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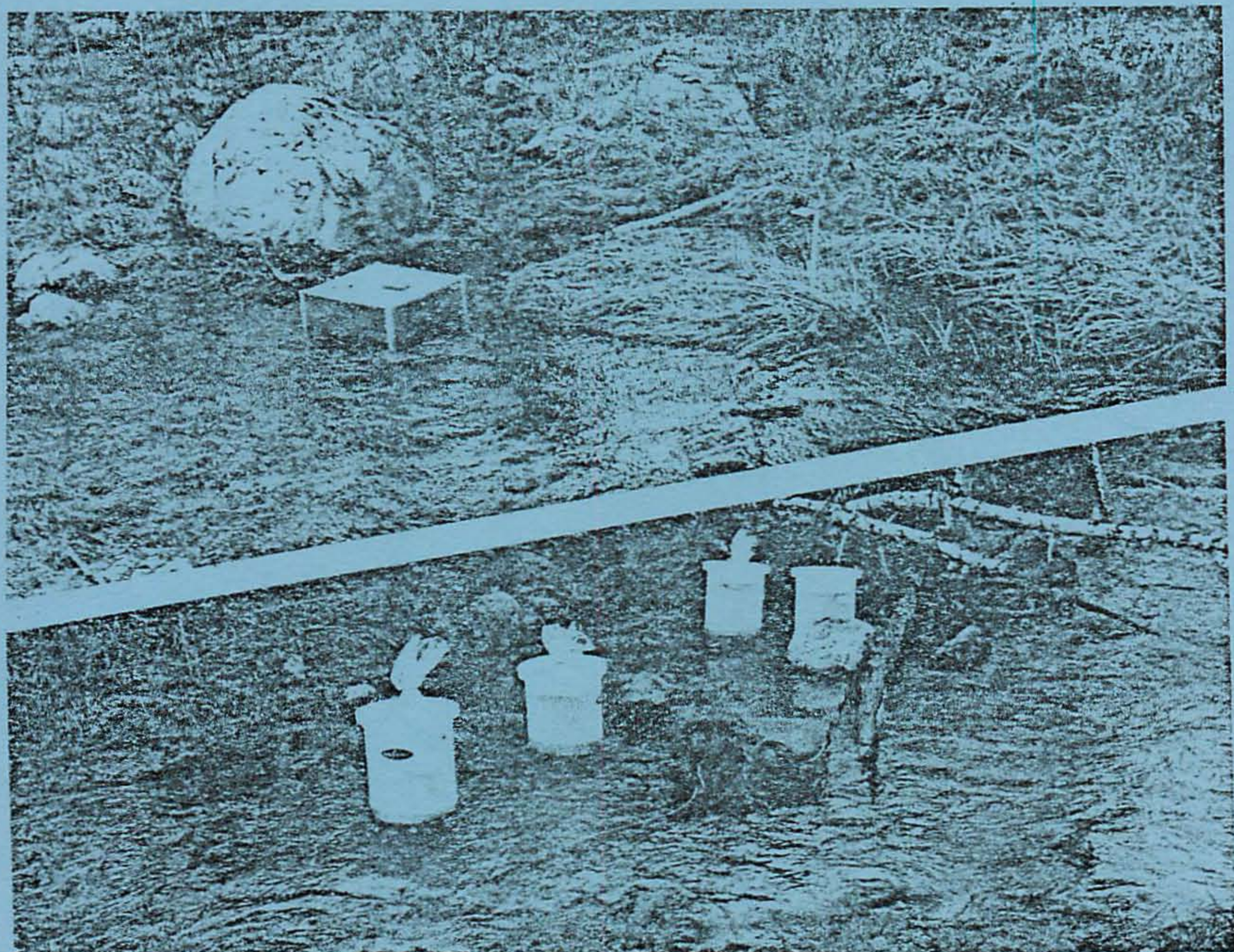
FIELD EVALUATION OF THE ENVIRONMENTAL IMPACT OF NONYL PHENOL

Edited by J.A. Armstrong and P.D. Kingsbury

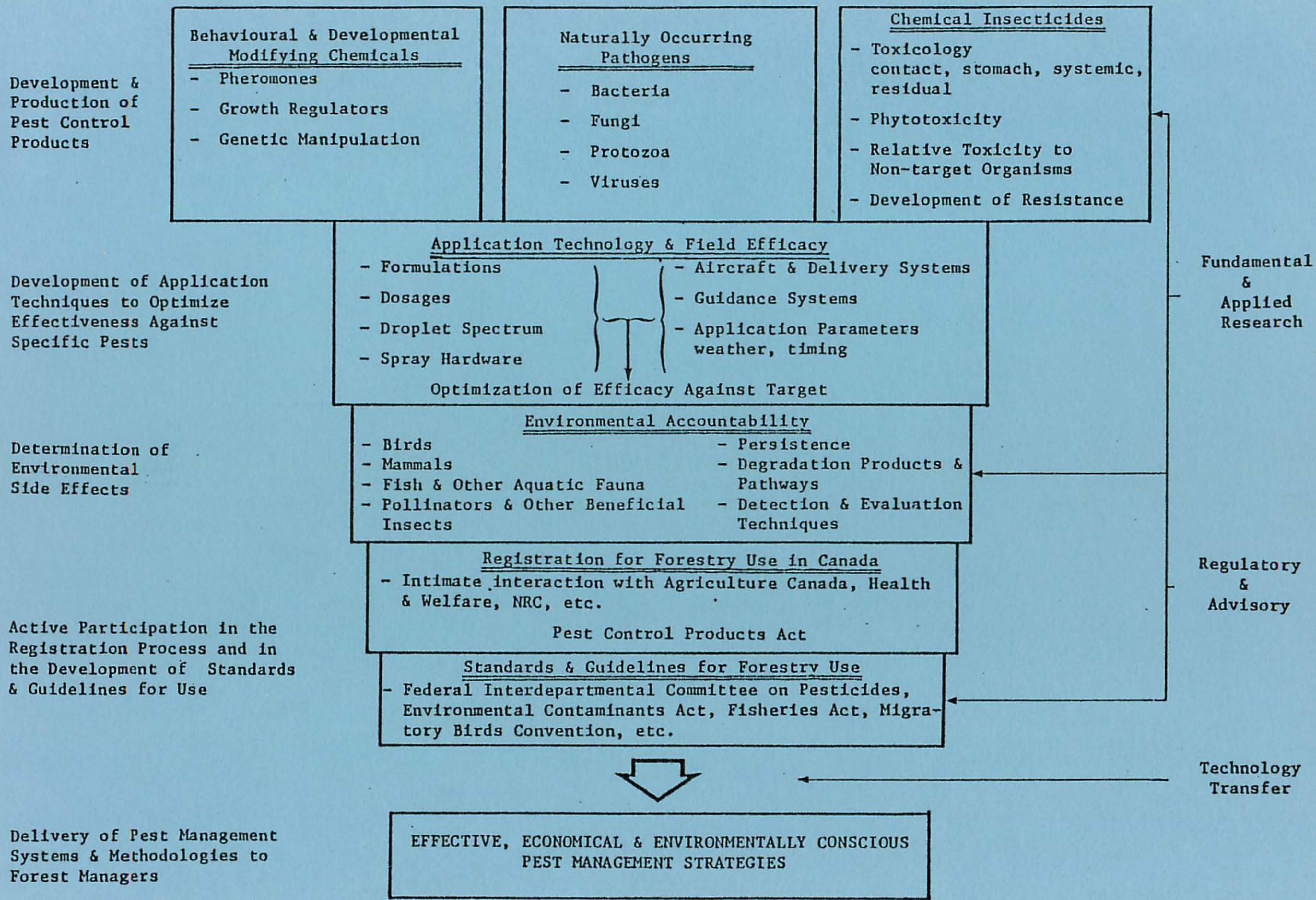


FOREST PEST MANAGEMENT INSTITUTE
CANADIAN FORESTRY SERVICE
SAULT STE. MARIE, ONTARIO

INTERIM PROGRESS REPORT

SEPTEMBER, 1979

THE PROGRAM OF THE FOREST PEST MANAGEMENT INSTITUTE



FIELD EVALUATION OF THE ENVIRONMENTAL IMPACT OF NONYL PHENOL

AN INTERIM PROGRESS REPORT ON STUDIES CARRIED OUT BY

THE FOREST PEST MANAGEMENT INSTITUTE

IN 1979

EDITED BY J. A. ARMSTRONG AND P. D. KINGSBURY

SEPTEMBER 1979

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INTRODUCTION

Early in 1979 the Pesticides Section of Agriculture Canada received a report from fisheries researchers at the St. Andrews Biological Station dealing with laboratory toxicity studies on the carbamate insecticide aminocarb and its formulation. The researchers reported that nonyl phenol, a component of commercial aminocarb (Matacil®) formulations, was considerably more lethal to juvenile Atlantic salmon, *Salmo salar* L. than pure aminocarb, and on the basis of this finding raised objections to the use of aminocarb formulations containing nonyl phenyl in spruce budworm, *Choristoneura fumiferana* Clem., control programs. In response to these questions about the safety of current aminocarb formulations, officials of Agriculture Canada convened a meeting of representatives of concerned federal agencies and industry to develop a common understanding regarding the nonyl phenol toxicity question and to decide on necessary actions to be taken. Following this meeting a policy statement was issued by the Pesticides Section indicating that, based on an assessment of currently available data it had been decided that the registration status of Matacil® formulations would not be altered. The main factors taken into account in making this decision were: the extensive field monitoring data available demonstrating the lack of harmful effects on both aquatic and terrestrial ecosystems from aminocarb formulations applied in actual spray operations, the calculated safety factors between toxic levels and potential nonyl phenol residues in water under "worst case" conditions, the desirable quality of nonyl phenol's low toxicity to mammals and safety to humans, and the difficulties involved in changing existing aminocarb formulations and adequately testing new formulations for their safety and effectiveness prior to 1979 control operations with Matacil®. At the same time, steps were taken to initiate further investigations by industry and government agencies into the behavior of nonyl phenol in aquatic systems, in order to provide more information upon which the potential hazards of this material could be adequately evaluated.

The Forest Pest Management Institute has played a lead role in generating field data applicable to Canadian forestry situations on the environmental impact of insecticides of current or potential usefulness in forest pest control programs. In light of the concern over possible effects of nonyl phenol, this Institute carried out a field evaluation in 1979 of the environmental impact of this material with the objective of providing pertinent data to better evaluate the hazard of continued use of this solvent in insecticide formulations applied to Canadian forests. The main focus of the study was to document the fate, persistence and biological significance of nonyl phenol applied to an aquatic system under conditions similar to actual forest pest control operations. Studies on the fate of nonyl phenol in terrestrial systems and its effects on terrestrial fauna were also carried out.

The studies reported herein represent the combined efforts of numerous members of the Forest Pest Management Institute staff. Plot layout, spray formulation, deposit assessment and spruce budworm sampling were organized and conducted by A. Obarymskyj, L. Smith, B. Tomkins and B.F. Zylstra; meteorological assessment was conducted by J.W. Beveridge; spray application was carried out by L.B. Pollock. Details of these aspects of the field application procedures were assembled and reported by B.F. Zylstra. Residue studies were planned and carried out by K.M.S. Sundaram, S. Szeto, R. Hindle and D. MacTavish and reported by S. Szeto. Environmental impact studies were planned and co-ordinated by P.D. Kingsbury and B.B. McLeod with S.B. Holmes responsible for aquatic studies, R.L. Millikin responsible for bird and terrestrial insect knockdown work and K.L. Mortensen responsible for the honeybee program. G.M. Howse of the Great Lakes Forest Research Centre co-operated in this program by supervising spruce budworm counting and defoliation assessment in the laboratory and making the results available for inclusion in this report.

STUDY SITE

Field studies were carried out in Algoma District, Ontario just north of the Dubreuilville airstrip, which was used for mixing and loading operations (Fig. 1). A small, unnamed stream approximately 3 km in length flowing into the Magpie River 3 km north of the town of Dubreuilville was used as the nonyl phenol treatment stream. A 40 ha rectangular block centred on the lower portion of the stream and bisected by an access road was the site of the terrestrial studies (Fig. 2).

For descriptive purposes the nonyl phenol treatment stream is best divided into two sections. The lower section, which is wholly within the spray block, has two distinct riffle areas separated by an old beaver pond. The riffle areas have moderately fast currents over boulder and coarse gravel bottoms. Siltation is evident in areas where the current is reduced by natural obstructions and small pools. In the pond the current is very slow and silt and organic detritus has been deposited to a considerable depth over the boulder bottom. The area around the stream in this section has been almost completely cut over leaving only a fringe of small trees bordering the stream. Alder, *Alnus rugosa* (DuRoi) Spreng., and white birch, *Betula papyrifera* Marsh., are the predominant species with a few white spruce, *Picea glauca* (Moench) Boss., and jack pine, *Pinus banksiana* Lamb. The upper section of the stream, which is only partly within the spray block, meanders slowly over a fine gravel and sand bottom. Most of the stream bed is covered to varying depths by silt and organic detritus. The bank vegetation in this section is primarily low grasses and sedges and the stream is completely open to the sky.

