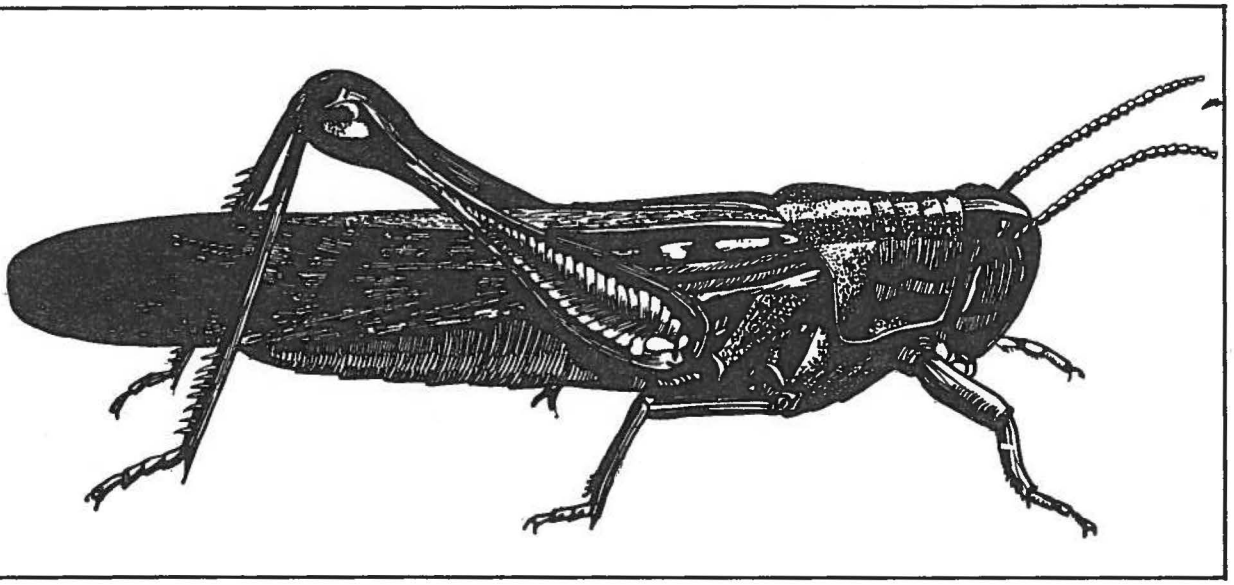

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THE MANITOBA ENTOMOLOGIST

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THE MANITOBA ENTOMOLOGIST

An official publication of the Entomological Society of Manitoba, an organization to foster the advancement, exchange and dissemination of entomological knowledge

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THE CO-ORDINATION OF RESEARCH ON A CONTROL PROGRAM FOR SPRUCE BUDWORM

C.H. BUCKNER, Moderator

Recent legislation has placed increasingly strigent controls on the use of pesticides. While the principal documents governing the use of insecticides are in federal legislation, it rests in the hands of most of the provinces to enforce the various acts pertaining to their use. In this regard, the Province of Manitoba has been one of the first to set up a structure directed towards adequate control of the use of pesticides. The studies reported in this symposium represent the combined co-ordinating efforts of the Province of Manitoba, The University of Manitoba and Federal Government agencies, and represents the first concentrated and systematic Canadian effort to examine the intricate environmental mechanisms influenced by areal dissemination of forest insecticides.

For a first attempt, the breadth of the studies are remarkably complete. Few important areas of the environment were overlooked, and many of the component projects exhibited a high degree of sophistication. Superficial studies were inevitable: lack of personnel and technology were chiefly responsible for gaps in information. As a first attempt at a comprehensive environmental scrutiny of a program of this nature organizers and scientists are to be congratulated. This is not to indicate that we become complacent, because much remains to be learned, but with the pattern and guideline developed in this project, advances appear inevitable.

AERIAL APPLICATIONS OF CHEMICAL INSECTICIDES AGAINST THE SPRUCE BUDWORM IN MANITOBA, 1973

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ABSTRACT. The first aerial spray program to control spruce budworm in Manitoba was carried out in 1973 in the Spruce Woods Provincial Forest and Park. Appoximately 8,300 acres of white spruce in prime recreational areas were selected for treatment on the basis of previous budworm injury, with the objective of preventing further stand deterioration and loss of aesthetic values. Sprays of fenitrothion at 4 oz active ingredient per acre and carbaryl at 16 oz active ingredient per acre effectively reduced budworm numbers and afforded good foliage protection when applications were made in the 4th and early 5th larval instars. Effectiveness varied, however, with the type of aerial application equipment used. Results were more consistent with boom and nozzle apparatus than with Micronair atomizers, probably because the smaller droplets produced by the latter equipment were more prone to evaporation and drift.

INTRODUCTION

Outbreaks of the spruce budworm *(Choristoneura fumiferana* Clem.) and the devastation they cause in spruce-balsam forests have been well documented for many years in eastern Canada (Blais 1954, Elliott 1960, Webb *et al* 1961, Brown 1970). Early records, however, provide only limited information on the history of the insect in Manitoba. Fragmentary reports indicate that it occurred abundantly in the central part of the province about 1907 (Hewitt 1911) and at damaging outbreak levels in white spruce *(Picea glauca* (Moench) Voss) and balsam fir *(Abies balsamea* (L.) Mill.) stands in the Lake Winnipeg region some 20 years later (Watson 1927).

Despite this apparent lack of continuity in early infestation history, three major outbreaks of the spruce budworm have been reported since continuing forest insect surveys were commenced in 1938: in the Spruce Woods Provincial Forest (Brown 1940), in the Namew Lake area on the Manitoba-Saskatchewan border (Wong *et al* 1952), and along the southern portion of Lake Winnipeg in eastern Manitoba (Prentice and Hildahl 1954). In addition, localized infestations ranging from 3 to 6 years' duration occurred in several other parts of the province (Fig. 1). Although these smaller infestations subsided before causing serious injury in affected stands, the Namew Lake and eastern Manitoba infestations (which persisted some 18 and 9 years respectively) caused notable tree mortality in localized areas (Prentice and Hildahl 1959) before being terminated by adverse weather, mainly late frosts and prolonged periods of below normal spring temperatures (Prentice and Hildahl 1960, Cayford *et al* 1959, Ives *et al* 1969).

The outbreak in the Spruce Woods Provincial Forest and Park — an isolated island of white spruce containing no balsam fir — has followed a long period of activity, at least since 1938. During this time, budworm populations have fluctuated widely, but until recently

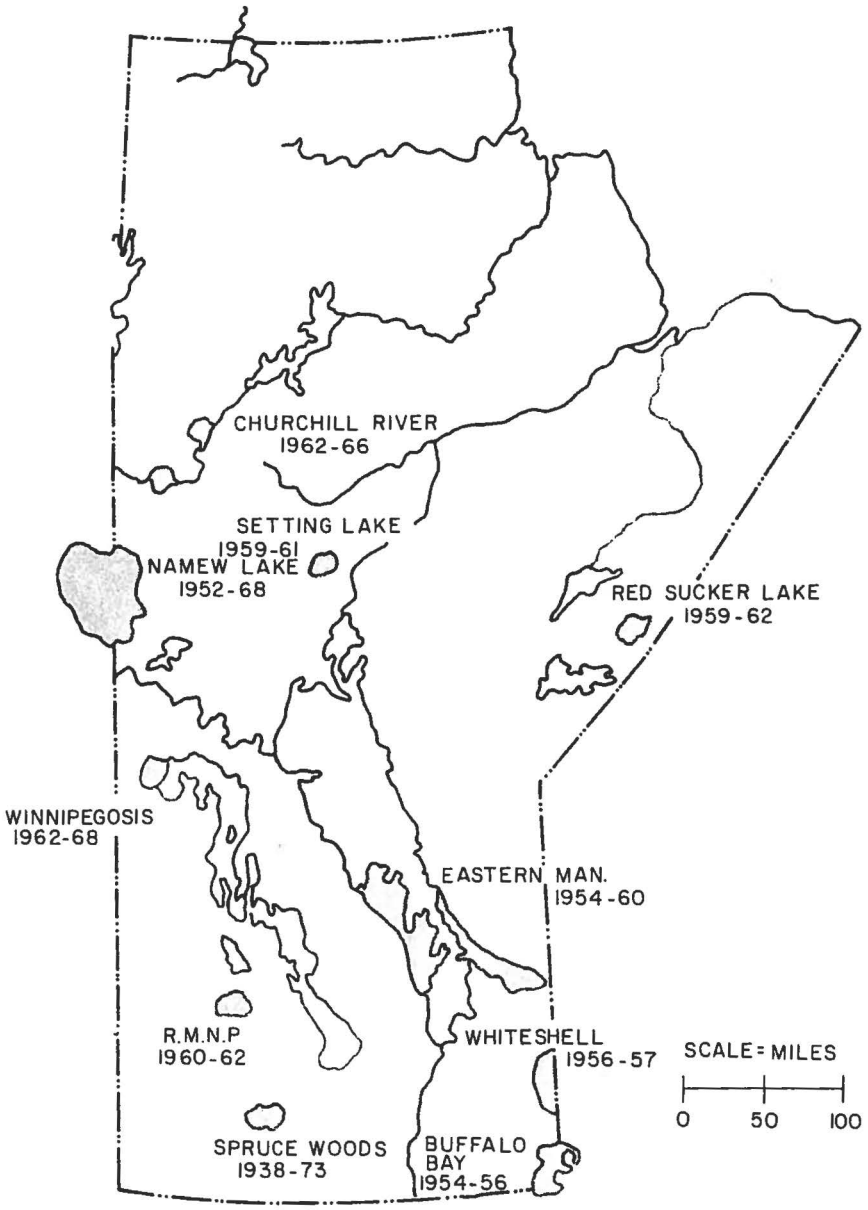

Figure 1. Spruce budworm outbreaks and their duration in Manitoba, 1938-1973.

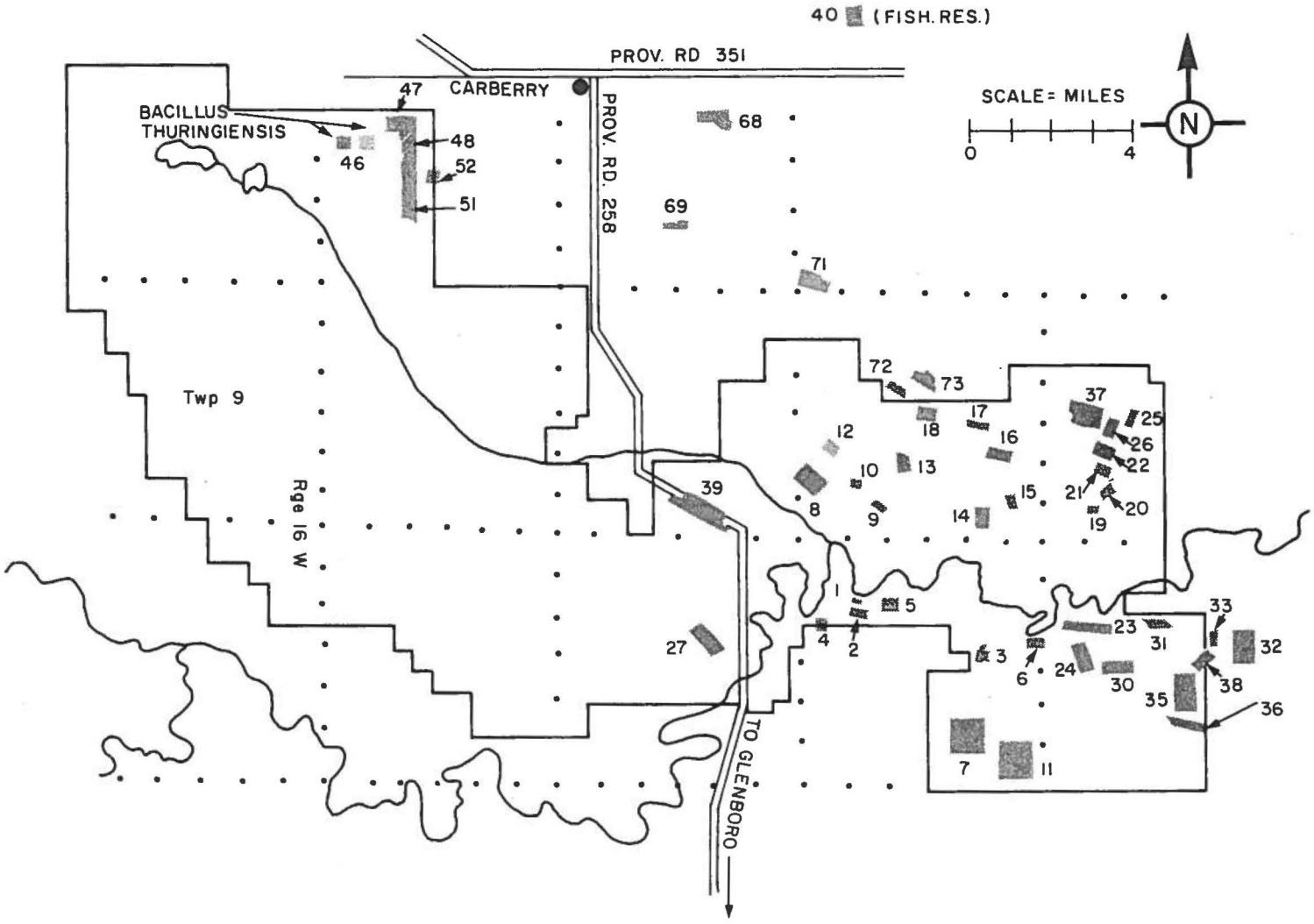

Figure 2. Areas of the Spruce Woods Provincial Forest and Park sprayed with fenitrothion and carbaryl for spruce budworm control in 1973.

serious tree injury has been negligible. Current infestations, encompassing about 115 sq miles, developed in 1967 (Ives *et al* 1967), and since 1970 have caused rapid deterioration of nearly mature and mature white spruce stands and almost complete destruction of regeneration in several localized areas.

Spray Program and Application Methods

Because the Spruce Woods Provincial Forest and Park are prime recreation areas, in 1973 the Manitoba Department of Tourism, Recreation and Cultural Affairs undertook an aerial spray program to control the budworm in order to maintain the aesthetic values of the area. The spray program was approved and licensed through an order of the Clean Environment Commission under the Clean Environment Act of Manitoba. The Canadian Forestry Service advised on spray techniques and determined the effectiveness of the spray operation. Other agencies of Environment Canada and the University of Manitoba were involved in environmental impact studies, especially as they applied to fish, birds and wildlife of the area.

Approximately 8,300 acres of white spruce forest were sprayed during the budworm control program. This consisted of 45 individual areas, ranging in size from 28 to 654 acres, selected on the basis of previous budworm injury and their importance to future park development (Fig. 2). Spraying was carried out from June 7 to June 11 and limited to early morning (0530-0930 h) and late afternoon periods (1700-2130 h) when winds were less than 8 mph and humidity was highest, to minimize spray drift and reduce evaporation of spray droplets. Originally applications of the insecticides were planned to commence June 4 to coincide with the period when the majority of the budworm larvae were in the 4th instar. However, due to a sustained period of high winds and rain the spray operation was delayed 3-6 days; only about 20 h of suitable spraying weather occurred over a two-week period.

Fenitrothion (0,0-dimethyl 0-(4-nitro-m-tolyl) phosphorothiote) was applied to approximately 8,000 acres in an oil-in-water emulsion spray at the rate of 4 oz active ingredient in 1 U.S. gal of water per acre. The fenitrothion was formulated prior to application by weight as follows: fenitrothion (97.1%) = 77.5%, Atlox 3409 emulsifier = 11.6%, and Texaco aerotex 3470 oil = 10.9%. Carbaryl (1-naphthyl methylcarbamate) in the commercial formulation Sevin-4-oil, and diluted with No. 2 fuel oil, was applied to 125 acres at the rate of 16 oz active ingredient in 40 U.S. oz total solution per acre. An additional 200 acres of infested forest were treated with a microbial insecticide "Dipel," containing *Bacillus thuringiensis* var. *alesti*, at the rate of 1/2 lb wettable powder equivalent to 4 billion International Units of potency per acre; results have been reported by DeBoo and Campbell (1974).

Spraying was carried out with two Cessna Ag-Wagon aircraft equipped with Micronair 2000 and 3000 spray systems, and one Piper Pawnee 235B aircraft equipped with conventional boom and nozzle (DeBoo and Hildahl 1967). The aircraft each covered an effective swath width of 50 ft flying at a height of 8-15 ft above the treetops, and were guided upwind along successive flight paths by flagmen with white markers or colored helium-filled weather balloons on aluminum poles. Oil- and water-sensitive Kromekote cards on aluminum stands were placed in openings and near trees along a transect at right angles to the swath direction to determine spray deposit pattern (7-10 sampling stations per block).

Control Assessment Methods

Control was assessed using the standard branch-sampling procedure (Miller *et al* 1972). The sample unit consisted of two 18-in. branch samples per tree collected from mid-crown regions of sample trees selected at right angles to the spray swaths. Assessments included (1) pre-spray and post-spray larval populations (DeBoo *et al* 1973, Martineau and Benoit 1973), (2) egg-mass density following adult emergence in early July, and (3) foliage protection achieved by the application of the different insecticides as measured by percent defoliation.

Larval density counts commenced 6 days before spraying to determine pre-spray population levels and natural mortality trends. The control achieved was based on the

number of surviving larvae on twenty 18-in. branch samples taken from each treated block and compared with a similar number of samples from untreated stands 5 days after spray application. Egg-mass density was determined by sampling foliage after egg deposition was complete (late July and early August). Foliage protection was assessed using two 18-in. branches from 10 trees in each of the treated and adjacent untreated areas in early November. Each individual current shoot on the sample branches was assessed on the basis of foliage consumed.

RESULTS AND DISCUSSION

The effectiveness of the spray program was based on assessments made in seven representative treated blocks ranging from 75 to 443 acres, and compared with adjacent untreated stands.

At the rates applied, both chemicals reduced spruce budworm larval population levels, but the effectiveness varied with date of spraying, type of treatment, and aerial application equipment (Table 1). Population reductions of 86% were achieved in the block treated with carbaryl and from 70% to 85% in three blocks treated with fenitrothion using boom and nozzle spray equipment. Fenitrothion was less effective when emitted from Micronair equipment, reducing larval incidence by only 29% and 51% respectively in two treated blocks. However, the same chemical plus "Target-E" (a molasses-base adjuvant added at the rate of 2 oz per U.S. gal of insecticide mixture) gave 86% population reduction in one plot. The relatively poor results obtained with fenitrothion without the adjuvant may be associated with increased drift and evaporation due to the smaller droplets produced with atomizing equipment (approximately 100μ compared with 150-200μ produced by boom and nozzle). The number of droplets reaching spray deposit cards in the blocks sprayed with boom and nozzle equipment was fairly uniform, ranging from 12 to 15 droplets per cm^2, but was variable with Micronair equipment without an adjuvant in the spray formulation (13-26 droplets per cm^2). With "Target-E" added the number of droplets increased to 44 per cm^2. These results indicated that the heavier droplets produced by the adjuvant increased the numbers reaching the target area.

Because the spray program was delayed several days due to adverse weather, foliage protection afforded by the treatments was less than desirable in some areas (Table 1). In the blocks where the sprays were applied on June 7, when the majority of the larvae were in the 4th and early 5th instars, feeding injury was only 15% or less. On the other hand, areas sprayed on June 10 and 11 showed little reduction in defoliation compared to adjacent unsprayed stands. To make an accurate comparison of foliage injury between sprayed and unsprayed stands, however, was difficult because the areas supporting the highest budworm populations were invariably selected for treatment.

Investigations in late July and early August showed wide variation in egg populations in treated and untreated stands, ranging from 26 to 379 egg-masses per 100 ft^2 of foliage in the former and from 79 to over 2,000 per 100 ft^2 in the latter (Table 2). Substantially fewer egg-masses were recorded in the blocks where the spray treatments were most effective (greatest larval population reduction and foliage protection). In three treated areas, egg-mass density was greater than in adjacent check plots, probably reflecting the movement of moths from untreated areas as well as the ineffectiveness of some of the treatments. These egg-mass densities were considered high enough (over 300 per 100 ft^2 of foliage) to cause noticeable defoliation in 1974.

CONCLUSIONS

The aerial spray program in 1973 showed that either fenitrothion or carbaryl sprays can reduce budworm populations to tolerable levels in scattered white spruce stands such as are found in the Spruce Woods Forest and Park areas of southwestern Manitoba. Boom and nozzle distributing apparatus, delivering larger droplets, provided more consistent results than the more sophisticated Micronair atomizers. The latter should be ideal for budworm sprays, but the small droplets produced by this type of equipment are extremely prone to

Table 1. Spruce budworm larval population reductions and foliage protection achieved with aerial applications of fenitrothion and carbaryl in the Spruce Woods Provincial Forest and Park in 1973

Block # (Fig. 2)	Spray Date	Spray Equipment	Treatment	Avg. no. larvae per 18" branch [a]		% Population [b] reduction	Avg. % defoliation [c]	
				Treated	Untreated		Treated	Untreated
5	6-7	Boom & nozzle (#8003)	Carbaryl	4.0	27.5	86.3	15	25
40	6-7	Boom & nozzle (#8004)	Fenitrothion	6.5	27.0	75.9	14	78
8	6-10	Boom & nozzle (#8004)	Fenitrothion	4.0	27.0	85.2	32	47
13	6-11	Boom & nozzle (#8004)	Fenitrothion	8.0	27.0	70.4	61	45
20	6-10	Micronair (2000 & 3000)	Fenitrothion	9.6	19.5	50.7	38	41
37	6-10	Micronair (2000 & 3000)	Fenitrothion	13.9	19.5	28.7	36	45
35	6-11	Micronair (2000 & 3000)	Fenitrothion + "Target-E"	2.5	18.0	86.1	31	24

[a] Based on larval counts taken 6 days before and 5 days after spray application.