Fig. 1 NONYL PHENOL BLOCK LOCATION

Aerial View

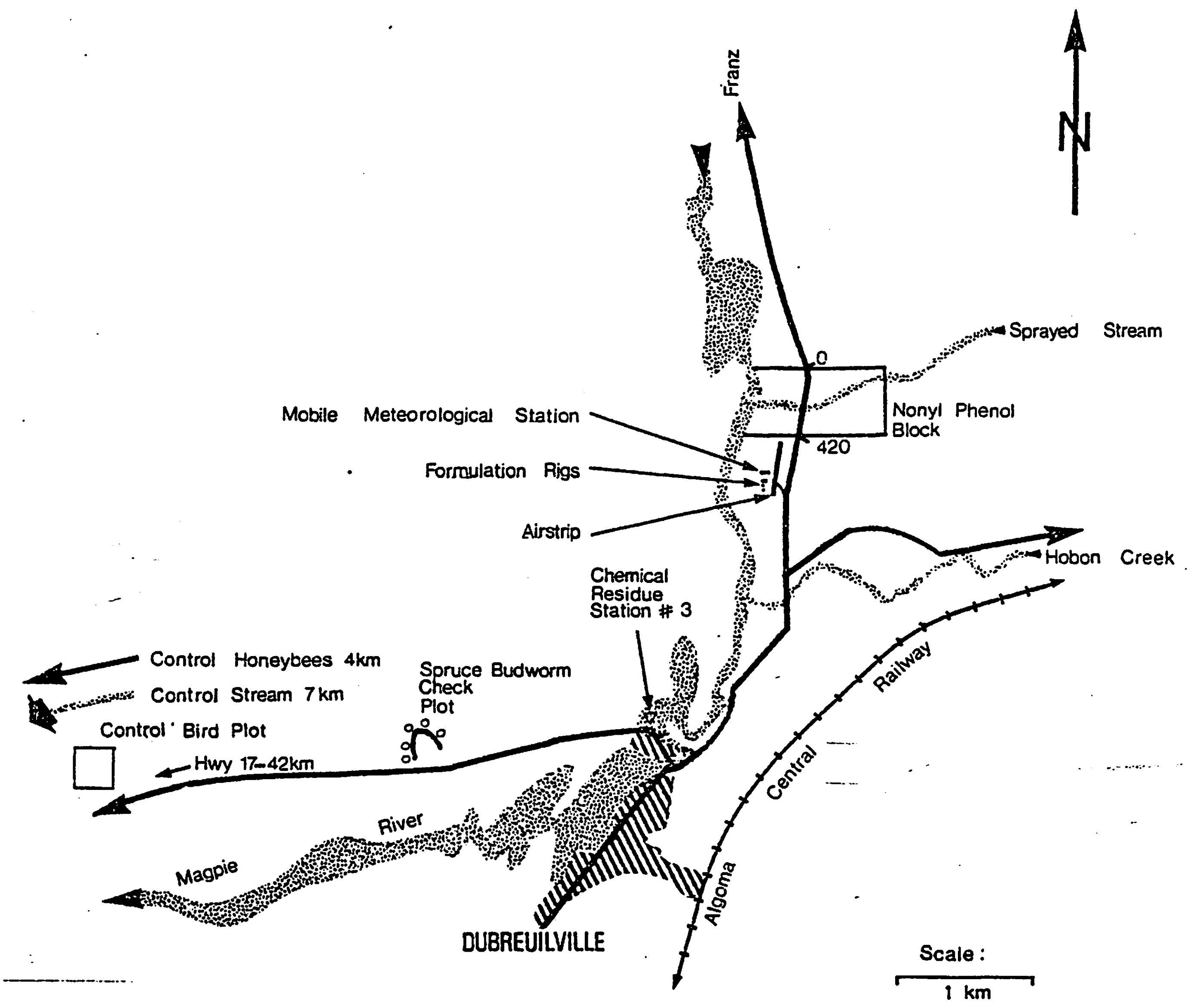
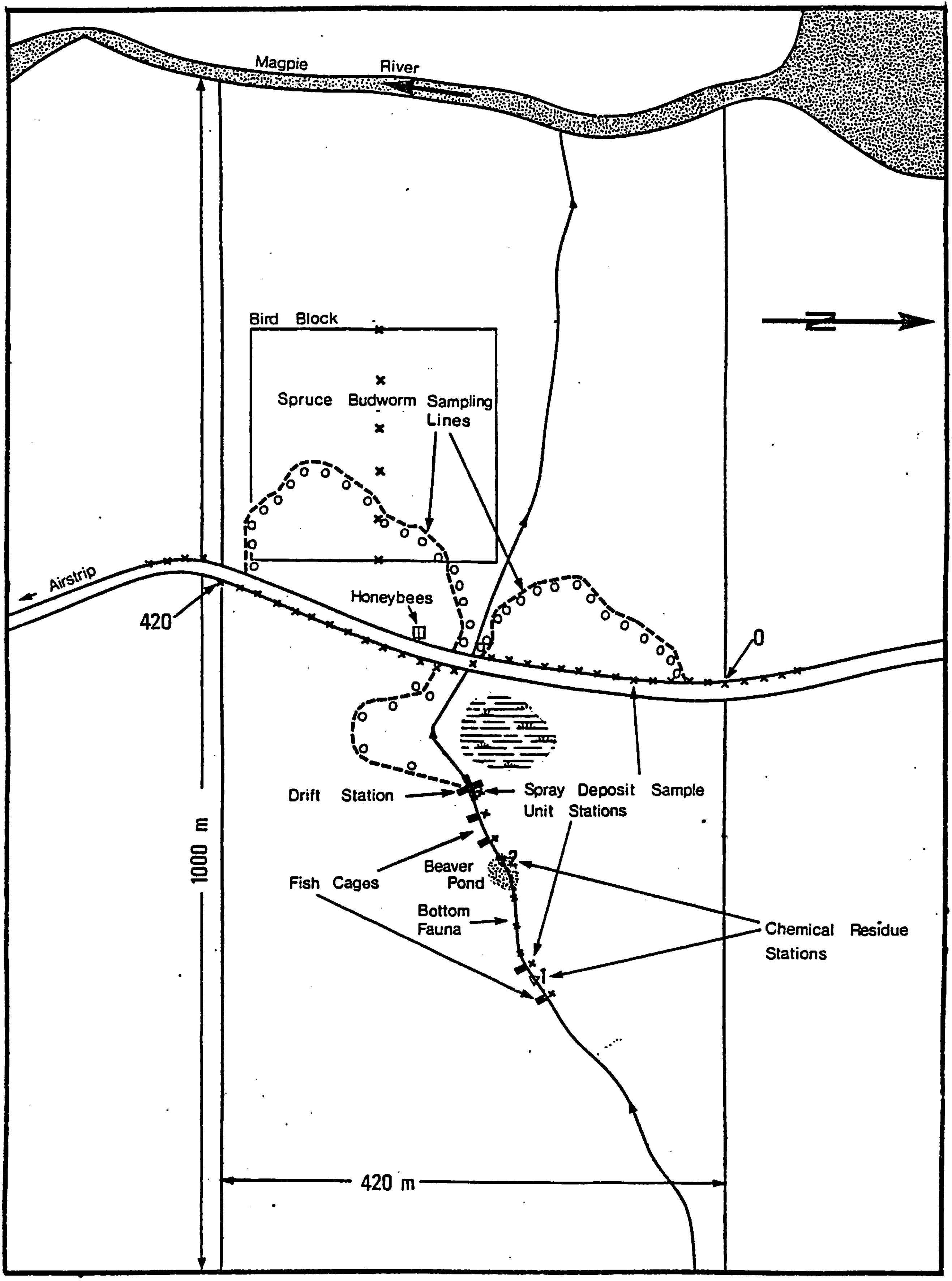


Fig. 2 DIAGRAM OF EXPERIMENTAL BLOCK



An unnamed stream approximately 12 km west of Dubreuilville was used as the untreated control for aquatic studies. This stream flows out of an old beaver pond through a stretch of riffle area approximately 200 m in length and into a lake. The riffle portion has a moderately fast current over a boulder and coarse gravel bottom, and is bordered by alder, black spruce, *Picea mariana* (Mill.) B.S.P., beaked hazelnut, *Corylus rostrata* Ait., honeysuckle, *Lonicera* sp. L., and elderberry, *Sambucus* sp. (Tourn.) L. In the pond the current is very slow and the boulder bottom is covered with a thick layer of silt and organic detritus.

Total streamflow measurements taken on 30 May 1979 were 0.19 m^3/sec in the nonyl phenol treatment stream and 0.07 m^3/sec in the nonyl phenol control stream.

A 4-hectare bird census plot was set up within the nonyl phenol treatment block on the south side of the treatment stream between the Magpie River and the access road (Fig. 2). The bird plot was located in a mixed stand of second growth jack pine and trembling aspen, *Populus tremuloides* Michx., with scattered white birch and black spruce. An untreated control bird census plot was set up about 6 km southwest of the treatment plot along the Dubreuilville road. The plot was located on uneven terrain, falling off to a stream on the north and west sides. The stand had been selectively cut; second growth species were white birch, black spruce, balsalm fir, *Abies balsamea* (L.) Mill., and speckled alder in order of predominance.

Two colonies of domestic honey bees, *Apis mellifera* L., were set up near the centre of the nonyl phenol treatment block and two colonies were set up along the Dubreuilville road about 10 km to the west to serve as untreated control.

Although the treatment block was selected primarily to conform to requirements for environmental impact studies, there was enough balsam fir and white spruce within the block that 25 trees of each species could be sampled to monitor spruce budworm population levels. These fifty trees ranged between 8 and 20 m in height and were located along three sample lines within the boundaries of the spray block. Spruce budworm samples were also collected from fifteen balsam fir and fifteen white spruce located on an untreated check plot 4 km away along the Dubreuilville road.

SPRAY APPLICATION

The environmental impact studies carried out were designed to evaluate the effects on non-target fauna of exposure to the highest dosage of nonyl phenol that would be applied to forested areas under actual operational pest control programs. To this end, the dosage of nonyl phenol applied to the treated stream and plot was 0.47 ℓ/ha, equivalent to the quantity of nonyl phenol applied to an area receiving the seasonal maximum allowable dosage (a total of 0.175 kg/ha applied in two 0.088 kg/ha applications) of aminocarb formulations containing this solvent. The conventional total emission rate of 1.46 ℓ/ha was duplicated by mixing the nonyl phenol in an appropriate quantity of insecticide diluent 585. A small quantity of Automate "B" red dye was added to the spray mixture to facilitate deposit assessment.

The actual spray mixture consisted of:

Nonyl phenol[1]	30.28 ℓ	(32% by volume)
insecticide diluent 585[2]	62.46 ℓ	(66% by volume)
automate "B" dye	1.89 ℓ	(2% by volume)

Spraying was carried out using a Cessna 185 Sky Wagon equipped with micronairs. The aircraft delivery system had been previously calibrated to emit 1.46 ℓ of spray liquid per hectare based on a swath width of 60 m; aircraft speed of 177 km/hr; spray pressure of 40 p.s.i.; and a variable restrictor unit (VRU) setting of 8.

Spraying commenced on 29 May, 1979 at 0710 hours on the south (down-wind) side of the block and then progressed at 60 m swaths to the north (upwind) side of the block. After completion of the block, a single swath application was emitted over a portion of the treatment stream outside the block starting at the eastern block boundary and for a distance of about 0.5 km upstream.

Meteorological measurements taken at canopy height (12 m) the morning of spray application are presented in Table 1. The complete cloud cover and cool, calm conditions favored a good deposit of the emitted formulation.

DEPOSIT ASSESSMENT

Deposit sampling stations were placed in a deposit assessment line along the access road bisecting the nonyl phenol treatment block and at suitable locations in the vicinity of environmental impact study sites within the block (Fig. 2). Deposit stations along the deposit assessment line consisted of 30 cm long aluminum stakes with a 10 x 10 cm aluminum tray fastened to the top of the stake. Similar stakes were used for stream bank deposit stations while 1 m stakes were used to place deposit stations in midstream. Sampling stations along the deposit assessment line were laid out at 15 m intervals beginning at the north boundary of the block (zero, start) and ending at 420 m, the south boundary of the block. Spray drift deposit stations were placed at - 15 m beyond the zero station and 435 to 480 m

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[1]Rohm and Haas Canada Ltd., West Hill, Ontario.
[2]Shell Canada Ltd., Toronto, Ontario.

Table 1

Meteorological measurements at canopy height on 29 May 1979*, Dubreuilville airstrip

Time	Temperature °C	Relative Humidity %	Wind m/sec	Wind Direction**	Pressure mb	Solar radiation cal/cm^2
05:00	8.0	98	0.9	18°	973.8	-
05:15	8.0	98	0.4	30°		-
05:45	8.0	98	0.3	10°		-
06:00	8.2	98	0.9	22°	974.0	-
06:15	8.2	98	0.4	45°		-
06:30	8.2	98	0.7	33°		-
06:45	8.2	98	0.2	12°		-
07:00	8.4	98	0.4	6°		-
07:15	8.5	98	1.0	18°	974.4	0.10
07:30	8.7	98	0.8	28°		0.10
07:45	8.8	98	0.7	1°		0.15
08:00	9.0	98	0.4	39°	974.6	0.15

* nonyl phenol treatment applied between 7:10 and 7:22.

** magnetic north with no correction.

inclusive beyond the south block boundary. Sample units were coded - 15, 0, 15, 30 etc. according to their distance from the start or zero station.

Each deposit sampling unit consisted of a 10 cm x 10 cm Kromekote® card and either paired glass slides or a stainless steel plate. Spray droplets deposited on the Kromekote® cards were sized and counted using a NCR microcard reader. Droplet density (drops/cm^2) were determined for each drop size class and then totalled to provide a drop density figure for the spray card. The glass slides and stainless steel plates were washed with toluene and the quantity of dye rinsed off them was measured using a Baush and Lomb Spectronic 100 spectrophotometer. The equivalent deposit in ℓ/ha was calculated by comparison with the quantity of dye measured in a standard made up from the original dyed tank mix.

A summary of deposit measured in the nonyl phenol treatment block is presented in Table 2. Relatively high levels of the emitted spray products were measured on deposit samplers in the various study areas, especially those in open areas like the mid-stream samplers. Deposit across the block as measured along the deposit assessment line (Fig. 3) is reasonably uniform from deposit stations 225 to 420, the portion of the block treated in the first three passes of the aircraft. At this point in the treatment, one of the Micronair units malfunctioned and began producing large drops, resulting in lower drop densities but greater volumes per unit area being deposited in the portion of the block between deposit stations 90 and 210.

RESIDUE STUDIES

Sample collection

Nonyl phenol residues were measured in samples of water and sediment taken from the treatment stream, and white spruce foliage and soil collected from the treated plot. Each of these substrates were sampled one day prior to treatment and 1h, 3h, 4h, 6h, 24h, 2 days, 3, 4, 5, 9, 14, 30 and 62 days thereafter. Water and sediment samples were collected at two stations in the treatment stream: Station 1 in the steady flowing upper portions of the stream and Station 2 in the extremely slow flowing water behind a beaver dam. At Station 1, samples from four sub-stations 10 m apart were combined to give one amalgamated sample representative of flowing portions of the stream.

Water samples were also collected from the Magpie River 3 km southwest of the spray block where the Dubreuiville road crosses the river (designated Station 3). Samples consisted of approximately 5 litres of water collected from the stream surface (including the surface film) or 500 g of sediment scooped from the streambed. After

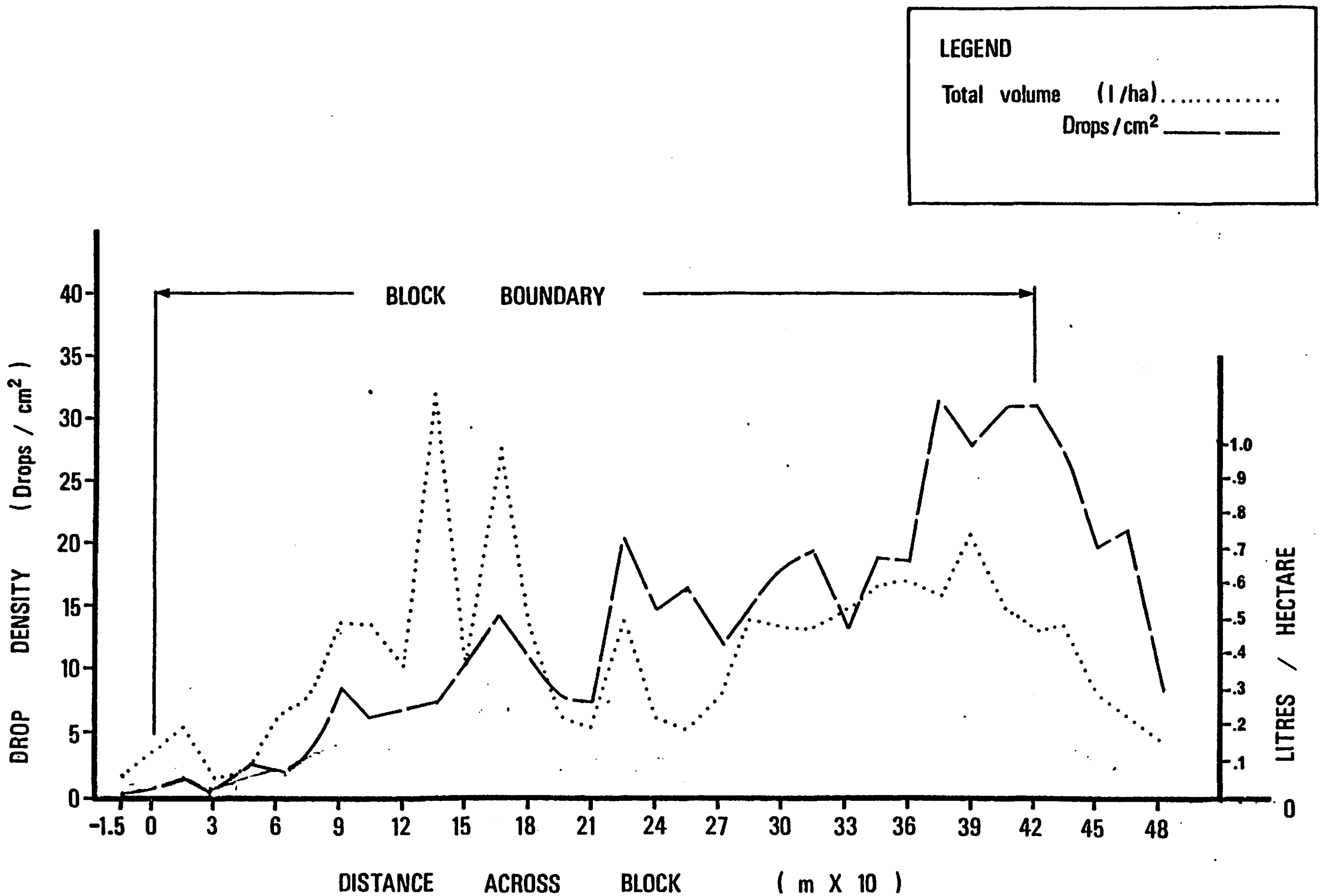
Table 2

Deposit assessment summary from the nonyl phenol treatment plot* sprayed 29 May 1979, Dubreuilville, Ontario

	No. of deposit samplers	Mean drop density drops/cm^2	Mean volume deposited l/ha	Mean % of emitted volume recovered
Deposit assessment line	34	13.29	0.41	27.9
Treatment stream				
Mid-stream samplers	4	14.72	0.66	45.2
Stream bank samplers	5	11.14	0.28	18.9
Fish Cages	4	10.90	0.18	12.4
Bird Plot	6	19.72	0.60	40.7
Bee hives	2	10.72	0.38	25.8

* spray emission rate 1.46 l/ha (20 fl. oz./acre).