[b] Pre-spray larval counts averaged 45 larvae per 18-in. branch sample — population reduction curves corrected using Abbott's formula (Abbott 1925).

[c] Average % defoliation based on detailed examination of new shoots.

Table 2. Spruce budworm egg populations in treated and untreated stands following spray applications in the Spruce Woods Provincial Forest and Park in 1973

Block # (Fig. 2)	Treatment	Spray Equipment	Avg. no. egg-masses per 100 ft^2 foliage [a]	
			Treated	Untreated
5	Carbaryl	Boom & nozzle	26 [b]	1499
40	Fenitrothion	Boom & nozzle	117 [b]	2072
8	Fenitrothion	Boom & nozzle	173 [b]	259
13	Fenitrothion	Boom & nozzle	370 [b]	609
20	Fenitrothion	Micronair	372 [c]	93
37	Fenitrothion	Micronair	379 [c]	169
35	Fenitrothion + "Target-E"	Micronair	92 [c]	79

[a] Based on counts taken from ten 18-in. branch samples per treated and untreated area.

[b] Significantly different from untreated check at 1% confidence level — statistical analysis by t test per treatment.

[c] More eggs in treated than untreated area.

drift and evaporation, thus requiring much more exacting weather conditions during application. Even at wind speeds of less than 5 mph, it may be difficult to achieve satisfactory deposition on the target area in regions like Manitoba with low humidities.

Sprays of fenitrothion at 4 oz active ingredient per acre reduced spruce budworm numbers to the extent of 85-86%, and afforded good foliage protection when the applications were made during the 4th and early 5th larval instars. Similarly, sprays of carbaryl at 16 oz active ingredient per acre in Sevin-4-oil commercial formulation gave 86% larval population reduction applied under the same conditions. These results indicate that carbaryl may be an acceptable insecticide for budworm control by aerial application because of its lower evaporation rate, but further tests would be desirable to determine the minimum dosage required to provide satisfactory foliage protection.

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FIELD AND LABORATORY STUDIES OF THE EFFECT OF EXPOSURE TO FENITROTHION ON FRESHWATER AQUATIC INVERTEBRATES

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ABSTRACT. Drift and Ekman grab samples were taken to determine the effect upon stream invertebrates of an aerial application of fenitrothion at 4 oz a.i./acre to a spruce forest. Laboratory studies were carried out in conjunction with the field program to investigate the lethal and delayed lethal effects of this chemical. In Pine Creek, Manitoba, the site of the experimental treatment, a large increase in drift of the most common animals was recorded immediately following the spray, both at a station within the spray area and at a downstream station. No related decreases in standing crop were recorded possibly due to recolonisation from upstream or from eggs, or because the chemical apparently never deeply penetrated the sediments. Laboratory tests indicate that there is both an immediate and a delayed lethality confirming previous observations by other authors. It is concluded that spraying of a whole prairie stream system could well have disastrous effects on the invertebrate population.

INTRODUCTION

Since 1968, fenitrothion, an organophosphate insecticide, has replaced D.D.T. in forest insect control operations in Eastern Canada (Elson *et al* 1972). Field studies of the effects of these control operations on non-target aquatic animals have generally concluded that the concentrations used (usually 2 or 3 ozs active ingredient/acre and one or two applications/year) produce no acute toxic effect on fish. An exception is a report by Hatfield (1969) of salmon fry mortalities following two, 2 oz/acre spray applications in Newfoundland. Effects of fenitrothion on aquatic invertebrates are variable: Penney (1971) was not able to show an effect on the biomass of aquatic insects of an application of 3 oz active ingredient (a.i.)/acre followed by application of 2 oz a.i./acre in one small stream, but in another study involving two sprays of 2 oz a.i./acre he was able to show a 27% decrease in biomass. Laboratory studies by Wildish and Phillips (1972) indicate that all aquatic insects with the exception of Diptera could be killed following an operational spray of 2 oz a.i./acre; their 24 hr LC50's for fenitrothion (mg/l) vary from 0.002 with *Acroneuria* sp. (Plecoptera) to 40 for *Eriocera spinosa* (Diptera). Tadano (1970), however, presented evidence that not all Diptera were as resistant as *Eriocera*. In studies of the genetics of cross resistance using several strains of the mosquito larva *Culex pipiens pallens* he indicates LC50 levels of from about 0.006 mg/l to about 0.17 mg/l fenitrothion.

The present study, which includes a field investigation of the effects of a spruce budworm control operation at 4 oz a.i./acre on the morning of June 7, 1973 (see Hildahl and DeBoo 1973) on the drift and biomass of aquatic invertebrates in a small stream in West central Manitoba, and subsequent laboratory studies on lethality and delayed lethality of fenitrothion, was carried out in conjunction with several other impact studies (Hildahl and DeBoo, 1973, Leonhard a, b, in preparation; Findlay *et al* 1974; Lockhart *et al* 1973). These studies were carried out, under license of the Manitoba Clean Environment Commission, in two areas: several blocks of the Spruce Woods Provincial Forest, Manitoba, and several small plots a few miles north of this Forest (see Hildahl and DeBoo 1973, Fig. 1). Pine Creek, a small spring-fed stream, about 12 - 18" deep and 7 - 8 feet wide, runs through a small (125 acre) plot in the latter area (Fig. 1), and was the stream on which both the fish and aquatic invertebrate studies were carried out.

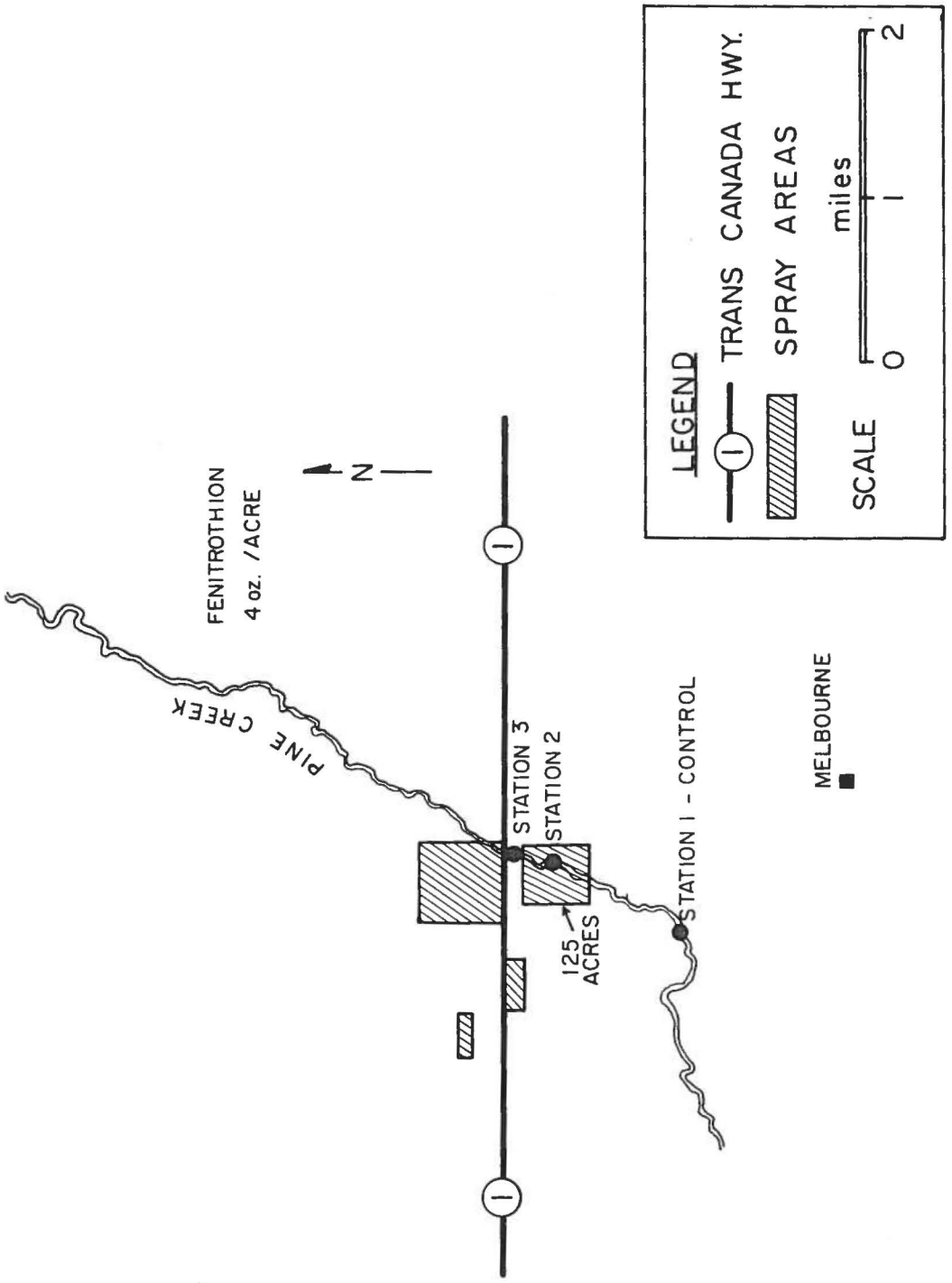

Fig. 1. Map of experimental area.

METHODS

Three stations were established: Station 1 about 1 mile upstream from the test spray area; Station 2 in the middle of the area; Station 3 about 1/4 mile downstream from the spray area and about 100 yds south of the Trans-Canada Highway (see Fig. 1). All three of these stations were similar in width, depth, water flow speed and the substrate which consisted of a relatively coarse, rather unstable, sandy material. The invertebrate population was essentially limited to chironomids and oligochaetes presumably because of the substrate type. A few mayfly, beetle, and caddisfly larvae were associated with submerged tree roots, etc. in the stream.

Temperature, pH, dissolved oxygen and fenitrothion (both substrate and water column) samples were taken every two hours starting 24 hours before the spray and continuing for 48 hours after the spray at each station. Four, 1 ft^2, 200 micron mesh drift nets, set to sample 0.5 ft^2 (including the water/air interface) of the water column were positioned at each station and emptied every two hours. Three sets of grabs (improved Ekman; Burton and Flannagan 1973) were taken from the substrate at each station one day before, and on the third day after the spray. Each grab sample was sieved through a 600 micron mesh sieve and then sugar floated (Flannagan 1973); all samples were sorted using a dissecting microscope (x20).

The laboratory lethality tests were carried out using temperature controlled (20°C) dechlorinated Winnipeg tap water employing a Mount and Brungs (1967) proportional dilutor to deliver the fenitrothion concentrations. In these tests the animals were exposed to the various concentrations of fenitrothion for 24 hours, removed from the test chambers and observed in their normal laboratory habitat for a further 72 hours. The fenitrothion samples, water and substrate were analyzed by Mr. B. Grift (Grift and Lockhart *in press*) of the Freshwater Institute. The mosquito larvae used in the lethality tests were kindly provided by Dr. R. Brust of the Entomology Department, University of Manitoba. The remaining animals were taken from cultures held at the Freshwater Institute.

RESULTS

Temperatures in the stream fluctuated diurnally from about 16°C in the late afternoon to about 11 or 12°C in the early morning. These temperatures are about 4 - 5°C lower than is typical for streams in Manitoba, at this time of the year, presumably due to the fact that the stream is mainly spring-fed and has exceptionally heavy tree cover along most of its length. Water flow was consistent during the experimental periods at around 10 cu. ft sec. Dissolved oxygen levels remained relatively constant at or slightly above saturation levels and pH was consistent also at pH 6.6 for stations 1 and 2 and pH 6.5 at station 3.

Fenitrothion analyses of the stream samples indicated that the chemical was not at anytime detectable in the stream substrate. However, it was detected in small quantities in the water column at station 1 (0.52 µg/l) two days after the spray and in relatively large quantities in the water at stations 2 and 3 (Fig. 2). It is believed that the peak concentration was missed at station 2 since the station was not sampled until all of the spray appeared to have settled (for reasons of safety). However, station 3 probably indicates a reasonably accurate picture of actual concentrations in the stream. A heavy rainfall on the afternoon of June 7 probably caused the second peak shown on Fig. 2. It is obvious from these results that the fenitrothion was very quickly washed downstream and that very little of it remains in the stream twenty-four hours after the application. The small amount of fenitrothion detected at station 1 was presumably due to aerial drift of small particles from the spray.

The drift samples at station 1 (control station) and the pretreatment samples at the other two stations, indicated that the normal animal drift in this stream, as one would expect, is largely composed of chironomidae (Fig. 3). The normal drift pattern of Chironomidae and terrestrial invertebrates appears to be largely diurnal and composed of small numbers of animals. However, on the night of the 7-8th the pattern appeared to be upset since the normal early morning decrease in chironomid drift did not occur. This is

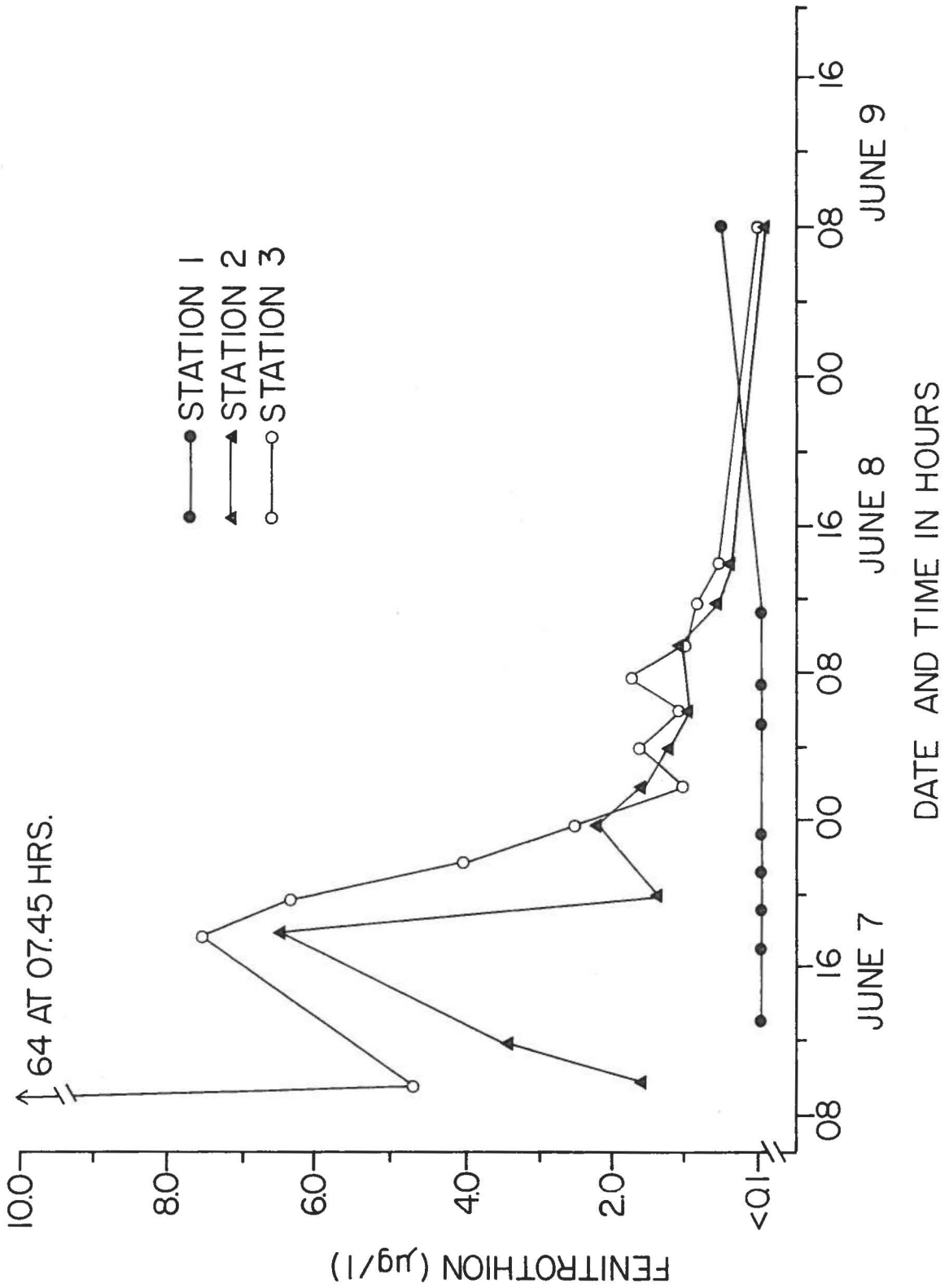

Fig. 2. Fenitrothion concentrations (mg/l) in the stream water at the various stations in the three days following the treatment.

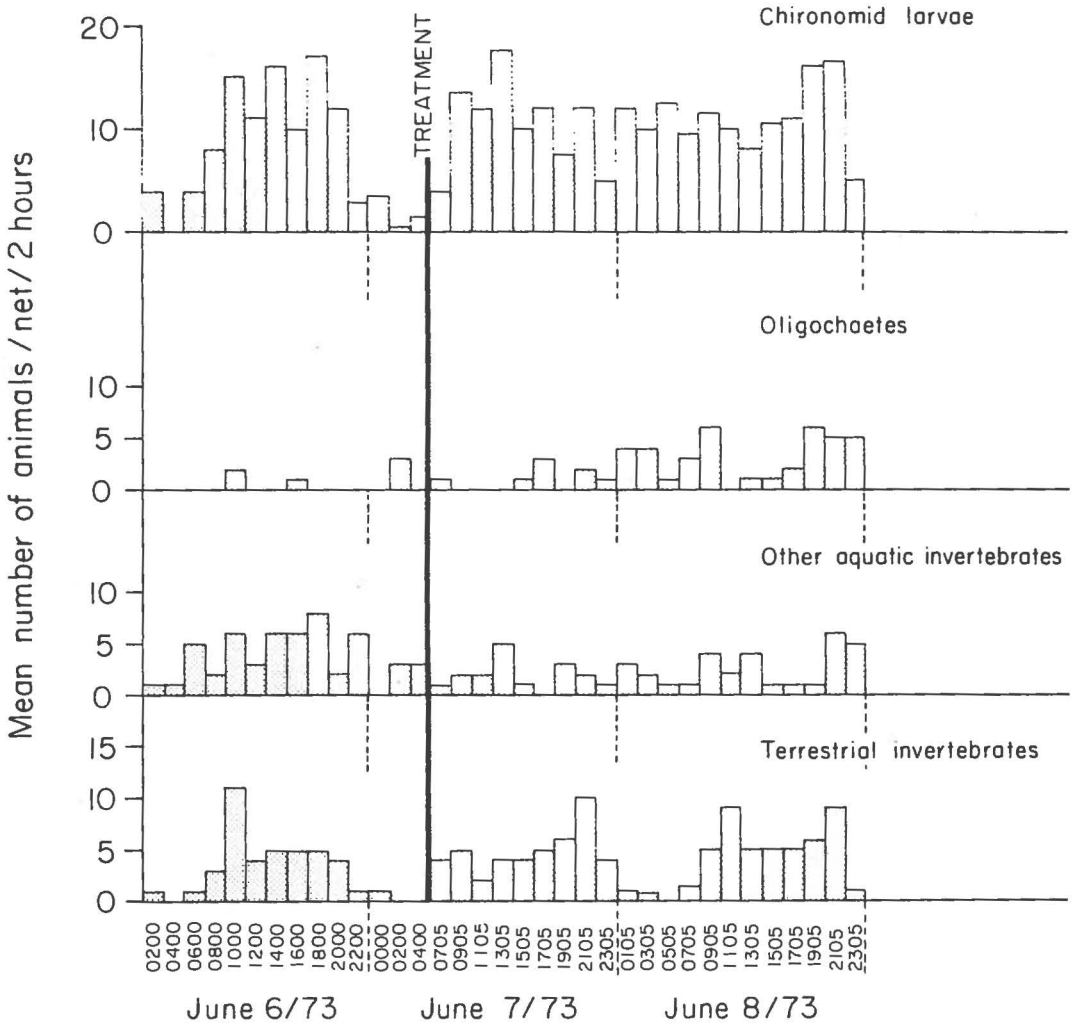

Fig. 3. Histograms of the drift of the various invertebrate groups collected at station 1 (control) for the period starting one day before and continuing for two days after the spray.

probably related to the appearance of small quantities of fenitrothion in the water at this time. No change is apparent in the pattern of the other groups of invertebrates studied and only small change in total numbers of animals/day was recorded. Station 2 samples (Fig. 4) indicate that the chironomid drift pattern was upset and increases of 700 - 800% in total numbers/net for a 24 hour period following the fenitrothion application were recorded. A large increase in the number of other aquatic invertebrates (mainly mayfly and Coleoptera larvae) and of drifting terrestrial invertebrates is also evident. A similar, more pronounced and longer lasting effect, was evident at station 3 (Fig. 5) which was located outside, although downstream from the spray area.

t-tests for difference between means of the pre- and post-treatment standing crops of animals as evidenced by the Ekman grab samples (Table 1), indicate that there was a significant ($P < 0.05$) increase in mean numbers of chironomids at station 1 and station 3 and a significant decrease in oligochaetes at station 1 only. The increase in numbers of chironomids may be due to increased colonisation from the drift of animals from less seriously affected areas upstream or to recruitment from newly hatched eggs. It is considered that the decrease in oligochaetes at the control station is unlikely to be related to the fenitrothion spray as it is probably part of the normal annual cycle.

Table 1. **Mean numbers, standard deviation, and t-values (df+5) for the oligochaetes and chironomids collected in the Ekman grab samples at the three stations before and after the fenitrothion spray.**

	Pretreatment Mean No/grab± S.D.	Post Treatment Mean No.grab± S.D.	t-value Pre vs Post Treatment
Station 1			
Oligochaeta	42 ± 5.66	22.33 ± 8.14	2.9079*
Chironomidae	5.5 ± 2.12	14 ± 5.57	2.2104*
Station 2			
Oligochaeta	3 ± 3.61	2.67 ± 1.15	0.1525
Chironomidae	10.33 ± 7.51	25 ± 20.30	0.1739
Station 3			
Oligochaeta	18 ± 6.56	12.33 ± 16.20	0.5617
Chironomidae	2.33 ± 0.58	7.0 ± 3.61	2.2138*

* Difference significant at $P < 0.05$ (single tailed tests)

The laboratory studies clearly show that exposure to levels of fenitrothion below those found in the stream are immediately toxic to many aquatic invertebrates and that in a twenty-four hour period these animals can receive a dose which will produce death later (Fig. 6).

DISCUSSION

The aerial spraying of fenitrothion had a marked effect on the drift of both aquatic and terrestrial animals in the stream and while it is reasonably certain that most of the terrestrial animals were dead or moribund, the same cannot be directly assumed in the case of the aquatic ones, especially since the standing crop estimates did not show a decrease at the two experimental stations. However, it can be generally assumed that where a very large number of live animals are displaced from their niche, the displaced animals are "ecologically dead", i.e. any similar ecological niche downstream is already occupied. Also

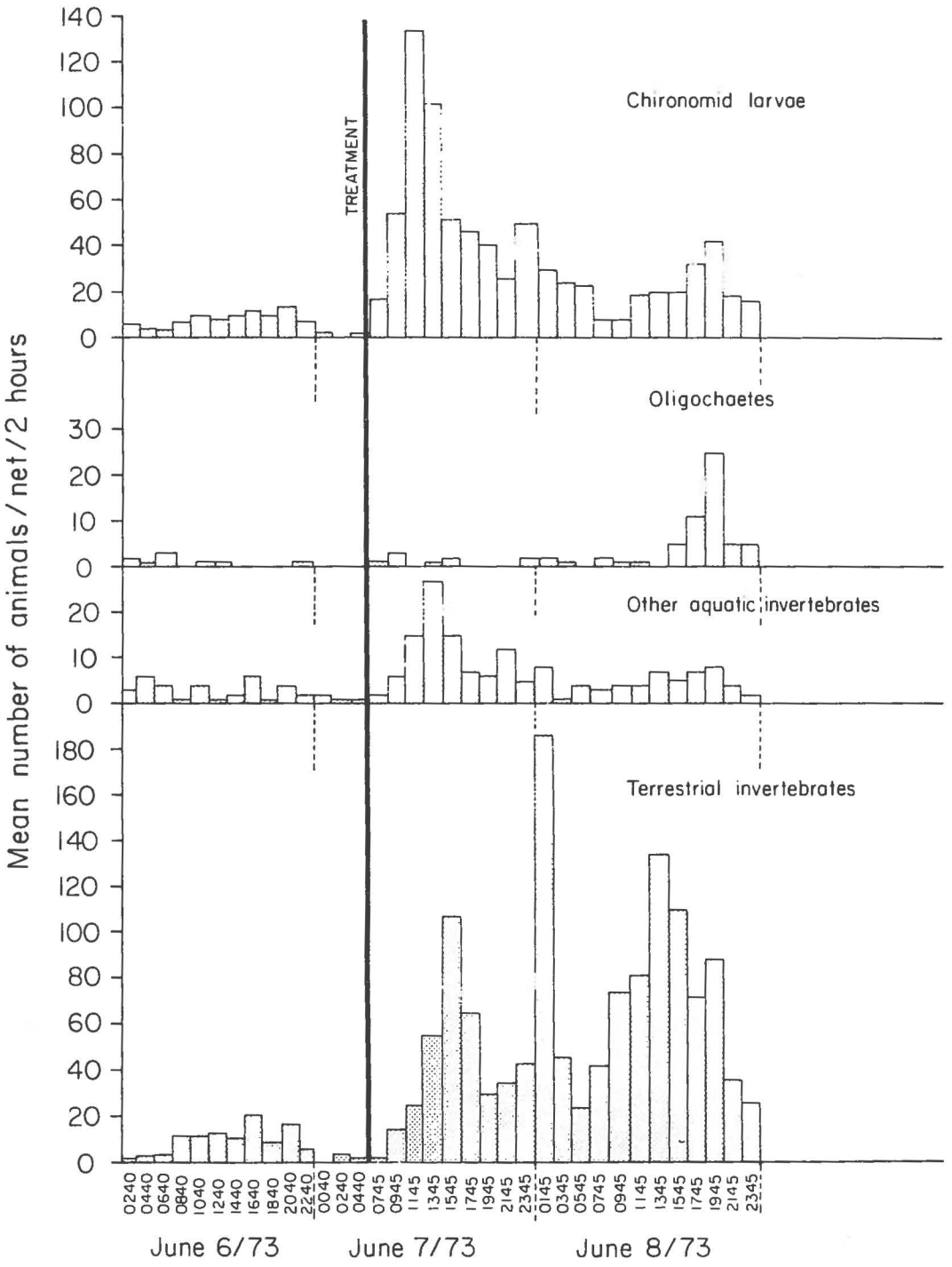

Fig. 4. Histograms of the drift of the various invertebrate groups collected at station 2 for the period starting one day before and continuing for two days after the spray.

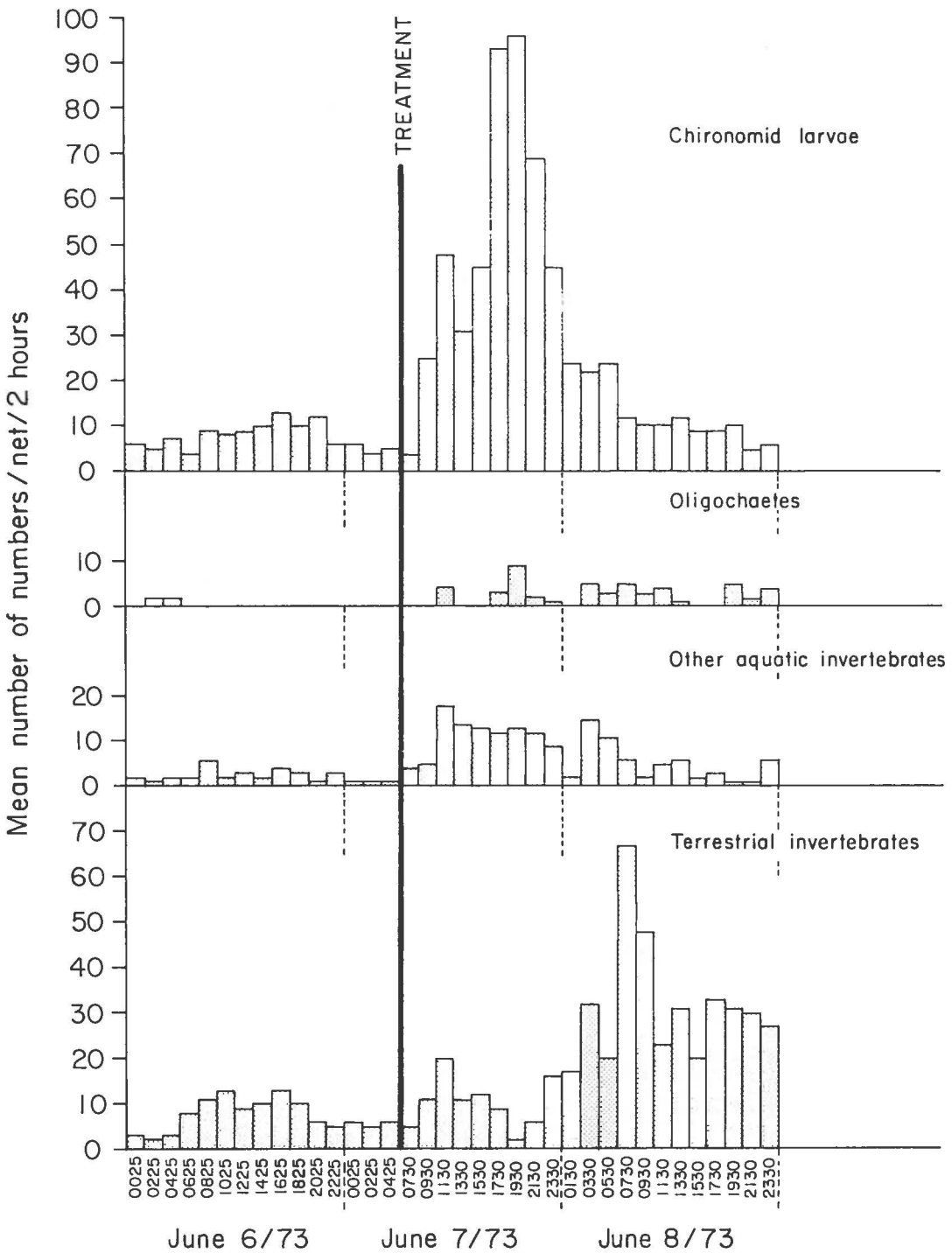

Fig. 5. Histograms of the drift of the various invertebrate groups collected at station 3 for the period starting one day before and continuing for two days after the spray.

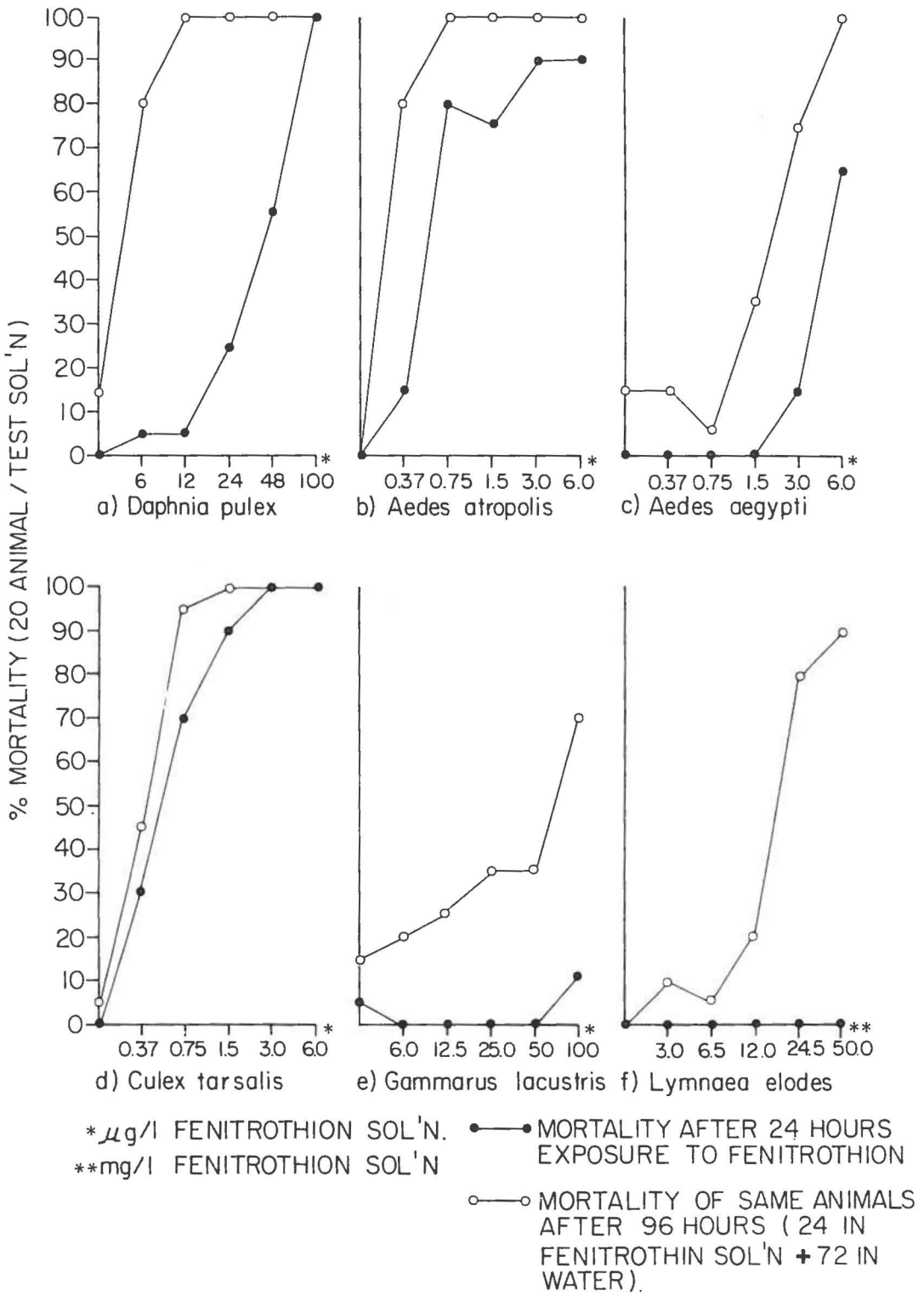

Fig. 6. Curves indicating 24 hour and 96 hour cumulative mortality of six aquatic invertebrates species exposed to various concentrations of fenitrothion for 24 hrs then returned to clean water conditions.

in this particular study only about 1/4 mile of the stream was directly under the spray leaving many miles of stream above the affected area from which recolonisation could take place. The "aerial drift" fenitrothion detected at station 1 is unlikely to be of sufficient concentration to kill animals, however it appeared to upset the normal animal drift pattern indicating that it also occurred further upstream. The increase in animal drift from these areas, recorded at station 1, though small, will undoubtedly assist in the recolonisation process. This drift plus new recruitment from hatching eggs etc. probably masked the effects of the loss of chironomids from the system and may therefore account for our failure to record decreases in standing crop of aquatic invertebrates after the treatment. However, since fenitrothion was never recorded in the substrate the possibility that animals buried in the substrate would not be directly exposed to fenitrothion and thus would not be killed must remain.

The laboratory studies indicate both an immediate mortality and a delayed mortality after 24 hours exposure to various concentrations of fenitrothion solution. A similar delayed mortality after only one hour exposure to 0.066 mg/l fenitrothion has been recorded by Wildish and Phillips (1972) for one species of stonefly. Rorke, Gardner and Greenhalgh (1974), using lethality and two different behavioural symptoms of the snail *Helix aspersa* in response to exposures to compounds which are known to have an anticholinesterase effect (including fenitrothion) and compounds which did not inhibit cholinesterase activity, suggested that only part of the toxic effect of fenitrothion is associated with cholinesterase inhibition, the remainder being due to some other cause. It therefore seems possible that the immediate and delayed mortality recorded in the laboratory experiments are related to these two causes. Other factors, such as changes in the rate of absorption or relocation within the animals tissues and changes in the excretion rate of fenitrothion, associated with the change when the animals are moved from the fenitrothion to the clean water situation, could also be involved in this delayed lethality problem.

The laboratory tests while carried out on species of invertebrates not present or not numerically important in the experiment stream and at temperatures higher than those recorded in the stream, do still serve to indicate that fenitrothion is extremely toxic to a wide range of aquatic invertebrates at low concentrations and that only short periods of exposure are needed to bring about eventual mortality.

CONCLUSIONS

It is concluded that the extremely large drift recorded represents a considerable loss to the stream system and that had the whole stream been sprayed, the results could have been very serious. Further research is necessary, to establish the long-term effects of such spray operation on the total stream fauna, and to assess the extent of downstream damage. The "delayed" lethality problem and its possible effects on living drift and standing crop of invertebrates also requires further investigation.

ACKNOWLEDGEMENTS

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BIOCHEMICAL AND RESIDUE STUDIES ON RAINBOW TROUT *(SALMO GAIRDNERI)* FOLLOWING FIELD AND LABORATORY EXPOSURES TO FENITROTHION

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ABSTRACT. Fenitrothion residues and several biochemical parameters were determined in yearling rainbow trout caged in a stream during aerial application of fenitrothion (0,0-dimethyl-0-(4-nitro-m-tolyl) phosphorothioate) at four ounces per acre. Samples of fish were taken one and four days after spraying and whole body residue concentrations averaged 0.44 and 0.63 µg/g on the first day after treatment; however, by the fourth day only 3 of 22 fish still contained measurable amounts. Biochemical measurements on treated fish were compared with other fish held upstream from the spray plot and no treatment effects were detected at a probability level of 0.05. Laboratory studies confirmed a lack of effect on serum chemistry and showed that the pesticide was distributed to internal organs.

INTRODUCTION

Fenitrothion (0,0-dimethyl-0-(4-nitro-m-tolyl) phosphorothioate) has been used widely in eastern Canada for control of spruce budworm *(Choristoneura fumiferana* Clem.) but not in prairie regions. Early in 1973 fenitrothion was selected to control a severe local outbreak of spruce budworm in a parkland region of south western Manitoba. Several small plots totalling 3,400 hectares were treated by aircraft at 280 g/ha (4 oz/acre) on June 7, 1973. Since spray plots included sections of two creeks and the Assiniboine River, there was concern for the safety of fish and other aquatic organisms, and legal authority permitting use of fenitrothion required that field research accompany treatment in order to assess possible effects on non-target organisms.

Previous field studies (Hatfield and Riche 1970) indicated that little if any fish mortality might be expected; however, recent reports of fish kills (Coté and Tétreault 1973; Kingsbury 1973) have forced re-examination of this pesticide for non-target effects. Experimental exposures of salmonids to fenitrothion have demonstrated a variety of sub-lethal effects including depressed learning ability (Hatfield and Johansen 1972), increased susceptibility to predation (Hatfield and Anderson 1972), and reduced brain acetylcholinersterase activity (Zitko, Carson and Finlayson 1970); however, these effects have not appeared at the low treatment levels typical of field use. Brain acetylcholinesterase activity was selected as the criterion of poisoning most readily applied to our situation. In addition we analyzed serum for a number of biochemical parameters since this procedure has been successful in detecting experimental poisoning in fish (Eisler 1967; Holmberg *et al* 1972).

MATERIALS AND METHODS

Yearling rainbow trout (20 - 60g) of a single genetic stock were placed in cages in Pine Creek near Carberry, Manitoba, on May 23, 1973. The cages, approximately 1.2m x 1.5m made of 2.5cm wire mesh lined with 0.63cm nylon mesh extending to the stream bottom, were located in areas of slow water in a 50ha spray plot and at a second site about 1 mile upstream from the nearest point of treatment. There were no other spray plots between the one under study and springs forming the source of Pine Creek about 16km upstream. The plot was treated by aircraft (Hildahl and DeBoo 1973) in a flight pattern generally parallel

to the stream and no attempt was made to avoid spraying over the water. Spraying occurred from 0600 to 0630 h on June 7, 1973 and fish were taken from cages on June 5, 8 and 11. Immediately upon removal from the water, blood was drawn from caudal vessels (Steucke and Schoettger 1967) of each fish and brains were removed. The brains and carcasses were frozen immediately on dry ice in individual plastic bags. Blood was allowed to clot on ice and serum was removed and frozen until analyses were begun one week from the dates of collection. Water samples were frozen in brown glass bottles. Wild fish obtained from minnow traps were either frozen for fenitrothion residue analyses or preserved in formalin.

Analyses of sera were carried out with only minor modifications to standard techniques of microanalyses (Mattenheimer 1970) generally using prepared reagents from Boehringer Mannheim Corporation. Serum cholinesterase was determined by the procedure of Ellman *et al* 1961. All serum enzymes were measured from reaction rates at 25°C recorded with a Zeiss PMQ-II spectrophotometer equipped with a 6-cuvette automatic sample changer and a multipoint recorder. Reproducibilities of serum analyses are expressed below as relative standard deviations (standard deviations of the mean of a series of replicates — usually 10 — expressed as a percentage of the mean).

Analysis	Method	Relative Standard deviation (%)
Inorganic phosphate	Molybdate	1.3
Protein	Biuret	2.0
Sodium	Flame emission	2.3
Chloride	Microtitration	1.8
GOT[a]	Rate of oxidation of NADH[b]	6.1
LDH[c]	Rate of oxidation of NADH	2.8
Alkaline phosphatase	Rate of hydrolysis of p-nitrophenyl phosphate	3.8
Cholinesterase	Rate of hydrolysis of acetylthiocholine	2.3
Glucose	Glucose oxidase	0.8

Brains were analyzed for cholinesterase activity at 15°C by a manometric procedure (Knowles and Casida 1966; Hogan and Knowles 1968) using a Warburg apparatus with acetylcholine chloride as substrate. Protein in brain homogenates was determined with a modification of the Folin-Ciocalteau procedure (Miller 1959). Relative standard deviations of replicates of the two brain measurements were 2.0% and 5.0% respectively.

Fenitrothion residues in water and fish were measured by the gas-liquid chromatographic procedure of Grift and Lockhart (1974).