Fig. 3 SPRAY DEPOSIT ACROSS BLOCK



collection samples were stored in clean glass containers and packed with ice in styrofoam coolers until transported to the laboratory, where they were held at 0°C until analyzed.

White spruce foliage samples consisted of current year's foliage cut from branches taken from mid-crown portions of ten selected dominant trees to give an amalgamated sample from within the treatment block. A fully exposed plot (4m x 4m), about 10 m away from the nearest tree canopy, was selected for the sampling of forest soil. The acidity of the fine sand was about pH 6.4. At each sampling, 20 cores (2.5 cm in diameter) were taken from the top 5 cm layer randomly, wrapped in aluminum foil, and further handled as described for other substrates.

Extraction of stream water

Nonyl phenol residues were extracted from water samples in the laboratory by measuring individually four 250 ml aliquots of stream water into a 500 ml separatory funnel. Nonyl phenol residues were partitioned into dichloromethane (3 x 50 ml) by counter-current extraction. This procedure was repeated until all 5 liters were extracted. The combined extracts were dried on anhydrous sodium sulfate (10 g) and then evaporated just to dryness in a flash evaporator at 38°C. The residues were dissolved in 2 ml methanol (HPLC-grade) for analysis by reverse phase high-performance liquid chromatography (HPLC) without further clean-up.

Extraction and clean-up of foliage

The cut-up foliage samples collected from each sampling were well mixed and a 50 g aliquot was extracted by washing four times with ethyl acetate (100, 50, 50 and 20 ml) in a 500 ml glass-stoppered graduated cylinder. The combined washings were dried by passing through anhydrous sodium sulfate column (10 g) and evaporated just to dryness in a flash evaporator at 38°C. The residues were dissolved in about 3 ml acetone and then transferred quantitatively into a 250 ml separatory funnel with 100 ml of aqueous $NaOH/NH_4Cl/NaCl$ solution (6 g of NH_4Cl and 3 g of NaCl in 1,000 ml of 0.1 M NaOH). Nonyl phenol residues were extracted from the aqueous solution by partitioning 4 times with hexane (50, 25, 25 and 25 ml). The combined hexane extracts were dried on anhydrous Na_2SO_4 (10 g) and concentrated to about 5 ml in a flash evaporator at 38°C for further clean-up by Florisil column.

Chromatographic columns (500 mm x 22 mm o.d.) with Teflon stopcocks were packed with 3 g of activated Florisil which were kept at 130°C for at least 48 h prior to use. The columns were washed with 20 ml of benzene and followed by 20 ml of hexane. Crude foliage extracts were quantitatively transferred to the columns by rinsing with hexane (5 ml), followed by elution with hexane (100 ml) and the eluates were discarded. Nonyl phenol residues were eluted with benzene (250 ml) and the eluates were evaporated just to dryness in a flash evaporator at 38°C. The residues were then dissolved in methanol (HPLC-grade) and analyzed by reverse phase HPLC.

Extraction and Clean-up of forest soil and sediment

Aliquots of 100 g (wet weight) of the well mixed soil and sediment (excess water had been removed by filtration) were homogenized twice with ethyl acetate (2 x 100 ml) for 5 min in a Sorvall Omni mixer. Anhydrous sodium sulphate (50 g) was added to the sample for homogenization. The homogenates were filtered through a layer of anhydrous sodium sulphate (3 cm) over Whatman No. 1 filter paper in a Buchner funnel, followed by rinsing with ethyl acetate (3 x 10 ml). The combined filtrates were evaporated just to dryness in a flash evaporator at 38°C and the residues were dissolved in about 2 ml dichloromethane for further clean-up.

Extracts of soil and sediments were cleaned on Florisil columns (2 g) in a similar manner as described for foliage. Nonyl phenol residues were eluted from the columns with dichloromethane (300 ml) immediately after the applications of crude extracts. The eluates were evaporated just to dryness and the residues were dissolved in 2 ml methanol (HPLC-grade) for analysis by HPLC.

High performance liquid chromatographic (HPLC) analysis

High performance liquid chromatographic analysis of extracted nonyl phenol residues was performed using a Hewlett Packard Model 1084B HPLC equipped with a variable wave-length UV detector. The operating parameters were as follows:

Column	Partisil PXS 10/25 ODS-2 from Whatman
Column Pressure	35 bar
Mobile Solvent System	95% methanol in water at 1 ml/min
Oven Temperature	40°C
UV Detector Wave-length	278 nm

Under these conditions nonyl phenol gave a major peak with a retention time of 5.50 min and a minor peak of 5.10 min. The major peak accounted for about 95% of the total peak area. Calibration curves were prepared daily before and after sample analysis to confirm the stability of the instrument. Quantification was done by external standardization based on peak area.

Recovery studies

Prespray samples of foliage, forest soil, stream water and sediment were fortified with nonyl phenol at two different levels and subsequently analyzed by the described methods. The percent recoveries are given in Table 1. No significant interference for nonyl phenol analysis was detected in any of the prespray sample. The detection limits were 0.2 ppm for foliage, 0.1 ppm for forest soil, 0.05 ppm for sediment and 1.0 ppb for stream water.

Table 3. Recovery of nonyl phenol from fortified foliage, forest soil/sediment and stream water samples.

Substrate	Fortification	Percent Recovery ($\bar{X}$ ± S.D., n = 2)
Foliage	1.0 ppm	83.7% ± 1.8%
	10.0 ppm	103% ± 4.9%
Soil/Sediment	1.0 ppm	97.0% ± 4.1%
	10.0 ppm	92.6% ± 0.4%
Stream Water	0.1 ppm	107% ± 0.1%
	1.0 ppm	95.6% ± 2.3%

Nonyl phenol residues in aquatic samples

The results of analyses of stream water samples collected from Station 1 demonstrate a rapid disappearance of nonyl phenol residues from flowing water (Table 4). The highest residues measured at this station were 9.1 ppb one hour after treatment of the stream, and these decreased to trace levels (< 2.0 ppb) after six hours and non detectable levels within 24 hours. Residues detected in Station 2 represented an extreme case as the water there was almost stagnant. At the time of first sampling, there was a pool of Automate "B" Red dye floating on the surface. The concentration of nonyl phenol in water from that station four hours after spraying was 1,100 ppb, almost 500 times higher than that of Station 1. However, the residues declined rapidly to 110 ppb in the next two hours, 12 ppb within 24 hours and non detectable levels within three days.

Trace amounts of nonyl phenol were found three hours after spraying at Station 3 in the Magpie River downstream of the treatment stream. Trace amounts of residues were also detected at Station 1 and 2 after four days and at all three stations after five days following heavy rainfalls. Nonyl phenol was not detected in any water sample taken beyond this time.

TABLE 4

Nonyl phenol residues in stream water collected from the treatment stream*, Dubreuilville, Ont.

Time after spraying	Nonyl phenol residue (ppb) Station 1	Station 2	Station 3
1 hour	9.1	-	-
3 hours	4.3	-	Trace
4 hours	2.3	1,100	-
6 hours	Trace	110	-
24 hours	N.D.	12	-
2 days	N.D.	2.4	-
3 days	Trace	N.D.	N.D.
4 days	Trace	Trace	-
5 days	Trace	Trace	Trace
9 days	N.D.	N.D.	N.D.
14 days	N.D.	N.D.	N.D.
30 days	N.D.	N.D.	N.D.
62 days	N.D.	-	N.D.

Trace = < 2.0 ppb

N.D. - not detectable (detection limit was 1.0 ppb)

* - treated with 0.47 ℓ/ha nonyl phenol on 29 May 1979.

Nonyl phenol residues above the detectable limits for sediments (0.05 ppm) were only found in one sediment sample collected from the treatment stream. Trace levels (< 0.1 ppm) were detected in the four hour post-spray sample from Station 1 (Table 5).

Nonyl phenol residues in terrestrial samples

Nonyl phenol residues persisted in white spruce foliage for about 30 days. The highest concentration was 18.9 ppm detected one hour after spraying. It declined to 0.54 ppm in 30 days and was no longer detected 62 days after treatment (Table 5). Levels of nonyl phenol in soil never exceeded the limit of detection (0.1 ppm).

AQUATIC IMPACT STUDIES

Drift netting studies

Drift sampling was done each morning and evening from 24 May - 2 June 1979 (five days before to four days after the nonyl phenol application) in the nonyl phenol treatment and control streams. Five and 20 minute drift samples were taken using a standard 0.47 m x 0.32 m drift net. A drift net fitted with a restrictor to reduce the size of the opening to 0.48 m x 0.025 m was used for 2 hour drift sampling. In both cases drift nets were placed in the stream to sample a column of water from surface to bottom, including the surface film. Current speed was measured at the opening to each drift net half way between the surface and bottom using a Teledyne Gurley No. 625 Pygmy Current Meter. Using the above information the following could be calculated:

depth at station (m) x width of drift net opening (m)
x current speed (m/sec) x duration of drift sample (sec)
= m^3 of water in drift column.
width of drift net opening (m) x current speed (m/sec)
x duration of drift sample (sec) = m^2 of surface area
of drift column.

Drift samples were either picked immediately and preserved in a 30% methanol solution or preserved in their entirety in a 10% solution of formaldehyde. Invertebrates were counted and identified to order or family and the results expressed as:

number of invertebrates/m^3 of water in drift column
(for aquatic invertebrates)
number of invertebrates/10 m^2 of surface area of drift
column (for terrestrial invertebrates).

TABLE 5

Nonyl phenol residues in foliage, forest soil and sediment from the treatment plot*, Dubreuilville, Ont.

Time after spraying	Nonyl phenol residue (ppm)		
	Foliage	Forest soil	Sediment**
1 hour	18.90	N.D.	N.D.
3 hours	7.70	N.D.	N.D.
4 hours	4.86	-	Trace
6 hours	4.44	N.D.	N.D.
24 hours	4.06	N.D.	N.D.
2 days	3.54	N.D.	N.D.
3 days	2.06	N.D.	N.D.
4 days	1.49	N.D.	N.D.
5 days	1.70	N.D.	N.D.
9 days	1.23	N.D.	N.D.
14 days	1.43	N.D.	N.D.
30 days	0.54	N.D.	N.D.
62 days	N.D.	N.D.	N.D.

N.D. = not detectable (detection limits were 0.20 ppm for foliage, 0.10 ppm for forest soil and 0.05 ppm for sediment).

Trace = < 0.10 ppm (detected in sediment collected from station 1).

* treated with 0.47 ℓ/ha nonyl phenol on 29 May 1979.

** sediment samples were collected from both station 1 and 2 and analyzed individually.

Additional 5 and 20-minute drift samples were taken on spray day to determine any immediate effects of the treatment.

The results of drift netting are presented in Tables 6 to 13. Numbers of most groups of aquatic invertebrates remained fairly constant over the course of the study. There was a significant (> one order of magnitude) increase in the number of blackfly larvae (Diptera:Simuliidae) caught in the 2-hour drift net set at the treatment station one day after the nonyl phenol application (Table 8), but no indication of any effect on this group in the 20 minute drift net set begun at the same time (Table 6). Blackfly larvae were caught in large numbers in drift sets in the control stream at the same time (Tables 7 and 9), but the proportional increase was smaller than in the 2 hour drift net set in the treatment stream. This increase may have been a response to warming stream temperatures as suggested by the increased drift of blackfly larvae in the control stream. It may also have resulted from mechanical disturbance of the bottom due to sampling activities above the drift station some time after the 20-minute drift had been removed.

There were no indications of increases in the numbers of terrestrial organisms drifting on the stream surface after the nonyl phenol application (Tables 10 to 13).

Bottom fauna

Bottom fauna populations were sampled before and after the nonyl phenol application using a standard 0.093 m^2 Surber sampler (Surber, 1936). Supplemental information was obtained by collecting organisms from four randomly chosen rocks (approximately 20 cm in diameter). Samples were either picked immediately and preserved in a 30% methanol solution, or preserved in their entirety in a 10% solution of formaldehyde. Benthic organisms were subsequently counted and identified to order or family in the lab. For each sampling date, results were expressed as mean number and standard deviation of aquatic organisms of a particular group collected in four Surber samples or from four rocks.