Laboratory exposures of rainbow trout were carried out during the winter of 1973-74 in a temporary building covered with translucent plastic. Rainbow trout (230 to 460g) were maintained in circular polyethylene-lined pools 3m in diameter kept at a depth of 61 cm with continuously flowing water. No attempt was made to control photoperiod, water temperature (6-9°C) or water chemistry. An experimental exposure of 46 µg/l corresponding to a surface spray of 280g/ha was carried out by treating a pool with the mixture below:

205 mg fenitrothion (97.1% sumithion)
30.6 mg Atlox 3409 emulsifier
28.8 mg Texaco Aerotex solvent
Sufficient water to make 6.8 ml

[a] Glutamic oxalacetic transaminase (EC No. 2.6.1.1)

[b] Nicotinamide-adenine dinucleotide (reduced)

[c] Lactate dehydrogenase (EC No. 1.1.1.27)

Table 1. Brain acetylcholinesterase activity (micromoles acetylcholine hydrolysed per mg brain protein per hour at 15°C) in rainbow trout before and after aerial application of fenitrothion at 4 oz/acre. June 1973.

Fish Group		Brain acetylcholinesterase Activity		
		Two Days Before Spray	One Day After Spray	Four Days After Spray
Sprayed	$\bar{X}$	6.97	6.84	6.86
	S.D.	0.71	0.84	0.52
	n	20	20	12
	range	5.54—8.42	5.37—8.94	6.18—7.86
Unsprayed	$\bar{X}$	6.50	6.64	6.87
	S.D.	1.12	1.19	0.81
	n	20	20	12
	range	4.89—8.69	4.08—8.91	5.69—8.52

Table 2. Serum chemistry of rainbow trout in Pine Creek, Manitoba, June 1973. Treated fish were held in an area sprayed with Fenitrothion at 4 ounces per acre and untreated fish were held 1 mile upstream from the nearest application. Tabulated statistics were calculated using a transformation to natural logarithms and the means and standard errors are presented in that form except that means (geometric) were also antilogged and presented in parentheses in original concentration units as indicated.

		Untreated Two Days Before	Treated Two Days Before	Untreated One Day After	Treated One Day After	Untreated Four Days After	Treated Four Days After
Sodium	$\bar{X}$(1n)±S.E. (1n)	5.783±0.022	5.763±0.018	5.605±0.042	5.684±0.040	5.778±0.016	5.769±0.016
	$\bar{X}$(mg/dl)	(325)	(318)	(272)	(294)	(323)	(320)
	range	250-380	275-360	190-365	183-345	286-351	191-351
	n	20	20	20	20	12	12
Chloride	$\bar{X}$(1n)±S.E. (1n)	5.927±0.021	5.938±0.020	5.976±0.033	6.023±0.039	6.190±0.005	6.155±0.004
	$\bar{X}$(mg/dl)	(375)	(379)	(394)	(413)	(488)	(471)
	range	312-425	326-425	275-521	246-463	477-506	463-477
	n	20	20	20	20	12	12
Protein	$\bar{X}$(1n)±S.E. (1n)	0.981±0.044	0.931±0.032	0.832±0.044	0.987±0.053	1.101±0.017	1.107±0.024
	$\bar{X}$(g/dl)	(2.67)	(2.54)	(2.30)	(2.68)	(3.01)	(3.03)
	range	1.56-3.92	2.02-3.15	1.46-3.02	1.41-3.89	2.70-3.39	2.68-3.49
	n	20	20	20	20	12	12
Inorganic Phosphate	$\bar{X}$(1n)±S.E. (1n)	2.684±0.040	2.552±0.031	2.524±0.037	2.518±0.042	2.557±0.027	2.594±0.027
	$\bar{X}$(mg P/dl)	(14.6)	(12.8)	(12.5)	(12.4)	(12.9)	(13.4)
	range	10.1-19.1	11.1-16.2	8.15-16.6	7.01-15.2	10.9-15.1	11.4-16.2
	n	20	15	20	20	12	12
Alkaline Phosphatase	$\bar{X}$(1n)+S.E. (1n)	2.853±0.131	2.835±0.134	2.434±0.117	2.265±0.102	2.641±0.112	2.531±0.145
	$\bar{X}$(mU/ml)	(17.3)	(17.0)	(11.4)	(9.63)	(14.0)	(12.6)
	range	6.83-73.6	8.08-78.6	4.68-38.9	2.39-18.4	10.5-31.6	7.13-37.7
	n	20	20	20	20	12	12

(Continued)

Table 2 (continued)

		Untreated Two Days Before	Treated Two Days Before	Untreated One Day After	Treated One Day After	Untreated Four Days After	Treated Four Days After
Acetyl-Cholinesterase	$\bar{X}$(1n)±S.E. (1n)	3.738±0.069	3.642±0.063	3.554±0.077	3.625±0.105	4.103±0.057	4.132±0.079
	$\bar{X}$(mU/ml)	(42.0)	(38.2)	(34.9)	(37.5)	(60.5)	(62.3)
	range	22.4-78.1	23.6-62.8	13.3-56.2	13.3-68.3	44.2-81.5	45.7-92.2
	n	20	20	20	20	12	12
Lactate Dehydrogenase	$\bar{X}$(1n)±S.E. (1n)	7.062±0.106	6.903±0.068	6.771±0.159	7.274±0.101	7.202±0.110	7.367±0.143
	$\bar{X}$(mU/ml)	(1170)	(994)	(872)	(1440)	(1350)	(1580)
	range	659-3500	551-1900	270-4080	741-2880	907-3340	706-3340
	n	20	20	20	20	12	12
Glutamic-Oxalacetic Transaminase	$\bar{X}$(1n)±S.E. (1n)	4.880±0.078	4.785±0.131	4.655±0.074	5.246±0.134	5.194±0.083	5.209±0.072
	$\bar{X}$(mU/ml)	(131)	(120)	(115)	(190)	(180)	(183)
	range	84.9-344	65.1-803	53.8-242	73.5-841	128-316	120-268
	n	20	20	20	20	12	12

This formulation was identical to that used commercially at Pine Creek. A second exposure was carried out using a 100-fold higher treatment level; in both cases control pools were treated with appropriate quantities of solvent and emulsifier.

RESULTS

Fenitrothion was determined in all post-spray trout taken from two cages in the sprayed section of Pine Creek; none was detected in samples prior to treatment. The results for homogenized whole fish carcasses taken on June 8, one day after spraying, are shown below:

		Fenitrothion Content (µg/g wet tissue)	
	Number Analyzed	mean ± SD	range
Cage 1	20	0.44 ± 0.08	0.28 — 0.57
Cage 2	15	0.63 ± 0.46	0.21 — 1.84

A second post-treatment sample obtained June 11, 4 days after spraying revealed that fenitrothion residues had fallen below detection limits (about 0.02 µg/g) in 19 of 22 fish sampled. The remaining three had fenitrothion concentrations of 0.03, 0.12 and 0.15 µg/g.

Brain cholinesterase activities from sprayed and unsprayed trout are shown in Table 1 and serum parameters in Table 2. For statistical treatment the serum data were first transformed to natural logarithms and then analyzed by two-way analysis of variance to detect time and treatment effects. There were no treatment effects at a probability level of $P = 0.05$. However, GOT and LDH activities may have indicated some stress due to treatment since sprayed fish differed from unsprayed ones, if probability levels of 0.057 and 0.086 respectively are considered significant. Furthermore, both these enzyme activities and serum protein had significant interaction between time and treatment effects. Time effects were significant ($P < 0.05$) in all cases, chlorides showing a particularly striking upward trend. It would seem that the caging experience had a much greater effect on the biochemical parameters of these fish than did the fenitrothion application.

Some preliminary analyses were made on water and on wild fish taken from Pine Creek at sites of trout cages and from a stagnant pool a short distance from the sprayed cages. Minnow traps beside sprayed cages yielded several small wild fish with fenitrothion residues within the ranges for caged trout. Dissection revealed that stomachs of wild fish were filled but that those of caged trout were empty. Water in the stagnant pool was of some interest since it provided an opportunity to determine the persistence of fenitrothion in the absence of dilution by inflow of clean water. Concentrations of fenitrothion from this pool are shown below:

	Sample time	Fenitrothion concentration (µg/l)
June 7,	0600 — 0630	Spraying in progress
June 7,	1600 hr	51.5
June 7,	2300 hr	75.5
June 8,	0900 hr	38.8
June 11,	1600 hr	2.14
June 15		<0.10
June 26		<0.10

The disappearance of fenitrothion from the water was surprisingly rapid; however, this was confirmed experimentally by making distilled water to 200 µg/l with fenitrothion and by then measuring the pesticide remaining at 4 time periods. Two pyrex flasks were wrapped with aluminum foil; two were not wrapped, and all were allowed to stand outdoors for 8 days. The results below indicate that photodecomposition was probably an important mechanism of fenitrothion disappearance. The half time of fenitrothion was under one day in both field and lighted flasks.

Table 3. Mean fenitrothion residues in tissues of rainbow trout experimentally exposed to pulses of 46.1 µg/l and 4.61 mg/l in a continuously flowing system. Figures are means of analyses from 6 fish except as noted.

Exposure Level	Time After Treatment	Liver Fenitrothion (µg/g)	Kidney Fenitrothion (µg/g)	Muscle Fenitrothion (µg/g)
46.1 µg/l	1 day	1.17	1.60	1.56
46.1 µg/l	4 days	1.29 [a]	1.09 [a]	1.34 [a]
4.61 mg/l	1 day	68.9	56.6	91.7
4.61 mg/l	6 days	94.7	107	144

[a] 5 samples

Table 4. Serum and brain acetylcholinesterase activities of rainbow trout exposed to two levels of fenitrothion at time intervals before and after exposure. Geometric means are calculated from logarithms of assay values.

Group and time		Serum cholinesterase (mU/ml)	Brain acetylcholinesterase (µmol substrate hydrol/mg brain protein/hr at 15°C)
Untreated	$\bar{X}$	30.8	4.83
2 days before exposure	range	24.0 - 53.6	3.78 - 5.48
Untreated	$\bar{X}$	25.5	4.23
1 day after exposure	range	19.9 - 36.4	3.78 - 4.86
Untreated	$\bar{X}$	25.7	5.89
4 days after exposure	range	16.6 - 37.6	5.25 - 6.23
Treated at 46.1 µg/l	$\bar{X}$	25.3	4.62
2 days before exposure	range	15.3 - 45.1	3.12 - 5.32
Treated at 46.1 µg/l	$\bar{X}$	22.9	5.57
1 day after exposure	range	13.9 - 29.5	5.23 - 6.05
Treated at 46.1 µg/l	$\bar{X}$	24.2	5.74
4 days after exposure	range	14.9 - 33.2	4.61 - 6.66
Treated at 4.61 mg/l	$\bar{X}$	12.2	3.26
1 day after exposure	range	7.20 - 17.9	2.57 - 3.88
Treated at 4.61 mg/l	$\bar{X}$	11.2	3.62
6 days after exposure	range	8.00 - 14.1	2.64 - 4.24

Sample time	Fenitrothion remaining (µg/l) Dark flasks	Lighted flasks
Start	200	200
Day 1	192	10.4
Day 2	182	2.1
Day 4	163	0.6
Day 8	142	0.7

Fish captured in the stagnant pool contained the highest concentrations of fenitrothion recorded in the field (Hatfield and Riche 1970). Brook sticklebacks *(Culaea inconstans* Kirtland) and two species of dace *(Chrosomus eos* Cope and *Chrosomus neogaeus* Cope) were among those analyzed. Fish removed from the water on June 7 at 1430h contained fenitrothion at 4.77 µg/g. By 0900h the following morning a pooled sample of several fish had an even higher residue level of 13.7 µg/g. By June 11 fenitrothion concentration was down to only 0.19 µg/g and by June 15 residues were below detection. Dace formed the majority of fish trapped in the stagnant pool prior to treatment but few were caught after spraying. At an untreated site dace were predominant in the catch before and after treatment.

Laboratory exposures of rainbow trout were conducted during the winter of 1973-74 so that fenitrothion residues could be determined in internal organs. Table 3 lists fenitrothion concentrations found in liver, kidney and muscle tissues for fish exposed to pulses of 46.1 µg/l and 4.61 mg/l. It is evident that fenitrothion was taken up from water and distributed to these internal organs. Residue levels following exposure to 46.1 µg/l were somewhat higher than those found in trout caged in Pine Creek and neither serum nor brain cholinesterase was affected (Table 4). At the 100-fold higher treatment level, however, both these enzyme activities were reduced ($P \leq 0.05$). With the exception of cholinesterase at the higher treatment level, the same serum chemistry profiles as performed on fish from Pine Creek indicated that neither treatment had an effect at the $P = 0.05$ level. Serum glucose was added to these profiles and concentrations from before and after treatment were unchanged, although they differed from controls. Untreated fish showed a sharp drop in blood sugar during the experiment but treated fish remained at pretreatment levels.

No fish died even in the high treatment (4.61 ppm) although their behaviour was remarkably affected. Normally these fish actively avoided capture with a dip net but within hours of exposure all assumed an upright motionless position on the water surface and made no movement to avoid hand contact. By the fourth day after treatment long pieces of intestinal mucosa were shed intact. After several days in the motionless position, fish appeared to recover; feeding was resumed and 7 months of post treatment observation have noted no abnormalities.

DISCUSSION

From previous field studies (Hatfield and Riche 1970) and laboratory toxicology (Nishizawa *et al* 1961) we did not expect fish mortality following forest spraying at 4 oz/acre and we observed none which we could relate to treatment. Fenitrothion was taken up by fish, apparently directly from water since caged trout were unfed; furthermore wild fish with full stomachs captured adjacent to cages had residue concentrations in the same range as caged trout. Laboratory studies (Wildish and Lister 1973) have indicated that consumption of contaminated food is not a significant cause of fenitrothion poisoning in fish. Fenitrothion concentrations in fish on June 8 agree very closely with results from Newfoundland following a treatment in 1968 (Hatfield and Riche 1970). The pesticide was short lived in both water and fish; in trout it appears that fenitrothion has a half time of less than one day rather like malathion in carp (Bender 1969). In water, photodecomposition to unidentified products seems to explain the disappearance of fenitrothion. Fenitrothion in laboratory exposures was more persistent in both water and fish than it was in the field. The greater persistence in water in the laboratory is unexplained although the polyethylene plastic cover over the exposure building would not transmit all spectral components in

sunlight. The greater persistence in fish (Table 3) may be due to several factors; the greater persistence in water, the cooler water temperatures (6-9°C as compared with 11-21°C in the field) and the use of larger fish. The residue measured in fish must represent the sum at any time of uptake and any losses through elimination or chemical alterations. Uptake from water is obviously more rapid than the sum of all losses, or fenitrothion residues would not be observed, and it seems likely that the persistence in laboratory fish is due to more prolonged opportunity for uptake. Since none of the exposures resulted in fish mortality even when laboratory residue levels exceeded by several fold those from the field, it seems most unlikely that fenitrothion at 4 oz/acre kills mature fish.

The best known tests of sub-lethal poisoning of fish by organophosphorus compounds are cholinesterase measurements (Coppage and Matthews 1974); brain has been the tissue most frequently analyzed but serum has also been used (Hayama and Kuwabara 1962). Neither serum nor brain tests indicated poisoning in trout caged in Pine Creek; however, poisoning was detected in the high-level laboratory exposure, and serum activity was more sensitive than brain (Table 4). Serum cholinesterase was also found to be more sensitive than brain in Japanese quail caged in the spray plot (Findlay *et al* 1974). Gibson *et al* 1969, reported fish recovering from as much as 90% inhibition of brain cholinesterase and so even positive test results are difficult to interpret. Recent observations on snails (Rorke *et al* 1974) have suggested that fenitrothion elicits two types of symptoms of toxicity and that only one type is associated with cholinesterase inhibition.

Additional tests of sub-lethal poisoning would be useful aids in judging the impact of fenitrothion since mechanisms of toxicity other than cholinesterase inhibition seem probable. The approach of biochemical "profiling" has shown promise in the detection of sub-lethal poisoning in fish (Eisler 1967; Holmberg *et al* 1972) and the parameters analyzed in this report have been used previously in various studies of stress in fish (Bell 1968; Conte 1965; Grant and Mehrle 1970; Nakatani 1957; Christensen *et al* 1972; Rasmussen and Rasmussen 1967). All parameters were found to have changed between sampling periods, but none differed between treated and untreated fish at the $P \leq 0.05$ level. This would argue that the parameters were more sensitive to stress associated with the experimental caging experience than to stress associated with fenitrothion exposure. The same parameters failed to indicate stress in the laboratory at the lower treatment level and even at the high treatment level of 4.61 mg/l, with residues exceeding 100 µg/g (Table 3), only cholinesterase was affected. These profiles, with the exception of cholinesterase, do not detect fenitrothion exposure; and it follows that mechanisms controlling normal serum concentrations of the measured components were apparently intact.

In conclusion, fenitrothion as used in this forest treatment reached water rapidly and was taken up by fish. Uptake was directly from water, consumption of contaminated food being of minor significance. Fenitrothion disappeared rapidly from both fish and water, with a half time of one day or less in both cases. Photodecomposition to new products adequately accounts for the disappearance from water. A toxic but still sub-lethal concentration of fenitrothion in laboratory water resulted in decreased activities of serum and brain cholinesterase; however, both cholinesterase tests and several other serum tests used in diverse studies of stress in fish failed to detect poisoning when fenitrothion was applied at 4 oz/acre either in the field or in the laboratory.

These data confirm that fenitrothion does not kill mature fish when applied at 4 oz/acre, however, they do not exhaust the search for possible effects, such as alteration of behaviour (Scherer 1974) or reduction in food resources (Flannagan 1973). If toxicity problems exist for fish with fenitrothion as used in these small plots, then they seem more likely to be with compounds formed from fenitrothion than with the parent material itself.

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THE EFFECTS OF PESTICIDES ON SMALL FOREST VERTEBRATES OF THE SPRUCE WOODS PROVINCIAL FOREST, MANITOBA

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ABSTRACT. The Spruce Woods Provincial Forest, Manitoba, was sprayed in June 1973 for control of the spruce budworm, *Choristoneura fumiferana* (Clemens). Populations of songbirds and small mammals were monitored on seven 20-acre study plots to determine effects of the applied insecticides on animals other than the target insect. Two of the study plots were on Fenitrothion spray blocks, two on Dipel spray blocks, one on a block sprayed with Seven-4-oil, and two outside the spray zone as controls. Bird populations were assessed before and after the insecticide applications by the singing male census technique, and small mammals were kill-trapped about 2 months after the spray to compare breeding conditions of sprayed populations with those on the controls. The observational data and analyses of variance showed no changes that could be attributed to the treatment.

The Spruce Woods Provincial Forest, Manitoba, was treated with insecticide by aerial application in June 1973 to reduce damage from epidemic populations of the spruce budworm *Choristoneura fumiferana* (Clemens) (see Hildahl 1974 for a description of the operation). Single applications of Fenitrothion in dosages of 4 ounces of active ingredient/acre were used predominantly, but Dipel and Sevin were applied to three small area experimental plots. Populations of songbirds and small mammals were monitored on seven 20-acre study plots. Two of these were located in Fenitrothion spray blocks, two on the Dipel blocks, one on the Sevin spray block and two plots were controls: one located near the Dipel plots to the north and the other in the vicinity of the Fenitrothion and Sevin blocks in the southern area (Figs. 1 and 2). Songbird populations were estimated immediately before, and at 1, 2 and 3-week intervals after insecticide applications. Small mammal populations were censused approximately 2 months after the spray dates.

The Spruce Woods Forest is located in the sand-dune area east-southeast of Brandon, Manitoba. The area is treed largely by white spruce with pockets of poplar, oak and brush wherever moisture accumulates. The natural forest of mature white spruce is interspersed with grassy fields and reforestation plantations ranging from seedlings to mature stands. Soils in the area vary from rich leaf and needle litter in heavily wooded areas to sandy loam in open areas. The seven study plots each contained mixed habitats including mature spruce, poplar bluffs, shrubs, and grassland.

The two Dipel plots and the northern control were generally more heavily wooded than the four plots in the southern area. The two fenitrothion plots and the Sevin plot were sparsely wooded, as was most of the southern control plot, excepting a low area with a natural spring in the southeastern corner of the control which had a dense jungle of poplar, oak, dogwood, and hazel, with higher bird populations.

METHODS

Populations of songbirds were measured on 20-acre (10 x 20 chain) study plots by mapping the breeding territories of singing males, following the technique described by Kendeigh (1944). In each plot, parallel longitudinal lines were marked at 2-chain intervals by following compass lines. Imaginary cross-sectional lines were established by hanging flagging tape every 2 chains along each longitudinal line. Populations were assessed by cruising the lines and recording the locations of each singing male on a map scale 1 in.:132 ft (2 chains). About 2 hours were spent making each map and each census required a minimum of 2 maps.

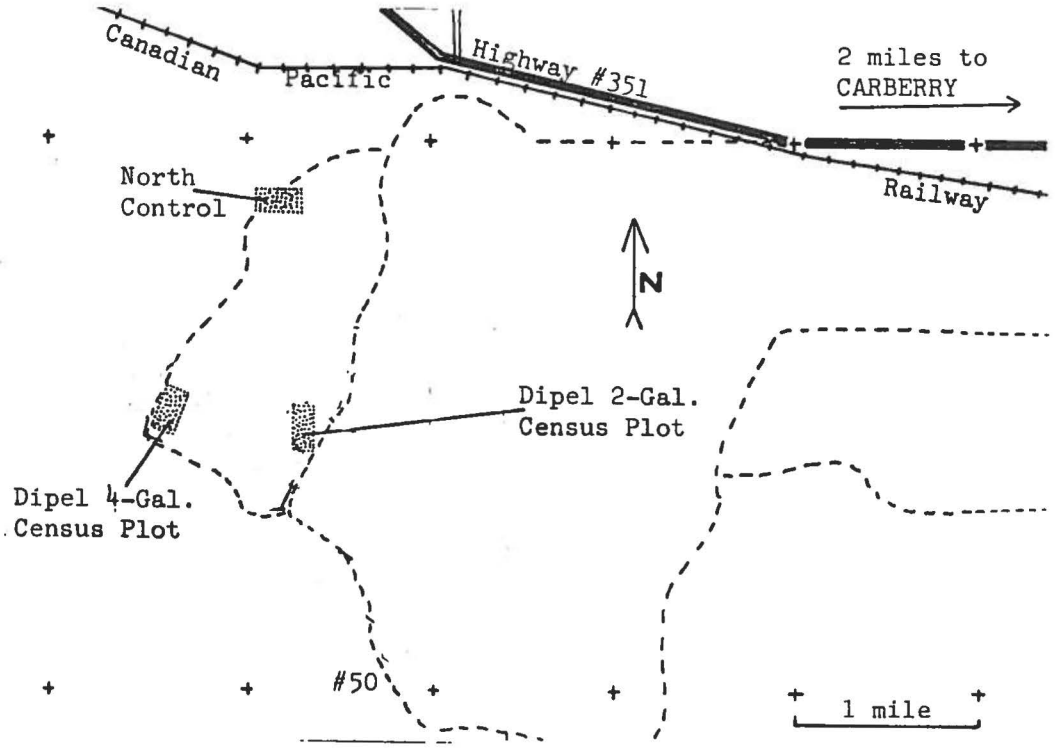

Fig. 1. Location of *Bacillus thuringiensis* (Dipel) plots, Spruce Woods Provincial Forest, Manitoba.

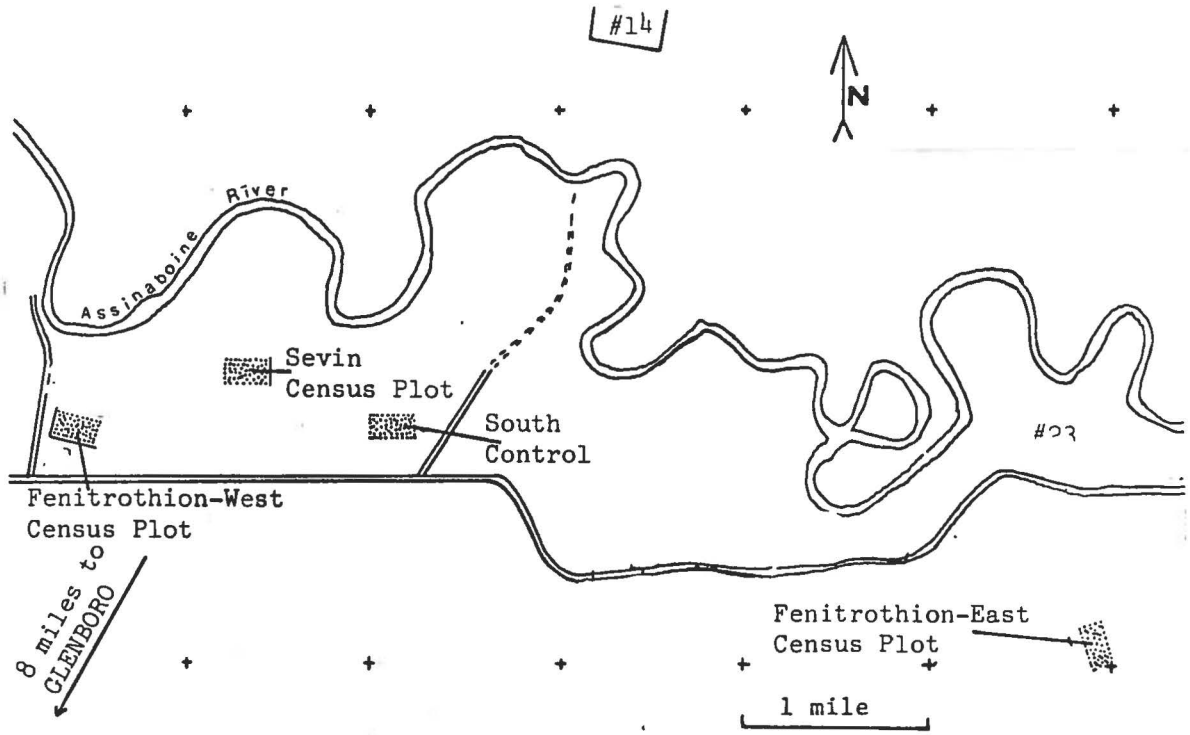

Fig. 2. Location of fenitrothion and sevin plots, Spruce Woods Provincial Forest, Manitoba.

Table 1. Population Summary in Pairs per 100 Acres for the Family Groups of Breeding Species Before, and at 1, 2, and 3–Week Intervals Following Applications of Dipel

Family	North Control			Dipel 2 gal			Dipel 4 gal			
	Pre-Spray	Post-Spray		Pre-Spray	Post-Spray		Pre-Spray	Post-Spray		
		Wk-1	Wk-3		Wk-2	Wk-3		Wk-1	Wk-2	Wk-3
TYRANNIDAE (Flycatchers)	5	0	0	0	0	5	15	0	0	5
PARIDAE (Chickadees)	10	0	10	5	5	20	5	5	5	5
SITTIDAE (Nuthatch)	5	0	5	10	5	15	5	0	0	0
SYLVIIDAE (Kinglets)	0	0	0	15	10	10	10	0	5	0
VIREONIDAE (Vireos)	0	10	0	10	0	0	10	0	5	10
PARULIDAE (Warblers)	20	20	20	80	20	45	65	25	15	35
FRINGILLIDAE (Sparrows)[a]	80	50	65	100	80	105	70	60	55	100
TOTALS	120	80	100	220	120	200	180	90	85	155

[a] *excludes transient Siskins, Goldfinches and Crossbills*

Table 2. Songbird Populations (Pairs per 100 Acres) Before and at 1, 2, and 3-Week Intervals Following Applications of Dipel

Family	Species	North Control			Dipel 2 gal			Dipel 4 gal			
		Pre-Spray	Post-Spray		Pre-Spray	Post-Spray		Pre-Spray	Post-Spray		
			Wk-1	Wk-3		Wk-2	Wk-3		Wk-1	Wk-2	Wk-3
COLUMBIDAE	Mourning Dove	10	5	0	0	0	5	0	5	5	5
CUCULIDAE	Black-billed Cuckoo	0	0	0	0	0	0	0	2	0	0
PICIDAE	Pileated Woodpecker	0	1	0	0	0	0	1	0	0	0
	Yellow-Shafted Flicker	0	0	0	0	0	0	1	0	0	0
	Yellow-bellied Sapsucker	0	0	0	0	0	2	0	0	0	0
TYRANNIDAE	Eastern Kingbird	5	0	0	0	0	0	0	0	0	0
	Great Crested Flycatcher	0	0	0	0	0	5	5	0	0	5
	Least Flycatcher	0	0	0	0	0	0	10	0	0	0
CORVIDAE	Gray Jay	1	0	1	2	10	5	0	0	0	0
	Blue Jay	0	2	1	4	0	2	1	2	0	1
PARIDAE	Black-capped Chickadee	5	0	5	0	0	5	5	5	5	0
	Boreal Chickadee	5	0	5	5	5	15	0	0	0	5
SITTIDAE	Red-breasted Nuthatch	5	0	5	10	5	15	5	0	0	0
TROGLODYTIDAE	House Wren	5	0	0	0	0	0	0	0	0	0
TURDIDAE	Eastern Bluebird	0	0	0	0	0	0	0	0	5	0
SYLVIIDAE	Golden-crowned Kinglet	0	0	0	5	5	0	5	0	5	0
	Ruby-crowned Kinglet	0	0	0	10	5	10	5	0	0	0
BOMBYCILLIDAE	Cedar Waxwing	1	46	17	0	5	0	0	0	0	0
VIREONIDAE	Red-eyed Vireo	0	10	0	10	0	0	10	0	5	10
PARULIDAE	Tennessee Warbler	0	0	0	0	0	5	0	0	0	0
	Orange-crowned Warbler	15	10	10	10	0	0	35	10	5	10
	Nashville Warbler	0	0	0	0	0	0	5	0	0	0
	Cape May Warbler	0	0	10	40	15	20	25	15	5	10
	Myrtle Warbler	0	5	0	25	5	20	0	0	5	15
	Ovenbird	5	5	0	5	0	0	0	0	0	0
ICTERIDAE	Brewer's Blackbird	0	0	0	0	0	0	4	0	0	0
	Brown-headed Cowbird	4	10	4	4	2	14	5	0	0	5
FRINGILLIDAE	Purple Finch	0	0	0	5	5	5	5	0	0	0
	Pine Siskin	14	0	8	11	30	8	60	0	0	4
	American Goldfinch	0	0	2	0	0	2	7	0	0	0
	Red Crossbill	0	0	8	75	0	42	0	125	85	0
	White-winged Crossbill	0	0	0	0	0	5	0	0	0	0
	Rufous-sided Towhee	5	0	0	0	0	0	0	0	0	0
	Vesper Sparrow	10	0	10	5	0	10	5	5	5	0
	Slate-coloured Junco	0	5	15	0	10	20	0	0	5	5
	Chipping Sparrow	60	45	35	90	60	65	60	55	45	90
	Clay-coloured Sparrow	5	0	5	0	0	5	0	0	0	0
	White-throated Sparrow	0	0	0	0	5	0	0	0	0	0

Table 3. Analysis of Variance for the Various Avian Populations Relative to the Insecticide Treatments

Treatment	Source of Variation	Sums of Squares	Degrees of Freedom	Mean Squares	F Ratio
Dipel 2-gal	Due to Treatments	442	2	221	.791
	Analytical Error	22609	81	279	
Dipel 4-gal	Due to Treatments	637	3	212	.572
	Analytical Error	40061	108	371	
Fenitrothion East Plot	Due to Treatments	115	2	58	.386
	Analytical Error	6707	45	149	
Fenitrothion West Plot	Due to Treatments	55	3	18	.133
	Analytical Error	14313	104	138	
Sevin	Due to Treatments	65	3	22	.203
	Analytical Error	16949	160	106	

Table 4. Population Summary in Pairs Per 100 Acres for the Family Groups of Breeding Species Before, and at 1, 2, and 3-Weeks Following Applications of Fenitrothion

Family	South Control			Fenitrothion East			Fenitrothion West			
	Pre-Spray	Post-Spray Wk-1	Post-Spray Wk-3	Pre-Spray	Post-Spray Wk-1	Post-Spray Wk-3	Pre-Spray	Post-Spray Wk-1	Post-Spray Wk-2	Post-Spray Wk-3
PICIDAE (Woodpeckers)	2	1	0	0	0	0	1	1	0	2
TYRANNIDAE (Flycatchers)	15	15	0	5	0	0	10	0	0	0
PARIDAE (Chickadees)	5	0	5	5	5	0	10	10	5	10
SITTIDAE (Nuthatch)	0	0	0	0	5	0	5	5	0	0
TROGLODYTIDAE (Wren)	0	0	0	0	0	0	15	10	10	15
SYLVIIDAE (Kinglets)	0	0	0	0	0	0	0	5	5	5
VIREONIDAE (Vireos)	35	50	50	5	0	5	15	10	25	20
PARULIDAE (Warblers)	120	95	120	30	35	30	40	25	10	20
FRINGILLIDAE (Sparrows)[a]	50	80	55	60	75	95	75	110	80	70
TOTALS	227	241	230	105	120	130	171	176	135	142

[a] *excludes transient Siskins, Goldfinches and Crossbills.*

Table 5. Songbird Populations (Pairs Per 100 Acres) Before and at 1, 2, and 3-week Intervals Following Applications of Sevin and Fenitrothion

"For each treatment, column headings are: Pre = before treatment census; 1, 2, 3 = censuses at 1, 2 and 3 weeks after treatment date."

	South Control			Sevin				Fenitrothion E.			Fenitrothion W.			
Family and Species	Pre	1	3	Pre	1	2	3	Pre	1	2	Pre	1	2	3
COLUMBIDAE														
Mourning Dove	0	0	0	0	5	5	5	0	0	0	0	5	0	5
CUCULIDAE														
Black-billed Cuckoo	0	0	0	0	1	0	0	0	1	0	0	0	0	0
PICIDAE														
Pileated Woodpecker	0	0	0	1	0	0	0	0	0	0	1	0	0	2
Yellow-bellied Sapsucker	2	0	0	1	0	1	1	0	0	0	0	0	0	0
Hairy Woodpecker	0	1	0	0	0	2	0	0	0	0	0	1	0	0
TYRANNIDAE														
Eastern Kingbird	0	0	0	0	5	5	0	5	0	0	0	0	0	0
Great Crested Flycatcher	0	10	0	0	5	5	0	0	0	0	5	0	0	0
Least Flycatcher	15	5	0	5	0	0	0	0	0	0	5	0	0	0
Olive-sided Flycatcher	0	0	0	0	5	0	0	0	0	0	0	0	0	0
HIRUNDINIDAE														
Barn Swallow	0	0	0	1	0	0	0	0	0	0	1	0	0	0
CORVIDAE														
Blue-Jay	0	2	0	0	4	0	0	0	0	0	0	1	0	0
Black-billed Magpie	0	0	0	0	3	3	0	0	0	0	0	0	0	0
PARIDAE														
Black-capped Chickadee	5	0	5	5	10	5	0	5	5	0	5	10	5	10
Boreal Chickadee	0	0	0	0	0	5	0	0	0	0	5	0	0	0
SITTIDAE														
Red-breasted Nuthatch	0	0	0	5	0	0	0	0	5	0	5	5	0	0
MIMIDAE														
Brown Thrasher	0	0	0	5	0	0	0	0	0	0	0	0	0	0
TROGLODYTIDAE														
House Wren	0	0	0	0	0	0	0	0	0	0	15	10	10	15
TURDIDAE														
American Robin	1	5	5	0	0	0	0	0	0	0	0	0	0	0
Veery	5	0	0	5	0	0	0	0	0	0	0	0	0	0
SYLVIIDAE														
Ruby-crowned Kinglet	0	0	0	0	0	0	0	0	0	0	0	5	5	5
BOMBYCILLIDAE														
Cedar Waxwing	7	9	5	1	19	9	10	0	10	0	0	0	2	2
VIREONIDAE														
Red-eyed Vireo	35	50	50	15	0	5	5	5	0	5	15	10	25	20

Table 5. (Continued)

Family and Species	South Control			Sevin				Fenitrothion E.			Fenitrothion W.			
	Pre	1	3	Pre	1	2	3	Pre	1	2	Pre	1	2	3
PARULIDAE														
Black-and-White-Warbler	5	5	10	0	0	0	0	0	0	0	0	0	0	0
Tennessee Warbler	0	0	0	5	5	5	0	0	0	0	0	0	0	0
Orange-crowned Warbler	10	10	15	30	15	15	10	20	25	20	35	20	10	20
Nashville Warbler	0	0	0	0	0	0	0	0	0	0	5	5	0	0
Parula Warbler	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cape May Warbler	5	5	10	0	0	0	0	0	0	0	0	0	0	0
Yellow Warbler	15	0	0	0	0	0	0	0	0	0	0	0	0	0
Myrtle Warbler	5	0	5	5	0	0	0	0	0	0	0	0	0	0
Chestnut-sided Warbler	20	15	15	0	0	0	0	0	0	0	0	0	0	0
Ovenbird	20	10	10	5	0	0	0	10	10	10	0	0	0	0
Canada Warbler	5	0	0	0	0	0	0	0	0	0	0	0	0	0
American Redstart	35	50	55	0	0	0	0	0	0	0	0	0	0	0
ICTERIDAE														
Red-winged Blackbird	0	2	0	0	9	5	1	0	0	0	1	0	0	0
Brewer's Blackbird	0	0	0	0	0	3	8	1	2	0	0	0	0	0
Common Grackle	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Brown-headed Cowbird	6	15	2	2	9	10	10	2	11	20	8	6	5	2
FRINGILLIDAE														
Rose-breasted Grosbeak	5	0	0	0	0	0	0	0	0	0	0	0	0	0
Purple Finch	5	0	0	0	0	0	0	0	0	0	0	0	0	0
Pine Siskin	7	0	2	18	3	3	11	7	9	10	7	0	9	0
American Goldfinch	1	0	2	3	5	6	6	0	0	0	3	0	0	0
Red Crossbill	0	0	0	29	0	0	0	0	0	0	4	0	0	0
White-winged Crossbill	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Rufous-sided Towhee	0	5	5	0	0	5	0	0	0	15	0	0	0	0
Vesper Sparrow	5	15	25	15	35	35	40	15	20	20	5	25	20	10
Lark Sparrow	5	0	0	0	5	5	0	5	0	0	10	5	0	0
Slate-coloured Junco	0	0	5	10	0	0	0	0	0	0	0	5	0	0
Chipping Sparrow	25	50	10	55	35	60	55	35	40	60	55	70	45	45
Clay-coloured Sparrow	5	10	10	10	5	25	20	5	15	15	5	5	15	15
White-crowned Sparrow	0	0	0	5	0	0	0	0	0	0	0	0	0	0
Fox Sparrow	0	0	0	0	5	0	0	0	0	0	0	0	0	0
Song Sparrow	0	0	0	5	0	5	0	0	0	0	0	0	0	0

Analyses of variance were performed to detect possible differences in bird population levels due to the treatments. Populations at the one, two and three week intervals were considered separately to detect immediate, delayed and fledgling impact.

Small mammal populations were censused by snap-back trapping. Each trapline consisted of a 90-yd baseline with 4-yd perpendicular lines, each bisected by the baseline, at 10-yd intervals along the baseline. Five traps were located 1 yd apart along each perpendicular. Age, sex, breeding condition, weight, and lengths were recorded for each specimen, and each female was dissected to count embryos and placental scars.

RESULTS

Songbirds on the Dipel Plots

Censuses of the Dipel plots and the north control include 38 species of birds representing 15 families. Table 1 summarizes, by family, the breeding residents, while Table 2 gives the populations for each species before and at 1, 2 and 3 weeks following the dipel treatments.

Population changes during the censuses on the Dipel plots approximately paralleled those on the control plots for most of the species that were present in numbers sufficient for comparison. Orange-crowned Warbler populations declined on both spray plots and the control; Cape May Warblers decreased slightly on the spray plots but were not found on the control until 3 weeks after the applications; while Flycatchers, Chickadees and Sparrows, the other major budworm-eating groups showed no differences that would indicate an effect from the treatment. Analysis of variance of the data showed no significant differences between the populations (Table 3).

Following the Dipel treatments, three Boreal Chickadee fledglings, a young Cowbird and three Chipping Sparrow fledglings were observed on the 2-gal plot, while three Chipping Sparrow fledglings appeared on the 4-gal plot.

Songbirds on the Fenitrothion Treatment Plots

The two fenitrothion plots and the south control had populations representing 45 species from 16 families. A population summary by family group, for the breeding residents, is shown in Table 4, while species populations before, and 1, 2 and 3 weeks following the fenitrothion treatment are listed in Table 5.

Population fluctuations on the fenitrothion treatment plots were not very different from fluctuations on the south control. While Orange-crowned Warbler populations dropped slightly on the west treatment plot, they increased slightly on the east plot (Table 5). The Boreal Chickadee populations decreased slightly on the west treatment plot while the Black-capped Chickadee numbers increased; and on both treatment plots, the Vesper, Chipping and Clay-colored Sparrows increased in numbers, as did those on the control. An analysis of variance performed on this data (Table 3) show none of these density changes was significant.

A Crow's nest with five eggs was recorded on the west treatment plot before the spray, and apparently-healthy Crow and Chipping Sparrow fledglings were observed 3 weeks after treatment. Censuses taken 3 weeks after treatment on the fenitrothion-east plot recorded a Chipping Sparrow nest with one egg and two nestlings, and a Rufous-sided Towhee nest with five eggs.

Songbirds on the Sevin Treatment Plots

On the "Sevin" treatment plot and the south control 49 species representing 15 families were encountered. Table 6 summarizes populations of breeding resident by family group while Table 5 shows populations for each species before, and 1, 2 and 3 weeks after the treatment.