Sampling indicated no significant overall effect of the nonyl phenol application on bottom fauna populations. Fluctuations in numbers of most groups of aquatic invertebrates over the course of the study were observed, but these generally corresponded quite closely between the treatment and control streams (Tables 14 to 17). There was an indication of a reduction (< one order of magnitude) in numbers of midge larvae (Diptera:Chironomidae) found in Surber and rock samples taken from the treatment stream three days after the spray. This reduction did not show up in the control samples. By ten days post-spray, numbers of chironomid larvae in bottom fauna samples from the treatment stream had increased to above the pre-spray levels. Since there was no apparent increase in number of chironomids in the drift

Table 6

Aquatic organisms caught in 20 min drift net sets*, Nonyl Phenol Treatment Stream, Dubreuilville, Ontario, 24 May - 2 June 1979

Days before or after application of 0.47 l/ha nonyl phenol **	-5		-4		-3		-2		-1		Spray Day						+1		+2		+3		+4	
	am	pm	am	pm	am	pm	am	pm	am	pm	Pre	0hr	+½hr	+1hr	+2hr	pm	am	pm	am	pm	am	pm	am	pm
Depth (m)	0.38	0.32	0.35	0.34	0.25	0.28	0.24	0.23	0.25	0.29	0.24	0.24	0.24	0.24	0.24	0.21	0.18	0.18	0.16	0.21	0.33	0.39	0.38	0.35
Current Velocity (m/sec)	0.56	0.56	0.45	0.51	0.75	0.69	0.42	0.36	0.66	0.75	0.81	0.81	0.81	0.81	0.81	0.45	0.78	0.66	0.81	0.81	0.66	0.63	0.60	0.51
Volume of drift column (m^3)	81.72	101.07	60.48	66.59	105.75	74.19	38.71	31.80	93.06	122.67	109.64	109.64	109.64	109.64	109.64	53.30	79.19	67.00	73.09	95.94	122.84	138.58	128.59	100.67
Nematoda					0.010																			
Oligochaeta											0.018										0.008			
Hirudinea	0.012		0.016						0.032															
Gastropoda												0.009	0.009							0.010		0.007		0.010
Pelecypoda			0.016																					
Arachnida																								
Acari		0.030	0.016	0.030	0.019	0.014	0.026	0.032		0.008		0.027				0.019		0.030	0.014					
Crustacea																								
Ostracoda	0.012	0.099	0.016	0.075	0.170	0.054			0.054	0.049		0.073	0.082	0.073	0.027	0.094			0.082	0.042	0.163			
Amphipoda	0.012	0.010								0.008							0.013							
Ephemeroptera																								
Heptageniidae		0.010			0.010	0.014																	0.008	
Baetidae	0.049	0.030	0.016	0.105	0.047	0.014	0.026	0.126	0.108	0.049	0.073	0.036	0.046		0.027	0.038	0.063	0.104	0.041		0.033		0.031	
Odonata																								
Aeschnidae					0.010																			
Libellulidae																0.019								
Plecoptera		0.010			0.010					0.008		0.009					0.025			0.010	0.016		0.016	
Hemiptera																								
Corixidae				0.015	0.010		0.026		0.011							0.019	0.025		0.014			0.007	0.008	
Gerridae		0.020									0.018			0.009	0.009					0.010	0.008			
Trichoptera																								
larvae	0.086	0.079	0.050	0.015	0.180	0.014	0.078	0.032	0.064	0.049	0.082	0.073	0.082	0.091	0.036	0.019	0.010	0.075	0.096	0.031	0.081		0.016	0.099
pupae				0.045	0.019	0.014	0.026		0.043		0.027	0.055	0.027		0.073		0.088		0.096		0.041			
Coleoptera																								
Haliplidae adults		0.010	0.016																					
Dytiscidae adults																		0.015						
Gyrinidae adults											0.009		0.018					0.015						
Hydraenidae adults																					0.008			
Helodidae adults																		0.015						
Elmidae larvae				0.015	0.010		0.026		0.011	0.008										0.010				
Elmidae adults																				0.010	0.008	0.007		
Chrysomelidae adults																		0.015						
Diptera																								
Tipulidae larvae					0.010			0.032	0.011		0.009	0.018			0.018			0.015			0.008			
Tanyderidae larvae												0.009												
Simuliidae larvae	0.098	0.050	0.033	0.120	0.095	0.135	0.155	0.314	0.140	0.160	0.027	0.073	0.219	0.301	0.110		0.088	0.149	0.192	0.052	0.098			0.010
Simuliidae pupae								0.032	0.022				0.009											
Chironomidae larvae	0.024	0.010			0.047	0.054	0.026	0.032		0.008	0.009	0.027	0.018	0.055			0.025	0.030	0.014	0.021	0.024	0.007	0.008	0.010
Chironomidae pupae	0.037	0.079		0.045	0.019	0.014		0.032					0.009		0.027							0.007	0.008	
Dixidae larvae				0.015																				
Fish																0.019								
Total Aquatic Invertebrates	0.330	0.435	0.182	0.481	0.662	0.324	0.339	0.629	0.494	0.295	0.274	0.410	0.520	0.529	0.328	0.206	0.429	0.463	0.347	0.198	0.497	0.036	0.093	0.129

* Expressed as number of organisms/m^3 of water passing through the drift net

** Application at 0707 on 29 May 1979

Table 7

Aquatic organisms caught in 5 min drift net sets*, Nonyl Phenol Control Stream, Dubreuilville, Ontario, 24 May - 2 June 1979

Days before or after application of 0.47 l/ha nonyl phenol**		-5		-4		-3		-2		-1		Spray Day						+1		+2		+3		+4	
		am***	pm	am	pm	am	pm	am	pm	am	pm	5am	6am	7am	8am	9am	pm	am	pm	am	pm	am	pm	am	pm
Depth (m)		0.40	0.30	0.30	0.31	0.29	0.29	0.25	0.26	0.22	0.24	0.21	0.21	0.21	0.21	0.21	0.20	0.17	0.18	0.14	0.15	0.18	0.18	0.19	0.22
Current velocity (m/sec)		0.36	0.36	0.42	0.36	0.33	0.42	0.33	0.33	0.30	0.24	0.54	0.54	0.54	0.54	0.54	0.44	0.33	0.27	0.21	0.21	0.26	0.26	0.24	0.30
Volume of drift column (m^3)		55.30	15.23	12.10	10.71	13.49	11.69	7.92	8.24	9.31	8.12	15.99	15.99	15.99	15.99	15.99	12.41	7.91	6.85	4.16	4.46	6.60	6.60	6.43	9.31
Nematoda		0.018											0.062			0.062									
Hirudinea			0.066																						
Gastropoda		0.036	0.066		0.187	0.074	0.086					0.062				0.062					0.225				
Pelecypoda								0.126																	
Arachnida																									
Acari		0.199	3.283	0.248	1.307	0.222	0.142	1.389	0.607	0.215	0.616	0.062	0.188	0.062	0.168	0.062			0.584		0.225	0.606		0.156	1.074
Crustacea																									
Ostracoda		0.181	7.420	1.653	3.735	3.336	1.026	0.252	2.184	1.719	4.064		0.062	0.125	0.625		2.579	0.126	5.110	1.932	2.703	0.758	3.939	2.955	5.263
Amphipoda		0.199		0.083		0.148				0.107															
Ephemeroptera																									
Heptageniidae											0.173														
Baetidae										0.107		0.062		0.062						0.242					0.107
Plecoptera														0.062											
Hemiptera																									
Corixidae		0.018								0.107				0.062										0.156	
Gerridae																							0.152	0.156	
Trichoptera																									
	larvae	0.036	0.197			0.148		0.126			0.493					0.062		0.126		0.242		0.303	0.606	0.467	
	pupae																								0.215
Coleoptera																									
Amphizoidae	adults									0.107															
Haliplidae	adults	0.054										0.062	0.062			0.062				0.242					
Dytiscidae	adults																								0.107
Hydrophilidae	adults																	0.126							
Elmidae	larvae												0.062												
	adults		0.066									0.062													
Diptera																									
Tipulidae	larvae											0.062													
	pupae								0.121																
Culicidae	larvae											0.125					0.081		0.146						
Simuliidae	larvae	2.021	1.904	3.802	2.148	8.377	0.941	4.546	5.218	28.371	12.315	15.572	15.072	12.320	11.445	7.755	5.077	32.617	4.018	43.961	14.640	0.454	1.364	5.910	0.967
	pupae	0.054		0.083	0.187			0.126	0.121																
Chironomidae	larvae	0.145		0.413	0.280	0.074	0.086	0.126			0.739	0.250	0.375	0.188	0.062	0.062				0.725	0.901				0.322
	pupae	0.054		0.248		0.222		0.126	0.485	0.322	0.370		0.062	0.062											0.107
Total Aquatic Invertebrates		3.834	12.804	6.529	7.843	12.602	2.481	6.818	8.738	31.257	18.719	16.323	15.948	12.946	12.320	8.130	7.736	32.896	10.657	47.343	18.694	2.121	6.061	9.798	8.163

* Expressed as number of organisms/m^3 of water passing through the drift net
** Application at 0707 on 29 May 1979
*** 20 min drift net set

Table 8

Aquatic organisms caught in 2 hr drift net sets*, Nonyl Phenol Treatment Stream, Dubreuilville, Ontario, 24 May - 2 June 1979

Days before or after application of 0.47 l/ha nonyl phenol**	-5		-4		-3		-2		-1		Spray Day		+1		+2		+3		+4	
	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm
Depth (m)	0.40	0.49	0.41	0.40	0.48	0.38	0.32	0.32	0.30	0.32	0.30	0.28	0.21	0.21	0.20	0.24	0.36	0.42	0.41	0.36
Current velocity (m/sec)	0.56	0.56	0.42	0.39	0.27	0.30	0.27	0.27	0.24	0.30	0.51	0.33	0.18	0.21	0.36	0.18	0.21	0.21	0.18	0.21
Volume of drift column (m^3)	40.32	48.38	31.00	28.08	23.33	20.52	15.55	15.55	12.96	17.28	27.54	16.63	6.80	7.94	12.96	7.78	13.61	15.88	13.28	13.61
Arachnida																				
Acari	0.025					0.049							0.147							0.074
Crustacea																				
Ostracoda														0.378						
Amphipoda	0.025																			
Ephemeroptera																				
Heptageniidae	0.025		0.032																	
Baetidae	0.099	0.021		0.036		0.098				0.058						0.257	0.220	0.063	0.075	
Trichoptera																				
larvae	0.025	0.062	0.097	0.071	0.043				0.077				0.294	0.126	0.154			0.063	0.075	
pupae	0.025																			
Coleoptera																				
Haliplidae adults													0.147	0.126						
Hydrophilidae adults														0.378						
Elmidae larvae			0.032																	
Diptera																				
Tipulidae larvae													0.147			0.128				
Simuliidae larvae	0.074	0.083	0.064	0.107		0.098			0.077		0.218		12.059	0.252	0.154	0.386	0.294	0.063	0.075	0.074
pupae	0.050				0.043															
Chironomidae larvae		0.041	0.032										0.147			0.128	0.147			
pupae	0.025	0.021																		
Fish				0.036												0.128				
Total Aquatic Invertebrates	0.372	0.227	0.258	0.214	0.086	0.195			0.154	0.058	0.218		12.941	1.260	0.309	0.900	0.661	0.189	0.226	0.147

*Expressed as number of organisms/m^3 of water passing through the drift net
** Application at 0707 on 29 May 1979

Table 9

Aquatic organisms caught in 2 hr drift net sets*, Nonyl Phenol Control Stream, Dubreuilville, Ontario, 24 May - 2 June 1979

Days before or after application of 0.47 l/ha nonyl phenol**	-5		-4		-3		-2		-1		Spray Day		+1		+2		+3		+4	
	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm
Depth (m)	0.40	0.48	0.32	0.31	0.32	0.32	0.25	0.25	0.20	0.23	0.20	0.19	0.14	0.17	0.14	0.15	0.19	0.19	0.19	0.21
Current velocity (m/sec)	0.36	0.36	0.42	0.39	0.45	0.39	0.36	0.42	0.24	0.21	0.54	0.42	0.30	0.36	0.21	0.18	0.26	0.26	0.24	0.36
Volume of drift column (m^3)	25.92	31.10	24.19	21.76	25.92	22.46	16.20	18.90	8.64	8.69	19.44	14.36	7.56	11.02	5.29	4.86	8.89	8.89	8.21	13.61
Nematoda	0.039																			
Oligochaeta						0.044														
Gastropoda	0.077	0.032	0.041		0.116								0.132			0.206	0.225			0.074
Arachnida																				
Acari	0.116	0.997	0.041	0.873		0.134		0.106	0.694	0.345	0.103	0.139		0.363	0.189		0.112	0.675	0.365	0.588
Crustaceae																				
Ostracoda	0.154	3.183	0.455	1.654	0.154	2.158	0.309	0.423	1.157	1.611	0.309	1.114		3.085	1.134	1.646	2.025	3.037	1.949	2.939
Amphipoda	0.039																			
Ephemeroptera																				
Heptageniidae		0.032																		
Baetidae		0.032											0.265		0.189				2.436	
Plecoptera		0.032																0.225		
Hemiptera																				
Corixidae	0.039	0.032		0.046															0.122	
Corridae																		0.112		
Trichoptera																				
larvae	0.077	0.161					0.062		0.347		0.051			1.180		0.206	1.237	0.338	0.609	0.294
Coleoptera																				
Haliplidae adults	0.039		0.041	0.046																
Dytiscidae adults														0.091				0.112		
Diptera																				
Simuliidae larvae	6.134	0.836	0.992	1.195	1.196	0.712	3.272	0.529	12.616	0.806	9.619	1.462	11.508	1.724	11.909	0.823	1.462	0.450	2.558	0.147
pupae	0.077																			
Chironomidae larvae	0.193	0.161	0.041		0.077	0.089	0.124	0.053	0.116	0.115	0.103	0.279		0.091			0.112	0.338	0.122	0.147
pupae	0.154	0.064	0.124	0.046	0.039	0.044				0.115				0.091				0.112		
Total Aquatic Invertebrates	7.137	5.563	1.736	3.860	1.582	2.137	3.765	1.111	14.931	2.992	10.185	2.994	11.905	6.624	13.422	2.801	5.174	5.399	8.161	4.188

* Expressed as number of organisms/m^3 of water passing through the drift net
** Application at 0707 on 29 May 1979

Table 10

Terrestrial organisms caught in 20 min drift net sets*, Nonyl Phenol Treatment Stream, Dubreuilville, Ontario, 24 May – 2 June 1979

Days before or after application of 0.47 l/ha nonyl phenol**		-5		-4		-3		-2		-1		Spray Day						+1		+2		+3		+4	
		am	pm	am	pm	am	pm	am	pm	am	pm	Pre	0hr	+½hr	+1hr	+2hr	pm	am	pm	am	pm	am	pm	am	pm
Current Velocity (m/sec)		0.56	0.56	0.45	0.51	0.73	0.69	0.42	0.36	0.66	0.73	0.81	0.81	0.81	0.81	0.81	0.45	0.78	0.66	0.81	0.81	0.66	0.63	0.60	0.51
Surface area of drift column (m^2)		215.04	315.84	172.80	395.84	423.00	264.96	161.28	138.24	372.24	423.00	456.84	456.84	456.94	456.84	456.84	253.80	439.92	372.24	456.84	456.84	372.24	355.32	338.40	287.64
Arachnida																									
Araneida				0.058																					
Collembola		0.418			0.102	0.496	0.076	0.062	0.145	0.081			0.022	0.131	0.110	0.022					0.066	0.027			
Plecoptera	adults																								0.104
Trichoptera	adults					0.047				0.054			0.131			0.044				0.022		0.054			
Hymenoptera																									
Formicidae	adults				0.051														0.027				0.028	0.030	
Other	adults		0.031		0.051						0.024		0.044		0.022										0.035
Coleoptera																									
Staphylinidae	adults		0.031		0.102												0.039		0.134						
Other	adults											0.022													
Diptera	adults	0.093	0.380	0.116	1.430		0.038		0.579		0.047	0.022	0.131	0.088	0.066	0.044		0.023	0.108		0.044	0.054			
Total Terrestrial Organisms		0.512	0.443	0.174	1.736	0.544	0.113	0.062	0.723	0.134	0.071	0.044	0.328	0.219	0.197	0.110	0.039	0.023	0.269	0.022	0.110	0.134	0.028	0.030	0.139