The populations of the eight families of budworm-eating birds varied considerably between censuses (Table 6) but no significant differences were shown using analysis of

Table 6. Population Summary as Pairs Per 100 Acres for the Family Groups of Breeding Species Before, and at 1, 2, and 3-Week Intervals Following an Application of Sevin

Family	South Control			Sevin Treatment			
	Pre-Spray	Post-Spray		Pre-Spray	Post-Spray		
		Wk-1	Wk-3		Wk-1	Wk-2	Wk-3
PICIDAE (Woodpeckers)	2	1	0	2	0	3	1
TYRANNIDAE (Flycatchers)	15	15	0	5	15	10	0
PARIDAE (Chickadees)	5	0	5	5	10	10	0
SITTIDAE (Nuthatch)	0	0	0	5	0	0	0
TURDIDAE (Thrushes)	6	5	5	5	0	0	0
VIREONIDAE (Vireos)	35	50	50	15	0	5	5
PARULIDAE (Warblers)	120	95	120	45	20	20	10
FRINGILLIDAE (Sparrows)[a]	50	80	55	100	85	135	115
TOTALS	233	246	235	182	130	183	131

[a] *excludes transient Siskins, Goldfinches and Crossbills.*

Table 7. Small Mammal Species Trapped on Treatment and Control Plots in the Spruce Woods Provincial Forest, Manitoba, 1973

Species	PLOT							
	Control	Dipel 2-gal/ac.	Dipel 4-gal/ac.	Sevin Line 1	Sevin Line 2	Fenitro-thion Line 1	Fenitro-thion Line 2	Total
Peromyscus maniculatus	0	1	3	3	0	0	1	8
Clethrionomys gapperi	0	0	0	3	3	0	1	7
Sorex cinereus	0	0	0	1	2	0	0	3
Eutamias minimus	0	1	0	0	0	0	0	1
Zapus hudsonicus	0	0	0	0	0	1	0	1
TOTALS	0	2	3	7	0	1	2	20

Table 8. Small Mammal Populations on Treatment and Control Plots in the Spruce Woods Provincial Forest, Manitoba, 1973

Plot	Male[a]			Female[b]				Total Both Sexes
	Sub-Adults	Adults	Total	Pregnant With Scars	Scars Only	Not Pregnant	Total	
Control	0	0	0	0	0	0	0	0
Dipel 2-gal	0	2	2	0	0	0	0	2
Dipel 4-gal	0	2	2	0	1	0	1	3
Sevin, Line 1	0	4	4	1	0	0	1	5
Sevin, Line 2	1	2	3	3	0	1	4	7
Fenitrothion, Line 1	0	0	0	0	1	0	1	1
Fenitrothion, Line 2	0	1	1	1	0	0	1	2
Total	1	11	12	5	2	1	8	20

[a] *No juvenile males were trapped.*

[b] *No trapped females were juveniles, sub-adults or pregnant without placental scars.*

variance (Table 3). Table 5 illustrates that species such as Red-eyed Vireos, Orange-crowned Warblers and Chipping Sparrows showed a slight decline following the application, while the Chickadees, Vesper Sparrows and Clay-colored Sparrows increased in numbers following the spray.

Observations of nests found after the Sevin treatment included: (a) the successful hatch of eggs in Vesper Sparrow nests and a Black-billed Magpie nest; (b) the apparently healthy condition of nestlings in the Black-billed Magpie nest, a Chipping Sparrow nest; and (c) the survival of nestlings in 2 out of 3 Vesper Sparrow nests (the third nest had three eggs at week 1, three nestling at week 2, but had "nestlings abandoned" at week 3). These observations indicate that the Sevin treatment had no apparent effect on eggs and nestling survival.

Data from previous studies (Buckner, McLeod and Ray 1973) indicated that the most vulnerable species of birds are those inhabiting the most exposed habitats. The Nashville Warbler, Orange-crowned Warbler, Cape May Warbler and Chipping Sparrow would be most likely indicators of impact, but populations of these were clearly unaffected.

Small Mammal Populations

Small mammal populations were very low throughout the Spruce Woods area in 1973. A total of 20 animals representing 5 species were taken on the consecutive trap nights, August 4, 5, and 6 (Table 7).

Of the 20 animals trapped, 8 were females and 12 males. No juvenile animals and only a single sub-adult specimen was trapped. All animals appeared healthy. Dissections revealed that 7 of the 8 females were either pregnant or contained placental scars indicating an uninterrupted breeding cycle (Table 8). Many of the males also were in breeding condition. Recent studies indicate that the initial impact of a pesticide on wild small mammal populations in an interruption in breeding (Buckner, McLeod and Ray 1973).

These data indicate that the low numbers encountered in the Spruce Woods treatment plots resulted from poor habitat and natural population fluctuations rather than from the insecticides applied.

CONCLUSIONS

Comparisons of census results for songbirds and small mammals from five treatment plots with those from the controls show few decreases on treatments that do not parallel decreases on the controls; analyses of variance of the songbird populations show that none of these decreases are significant. It can therefore be concluded that none of the five pesticide applications monitored during this investigation had any significant effect on any of the small forest vertebrates studied.

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CANADIAN CONTRIBUTIONS TO FOREST INSECT CONTROL TECHNOLOGY

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ABSTRACT. At present, Canada has a highly sophisticated program of forest protection. The use of new spray application techniques using multi-engined aircraft coincides with the development of new concepts in insecticide usage. The forest entomologist is deeply involved in all phases of these developments. The future appears to be both highly challenging and rewarding in this effort to conserve and protect one of Canada's most important natural resources.

INTRODUCTION

Forest insect control research in Canada is sponsored primarily by the Canadian Foresty Service at six regional laboratories and at two supporting institutes (Chemical Control, Insect Pathology), and through research contracts and grants to universities. The objectives of this Canadian involvement in forest entomology are directed primarily at the protection of the forest resource in the interests of man. Simply stated, these objectives involve "the derivation of information on methodology and materials for the protection of trees from pest attack while minimizing the disturbance to the environment."

Alongside classical and world-renowned studies of insect biology and population dynamics, Canadian forest entomologists during the past two decades have made significant contributions to science through very intensive studies of parasites and predators, sex attractants, juvenile hormones, disease organisms, and certain of the highly-potent but short-lived synthetic organic insecticides. Although much work remains in the development of appropriate tactics for the implementation of operational control programs for selected pests occurring within diverse forest types and varying stand conditions, some very encouraging results have been obtained from field experiments during the past five years. This paper has been prepared primarily to briefly outline to colleagues in agriculture and associated disciplines a few of the achievements during this period, which have given Canada a leading position in the field of forest insect control.

INSECTICIDE CONCEPTS AND DOSAGES

The selection of insecticides for operational programs involves a definitive sequence of planning and experimentation over a period of several years, usually as follows:

(1) Assimilation of background information regarding toxicology, residues, hazards, etc., as provided by industry and other research organizations.
(2) Laboratory toxicological experiments, usually with introduced insect populations on small potted trees.
(3) Small-scale field trials using either ground or aerial spray techniques. Treated areas normally do not exceed 100 acres.
(4) Semi-operational forest-block applications by aircraft. Treated areas usually range from 100 - 500 acres but occasionally may cover up to 10,000 acres. At this stage, studies of environmental impact usually are implemented.
(5) Recommendation of the most efficacious and environmentally acceptable insecticides for registration (through Canada Agriculture) and use in forest pest management.

Effective quantities of insecticides for application vary according to their chemical potency, the method of application, type of host tree, and the feeding habits of the target pests (Table 1). In most cases for defoliating insects (the most common pest group), for example, 10 ounces per acre or less of active ingredient will suffice for aerial application.

Table 1. Examples of insecticide dosages for control of some forest insects in Canada.

Insecticide	Dosage/acre (oz. active ingredient)	No. Applications	Method of Application	Target Insects
methoxychlor	40	1	Aircraft	white pine weevil
methoxychlor	16	1	Ground sprayers	white pine weevil
carbaryl	9.8	1	Aircraft	gypsy moth
fenitrothion	2-4	1 or 2	Aircraft	spruce budworm
phosphamidon	1-2	1 or 2	Aircraft	sawflies
aminocarb	0.75	2	Aircraft	spruce budworm

As alternatives to chemicals, biological insecticides have been costly and difficult to produce in quantity. During recent years, however, at least two dramatic advances have occurred in Canada which point to the possibilities of the future. Firstly, a polyhedrosis virus specific to the redheaded pine sawfly *(Neodiprion lecontei* L.) was developed for use in pine plantations by the Insect Pathology Research Institute. Small quantities of this virus (derived from larval cadavers) in aqueous suspension may be applied to sawfly colonies infesting individual trees or to whole plantations if warranted. The disease establishes rapidly in the resident population and may spread considerable distances to untreated trees. Foliage consumption is greatly reduced and larvae die due to the virus infection. Collections of cadavers for virus stockpiling is possible, and it is hoped that growers will soon be able to have their own supply for combatting future infestations. The self-propagating insecticide is inexpensive (involving only the labour in collecting infected larvae), and only a few hundred specimens (e.g. 2 gm. of freeze-dried stock will treat up to 20 acres of trees 4-10 ft. tall) are required to initiate the shelf stock. The combination of virus sprays and a high incidence of a natural hymenopterous parasite virtually eliminated this insect pest from many areas of southwestern Quebec during the period 1970-1973. Should reinfestations occur, virus stock will be made available to growers for spray treatments.

Secondly, commercial preparations of a potent strain of the bacterium *Bacillus thuringiensis* (B.t.) have shown considerable promise during the past three years for control of Canada's notorious spruce budworm, *Choristoneura fumiferana* (Clem.). Research has shown that quantities in the range of 4-6 billion International Units/acre will provide good protection of fir and spruce foliage under certain conditions. The use of B.t. would be ideal for certain valuable and threatened forest stands or individual trees where chemical treatment is not feasible. Also, studies by the Laurentian Forest Research Center and the Chemical Control Research Institute indicate that the efficacy of B.t. treatments may be significantly enhanced by the addition of either the enzyme chitinase or by small quantities of synthetic insecticides. The bacterium is highly selective and its use in spruce-fir forests presents no hazards to fish and wildlife.

OPERATIONAL SPRAY TACTICS

When forested areas requiring insecticide treatment are small or where only a few trees are infested, usually a single conventional agricultural spray plane or a groundspray unit will provide effective coverage during the critical time period for treatment. Where larger acreages are involved more aircraft may be required to ensure proper timing of sprays to all areas [1].

[1] See "Aerial applications of chemical insecticides against the spruce budworm in Manitoba, 1973" by V. Hildahl and R.F. DeBoo *Manitoba Ent. 7:* 6-14.

FIGURE 1. A contrast in electronically-guided aircraft: Midair International's converted Douglas DC-7B equipped with the Litton LNT51 guidance system and having a payload of approximately 4000 gallons of spray mixture; the experimental Cessna 185 of the Chemical Control Research Institute fitted with the Decca Agri-Fix swath tracking system and having a carrying capacity of 90 gallons of spray material.

Historically, the use of aircraft in Canadian forest pest control dates back for more than 50 years. The concept of large-scale operational sprays commenced, however, only during the 1950's and has been restricted for the most part to forests in eastern Canada infested by the budworm. For example, during 1957, more than 200 Stearmans and other small planes were mustered to treat 5.2 million acres of forest in New Brunswick. Larger aircraft, the Grumman Avenger (TBM), replaced the Stearmans during the 1960's. The TBM had approximately 4 times the load capability of the Stearman, and fewer were required to treat equal acreages.

In 1971, a Douglas DC-7B was fitted with tanks and booms for use in the spruce budworm operations in Quebec (Fig. 1). Also incorporated into the aircraft was an electronic guidance system designed to automatically compute designated spray swath patterns. The trial with the DC-7B was basically successful, and by 1973 spray operations in Quebec included DC-6's, Constellations, Super Constellations, Lodestars, and a few TBM's. Only 22 aircraft were used that year to treat a total of 9.7 million acres of infested forest. In comparison with 1957, then, about one-tenth the number of aircraft were used to treat almost twice the area of forest.

The Canadian Forestry Service, the Government of Quebec, Forest Protection Ltd. of New Brunswick, and the Canadian aviation industry are actively engaged in improving electronic guidance systems, spray emission systems, swath patterns, and spray formulations for these large aircraft. It is anticipated that night applications, to take full advantage of optimum weather conditions and insect behaviour will soon be operationally possible.

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THE BEHAVIOUR OF HONEY BEES (*APIS MELLIFERA* L.) IN FLIGHT AND REARING ROOMS

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ABSTRACT. The behaviour of honey bees in flight and rearing rooms is influenced by intensity and quality of light, photoperiodism and temperature. All of these variables must be controlled to provide a suitable environment for natural flight and in-colony behaviour. Responses of bees to environmental stimuli in various flight rooms are reviewed and suggestions made for improved results in future flight room studies.

Bee flight and rearing rooms provide for year-round research in temperate areas. In addition, such rooms allow one to conduct bee flight behaviour studies under rigidly controlled environmental conditions. To date, attempts to maintain colonies of honey bees for extended periods of time in flight and rearing rooms have met with limited success. Abnormal flight behaviour, presumably caused by a failure of flight rooms to provide conditions for successful flight, has been common. The factors influencing flight, foraging and in-colony behaviour of bees in flight rooms are reviewed here. Suggestions are made for obtaining improved results in future flight room studies.

TYPES OF BEE FLIGHT AND REARING ROOMS

Honey bees have been maintained in various types of restricted enclosures, including outdoor screen cages, glass houses, indoor screen cages, and controlled environment chambers. Of these only the last two provide for the possibility of an environment with controlled temperature, humidity, light intensity and photoperiod.

Modern bee flight rooms can be grouped into three convenient classifications:

1) Screen cages installed in pre-existing rooms. Such rooms may have relatively simple environmental controls (Cornaire and Wilcox 1968) or may be very sophisticated with precise control of most environmental factors (Praagh 1972).
2) Modifications of existing rooms with the addition of lights and diffusion screens, temperature control (usually through the addition of an electric heater) and some form of humidity control (Chauvin 1953, Renner 1955, Smith 1961).
3) Rooms specifically designed as bee flight and rearing rooms (Nye 1962, Jay 1964). Such rooms have sophisticated controls on all environmental factors.

While all of the bee flight rooms mentioned above vary in size and detail, most attempt to attain conditions as similar as possible to the "normal" outdoor environment of bees.

FLIGHT-ORIENTATION AND DRIFTING

Orientation within a flight room can be divided into two inter-related categories — (1) spatial orientation by individual bees and, (2) drifting of bees between hives if more than one colony is present.

Spatial orientation is affected by the quality and quantity of light present. Bees were unable to orient in a room lit by undiffused incandescent bulbs with an intensity of 50 lux

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at the hive entrance (Chauvin 1953). This failure was possibly due to an unnatural angular radiance distribution (Verheijen 1958, Velthuis and Verheijen 1963), faulty spectral range for bee vision (Berthold 1931, Daumer 1958, Autrum and von Zwel 1964) and an insufficient light intensity (Nye 1962, Nelson 1966). More recent studies indicate that bees had little difficulty orienting in rooms with light intensities ranging from 500 to 4250 lux at 1 m above the floor when diffused fluorescent lamps were the light source (Renner 1955, Smith 1961, Nelson 1966, Nelson and Jay 1967a, Kefuss and Nye 1970).

Praagh (1972) reported that under white light bees congregated on or near the ceiling but dispersed when ultra-violet (UV) radiation was increased. He also noted that a UV lit area of the room attracted large numbers of bees, even though the light intensity did not appear as bright to human eyes as that in the area with white light.

The angular radiance distribution of light in the flight room may affect spatial orientation (Praagh 1972). In rooms lit with undiffused incandescent bulbs, bees tended to circle and crash into the bulbs (Chauvin 1953), a phenomenon described as "trapping" by Verheijen 1958. In flight rooms with highly reflective floors, flying bees become totally disoriented (Nelson 1966), a response similar to that reported for bees flying over snow (Velthuis and Verheijen 1963). Nelson reported that such behaviour ceased after the floor became stained with feces.

In rooms with white, reflective walls (Renner 1955, Nye 1962, Jay 1953), illuminated with diffused fluorescent lights of 500 or more lux in intensity, ceiling trapping has not been reported, although Nelson (unpublished) found that in such a room ceiling trapping occurred immediately adjacent to 100 watt incandescent bulbs used for auxiliary lighting in the flight room. Trapping ceased when these bulbs were removed. Rooms with non-reflective screen walls and ceilings and with indirect fluorescent lighting of relatively low intensity (Praagh 1972) required supplementary side lighting to prevent trapping and concentrations of flying bees in the upper third of the room.

Orientation of bees in a flight room becomes progressively more difficult when more than one colony is present, and severe drifting between colonies may occur. Chauvin (1953) found that under these conditions bees aggregated at one colony and ignored their home hive, a situation aggravated by the tendency of the newcomers to fan at the entrance with exposed Nassanof glands, increasing the attractiveness of the favored hive and resulting in a corresponding positive feedback response. Jay (1964) suggested that coloured flight room walls should reduce intercolony drifting, and Nelson and Jay (1967b) found that coloured entrance boards, objects placed near the entrance, and coloured walls all reduced intercolony drifting to a favored hive. Objects near the entrance and coloured walls were the most effective but drifting was not reduced to the level where multiple colonies could co-exist in the flight room. Selective intercolony drifting was not reported by Nye (1962) or Kefuss and Nye (1970).

FLIGHT-INTENSITY AND CYCLES

Flight activity in rooms (as in the field) is influenced by light intensity, photoperiodism and temperature.

Under field conditions, increased light intensity is a factor in initiation and termination of flight (Butler and Finney 1942, Ribbands 1953, Schricker 1965). The number of bees flying in a flight room on a given day correlated closely with the light intensity (Nelson and Jay 1967a), and at low levels flight activity ceased. Cornaire and Wilcox (1968) found that at continuous low light levels in a flight room flight activity was very limited. Flight activity under conditions of low light intensity may increase with increased UV light and a flicker frequency of 300/sec as opposed to the more common 120/sec (Praagh 1972).

Bees adapted quickly to various photoperiod regimes which were based on a 24 hour cycle in flight rooms. Flight activity fell rapidly in the period just prior to the time the lights were extinguished (Nelson 1966, Kefuss and Nye 1970) and sunset effects produced by utilization of several phototimers were effective in encouraging flying bees to return to the hive (Nye 1962, Jay 1964).

A 12 hour period of light resulted in bimodal patterns of flight activity, with "morning" and "afternoon" flight peaks and a "midday" lull (Nelson and Jay 1968). Similar patterns of flight activity occurred with free flying bees under outdoor conditions (Faberge 1943, Domagala-Lipin'sha 1962, Gary 1967). Decreasing the length of the photoperiod to 2 hours resulted in increased flight activity during the light period (Kefuss and Nye 1967) and colonies so conditioned continued to fly at a high rate during this period after the photoperiod was extended. As the day length was extended to 16 hours the bimodal pattern of flight activity decreased, as did flight activity peaks (Kefuss and Nye 1970). Under continuous light, the flight activity pattern degenerates, with random small bursts of activity (Bennet and Renner 1963).

Temperature limits flight activity (Ribbands 1953) and bees can perceive temperature variations as low as 0.25° C. Temperature also interacted with light intensity under field conditions (Corkins 1930, Walker 1945) to limit total flight activity.

Temperature variations in a flight room resulted in temperature correlated cycles superimposed over daily diurnal cycles of activity (Nelson 1964). Entrance activity studies showed significant interaction between temperature and light intensity with more bees leaving the hive at lower light intensities with increasing temperature (Nelson and Jay 1967a). Seasonal variations of flight activity also occur in response to temperature and light with more "winter" bees leaving the hive at lower temperatures for a given light intensity than do "summer" bees (Nelson and Jay 1968). Such seasonal differences were probably related to seasonal physiological differences (Maurizio 1961) and have been observed in the field (Lundie 1925).

Woodrow (1935) reported that high humidities caused a shortening of honey bee life spans but relative humidity within ranges of 15 to 90% did not affect flight activity in flight rooms (Nelson 1966).

FORAGING ACTIVITY

Foraging behaviour in flight rooms has not been extensively studied. Renner (1955) found that colonies foraged best on natural forage placed in water tumblers or on scented sugar syrup. Nye (1962) and Jay (1964) reported that bees would forage on ground pollen, pollen substitute and unscented sugar syrup presented in trays and dishes. With one exception (Praagh 1972), colonies in flight rooms have not been able to maintain themselves by foraging, and supplementary sources of pollen, sugar syrup and/or honey have been required in the hive. With such supplementary feeding, colonies have been maintained in flight rooms for periods of from 3 to 6 months (Nelson 1966, Kefuss and Nye 1970) and up to two years (Renner 1955).

Nelson (1966) found that the total number of successful foragers remained small in relation to the total bee flight with little correlation between flight activity and foraging activity. The number of foraging bees decreased when light intensities were lowered. Nye (1962) found that the number of bees collecting pollen dropped if the light intensity was lowered or if they were forced to fly across an area of lower light intensity.

While flight activity cycles apparently were determined by exogenous factors such as light and temperature, as well as by an internal clock (Bennet and Renner 1963, Kefuss and Nye 1970), foraging rhythms in flight rooms were endogenously controlled. Bees could only be trained in flight rooms to 24 hour foraging cycles (Beling 1929, Renner 1957, 1959, 1960). Foraging bees could be time trained, and their internal clock reset (within the limits of the 24 hour cycle) using artificial lighting (Beier and Lindauer 1970). This is difficult to accomplish in the field due to the so-called "sundial effect" (i.e. adjustment based on the position of the sun).

Pollen is usually fed in the flight room as ground-up bee collected pellets in a free access tray (Renner (1955, Nye 1962, Jay 1964, Nelson 1966, Praagh 1972). Pollen substitutes have been collected by bees, but these were nutritionally inadequate and significant reductions in brood rearing resulted (Nelson 1966). Given a choice of foraging for loose pollen in a tray, pollen under an 8 mesh wire screen, or pollen under perforated

metal (2.5 mm holes), bees preferred to forage through the screen and collected more pollen (Nelson 1966) due possibly to the solid surface which provided an adequate footing for packing corbiculae (Haydak 1963).

IN-COLONY BEHAVIOUR

Little research has been conducted on the behaviour of bees inside a colony in a flight room, the assumption being (perhaps over-simplified) that in-colony behaviour is to a large extent, not a function of the colonies exterior environment.

Bees reared brood in colonies in flight rooms (Renner 1955, Nye 1962, Jay 1964, Nelson 1966, Praagh 1972), but supplementary feeding is usually required to maintain brood rearing. Both drones and queens have been reared in colonies in the flight room during the winter (Jay 1964). Jay's colonies accepted over 50% of the worker larvae grafted into artificial queen cells. A queen has been both reared *and* mated in a flight room (Smith 1961) but this event has not been duplicated. While Nelson (1966) was unable to relate humidity inside the colony to either in-colony activities or flight activity, Praagh (1972) reported that successful brood production required high in-colony humidity.

GENERAL CONCLUSIONS

A controlled environment bee flight room must produce conditions which permit behaviour as close as possible to that obtained under field conditions. This is essential if the flight room is to be used either to study behaviour as influenced by environmental factors, or as a research tool during the winter in temperate areas.

While definite conclusions cannot be drawn, the following considerations appear valid and should be taken into account by those contemplating flight room research:

(1) Comparisons of behavioural data are valid *only* for flight rooms or chambers of similar design. Different types of rooms (e.g. walk-in environmental chambers vs indoor screened cages) may result in very different responses by bees.

(2) Initial environmental conditions for normal flight behaviour should be approximately: temperature 24-26° C; light intensity 2000-4000 lux with a flicker frequency of 100-120/sec (50-60 cycle/sec alternating current), relative humidity of 50-60% (although higher humidities may increase brood rearing).

(3) Reduced light intensities may be satisfactory if lights with high UV intensity and a flicker frequency of 300+/sec are used.

In spite of the number of studies conducted in flight rooms, the ideal of totally normal flight behaviour has not yet been realized. Stresses caused by size restrictions, lighting, etc., in rooms may make this goal unattainable.

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SOLUBLE ACETYLCHOLINESTERASE ACTIVITY IN THE BRAINS OF DISEASED WORKER AND DRONE HONEY BEES (*Apis mellifera* L.)

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ABSTRACT. Soluble acetylcholinesterase (AChE) activity in whole brains of virus-infected and uninfected *Apis mellifera* workers and drones was determined. Uninfected workers had about three times the AChE activity of infected workers. Uninfected drones also had greater AChE than infected drones.

INTRODUCTION

A disease of honey bees, tentatively identified as "hairless-black syndrome" and associated with four virus-like particles has been described (Kulincevic *et al* 1969) and the histological pathology (Horvath and Rothenbuhler 1972) and effect on fatty acids (Nelson *et al* 1971) determined. While virus infections are known to alter the activity of respiratory enzymes in insects (Bergold 1959), data are not available on the effect of viral pathogens on acetylcholinesterase (AChE) activity. A preliminary study was initiated to determine the activity of AChE in the brain of virus infected and healthy worker and drone honey bees.

MATERIALS AND METHODS

Honey bees were collected from two locations; infected workers and drones from the Ohio State Bee Laboratory, Columbus, and healthy workers and drones from the apiary of Ohio Northern University, Ada. Workers and drones selected as infected were taken from known diseased hives and demonstrated symptoms of the disease, but were not moribund. Bees from Ada were used as controls because the area is apparently free from the disease and is close enough to Columbus to minimize differences due to location. Bees of approximately the same age were used to minimize differences in AChE activity due to age differences as reported by Rockstein (1950). Worker bees used for the tests were held overnight in lots of 50 in cages (Kulincevic and Rothenbuhler 1973) at 22° C. Drones were likewise held in cages in lots of 20 with 50 healthy workers to maintain them.

Brains were isolated in chilled dry watch glasses without narcosis from infected and healthy workers and drones. All isolates were prepared between 0900 and 1300 hours to reduce expected circadian effects on AChE atctivity (Cymborowski *et al* 1970).

The soluble AChE was extracted according to the method of Beckendorf and Stephen (1970) as modified for whole brains by I. Cheldelen (personal communication 1971). Single whole brains were soaked to 0.025 ml of 0.25 M sucrose for 2 hours at 4° C. The total extracting solution was then drawn off in a micro-capillary tube and used in the enzyme

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assay. All brains and extracts were held at 0° C until incubation to minimize possible loss of enzyme activity. Acetylcholinesterase activity was determined according to the method of Ellman *et al* (1961). The reaction mixture consisted of 6 ml 0.1 M pH 8.0 sodium/potassium phosphate buffer, 40 µl acetylthyocholine iodide substrate, 200 µl dithiobisnitrobenzoic acid and 20 µl enzyme extract. The total reaction mixture was incubated in a water bath for 2 hours at 37° C after which the reaction was stopped by the addition of eserine sulphate to a final concentration of 10^{-3} M. A blank containing the reaction mixture without enzyme was run simultaneously. Optical density was determined at 412 nm. Results were calculated on a per brain basis (Cymbrowski *et al* 1970) in µ moles substrate split/min./brain.

RESULTS AND DISCUSSION

Healthy worker bees had approximately three times as much easily extractable AChE activity as worker bees infected with the virus ($P < 0.01$) (Table 1). Results with drones were less distinct although the healthy drones again showed more AChE activity than infected ones ($P < 0.05$). This reduction of AChE activity in virus-infected insects has not, to our knowledge, been reported previously. The higher AChE activity in drones, as compared to workers, is probably due to the larger size of drone brains and the proportionately larger optic lobes, which contain large amounts of AChE (Smallman and Mansingh (1969).

Table 1. Comparative AChE activity (u moles acetylcholine split per minute by one brain) in the brains of healthy and virus-infected worker and drone bees. Means and standard errors based on measurement of 8 workers or 4 drones

	AChE Activity	
	Healthy	Diseased
Workers	19.5 ± 3.3	6.5 ± 1.2
Drones	73.3 ± 10.2	45.3 ± 9.6

Horvath and Rothenbuhler (1972) describe basophilic granules in the neuropile area of abdominal and thoracic ganglia of infected bees which may be produced by a breakdown in normal physiological cellular activities. The concentration of AChE is directly related to quantity and heterogenity of RNA in insects (Smallman and Mansingh 1969, Grezelak *et al* 1974). A viral induced change in cellular activities or in RNA content in the cells may therefore be responsible for the lowered AChE activity. Other factors responsible for reduced enzyme activity could be some form of inhibition of the enzyme in diseased bees or differences in the extractability of AChE caused by virus infection.

Work is continuing to determine the cause and significance of the observed reduction in AChE activity to the pathology of the disease.

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A LABORATORY STUDY OF FAUNA AND FLORA IN AN AGRICULTURAL SOIL IN MANITOBA[1]

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ABSTRACT. Faunal, microbial and chemical analyses were done on black clay soils in which wheat, oats, and barley were grown in the laboratory under controlled conditions of light, temperature and moisture. Changes in faunal, floral and chemical components in unfertilized and fertilized soils and also in soils in which cereals were grown were determined. Fifteen species of mites including *Siteroptes,* which is a vector of the cereal pathogens *Fusarium* and *Nigrospora,* are reported for the first time in an agricultural soil in Manitoba. Each type of cereal created a characteristic soil environment in which populations of Collembola, mesostigmatic, pyemotid, and acarid *(Tyrophagus)* mites either increased or decreased. Their relative abundance was related to the type of cereal planted and the duration of incubation periods. Bacterial levels were high in soil in which mature wheat was grown. Growing of cereals did not affect the chemical components of the soil. The ecological implications of faunal changes associated with the use of fertilizer and cereal growing in agricultural soil are discussed.

For nearly a century, cereals have been the major crops in the southern part of the Prairie Provinces. Prior to man's exploitation through monoculture, this land was covered with wild grasses for thousands of years. In the natural Prairie grassland ecosystem, stability was maintained by nature's own checks and balances. Although farming on the Prairies has been a highly successful venture through production of large quantities of wheat, oats and barley, its long-term effect on the land environment is not clearly understood. One way to understand this environment is to study the faunal, floral and chemical components of the soil. To our knowledge the invertebrate fauna of agricultural soils and their relations to the floral and chemical components have not been explored in Manitoba. A comprehensive review of published work on interactions between soils and their modification by management practices in Canada has been prepared by Mills and Alley (1973).

The aim of this project was to identify and provide a quantitative estimate of fauna, microbial flora and chemical components in agricultural soil samples in which fertilizers were applied and cereals were grown under artificial conditions.

MATERIALS AND METHODS

Black clay soil, typical of the heavy soils of the Red River Valley with a known history of cultivation, was collected from a farming area near Winnipeg and stored indoors (ca 15 C) at the Research Station, Agriculture Canada, Winnipeg. A summary of materials and methods used in this study is given in Table 1. The experimental conditions used were identical to those used in the cereal breeding programs at the Agriculture Canada Research Station, Winnipeg. The clay soil was mixed with additives and fertilizer to ensure optimum plant growth (Table 1). A soil amendment ("Turface" — crushed rocks with no moisture) was added to the soil (1:2) to make it more porous; for the oats and barley plants, sand was also added (Table 1). The fertilizers used were 27-14 (27/100 lb N + 14/100 lb P_2O_5) for

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Table 1. Summary of materials and methods used in the experiments on monoculture effects in an agricultural soil

Expt No.	Sample group	No. of samples	Soil mixture ratio (% moisture content)	Fertilizer[a]	Incubation temperature	Plant height (cm)	No. of plants per plot	Planting/sampling date
1	Control	16	black clay soil + soil amendment 1:2 (20.1)	None	No incubation	—	—	2 March 72
2	Control	2	black clay soil + soil amendment 1:2 (23.7)	None	18 ± 2 C	—	—	2 June/ 1 Sept. 72
3	Control	2	black clay soil + soil amendment 1:2 (23.7)	27—14	18 ± 2 C	—	—	2 June/1 Sept. 72
4	Wheat, young plants	4	black clay soil + soil amendment 1:2 (40.5)	27—14	18 ± 2 C	40.6	1	10 Nov. 1971/ 3 March 72
5	Wheat, mature	20	black clay soil + soil amendment 1:2 (23.7)	27—14	18 ± 2 C	91.4	1	3 Dec. 1971/ 10 April 72
6	Control[b]	18	black clay soil + soil amendment + sand 3:1:1 (13.4)	None	No Incubation	—	—	20 April 72
7	Oats, mature	18	black clay soil + soil amendment + sand 3:1:1 (20.4)	27—14 RX—15	18 ± 2 C	76.2	2	1 Feb./20 April 72
8	Barley, mature	18	black clay soil + soil amendment + sand 3:1:1 (12.0)	27—14 RX—15	18 ± 2 C	76.2	3	1 March/27 April 72

[a] See text for explanation.

[b] Separate control for oats and barley (different soil mixture).

wheat; and a mixture of 27-14 and RX-15 (15 lb/ton N, 30 lb/ton P_2O_5, 15 lb/ton K_2O) for the oats and barley. Soil mixtures (500 cc per pot for expt 1-5 1500 cc per pot for expt 6-8) were placed in clay or pressed paper pots (clay for oats and barley samples, pressed paper for wheat) in growth chambers or in the greenhouse under controlled light, moisture, and temperature conditions. Four sets of soil mixtures identical to those in which the plants were grown were used as controls (Table 1). Hard red spring wheat, oats, and 2-row hybrid barley were planted in the soil-filled pots after they were kept at 18 ± 2 C for 1-3 days. Plants were exposed to continuous lighting from incandescent and fluorescent bulbs (2600-3600 ft cdl) except barley, which was grown in a greenhouse exposed to artificial light for 18 h and to sunlight during the brightest part of the day. They were watered automatically or once or twice daily, as required. The numbers of replicates for each experiment are given in Table 1.

The invertebrates in approximately 500 g of soil from each pot were extracted into 70% ethyl alcohol over a period of 7 days using an air-conditioned extraction device (Macfadyen 1962). The remaining soil in each pot within a group was pooled and two to five 20 g-samples and two 500 g samples were used for microbial and chemical analysis respectively.

Twenty-five g, dry weight, of each soil sample was serially diluted to 10^{-6} and plated out on Waksman's standardized medium (Waksman 1952) for bacteria, on rose bengal agar with mystatin for fungi, and on caseinate agar with mystatin for actinomycetes. Colonies were counted after 7 days' incubation at 21 ± 2 C.

Samples for chemical analysis were dried for 1 day at room temperature before testing. Analyses for texture, pH, electrical conductivity (mm HOS), carbonate ($CaCO_3$), nitrate (NO_3-N), nitrogen, phosphorus (P), and potassium (K) content were obtained using the standard soil testing techniques of the soil testing laboratory, University of Manitoba, Winnipeg.

RESULTS AND DISCUSSION

Faunal analysis

The agricultural clay soil contained 17 species of Acarina (Table 2) of which 15 are previously unreported from Manitoba. Oswald and Minty (1971) reported *Oppia minus* (Paoli) and *Tectocepheus velatus* Michael from forest soils and riverbank sites in Manitoba.

The number of invertebrate groups represented in the samples varied from 4-6 in the unfertilized controls (expt no. 1, 2) to from 7-10 in the fertilized control (no. 3) and the groups in which plants were grown to maturity (Table 3). In soil mixtures before incubation (no. 1, 6) only 4 kinds of invertebrates were found: both samples contained low levels of Collembola and relatively higher levels of pyemotid mites which may have decomposer roles. After incubation (with or without addition of fertilizer), 6-8 kinds of invertebrates occurred; in these samples, Collembola, mesostigmatids and pyemotids were common.

In control sample (no. 3), the addition of fertilizer did not increase the population of most invertebrates. Mesostigmata appeared for the first time and Collembola populations increased in soils after incubation (Table 3).

In samples in which wheat and oats were grown until maturity (no. 5, 8), striking increases in the populations of *Tyrophagus* were observed. Such a rise was not seen in soil containing young wheat or mature barley plants (no. 4, 8). It appears that wheat plants, when allowed to grow until maturity, provide an excellent environment for rapid population build-up of *Tyrophagus*.

Pyemotids thrived in all soil mixtures in which cereals were grown to maturity (particularly in barley and oat samples): the maximum number of pyemotids per sample in which oats, barley and wheat were grown was 1168, 1032 and 274, respectively. Although a few scutacarid mites *(Heterodispus* sp.) were present, a pyemotid species *Siteroptes absidatus* (Cross) was the main species of this type of trombidiform mites. The role of pyemotids in agricultural soil is not clear, although a related species, *Siteroptes cerealium*, is

Table 2. A systematic list of invertebrates in black clay soil from a cultivated agricultural plot in Manitoba

ANNELIDA

ARTHROPODA

ARACHNIDA

Acarina

Astigmata

Sancassania michaeli (Oudemans)

Sancassania sp.

Tyrophagus longior Gervais

Tyrophagus perniciosus Zachvatkin

Tyrophagus sp.

Cryptostigmata

Tectocepheus velatus (Michael)

Oppia minus (Paoli)

Protooribates sp.

Heterostigmata

Microdispus (Premicrodispus) sp.

Siteroptes absidatus Cross

Heterodispus capensis Paoli

Mesostigmata

Digamasellus sp.

Hypoaspis aculeifer (Canestrini)

Lasioseius arboreus (Chant)

Parasitus sp.

Protogamasellus primitivus Karg

Protogamasellus dispus (Genus et al.)

INSECTA

Collembola

Hypogastrura sp.

Homoptera

Aphididae

Table 3. Number of invertebrates (Mean and S D) in each sample in soils in which cereals were grown under artificial conditions. Cereals were not grown in controls.

	Acari											Insecta		
Expt. No.	Annelida	Anoetidae	Orbateile	Mesostigmata	*Sancassania*	Pyemotidae and Scutacaridae	Stigmaeidae	*Tarsonemus*	*Tyrophagus*	*Tydeus*	Unknown	Collembola	Homoptera
1			1.1 (±0.4)		2.1 (±0.8)	19.8 (±2.4)						9.3 (±3.3)	
2			1.0 (±1.0)	27.5 (±25.5)		4.0 (±1.0)			0.5 (±0.5)		1.0 (±0.0)	48.5 (±12.5)	
3	2.5 (+2.5)			27.5 (±1.5)	3.5 (±1.5)	16.5 (±16.5)			1.0 (±1.0)		3.0 (±3.0)	37.5 (±4.5)	6.0 (±6.0)
4									10.3 (±4.3)				
5	1.0 (±0.5)		0.5 (±0.2)	47.8 (±11.7)	3.6 (±0.7)	84.0 (±20.0)		6.1 (±2.5)	119.1 (±74.4)		0.8 (±0.2)	73.1 (±44.5)	5.8 (±2.0)
6				0.3 (±0.1)	2.4 (±0.8)	43.1 (±4.5)						9.4 (±1.7)	
7	43.9 (±26.1)	0.8 (±0.5)		52.8 (±15.0)	3.2 (±1.4)	254.2 (±78.2)			48.3 (±6.2)			152.3 (±24.6)	
8					0.3 (±0.2)	211.9 (±83.3)	8.2 (±2.3)		7.0 (±3.1)	0.8 (±0.2)	0.3 (±0.2)	1.4 (±0.7)	

known to attack growing cereals and other members of the grass family and to transmit soil-borne fungal parasites of grain such as *Fusarium poae* (Peck) and *Nigrospora oryzae* (Berk. & Br.) (Cooper 1940, Alfaro 1946, Cross 1965). It is possible that the increase of this type of mite in soil where no biotic or abiotic checks are operative could increase the incidence of fungal pathogens of cereals, especially oats and barley.

The presence of larger numbers of mesostigmatids, especially *Hypoaspis aculeifer* (Canestrini), could be in response to larger numbers of prey species, probably pyemotids and acarids.

Soils in which oats were grown and harvested (no. 7), contained relatively large populations of Collembola (Table 2).

Incubation and addition of fertilizer in control sample (Expt no. 3) did not disrupt the population balance in terms of a sudden rise in the numbers of 1 or 2 species, as did the growing of 1 type of cereal or 1 group of cereals (monoculture of wheat, oats and barley).

Annelids (mostly enchytraeid worms) were abundant only in soil mixtures in which oats were grown (no. 7); the maximum number of annelids found in a sample was 416.

Microbial analysis

Microbial analysis did not reveal any appreciable difference in the levels of fungi and actinomycetes after growth of cereals (Table 4). Bacterial levels, however, were higher in soil with wheat grown to maturity.

Chemical analysis

The levels of chemical components in soils in which cereals were grown did not change appreciably from the control soils (Table 5).

Ecological considerations

Because the present investigation is exploratory, limited in scope, and carried out under artificial conditions, speculations around the findings must be tentative and will have to be verified by further studies in the field. Despite these limitations, we have learnt that incubation is likely to increase the population of annelids, mites and Collembola. Large increases of certain invertebrate taxa may occur when cereals are grown until maturity on fertilized soil mixtures under simulated growing conditions. Growing of oats in such soils results in a large increase in the numbers of Collembola, pyemotid, acarid and mesostigmatid mites, and annelid worms. Growing of barley causes a large increase only in the numbers of pyemotids. Growing of wheat causes a large increase in the numbers of Collembola, acarid *(Tyrophagus)*, pyemotid and mesostigmatic mites. Therefore, it is possible that monoculture of wheat, oats or barley in agricultural soil can bring about some changes in the composition of the invertebrate fauna in the soil. This change is considerably greater in magnitude than that caused by the warming up of the soil alone. It is well known that balanced numbers of a variety of microfaunal species in tilled soils contribute to soil fertility (Edwards 1969). Continued growing of a particular species of cereal on the same land may increase the numbers of certain species and deplete the numbers of other species of invertebrates, thereby disrupting the balanced faunal numbers. The healthy growth of our agriculture and the protection of agricultural land will require careful monitoring of the associations of invertebrate species for two reasons: (a) the species with exploding population could be a crop pest previously unknown to agriculture, (b) soil fertility may be reduced by the disruption of the natural balance of animal numbers.

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Table 4. Mean number of microorganisms per g dry weight in pooled soil samples in which cereals were grown under artificial conditions. No plant was grown in controls.

Expt. No.	Sample group	No. of samples analyzed [a]	Fungi ($x10^6$)	Actinomycetes ($x10^6$)	Bacteria ($x10^6$)
1	Control, not incubated	5	0.05	0.2	3.8
4	Wheat, young plants	2	0.06	0.5	1.5
5	Wheat, mature	3	0.08	0.2	9.9
7	Oats, mature	3	0.05	0.4	1.1
8	Barley, mature	3	0.05	0.3	0.7

[a] Pooled samples with a number of replicates.

Table 5. Chemical components of pooled soil samples[a] in which cereals were grown under artificial conditions. All samples had mixed texture and medium $CaCO_3$ content.

Expt. No.	Sample group	pH	mm HOS	NO_3–N(ppm)	P (ppm)	K ppm
1	Control, not incubated	7.5	2.2	132.0	31.5	697.5
4	Wheat, young plants	8.0	0.4	6.8	58+	325.0
5	Wheat, mature	7.7	1.5	114.5	58+	414.5
6	Control[b] not incubated	7.6	2.5	149.0	32.0	470.0
7	Oats, mature	7.8	0.8	90.5	58+	557.0
8	Barley, mature	8.0	0.5	3.3	58+	475.0

[a] Means of 2 replicates.

[b] Separate control for oats and barley (different soil mixture).

Ottawa, for identifying the mites; and MERC/LIP Employment Plan 1972, Winnipeg, for providing financial assistance to the senior author. This study was also supported in part by a National Research Council of Canada operating grant.

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EFFECT OF INSECTICIDES AND METHODS OF APPLICATION ON THE SUGAR-BEET ROOT MAGGOT, AND ON PLANT STAND, ROOT DAMAGE AND YIELD OF SUGAR BEETS IN MANITOBA[1]

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ABSTRACT. Several organophosphorous and organocarbamate insecticides, tested in 1971 and 1973 in Manitoba, effectively reduced infestations of the sugar-beet root maggot, *Tetanops myopaeformis* (Roeder), on sugar beets, *Beta vulgaris* L. Aldicarb, carbofuran, Counter and BAY 92114 were the most effective of the chemicals tested and were associated with yield increases of up to 10 metric tons per hectare. Chlorpyrifos, fensulfothion, fonofos, pirimiphos-ethyl, phorate, AC 64475, BAS 2353 I and N-2596 were not considered suitable because of phytotoxicity, ineffectiveness, or inconsistency of performance from year to year. Furrow treatments were superior to nonfurrow treatments.