* Expressed as number of invertebrates/$10m^2$ of surface area of drift column

** Application at 0707 on 29 May 1979

Table 11

Terrestrial organisms caught in 5 min drift net sets*, Nonyl Phenol Control Stream, Dubreuilville, Ontario, 24 May - 2 June 1979

Days before or after application of 0.47 l/ha nonyl phenol**	-5 am***	-5 pm	-4 am	-4 pm	-3 am	-3 pm	-2 am	-2 pm	-1 am	-1 pm	Spray Day 3am	Spray Day 6am	Spray Day 7am	Spray Day 8am	Spray Day 9am	Spray Day pm	+1 am	+1 pm	+2 am	+2 pm	+3 am	+3 pm	+4 am	+4 pm
Current velocity (m/sec)	0.36	0.36	0.42	0.36	0.33	0.42	0.33	0.33	0.30	0.24	0.54	0.54	0.54	0.54	0.54	0.44	0.33	0.37	0.21	0.21	0.26	0.26	0.24	0.30
Surface area of drift column (m^2)	138.24	50.76	40.32	34.56	46.53	40.32	31.68	31.68	42.30	33.84	76.14	76.14	76.14	76.14	76.14	62.04	46.53	38.07	29.61	29.61	36.66	36.66	33.84	42.30
Arachnida																								
Araneida																0.645		0.525						
Collembola											0.131													0.236
Hymenoptera																								
Formicidae																						0.273		
Diptera	0.072	0.391	0.248	3.472		0.496		0.316		1.182			0.131	0.131	0.131	0.484		0.263			0.273	0.273		
Total Terrestrial Organisms	0.072	0.391	0.248	3.472		0.496		0.316		1.182	0.131		0.131	0.131	0.131	1.128		0.788			0.273	0.546		0.236

* Expressed as number of organisms/10m² of surface area of drift column
** Application at 0707 on 29 May 1979
*** 20 min drift net

Table 12

Terrestrial organisms caught in 2 hr drift net sets*, Nonyl Phenol Treatment Stream, Dubreuilville, Ontario, 24 May - 2 June 1979

Days before or after application of 0.47 l/ha nonyl phenol**	-5		-4		-3		-2		-1		Spray Day		+1		+2		+3		+4	
	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm
Current velocity (m/sec)	0.56	0.56	0.42	0.39		0.30	0.27	0.27	0.24	0.30	0.51	0.33	0.18	0.21	0.36	0.18	0.21	0.21	0.18	0.21
Surface area of drift column (m^2)	100.80	100.80	75.60	70.20		54.00	48.60	48.60	43.20	54.00	91.80	59.40	32.40	37.80	64.80	32.40	37.80	37.80	32.40	37.80
Collembola	1.290	0.099	0.661		no data											0.617				
Hemiptera		0.099	0.132																	
Trichoptera															0.154					
Hymenoptera																				
Formicidae														0.265						
Coleoptera				0.285																
Diptera	0.099	0.099	0.132	1.567										0.529	0.154	0.926	0.265			0.529
Total Terrestrial Organisms	1.389	0.298	0.926	1.852										0.794	0.309	1.543	0.265			0.529

* Expressed as number of invertebrates/10m^2 of surface area of drift column

** Application at 0707 on 29 May 1979

Table 13

Terrestrial organisms caught in 2 hr drift net sets*, Nonyl Phenol Control Stream, Dubreuilville, Ontario, 24 May - 2 June 1979

Days before or after application of 0.47 l/ha nonyl phenol**	-5		-4		-3		-2		-1		Spray Day		+1		+2		+3		+4	
	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm
Current velocity (m/sec)	0.36	0.36	0.42	0.39	0.45	0.39	0.36	0.42	0.24	0.21	0.54	0.42	0.30	0.36	0.21	0.18	0.26	0.26	0.24	0.36
Surface area of drift column (m^2)	64.80	64.80	75.60	70.20	81.00	70.20	64.80	75.60	43.20	37.80	97.20	75.60	54.00	64.80	37.80	32.40	46.80	46.80	43.20	64.80
Coleoptera																				
Staphylinidae adults														0.154						
other adults				0.142																
Diptera																				
adults		0.154	0.256	1.282	0.370					0.529	0.103					0.309				
Total Terrestrial Organisms		0.154	0.256	1.424	0.370					0.529	0.103			0.154		0.309				

* Expressed as number of invertebrates/10m^2 of surface area of drift column
** Application at 0707 on 29 May 1979

Table 14

Bottom fauna populations*, Nonyl Phenol Treatment Stream
Dubreuilville, Ontario, 25 May – 15 June 1979

Days before or after application of 0.47 l/ha nonyl phenol**		May 25 -4	June 1 +3	June 8 +10	June 15 +17
Nematoda		0.25 ± 0.50			
Oligochaeta		0.25 ± 0.50		0.75 ± 1.50	0.75 ± 0.96
Hirudinea				0.75 ± 1.50	
Pelecypoda				0.75 ± 1.50	0.50 ± 0.58
Crustaceae					
Amphipoda		0.75 ± 0.96	0.50 ± 1.00	1.00 ± 0.82	0.25 ± 0.50
Collembola				0.50 ± 1.00	
Ephemeroptera					
Ephemeridae			0.25 ± 0.50		
Baetidae		1.00 ± 1.41	4.00 ± 6.00	3.25 ± 1.71	8.25 ±11.21
Odonata					
Cordulegastridae				0.25 ± 0.50	
Plecoptera		0.25 ± 0.50			
Trichoptera					
	larvae	4.25 ± 2.06	5.00 ± 4.55	11.25 ± 6.70	4.25 ± 3.86
	pupae				0.25 ± 0.50
Coleoptera					
Elmidae	larvae	0.50 ± 0.58		0.25 ± 0.50	
	adults	0.25 ± 0.50		0.50 ± 0.58	
Diptera					
Tipulidae	larvae		0.25 ± 0.50	0.25 ± 0.50	0.75 ± 1.50
Dixidae	larvae	0.25 ± 0.50			
Simuliidae	larvae	3.25 ± 5.19	1.00 ± 0.82	51.50 ±25.64	48.25 ±48.94
	pupae			0.75 ± 0.50	2.25 ± 3.86
Chironomidae	larvae	1.75 ± 0.50	0.25 ± 0.50	6.50 ± 3.87	2.25 ± 1.26
	pupae	0.50 ± 0.58	0.50 ± 0.58	3.50 ± 1.73	0.25 ± 0.50
Empididae	larvae			0.25 ± 0.50	
	pupae				0.25 ± 0.50
Total Aquatic Invertebrates		13.25 ± 8.34	11.00 ±10.52	81.50 ±28.87	68.25 ±53.97

* Mean numbers and standard deviations of organisms collected in four 0.093 m^2 Surber samples.
** Application at 0707 on May 29, 1979

Table 15

Bottom fauna populations*, Nonyl Phenol Control Stream
Dubreuilville, Ontario, 26 May - 16 June 1979

Days before or after application of 0.47 l/ha nonyl phenol**		May 26 -3	June 1 +3	June 9 +11	June 16 +18
Nematodmorpha				0.25 ± 0.50	
Oligochaeta		0.25 ± 0.50	0.25 ± 0.50	0.50 ± 0.58	0.25 ± 0.50
Hirudinea				0.25 ± 0.50	
Gastropoda		0.50 ± 0.58	2.50 ± 2.65	3.00 ± 3.16	
Pelecypoda				0.75 ± 0.96	
Arachnida					
Acari		2.25 ± 3.86	0.50 ± 1.00	0.50 ± 0.58	
Crustaceae					
Amphipoda		1.00 ± 1.16	7.75 ± 9.60	2.25 ± 0.96	
Ephemeroptera					
Ephemeridae			0.25 ± 0.50		
Heptageniidae		0.25 ± 0.50	1.00 ± 1.16	0.75 ± 0.96	1.00 ± 1.41
Baetidae		0.25 ± 0.50	1.25 ± 1.26	0.75 ± 1.50	13.75 ± 9.29
Plecoptera		0.50 ± 0.58	0.50 ± 1.00	0.25 ± 0.50	0.75 ± 1.50
Hemiptera					
Corixidae		0.25 ± 0.50	0.25 ± 0.50	0.75 ± 0.96	
Notonectidae					0.25 ± 0.50
Gerridae				0.25 ± 0.50	
Trichoptera					
	larvae	7.25 ± 4.92	5.50 ± 5.57	9.50 ± 5.00	6.75 ± 1.71
	pupae		0.25 ± 0.50	0.75 ± 1.50	0.50 ± 0.58
Coleoptera					
Haliplidae	adults				0.50 ± 1.00
Elmidae	larvae	1.25 ± 2.50	2.25 ± 3.86	0.75 ± 0.96	2.75 ± 2.06
	adults	0.25 ± 0.50		0.25 ± 0.50	1.25 ± 1.89
Diptera					
Simuliidae	larvae	1588.50 ± 1317.74	838.50 ± 1367.10	944.50 ± 1060.20	153.50 ± 148.57
	pupae	2.25 ± 2.63	221.25 ± 236.39	8.50 ± 6.66	7.50 ± 1.29
Chironomidae	larvae	6.50 ± 5.32	6.75 ± 12.84	10.25 ± 6.99	9.00 ± 9.66
	pupae	1.50 ± 2.38	3.00 ± 3.83	2.00 ± 1.83	0.25 ± 0.50
Heleidae	larvae		0.25 ± 0.50		
Empididae	larvae		1.00 ± 2.00		
Fish				0.50 ± 0.58	
Total Aquatic Invertebrates		1388.25 ± 1523.49	1093.00 ± 1580.28	986.75 ± 1071.79	197.75 ± 151.84

* Mean numbers and standard deviations of organisms collected in four 0.093 m^2 Surber samples

** Application at 0707 on 29 May 1979

Table 16

Bottom fauna populations*, Nonyl Phenol Treatment Stream
Dubreuilville, Ontario, 25 May - 15 June 1979

Days before or after application of 0.47 l/ha nonyl phenol**		May 27 -2	June 1 +3	June 8 +10	June 15 +17
Hirudinea			0.25 ± 0.50	0.25 ± 0.50	
Gastropoda				o.25 ± 0.50	
Ephemeroptera					
Heptageniidae		0.25 ± 0.50		0.25 ± 0.50	0.75 ± 1.50
Baetidae		5.75 ± 2.63	3.25 ± 2.63	6.00 ± 4.97	4.25 ± 4.19
Odonata					
Libellulidae				0.25 ± 0.50	
Plecoptera		0.25 ± 0.50	0.25 ± 0.50	0.50 ± 0.58	
Trichoptera					
	larvae	5.00 ± 5.23		3.25 ± 2.99	2.25 ± 3.86
	pupae	0.25 ± 0.50		0.25 ± 0.50	0.50 ± 1.00
Coleoptera					
Elmidae	larvae				0.25 ± 0.50
	adults	0.25 ± 0.50	0.25 ± 0.50	0.25 ± 0.50	
Diptera					
Simuliidae	larvae	0.75 ± 0.50	1.25 ± 1.89	0.75 ± 0.96	0.25 ± 0.50
	pupae	0.25 ± 0.50		0.25 ± 0.50	
Chironomidae	larvae	4.50 ± 1.92	0.50 ± 0.58	7.00 ± 2.94	7.25 ± 5.12
	pupae	0.25 ± 0.50		0.25 ± 0.50	
Total Aquatic Invertebrates		17.50 ± 6.61	5.75 ± 2.63	19.50 ± 1.92	15.50 ±13.30

* Mean numbers and standard deviations of organisms collected from four rocks
** Application at 0707 on 29 May 1979

Table 17

Bottom fauna populations*, Nonyl Phenol Control Stream
Dubreuilville, Ontario, 26 May - 16 June 1979

Days before or after application of 0.47 l/ha nonyl phenol**		May 26 -3	June 1 +3	June 9 +11	June 16 +18
Oligochaeta					0.25 ± 0.50
Gastropoda		0.25 ± 0.50	0.75 ± 0.96		
Pelecypoda				0.25 ± 0.50	
Arachnida					
Acari			2.25 ± 4.50		
Crustaceae					
Amphipoda			1.25 ± 2.50		
Ephemeroptera					
Heptageniidae					0.50 ± 1.00
Baetidae					0.75 ± 0.96
Plecoptera		0.25 ± 0.50			0.25 ± 0.50
Megaloptera					
Corydalidae					0.25 ± 0.50
Trichoptera					
	larvae	9.75 ± 17.52	0.50 ± 1.00	5.75 ± 4.57	8.25 ± 7.85
	pupae				0.50 ± 1.00
Coleoptera					
Elmidae	larvae	0.25 ± 0.50			0.50 ± 1.00
Diptera					
Simuliidae	larvae	3782.25 ± 2941.48	1877.00 ± 2742.46	2629.00 ± 2534.01	263.00 ± 110.02
	pupae	11.00 ± 19.37	89.75 ± 126.38	29.50 ± 34.04	9.00 ± 3.16
Chironomidae	larvae	12.50 ± 8.27	7.75 ± 8.10	11.50 ± 5.51	18.00 ± 8.52
Total Aquatic Invertebrates		3816.25 ± 2948.10	1979.25 ± 2777.60	2676.00 ± 2564.80	301.25 ± 109.04

* Mean numbers and standard deviations of organisms collected from four rocks
** Application at 0707 on 29 May 1979

at this time, it is unlikely that this short-term reduction in numbers can be attributed to the nonyl phenol application.

Caged fish

Four days before the nonyl phenol application, hatchery reared brook trout, *Salvelinus fontinalis* (Mitchell), were placed in cages in the nonyl phenol treatment and control streams. Two cages (1 and 2) were placed in the control stream and four (3, 4, 5 and 6) in the treatment stream, with between 20 and 26 fish in each cage (Table 18).

The results of caged fish studies are presented in Fig. 4 and Table 19. Up until 3 June, five days after the nonyl phenol application, there was no significant difference (95% confidence limits, Appendix 1) in mortality between fish caged in the treatment and control streams (17.7 and 16.3% respectively). At this time approximately half the fish were removed for residue analysis. By 6 June, eight days post-spray, mortality of remaining treatment fish was significantly higher than control fish (58.7% vs 24%). By 8 June, ten days post-spray, 93.5% of the fish caged in the treatment stream were dead compared to only 32.0% in the control. Although this data suggests that nonyl phenol may have had a delayed lethal effect on caged fish, mortality in the control was far too high to state this with any degree of certainty.

Bioassays

Bioassays were conducted to determine the toxicity of nonyl phenol to fingerling brook trout and rainbow trout, *Salmo gairdneri* Richardson. The rainbow trout bioassay was conducted prior to the field season in the laboratory under environmentally controlled conditions. Measured volumes of a stock solution of nonyl phenol dissolved in absolute ethanol (20 mg/ml) were added to tanks containing 20 litres of dechlorinated tap water to produce the desired range of four concentrations (100, 180, 300 and 1000 μg/ℓ). Ethanol was added to a fifth tank of water to serve as a control. Nine fingerling rainbow trout were introduced into each tank and held at 9.0 ± 1.0°C in an environmental chamber for 96 hours. At intervals the fish were observed for mortality and the dead fish removed.