INTRODUCTION

Several organophosphorous and organocarbamate insecticides have been shown to protect sugar beets, *Beta vulgaris* L., against infestation and damage by the sugar-beet root maggot, *Tetanops myopaeformis* (Roeder) in Manitoba (Allen *et al.* 1969, 1971). Protection of the plants from damage was generally associated with increased yields of sugar beets. However, for various reasons, such as phytotoxicity with some methods of application, or the necessity to apply the insecticides at high rates to obtain satisfactory control, many of the insecticides were not considered practical for use in the control of the sugar-beet root maggot. Currently, only carbofuran and carbophenothion are registered for the control of the insect in Manitoba. Carbofuran has been shown to be more effective than carbophenothion (Allen *et al.* 1969).

This is a report on experiments conducted in 1971 and 1973 to assess the effect of other organophosphorous and organocarbamate insecticides, applied by various methods, on the sugar-beet root maggot, and on plant stand, root damage and yield of sugar beets. These experiments were initiated by the late Willard Ross Allen.

MATERIALS AND METHODS

The insecticides were used either as granular (G) formulations containing 5 to 15% active ingredients (5 to 15G) or as liquids prepared from emulsifiable concentrates (E.C.) (Table 1). The specific insecticides and rates of application used in the experiments are shown in Tables 2 and 3.

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3 Deceased.

Table 1. Description and sources of insecticides

AC 64475, Cyanamid of Canada, Scarborough, Ontario.

Aldicarb (Temik), Union Carbide of Canada Ltd., Toronto, Ontario.

BAS 2353 I 3,5-diethyl-phenyl-N-methyl-carbamate, B.A.S.F. Canada Ltd., Montreal, Quebec.

BAY 92114, Chemagro Corp., Kansas City, Missouri.

Carbofuran (Furadan), Chem. Div. F.M.C. Corp., Middleport, New York.

Counter (AC 92100) S-(tert-butylthio) methyl O, O, diethyl phosphorothioate, Cyanamid of Canada, Scarborough, Ontario.

Chlorpyrifos (Dursban), Dow Chemical of Canada Ltd., Sarnia, Ontario.

Fensulfothion (Dasanit), Chemagro Corp., Kansas City, Missouri.

Fonofos (Dyfonate), Stauffer Chemical Corp., Middleport, New York.

N-2596 S — (P-chlorophenyl) O-ethyl ethyl phosphorodithioate, Stauffer Chemical Corp., Middleport, New York.

Phorate (Thimet), Cyanamid of Canada Ltd., Scarborough, Ontario.

Pirimiphos-ethyl (PP-211) O[2-(diethylamino)-6-methyl-4-pyrimidinyl] O, O-diethyl phosphorothioate, Chipman Chemicals Ltd., Hamilton, Ontario.

Table 2. Effect of insecticide granules, applied in the soil above the seed, on seedling establishment, maggot control and yield of sugar beets at Rosenort Village, Manitoba, 1971.

	Stand		Maggots		Yield of harvested beets		
Insecticide [1]	Pre-thinning [2]	Post-thinning [4]	per beet	Control (%)	Number in 30m row	Weight g/beet	Metric tons per hectare
Aldicarb 10G	23.7 abc[3]	86 a	8.6 a	86	85 a	639[5]	32.13 a
Counter 15G	23.4 abc	87 a	13.2 ab	79	83 a	621	30.20 ab
Phorate 10G	21.0 c	86 a	18.7 abc	70	88 a	553	28.61 abc
Carbofuran 5G	24.0 a	93 a	20.5 abc	66	88 a	567	29.42 ab
Carbofuran 10G	25.1 ab	89 a	23.3 abc	63	87 a	603	30.54 ab
Fonofos 10G	21.9 bc	86 a	24.4 abc	61	85 a	594	29.48 ab
Fensulfothion 15G	22.2 abc	85 a	25.5 abc	59	83 a	608	29.48 ab
Pirimiphos-ethyl 10G	25.9 a	89 a	27.3 abc	56	88 a	572	29.30 ab
Chlorpyrifos 10G	25.2 ab	84 ab	29.0 bc	53	88 a	565	28.36 abc
AC 64475 10G	14.7 d	73 b	30.4 bc	51	72 a	620	25.85 bc
N-2596 10G	24.6 abc	95 a	36.8 c	41	87 a	571	28.92 abc
Untreated	23.8 abc	87 a	62.4 d	—	79 a	531	24.50 c

1 Toxicants applied at 1.12 kg active ingredient per hectare.

2 Number of beets per 2.54 m length of row.

3 Means followed by the same letter are not significantly different at the 1% level (Duncan 1955).

4 Number of beets per 30 m of row.

5 Differences between means are not significant (P = 0.05).

Table 3. Effect of insecticides and placement on stand, numbers of maggots, maggot damage and yield of sugar beets at Kronstal Village, Manitoba, 1973

Insecticide	Type of Application	Toxicant (kg/ha)	Stand		Maggots per beet	Root damage rating	Yield of harvested beets		
			Pre-thinning[1]	Post-thinning[3]			Number in 30 m row	Weight g/beet	Metric tons per hectare
Aldicarb 10G	Furrow	1.12	13.8 ab[2]	75 abc	4.4	1.60 a	73 abc	771 a	32.44 a
Aldicarb 10G	Furrow	0.84	18.3 a	82 a	—	1.64 ab	79 ab	692 abcd	31.97 ab
Aldicarb 10G	Furrow	0.56	18.3 a	83 a	9.2	1.75 abc	81 ab	709 abc	33.27 a
Counter 15G	Furrow	1.12	18.9 a	84 a	12.5	1.85 abc	81 ab	673 abcd	31.63 abc
Carbofuran 5G	Furrow	1.12	18.1 a	78 a	7.6	1.90 abcd	78 ab	691 abcd	31.48 abc
BAY 92114 10G	Furrow	1.68	18.9 a	83 a	15.3	2.03 bcde	80 ab	652 bcd	30.51 abcd
BAY 92114 10G	Furrow	1.12	18.5 a	86 a	—	2.06 cde	83 a	636 cd	30.92 abcd
Fensulfothion 15G	Furrow	1.68	13.8 ab	78 ab	—	2.18 de	76 ab	631 cd	27.76 cde
Fonofos E.C.	Dribble	1.12	12.8 ab	64 bc	—	2.16 cde	62 c	689 abcd	24.82 ef
BAS 2353 I 10G	Furrow	1.68	10.9 b	61 c	—	2.15 cde	60 c	760 ab	26.20 ef
BAS 2353 I 10G	Furrow	1.12	14.4 ab	73 abc	—	2.40 ef	72 abc	654 bcd	27.35 de
Fonofos 10G	Furrow	1.12	18.3 a	83 a	17.5	2.30 def	80 ab	601 cd	28.29 bcde
BAY 92114 10G	Band	1.68	17.8 a	80 a	—	2.21 de	77 ab	632 cd	28.18 bcde
Fensulfothion 15G	Band	1.68	18.0 a	81 a	—	2.63 fg	79 ab	596 cd	27.31 de
Untreated	—	0	17.3 a	73 abc	23.8	3.00 g	68 bc	580 d	23.07 f

1 Number of beets per 2.54 m length of row.

2 Means followed by the same letter are not significantly different at the 1% level (Duncan, 1955).

3 Number of beets in 30 m of row.

In each of the experiments, monogerm seed of the sugar beet cultivar CS43 was seeded in plots arranged in a randomized block design. The blocks were replicated 6 times in 1971 and 8 times in 1973. Each plot consisted of 4 rows 18 m long spaced at 56 cm. Four guard rows were provided on each side of each block. The seed was released from seeder boxes of a 4-row planter, equipped with double-disc furrow openers, at a depth of 2 to 2.5 cm at a rate of 24 seeds per m of row. In both years ammonium phosphate (11-55-0) was applied to the seed furrow at 40 kg/ha. The plots were seeded on May 12 in 1971 and on May 28 in 1973.

In 1971, the insecticides were applied as granules in the furrow at 1.12 kg/ha. The granules were delivered from cone-seeders into tubes behind planter discs and released above the seed as the furrow closed, then lightly covered with soil subsequently firmed by press-wheels. Immediately after seeding, ammonium nitrate (34-0-0) was broadcast over the plot area to supply 95 kg of nitrogen per ha. In mid June the rows of seedlings were side-dressed to a depth of 15 cm with Triple superphosphate (0-46-0) at the rate of 123 kg/ha.

In 1973, insecticides were applied at various rates into the furrow and as bands above the furrow as follows:

Furrow treatment: Granules were applied as described for this method in the experiment in 1971.

Band treatment: Granules were released from spreaders suspended 2.5 cm above the closed seed furrow and applied in a 10 to 13 cm band, then firmed by press-wheels and lightly covered with soil by drag-bars.

Furrow dribble treatment: An emulsion concentrate of fonofos in 97 litres of water per ha was metered into the furrow, with the seed, by a "John Blue" squeeze pump. Ammonium nitrate (34-0-0) was broadcast over the plot area in mid June at the rate of 78.5 kg nitrogen per ha.

The amount and type of fertilizer used from year to year depended on soil requirements and the progress of the season. For example, Triple superphosphate was not applied in 1973 because there is little response by sugar beets to fertilizer applied after mid June.

In each experiment, pre-thinning stands of seedlings were determined by counting the number of beets in two 2.54 m lengths of row in each plot. In 1971, post-thinning stands were determined by counting the beets in a 7.6 m length of row from each of the two centre rows of each plot. In 1973, post-thinning stands were determined by counting the beets in two 15 m lengths of row. The beets in these lengths of row were subsequently harvested for yield.

In both years, the emergence and flight periods, of the adult of the sugar-beet root maggot, were determined with water traps (Harper and Story 1962).

In late August 1971, maggots were counted in soil samples, 20 cm^2 x 35 cm deep, under each of 10 beets in outside rows of each plot. In late August 1973, the infestations were similarly determined, but for 7 treatments only (Table 3).

Ratings of damage to roots by the maggot were made only in 1973. In that year, 10 roots per plot were rated in mid July according to the following categories: 1 = no damage; 2 = light damage, <3 small feeding scars; 3 = moderate damage, $<1/2$ root area scarred; 4 = heavy damage, 1/2 to all of root area scarred; 5 = severe damage, beet dying or dead. These damage ratings were based on methods of Y.M. Yun, Great Western Sugar Company, Longmont, Colorado and R.D. Frye, North Dakota State University, Fargo, North Dakota (Personal communication, 1972).

In 1971 and 1973, beets from two 15 m lengths of the two centre rows of each plot were harvested and weighed in late September to determine yield.

All data were examined by analysis of variance and the multiple range test (Duncan 1955); significance was tested at the 1% level unless otherwise indicated.

RESULTS AND DISCUSSION

Adult flight patterns and maggot infestations

In 1971, adults were collected in water traps from June 8 to August 3. The greatest number, 50-100 adults per day, was collected from June 18 to July 12 and conditions were favorable for oviposition and survival of maggots. In August, there was an average of 62 maggots per plant in plots that were not treated. Despite this heavy infestation, the beet roots were not malformed, nor were many plants destroyed, because the plants were large (10 mm dia.) and well established at the time of infestation in mid June. However, at harvest, beet yields in the untreated plots were severely depressed (Table 2).

In 1973, almost twice as many adults were collected as in 1971 even though the collection period, June 9th to July 23, was shorter. However, the intensive flight period lasted only a short time, June 12 to 23, and the cool, wet weather probably did not favor oviposition and maggot establishment. Consequently, an average of only 24 maggots was recovered from untreated beets. Nevertheless, as the beets were small (3 mm dia.) when oviposition commenced, there was severe root damage, plants were destroyed and weights of beets on untreated plots were depressed as compared to the better insecticide treatments (Table 3).

Phytotoxicity

In 1971, pre-thinning stands of beets were reduced significantly in plots treated with AC 64475 when compared with stands in untreated plots (Table 2). The phytotoxic effect of the chemical was still apparent in the post-thinning stand and at harvest. There was also a significant reduction in stands of beets on plots treated with phorate, compared to those treated with carbofuran, pirimiphos-ethyl or chlorpyrifos. There were significantly fewer beets in stands on the untreated plots than in stands on plots treated with the latter 3 insecticides, probably because some plants were lost due to maggot feeding in the untreated plots.

In 1973, pre-thinning stands of beets were reduced significantly in plots treated with a furrow application of BAS 2353 I at 1.68 kg/ha (Table 3). This was also evident in post-thinning stands and at harvest. Pre-thinning stand reductions of nearly 20% occurred in plots treated with a furrow application of aldicarb at 1.12 kg/ha, BAS 2353 I at 1.12 kg/ha, fensulfothion at 1.68 kg/ha and fonofos E.C. at 1.12 kg/ha. These differences were not statistically significant probably because of the high coefficient of variation in the experiment. More replication would have increased the likelihood of detecting significant differences (Burnett and Baker 1973). Aldicarb reduced pre-thinning stands at 1.12 kg/ha, but not at 0.84 or 0.56 kg/ha. Similarly, fonofos E.C. at 1.12 kg/ha markedly reduced the pre-thinning stand when dribbled into the furrow as a liquid, but it was not phytotoxic when applied in the furrow as a granular formulation at the same rate (Table 3). Fensulfothion applied as a band treatment on the soil surface was not phytotoxic, but when applied in the furrow there was a reduction of more than 20% in the pre-thinning stand. The phytotoxic effect of fonofos E.C., like that of BAS 2353 I, was evident at post-thinning and at harvest when beet stands were reduced by about 25%.

In plots where there was no obvious phytotoxic effect due to the insecticides, there were generally more plants than in the untreated check. As noted by Allen *et al.* (1969), this may have been due to control of other pests, such as cutworms, which contribute to loss in beet stands.

Effectiveness of treatments

In 1971, all the insecticides were applied in the furrow and all provided significant reductions ($P \leq 0.01$) in maggot infestations which were associated with increased yields (Table 2). Plots treated with aldicarb provided the best control of maggots and gave the highest yields of beets. There was a trend toward lower yields as numbers of maggots increased due to less effective control. With the exception of phorate, insecticides that

reduced the infestation to fewer than 29 maggots per beet were associated with yield increases ($P \leq 0.01$) of 5 to 7.5 metric tons/ha. Phorate was phytotoxic to the pre-thinning stand and the toxic effect appeared to have persisted and affected beet development. The weight per beet in plots treated with phorate was the lowest of those treated with insecticides and was not significantly different from that of the check. AC 64475 was phytotoxic to beet seedlings, but apparently did not affect subsequent development of the surviving beets as indicated by the high weight per beet at harvest (Table 2).

In 1973, all the insecticidal treatments, except the band application of fensulfothion, provided control of the maggot, as reflected by a significant reduction ($P \leq 0.01$) in root damage compared to that of the untreated check (Table 3). The reductions were associated with significant increases ($P \leq 0.01$) in yield, except with fonofos E.C. at 1.12 kg/ha and BAS 2353 I at 1.68 kg/ha, where initial severe phytotoxicity reduced the number of beets available for harvest. As in 1971, treatments with aldicarb were associated with the best controls and highest yields, even at the lowest rate of application where the yield per hectare was 10 tonnes greater than in controls. Counter and carbofuran were also effective. Again, as in 1971, there was a general trend to lower yields with treatments that provided less effective controls. The furrow treatment of BAY 92114 was superior to a surface band treatment of the same material. In general, the furrow treatments were more effective than the nonfurrow treatments.

CONCLUSIONS

Results of the present study confirm those of previous experiments (Allen *et al.* 1971) that a furrow treatment of aldicarb is superior to carbofuran, applied in the same manner, for the control of the sugar-beet root maggot. Nevertheless, carbofuran, currently the most commonly used insecticide for this insect, provided adequate control as indicated by the 25 to 36% gain in yield. Furrow treatments of Counter and BAY 92114 were in the same range of effectiveness as carbofuran.

Most of the other chemicals tested were not considered suitable because of phytotoxicity, ineffectiveness, or inconsistency of performance from year to year.

In this study, furrow treatments were superior to band treatments which Allen *et al.* (1969) had shown to be effective. The dry surface soil and wind conditions that prevailed in 1973 may have reduced the effectiveness of the band treatments.

It is concluded that the application of the lowest effective dose of an insecticide close to the target area, but not in direct contact with the seed, is the most effective method of control for the sugar-beet root maggot in Manitoba. Low dosages will reduce cost and the risk of contamination from chemical residues.

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BOOK REVIEW

"*Insect Population Ecology* an analytical approach." By G.C. Varley, G.R. Gradwell, M.P. Hassel. (Blackwell Scientific Publications: London, December 1973). Pp. X + 212, illus. £2.75; Can. $6.40.

Many methods of analysis are now available to ecologists. The correct analysis to measure insect population changes involves a combination of conjecture, skill and experience. This book is one of the few attempts to develop, in unison, an analytical approach which combines theory, experiments and observations. The basic theme of the book is to explain simply and clearly how census information of insect life stages (life tables) can be used in the interpretation of population changes; detailed and diverse statistical treatments of population numbers are excluded.

There is a balanced blend of theory, mathematics, and examples from field and laboratory studies of insect pests of agricultural and forest crops. Chapter 1 introduces the theme and shows some of the ways in which population figures can be expressed. Chapters 2 and 3 consider competition between individuals and between species for limited resources. Chapter 4 discusses predator-prey interactions and includes some of Hassell's elegant studies on parasite searching behaviour. Chapter 5 examines the effects of weather and climate on insect populations. Chapters 6, 7 and 8 develop the use of life tables from simple examples to more complex cases in which incomplete life table data can still help in the understanding of the outbreaks of forest insect pests. The last chapter gives a short review of the more classical examples of biological control and considers the practical application of natural enemies in pest control. A section on exercises and experiments for each chapter is appended at the back of the book.

The book is well organized, well written and well illustrated. Although the bibliography is not extensive it contains a wide variety of the relevant examples of the older and more recent published insect population studies. A synopsis at the beginning of each chapter is also helpful to the reader.

Life table analysis is a useful tool in understanding insect populations which commonly have 1 generation per year; the technique expresses mortality (K-values) from stage to stage on a logarithmic scale. As with most insect population studies in the field this analytical approach is limited by the amount and types of data collected. It tends to be a static analysis of population change because it lacks the dynamic aspect of incorporating and predicting how numerous biotic and abiotic factors may affect a population at any one time. In addition, this method of analysis is inapplicable to animals which have overlapping generations (e.g. Aphids), where many stages are found living in the same environment, or where animals migrate (e.g. locusts).

As a text for research students in ecology the book has deficiencies. It omits consideration of problems concerned with energy flows and other dynamic aspects of insect communities. Although strong on the clarification of basic classical principles it lacks any new concepts that would encourage students to pursue ecosystem analysis using such techniques as multivariate analysis and computer simulation of population models as predictive tools.

I recommend the book to students of ecology and population analysts who want to have a practical guide to the interpretation of insect population changes using the life table approach.

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ROBERT DONALD DIXON 1938 – 1974

Robert (Bob) Dixon passed away suddenly at age 36 in Edmonton, Alberta, the 22nd of November 1974.

Born in Edmonton in 1938, Bob at the age of 10 moved with his parents to Sheraton, Manitoba. After completing elementary school in 1954, he worked as a labourer until he joined the Canadian Army in 1955. After his discharge in 1958, he returned to Northern Manitoba where he worked as a fisherman and miner.

In September 1960 at the age of 22, he entered high school, graduating in 1963. Possessing an interest in entomology he then entered the Faculty of Agriculture, University of Manitoba, graduating in 1967 with the degree of B.ScA. and obtaining a M.Sc. in 1968. His graduate work involved research on mosquito behaviour and their control.

In 1968 he joined the Alberta Department of Agriculture as Entomologist working on a wide spectrum of entomological problems. In 1970 he became Head of the Plant Industry Laboratory, the position he held at the time of his death.

He was a member of the Entomological Society of Manitoba, Entomological Society of Alberta, and the Entomological Society of Canada. In addition he was an active member of The Canada Committee on Biting Flies, The Alberta Mosquito Control Association, and Northwest Mosquito and Vector Control Association, and many other provincial committees. He was Co-ordinator of Biting Fly Research in Alberta and a leader in the establishment of urban mosquito abatement programs in Western Canada.

Bob was a true agricultural entomologist in that he was always concerned with the agricultural community and its problems in relation to insects.

Bob enjoyed a short but fulfilling entomological career, and as a man was admired by many. He had a love for nature and enjoyed many outdoor activities. He is remembered with great affection by his many colleagues and friends.

M.G. Dolinski

NOTICE TO CONTRIBUTORS

1. The Manitoba Entomologist is printed annually and publishes articles on all phases of entomology. Each paper should contain the results of original research or review, in depth, some aspect of entomology. While the primary aims are to publish material of regional interest, papers of interest to other geographic areas or of general interest will be accepted.

2. Manuscripts should be prepared according to instructions described in the CBE Style Manual Third Edition, published by the American Institute of Biological Sciences, 3900 Wisconsin Ave., N.W., Washington, D.C. 20016, U.S.A.

3. Manuscripts should be submitted in duplicate, including the original and one carbon copy, on 8½ x 11 paper, double spacing the entire manuscript. For the correct format, please follow "Ecology". Each manuscript over two typescript pages should include an abstract not exceeding 200 words.

4. Tables and illustrations should be clear and concise, kept within reasonable limits, and should not repeat material presented in the text. Notations identifying the author and title should be made lightly in pencil on the back of each illustration. Tables should be typed separately, one to a page at the end of the manuscript.

5. Each manuscript is reviewed by at least one external referee, who will check for scientific content, originality, and clarity of presentation.