The brook trout bioassay was conducted in the field at the time of the nonyl phenol application. Measured volumes of a stock solution of nonyl phenol in ethanol (2 mg/ml) and ethanol alone were added to polypropylene bags containing 20 litres of water taken from the control stream to produce the desired series of four concentrations (100, 210, 460 and 1000 μg/ℓ) and a control. Nine fingerling brook trout were introduced into each bag. The bags were then closed with a wire tie, placed in large plastic buckets, and set in the control stream. In this way the photoperiod and fluctuations in water

Table 18

Brook trout, *Salvelinus fontinalis*, caged in nonyl phenol treatment and nonyl phenol control streams, Dubreuilville, Ontario

25 May - 8 June 1979

Cage Number	Number of fish in cage	Number of fish weighed and measured	Total Length (mm)		Fork Length (mm)		Weight (g)	
			Mean	Range	Mean	Range	Mean	Range
1	24	24	100.3	74-138	97.8	72-134	9.41	3.1-22.3
2	25	20	99.7	78-117	97.0	76-114	9.59	4.4-15.5
3	26	12	99.1	86-113	96.0	84-110	8.43	4.7-15.1
4	20	7	101.1	91-120	98.7	89-117	9.51	7.2-13.9
5	25	25	94.0	75-117	91.7	72-113	7.21	3.0-11.9
6	25	25	88.6	67-114	86.0	65-110	6.34	2.8-12.5
Total	145	113	96.2	67-138	83.5	65-134	8.18	2.8-22.3

Fig. 4

Cumulative mortality of caged Brook trout (*Salvelinus fontinalis*)
Dubreuilville, Ontario, 29 May - 8 June 1979

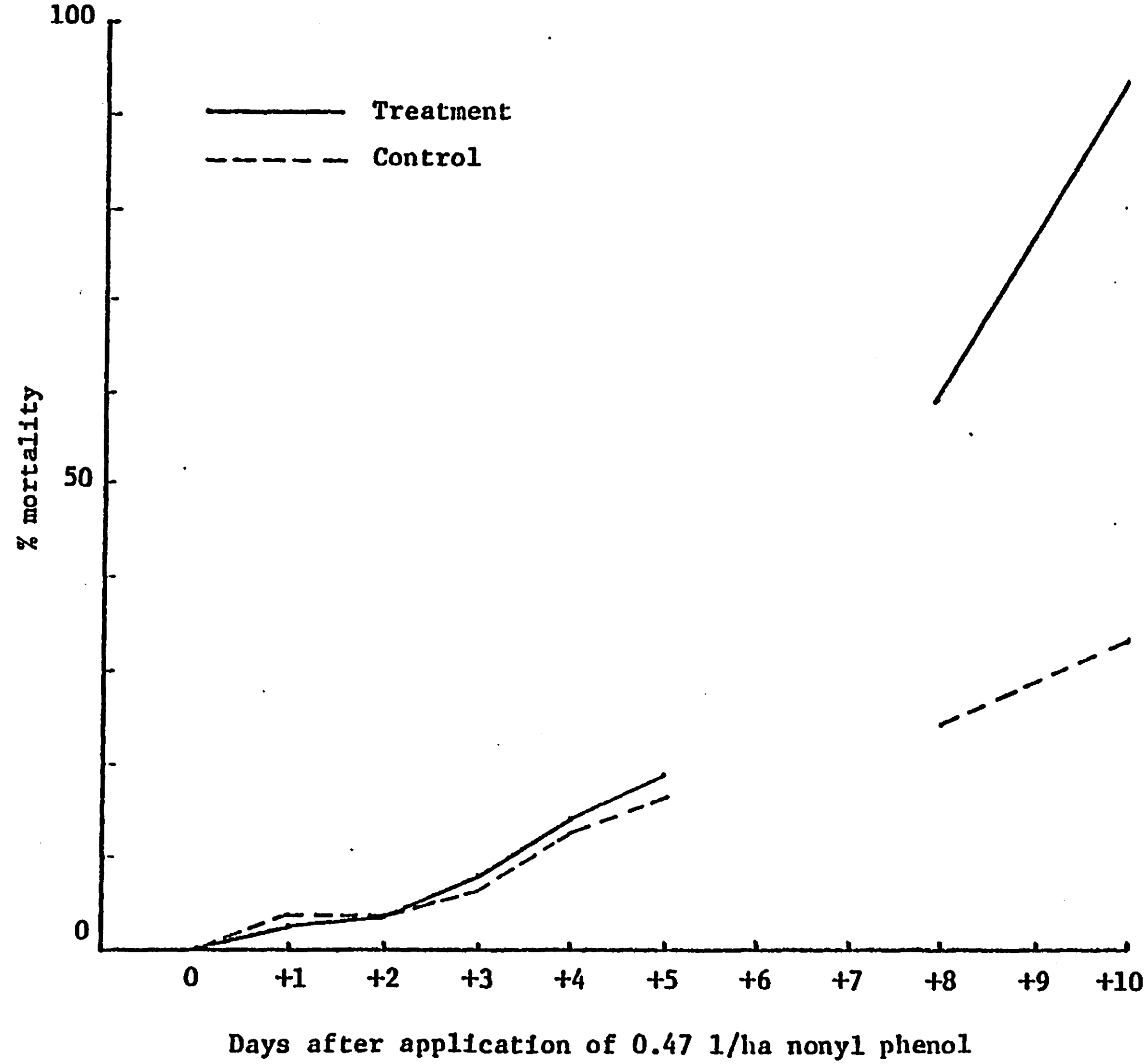


Table 19

Mortality/Survival of caged Brook Trout (*Salvelinus fontinalis*)
Dubreuilville, Ontario, 25 May – 8 June 1979

	May 25	May 26	May 27	May 28	May 29	May 30	May 31	June 1	June 2	June 3	June 4	June 5	June 6	June 7	June 8
Days before or after application of 0.47 l/ha nonyl phenol	-4	-3	-2	-1	0	+1	+2	+3	+4	+5	+6	+7	+8	+9	+10
Cage Number															
Control															
1	0/24	0/24	0/24	0/24	0/24	1/23	1/23	2/22	5/19	7/17*					
2	0/25	0/25	0/25	0/25	0/25	1/24	1/24	1/24	1/24	1/24			6/19		8/17
Treatment															
3	0/26	0/26	0/26	0/26	0/26	2/24	2/24	3/23	5/21	5/21			17/9		24/2
4	0/20	0/20	0/20	0/20	0/20	1/19	2/18	2/18	3/17	3/17			10/10		19/1
5	0/25	0/25	0/25	0/25	0/25	0/25	0/25	0/25	0/25	4/21*					
6	0/25	0/25	0/25	0/25	0/25	0/25	0/25	2/23	5/20	5/20*					

* On June 3, cages 1, 5 and 6 were removed and the fish collected for residue analysis. At this time some dead fish were removed which may not have been noticed when the daily checks were made.

temperature of a natural stream situation were reproduced. Fish were observed for mortality and the dead fish removed at intervals over a 96 hour period.

The results from these experiments (Tables 20 and 21) were used to construct toxicity curves for nonyl phenol on log-probit graph paper (Figs. 5 and 6). From these curves approximate 96 hour LC50's of nonyl phenol were interpolated as 230 μg/ℓ to fingerling rainbow trout under controlled laboratory conditions and 145 μg/ℓ for brook trout under actual field conditions.

An attempt was made to look for evidence of delayed lethal effects of nonyl phenol on fish following exposure to levels similar to those found in the field experiment. Measured volumes of a stock solution of nonyl phenol dissolved in absolute ethanol were added to tanks containing 20 litres of dechlorinated tap water to make a series of five concentrations (2.5, 5, 10, 20 and 40 μg/ℓ). Absolute ethanol equivalent to the amount added to the highest nonyl phenol concentration was added to the control. Eleven fingerling lake trout, *Salvelinus namaycush* (Walbaum), were placed in each tank and held at 12.0 ± 1.0°C in an environmental chamber. At the end of two weeks no mortality had been observed in any of the tanks with the exception of one fish which jumped out of the 10 μg/ℓ concentration. This bioassay is continuing.

TERRESTRIAL IMPACT STUDIES

Spruce budworm population sampling and assessment

Twenty-five balsam fir (*Abies balsamea* [L.] Mill) and 25 white spruce (*Picea glauca* [Moench] Voss) within the spray block were selected as sampling sites to measure spruce budworm population densities. Ground vegetation around each tree was removed to allow accessibility for the sampling crew and any adjacent or overstory trees which would screen out the spray were removed. Each sample tree was identified with a code number for reference. Branch tips, 46 cm in length were removed from the mid to upper crown region using pole pruners and the individual tips were placed in separate 25 lb. Kraft paper bags at the collecting station, coded appropriately to indicate place and date of sampling and then taken to the Great Lakes Forest Research Centre, Sault Ste. Marie, for budworm counting. All bagged samples were stored in a cold room at 6°C until the actual counts were made.

The sampling protocol followed was collecting one pre-spray sample immediately prior to spray application, a first post-spray sample 8 days after application and then a last sample at a stage when approximately 50% of the larvae had pupated. When pupation was complete, branch samples were also taken for defoliation determination.

Table 20

Mortality/survival of
laboratory bioassay fingerling rainbow trout, *Salmo gairdneri**
2 - 6 April 1979

	Time of Exposure							
Concentration	0 hr	3 hr	6 hr	12 hr	36 hr	48 hr	72 hr	96 hr
Control	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9
100 ug/1	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9
180 ug/1	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9
300 ug/1	0/9	0/9	0/9	0/9	8/1	8/1	9/0	9/0
1000 ug/1	0/9	1/8	9/0					

	Mean	Range
* Total length	0.52 cm	0.42 - 0.61 cm
Fork length	0.49 cm	0.40 - 0.59 cm
Weight	1.35 g	0.50 - 2.70 g

Fig. 5

Graphic estimation of 96 hr LC50 of nonyl phenol for fingerling rainbow trout, *Salmo gairdneri*, from laboratory bioassay data

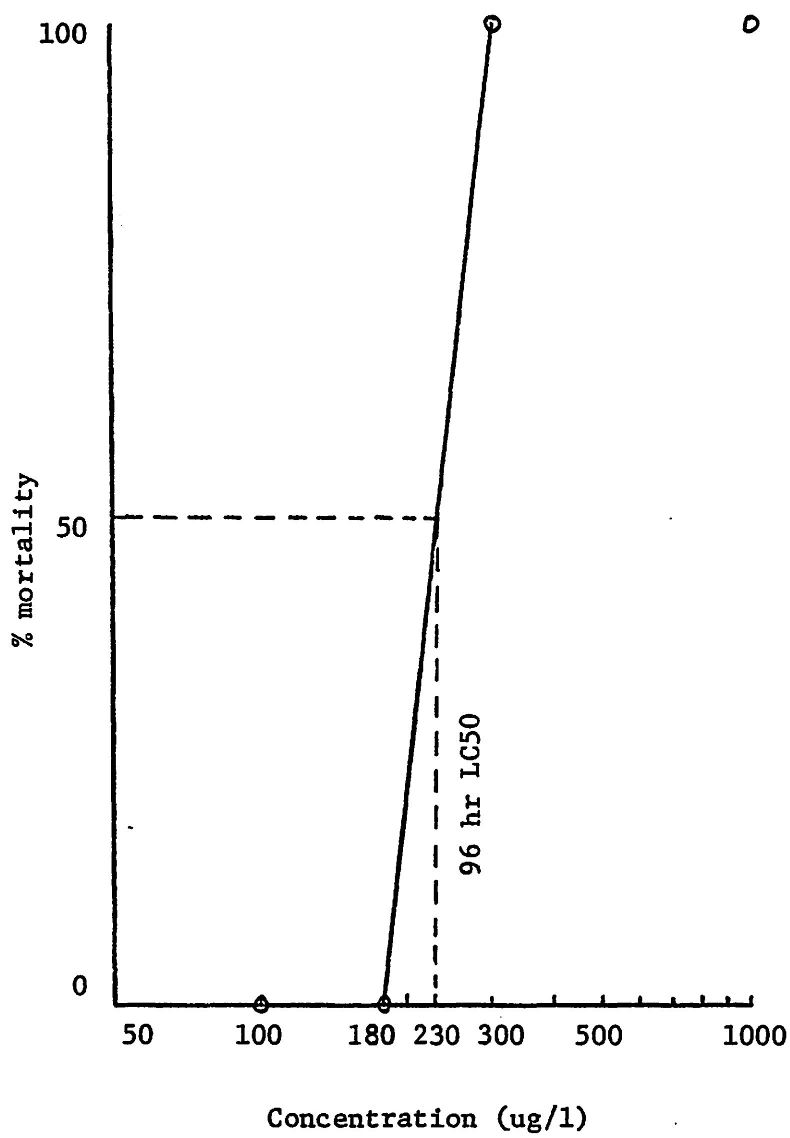


Table 21

Mortality/survival of
field bioassay fingerling brook trout, *Salvelinus fontinalis*
Dubreuilville, Ontario, 26 May – 30 May 1979**

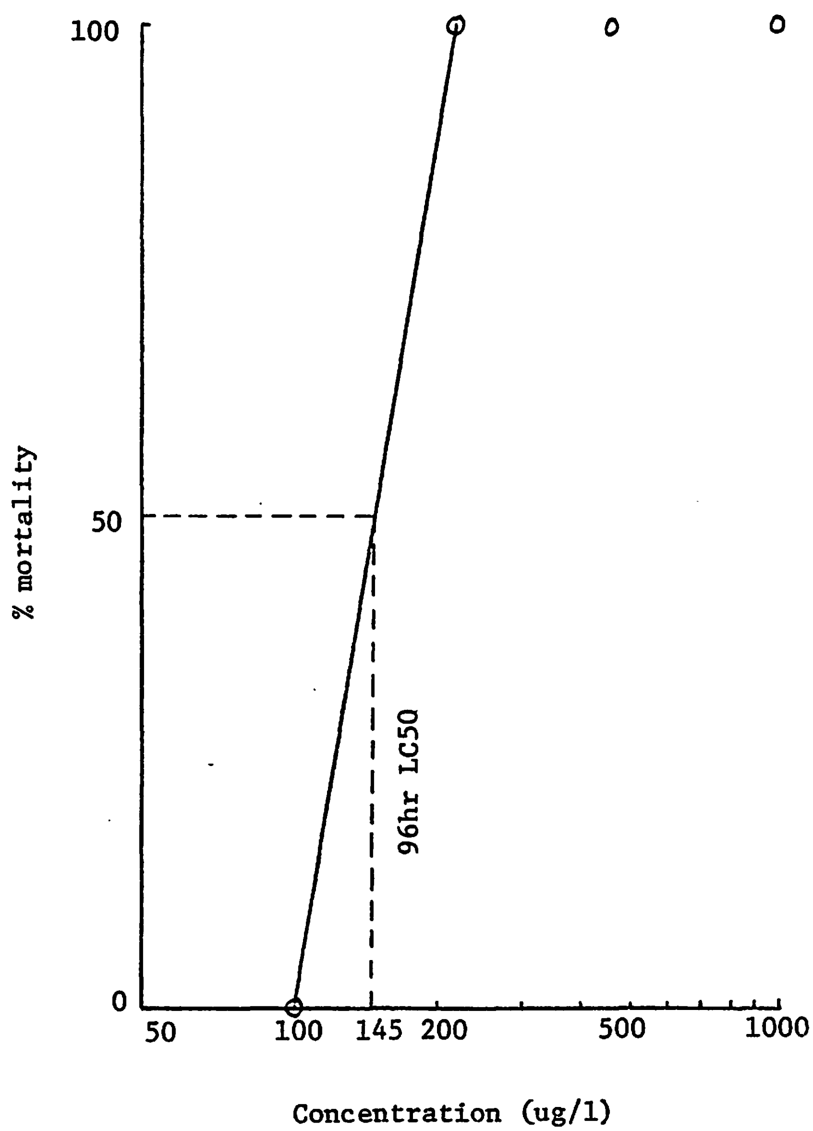
Concentration	Time of exposure									
	0 hr	8 hr	22 hr	30 hr	35 hr	46 hr	59 hr	69 hr	83 hr	96 hr
Control	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9
100 ug/1	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9
210 ug/1	0/9	0/9	0/9	0/9	0/9	0/9	0/9	1/8	1/8	9/0
460 ug/1	0/9	0/9	1/8	2/7	4/5	9/0				
1000 ug/1	0/9	9/0								

	Mean	Range
* Total length	0.49 cm	0.36 – 0.55 cm
Fork length	0.48 cm	0.35 – 0.54 cm
Weight	0.90 g	0.50 – 1.40 g

** The field bioassay was started at 0830 on 26 May 1979 and completed at 0830 on 30 May 1979

Fig. 6

Graphic estimation of 96 hr LC50 of nonyl phenol for fingerling brook trout, *Salvelinus fontinalis*, from field bioassay data



Fifteen balsam fir and 15 white spruce trees were identified and marked in the untreated check plot, and standard branch samples taken from them on the same days as the test trees were sampled. The data from these branches provided information to calculate the budworm population decline as occurred in an untreated population due to natural causes.

The results of the spruce budworm larval assessment study are presented in Table 22. Pre-spray larval densities on both the nonyl phenol and the untreated check plots were low, but increased on each by the next sampling date (1st post-spray sample). This apparent increase in the budworm population is the result of attempting to assess the numbers of early emerging instars using a sampling procedure developed for later instars. At the time the pre-spray sample was taken, not all larvae had emerged and migrated to the branch tip, thus a lower count was obtained using the 46 cm branch tip than if the whole branch had been taken and checked for L-2 budworm. Later samples taken when all emergence and migration was complete yielded a higher number and thus there is the indicated increase in larval density. It is thus impossible to determine whether there was any effect of the aerial spray on budworm larvae and with no accurate fix on the population the defoliation data are also meaningless.

Terrestrial invertebrate knockdown

Rectangular plastic washtubs 29 cm x 33 cm x 15 cm were placed under tree species typical of the forest stands on the treatment and control bird plots (Table 23) and of the shoreline cover along the treatment and control streams (Table 24). Invertebrates (live and dead) were collected from the buckets each evening, preserved in 70% methanol and later identified to order in the laboratory.

Terrestrial insect catches in all the areas sampled showed very similar patterns of increase and decrease, both between various areas sampled in each plot and between treatment and control plots (Fig. 7 and 8). There was a slight increase in the catch of spiders (Arachnida) and flies (Diptera) in the treated bird plot after the treatment, particularily under coniferous trees (Tables 25 and 26). A slight increase along the nonyl phenol treatment stream the day of spray application consisted mostly of mites (Acari), springtails (Collembola) and adult hymenopterans (Tables 27 and 28). In all cases these increases were no larger than similar increases seen in the control areas within the next few days. Catches on all plots were high on 1 June as the result of heavy rains which fell throughout the day.

Honeybees

Two colonies of domestic honey bees were set up in the nonyl phenol spray plot and in an untreated area about 10 km away on 25 May.

Table 22. Densities of living budworm on various sample dates, pupal survival and current defoliation for a plot sprayed with nonyl phenol and a check plot. Sprayed May 29/79 a.m. Treated plot n = 25, check plot n = 15.

	Prespray[1] Living L_2 per 46 cm branch tip		1st Postspray[2] Living L_{3-4} per 46 cm branch tip		2nd Postspray[3] Living L_6-p per 46 cm branch tip		% Successful pupal emergence[4]		% 1979 Defoliation	
	bF	wS	bF	wS	bF	wS	bF	wS	bF	wS
Nonyl phenol	2.8	4.5	27.4	33.3	8.0	4.8	68	50	74	80
Check	5.3	24.7	76.3	113.3	10.9	19.9	38	68	91	95

[1] Nonyl phenol plot sampled May 28/79
Check plot sampled May 29/79

[2] Both plots sampled June 6/79

[3] Nonyl phenol plot sampled July 6/79

[4] $\%\ \text{Successful pupal emergence} = \dfrac{\text{emerged budworm}}{\text{budworm alive on sample date}} \times 100$

TABLE 23

Tree species samples for terrestrial invertebrate knockdown on bird plots, Dubreuilville, Ontario, 25 May - 3 June, 1979

Nonyl phenol treatment plot

Bucket number	Species
1	*Pinus banksiana* Lamb.
2	*Abies balsamea* (L.) Mill.
3	*Abies balsamea* (L.) Mill.
4	*Abies balsamea* (L.) Mill.
5	*Picea mariana* (Mill.) B.S.P.
6	*Populus tremuloides* Michx.
7	*Corylus cornuta* Marsh.
8	*Populus tremuloides* Michx.
9	*Salix* L.
10	*Salix* L.
11	*Salix* L.
12	*Salix* L.

Nonyl phenol control plot

Bucket number	Species
1	*Abies balsamea* (L.) Mill.
2	*Abies balsamea* (L.) Mill.
3	*Abies balsamea* (L.) Mill.
4	*Picea mariana* (Mill.) B.S.P.
5	*Abies balsamea* (L.) Mill.
6	*Corylus cornuta* Marsh.
7	*Betula papyiifera* Marsh.
8	*Populus tremuloides* Michx.
9	*Salix* L.
10	*Salix* L.
11	*Salix* L.
12	*Salix* L.

TABLE 24

Tree species samples for terrestrial invertebrate knockdown along streams, Dubreuilville, Ontario
24 May - 2 June, 1979

Nonyl phenol treatment stream

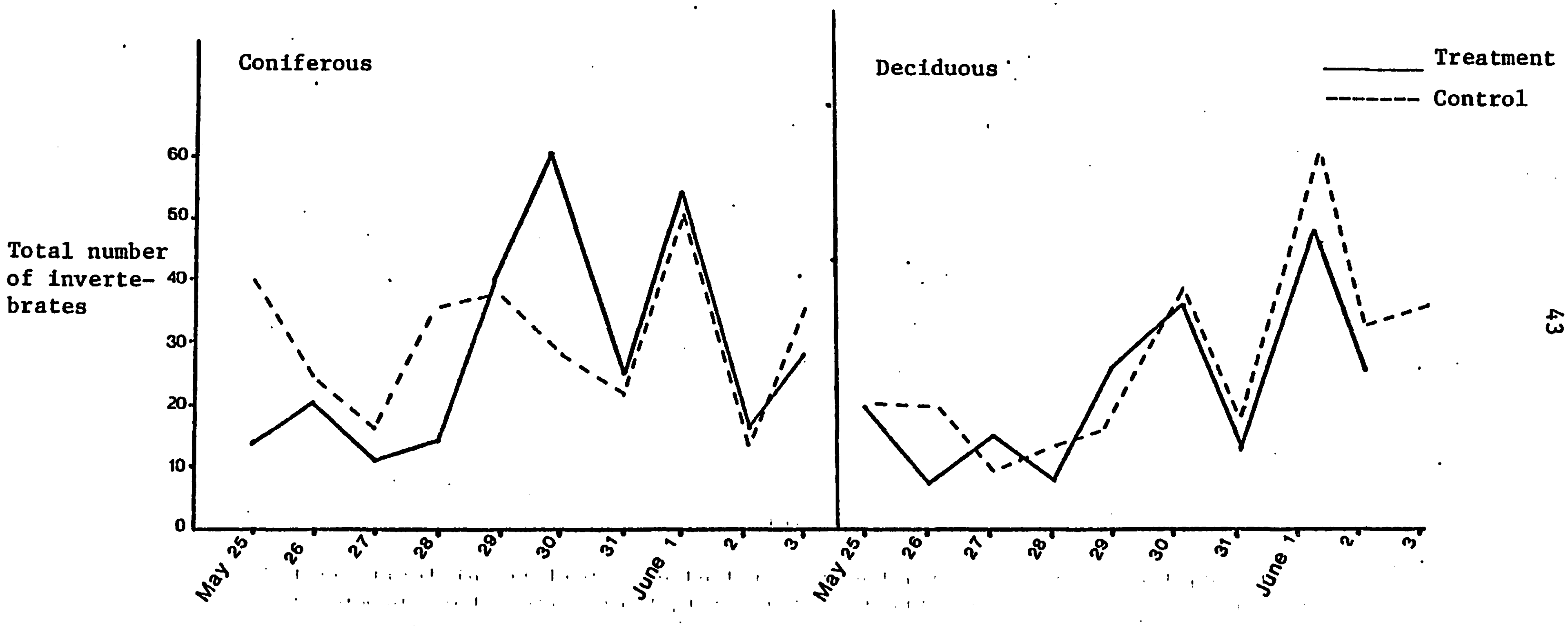
Bucket number	Species
1	*Picea glauca* (Moench) Voss
2	*Picea glauca* (Moench) Voss
3	*Alnus rugosa* (Du Roi) Spreng
4	*Alnus rusoga* (Du Roi) Spreng
5	open

Nonyl phenol control stream

Bucket number	Species
1	*Picea mariana* (Mill.) B.S.P.
2	*Alnus rugosa* (Du Roi) Spreng
3	*Lonicera* sp. L.
4	*Sambucus* sp. (Tourn.) L.
5	open

Fig. 7

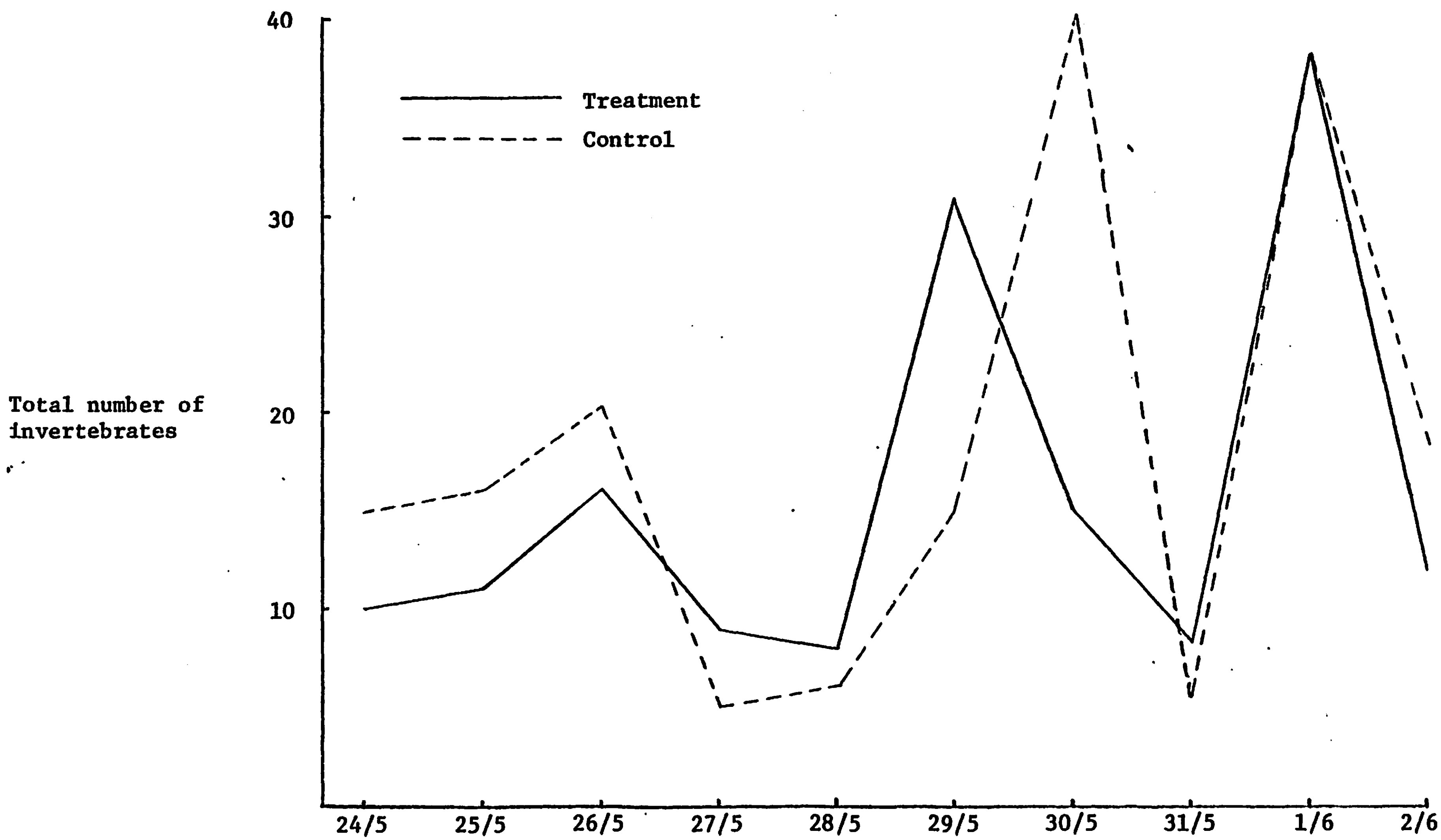
Terrestrial invertebrates in buckets set under trees in the nonyl phenol treatment* and control bird plots, Dubreuilville, Ontario. 25 May-3 June 1979



*treated at 0707 on 29 May 1979

Fig. 8

Terrestrial insect knockdown along the treatment* and control streams, Dubreuilville, Ontario, 24 May - 2 June 1979



* application at 0707 on 29 May 1979

Table 25

Terrestrial insect knockdown
Nonyl phenol treatment plot,
Dubreuilville, Ontario
25 May-3 June 1979

		May 25 -4	May 26 -3	May 27 -2	May 28 -1	May 29 +0	May 30 +1	May 31 +2	June 1 +3	June 2 +4	June 3 +5
Arachnida	Phalangida	0	0	0	0	0	0	0	0	1	0
	Araneida	2	3	3	1	7	8	5	2	2	4
	Acari	0	0	0	0	0	0	0	1	1	0
Collembola		1	0	0	0	0	0	1	1	0	0
Hemiptera		0	0	1	0	0	2	0	1	0	1
Coleoptera	Staphylinidae	0	0	0	0	2	4	3	3	5	4
	Other	0	0	0	0	4	3	1	5	0	0
Trichoptera		0	0	0	0	0	0	0	0	0	0
Lepidoptera	Larvae	0	0	1	0	0	0	0	1	0	0
Diptera	Chironomidae	0	0	0	0	0	1	0	0	0	0
	Other	7	6	4	9	24	31	8	39	14	27
Hymenoptera	Formicidae	0	0	0	1	0	6	1	0	0	1
	Other	2	0	1	2	0	0	2	9	3	1
Total Number of Organisms		12	9	10	13	37	55	21	62	26	38

Table 26

Terrestrial insect knockdown
Nonyl phenol control plot,
Dubreuilville, Ontario
25 May-3 June 1979

		May 25	May 26	May 27	May 28	May 29	May 30	May 31	June 1	June 2	June 3
		-4	-3	-2	-1	+0	+1	+2	+3	+4	+5
Gasteropoda		0	0	0	0	0	0	0	0	1	0
Arachnida	Araneida	3	0	1	1	1	3	1	2	1	2
Collembola		1	0	3	5	0	1	0	2	0	0
Hemiptera		0	0	0	0	1	0	0	0	0	0
Homoptera		0	0	0	0	0	0	0	0	0	1
Coleoptera	Staphylinidae	3	0	0	0	1	4	1	2	1	2
	Other	0	0	0	0	3	1	2	2	0	0
Lepidoptera	Larvae	1	0	3	0	1	0	0	0	0	0
Diptera	Chironomidae	0	0	0	0	1	1	0	2	2	4
	Other	13	16	4	10	18	27	19	51	28	30
Hymenoptera	Formicidae	0	0	0	0	0	0	0	0	1	2
	Other	4	1	0	3	4	3	0	6	2	2
Total Number of Organisms		25	17	11	19	30	40	23	67	36	43

Table 27

Terrestrial insect knockdown, nonyl phenol treatment stream
Dubreuilville, Ontario, 24 May - 2 June 1979

		May 24	May 25	May 26	May 27	May 28	May 29	May 30	May 31	June 1	June 2
Days before or after application of 0.47 l/ha nonyl phenol		-5	-4	-3	-2	-1	0	+1	+2	+3	+4
Arachnida											
Araneida		1		1	1	1	3	3			
Acari							5	2			1
Collembola					2		6	1		3	
Ephemeroptera											
	adults									1	
Hemiptera											
	nymphs	1									
	adults									1	
Lepidoptera											
	larvae						1				
Hymenoptera											
Formicidae			1				1	1	2		1
Other	adults	2	6	3	2	4	8	2	2	4	1
Coleoptera											
Staphylinidae	adults	1		1	2	1		1	1	4	1
Other	adults		2				2	3		2	1
Diptera											
	adults	5	2	10	2	2	5	2	3	23	12
Unidentifiable Insects				1							1
Total Insect Knockdown		10	11	16	9	8	31	15	8	38	18

Table 28

Terrestrial insect knockdown, nonyl phenol control stream
Dubreuilville, Ontario, 24 May - 2 June 1979

		May 24	May 25	May 26	May 27	May 28	May 29	May 30	May 31	June 1	June 2
Days before or after application of 0.47 l/ha nonyl phenol		-5	-4	-3	-2	-1	0	+1	+2	+3	+4
Arachnida											
Araneida		4	8	7			1	2		2	
Acari								1			
Collembola					2			1			
Plecoptera									1		
Hemiptera											
Gerridae			1				1				
Other	nymphs	2		1							
	adults	1					1			1	
Lepidoptera											
	larvae							1			
	adults									1	
Hymenoptera											
Formicidae			1					2		1	1
Other	adults			1	2		2	5	1	5	
Coleoptera											
Staphylinidae	adults	2	2	1			1	10	1	5	
Other	adults		3					5		2	
Diptera											
	adults	6	1	10		6	8	12	3	21	11
Unidentifiable Insects					1		1	1			
Total Insect Knockdown		15	16	20	5	6	15	40	6	38	12

The colonies were of Italian strain from stock overwintered at the FPMI apiary near Sault Ste. Marie. Once on site, all colonies were fitted with a dead bee box, an Ontario Agricultural College pollen trap and an electronic photo-electric cell equipped activity counter. Hive weights were taken with a bathroom scale placed under the edge of the hive. Monitoring was carried out daily from 27 May to 4 June. On 5 June the colonies were returned to the laboratory apiary to prevent their possible destruction by black bears. Brood measurements (square centimeters of capped brood) were made on 30 May, 6 June and 30 July. On 30 May a portion of a frame from each colony was delineated and the number of cells containing eggs was estimated. On 6 June these areas were resurveyed and the cell contents recorded.

The application of nonyl phenol had relatively little impact upon population or activity of domestic honey bees. A slight increase in the numbers of dead foraging bees was recorded from the treated hives on the day following the day of treatment, but subsided quickly to "normal" levels in subsequent days (Table 29). Activity patterns (flights to and from the hive) were relatively constant on the treated site but somewhat more variable on the untreated control site, probably reflecting local conditions such as hive location, shelter, micro-climatic conditions, etc. Pollen collections reflect weather patterns throughout the experimental period. Very little pollen was recorded at the treated site and the discovery of a virgin queen in one of the untreated hives would indicate that the colony had superceded an old failing queen which would account for the reduction in pollen collecting for brood rearing.

Hive weights remained relatively constant throughout the experimental period. The area of capped brood was slightly affected by the requeening as the area of brood declined slightly over a period of 9 days in the treated hives while a slight increase was recorded on the untreated hives. Measurements taken later in the season show a substantial increase of capped brood in hives from both treated and untreated sites. Of 340 cells counted in a marked portion of brood in the untreated hives, 295 contained eggs of which 292, or approximately 99%, reached the capped brood stage, while 400 cells out of 490, or 81%, reached the capped brood stage in the treated hives (Table 30).

Birds

Bird censuses were conducted on the nonyl phenol treatment and control bird plots for five days before and after treatment. Singing males were identified and their territories delineated for the pre-spray and post-spray periods.

Avian activity on both plots follow a similar pattern except for a noticeable decline in singing activity on the treatment plot during the spray operations when large numbers of people were engaged in various activities on or very near the bird plot (Fig. 9). None of

Table 29

Honeybee activity on nonyl phenol treated and untreated control plots

Dubreuilville, Ontario
27 May - 6 June
1979

(average of two colonies on each site)

	Untreated control beehives					Nonyl phenol treated beehives				
Date	Mortality (adult bees)	Activity bee/trips	Pollen (gm)	Hive weight (kg)	Brood (cm^2)	Mortality (adult bees)	Activity bee/trips	Pollen (gm)	Hive weight (kg)	Brood (cm^2)
27 May	4.5	-	-	-		18.0	-	-	-	
28 May	2.0	35520	-	-		3.5	2880	-	-	
29 May	2.0	7296	4.12	12.2	928	3.0	11776	0	12.2	1016
30 May	6.5	18688	21.85	12.4		23.5	23552	4.04	12.4	
31 May	4.0	10624	7.01	12.2		8.5	23552	0	12.4	
1 June	3.0	47232	4.91	12.2		5.0	23872	1.45	12.7	
2 June	1.0	8448	8.14	12.2		2.0	20416	1.80	12.4	
3 June	1.5	11648	16.24	12.2		3.5	20864	0	12.4	
4 June	0.5	8640	22.80	11.9		6.5	38976	1.91	12.4	
6 June	-	-	-	-	1168	-	-			944
30 July	-	-	-	-	2688	-	-			2296

Table 30

Honeybee brood development

Nonyl phenol experimental area

Dubreuilville, Ontario
30 May - 6 June, 1976

Untreated control hives	30 May	6 June	Treated hives	30 May	6 June
no. of cells examined	340	340	no. of cells examined	500	500
no. of cells containing eggs	295	0	no. of cells containing eggs	490	10
no. of cells containing larvae	0	0	no. of cells containing larvae	0	10
no. of cells containing capped brood	0	295	no. of cells containing capped brood	0	400
no. of empty cells	45	48	no. of empty cells	10	80

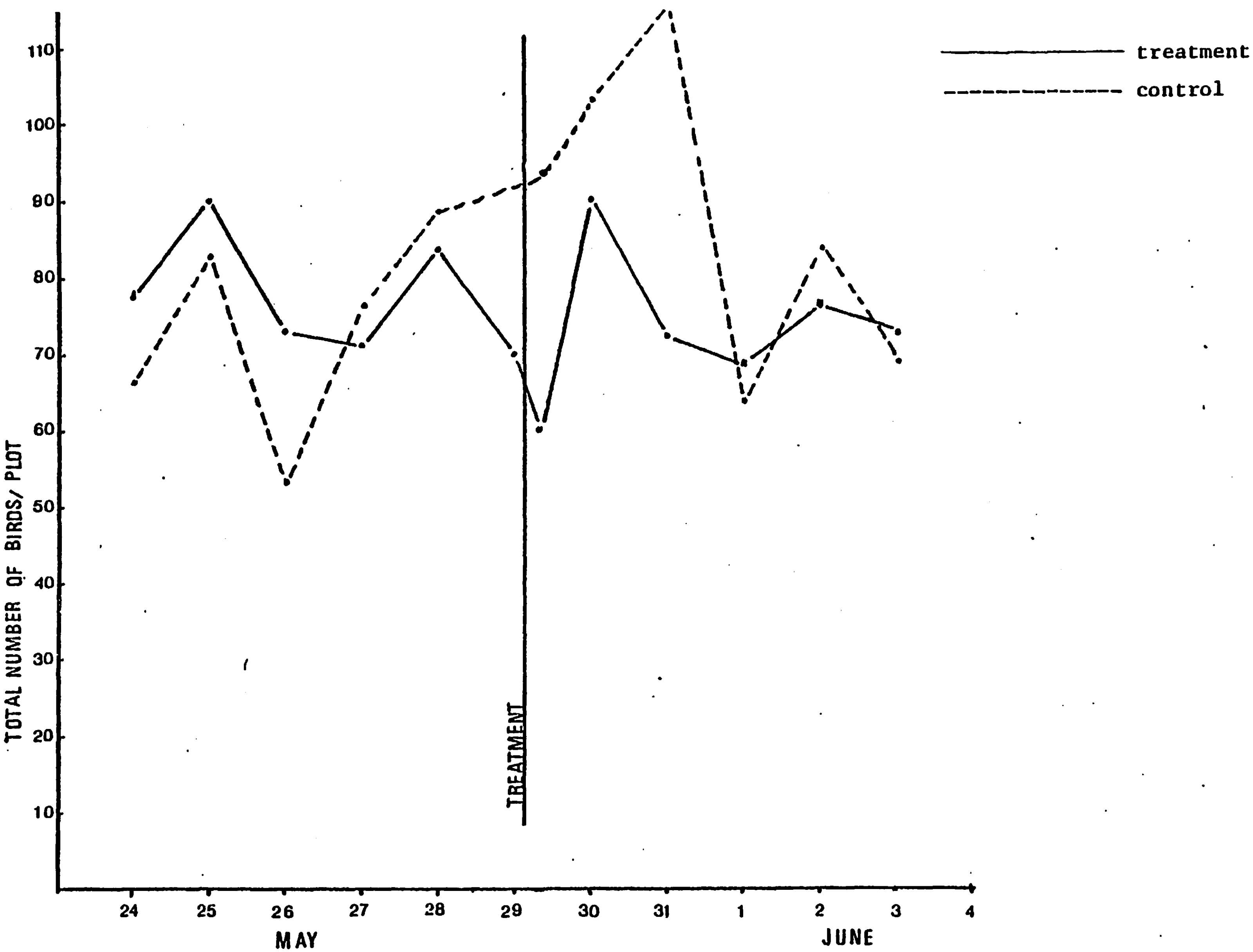


Fig. 9. Forest songbird activity on the nonyl phenol treated and control bird plots, Dubreuilville, Ontario

the major family groups were affected by the treatment and a recorded decline in the family Fringillidae on the treated plot also occurred on the untreated control plot (Tables 31 and 32).

Populations of the ruby crowned kinglet, *Regulus calendula* (Linnaeus), a small insectivorous bird known to be quite pesticide sensitive, were not affected. Activity of such species as the Nashville warbler, *Vermivora ruficapilla* (Wilson) and the yellow-rumped warbler, *Dendroica coronata* (Linnaeus) decreased following treatment while others like the Cape May warbler, *Dendroica tigrina* (Gmelin) and Tennessee warbler, *Vermivora peregrina* (Wilson) increased. White-throated sparrow, *Zonotrichia albicollis* (Gmelin) activity declined on both plots following treatment (Tables 33 and 34).

While the activity of some species were affected by human activity following treatment, breeding territories remained occupied. The breeding territories of six species of wood warblers (family Parulidae), Fig. 10, as well as territories for the white-throated sparrow, ruby-crowned kinglet and the hermit thrush, *Hylocichla guttata* (Pallus), Fig. 11, are illustrated and show that the experimental application of nonyl phenol did not force abandonment of territories.

Intensive plot searches were carried out for two days following the experimental treatment but no dead birds were recovered and no birds were observed exhibiting the typical symptoms of pesticide stress.

Table 31

Forest Bird Population Census
Nonyl phenol treatment plot
Dubreuilville, Ontario
24 May - 3 June 1979

Family	Prespray May 24 -5	May 25 -4	May 26 -3	May 27 -2	May 28 -1	May 29 -0	Daily ave.	Postspray May 29 +0	May 30 +1	May 31 +2	June 1 +3	June 2 +4	June 3 +5	Daily ave.
Tetraonidae	0	0	1	0	0	0	0.2	0	0	0	0	1	1	0.3
Alcedinidae	1	0	0	0	1	0	0.3	0	1	1	0	1	0	0.5
Picidae	1	3	3	4	0	0	1.8	0	0	0	2	0	0	0.3
Tyrannidae	0	0	0	2	0	0	0.3	2	2	4	0	4	2	2.3
Corvidae	4	2	2	2	2	0	2.0	0	1	0	0	3	0	0.7
Paridae	0	0	2	1	0	0	0.5	0	3	1	0	0	2	1.0
Sittidae	2	0	0	0	0	0	0.3	0	0	0	0	0	0	0.0
Troglodytidae	2	0	0	2	2	2	1.3	0	0	0	0	0	2	0.3
Turdidae	1	3	3	0	9	6	3.7	2	8	6	8	6	11	6.8
Sylviidae	6	8	6	8	4	6	6.3	6	8	4	2	2	2	4.0
Vireonidae	2	0	0	4	0	2	1.3	0	2	2	0	0	0	0.7
Parulidae	41	51	24	39	47	33	39.2	40	50	32	37	40	42	40.2
Icteridae	0	0	2	0	0	0	0.3	0	0	0	0	0	0	0.0
Fringillidae	17	23	30	9	18	21	19.7	10	15	22	19	19	11	16.0
Total Birds	77	90	73	71	83	70	77.3	60	90	72	68	76	73	73.2

Table 32

Forest Bird Population Census
Nonyl phenol untreated control plot
Dubreuilville, Ontario
24 May - 3 June 1979

Family	Prespray						Postspray						
	May 24	May 25	May 26	May 27	May 28	Daily	May 29	May 30	May 31	June 1	June 2	June 3	Daily
	-5	-4	-3	-2	-1	Ave	+0	+1	+2	+3	+4	+5	Ave
Tetraonidae	0	1	0	0	1	0.4	2	0	1	0	0	1	0.7
Picidae	2	1	2	0	2	1.4	5	1	2	0	1	0	1.5
Tyrannidae	2	0	0	0	0	0.4	0	4	2	0	6	4	2.7
Corvidae	2	0	0	1	0	0.6	1	4	2	0	0	0	1.2
Paridae	2	0	0	1	2	1.0	0	0	0	0	1	0	0.2
Troglodytidae	2	2	4	4	2	2.8	2	0	0	2	2	2	1.3
Turidae	4	4	0	6	4	3.6	2	9	6	3	6	5	5.2
Sylviidae	6	6	4	4	10	6.0	8	10	2	3	2	6	5.2
Vireonidae	0	0	0	0	0	0.0	0	4	4	2	2	2	2.3
Parulidae	42	51	38	49	54	46.8	69	59	68	51	53	40	56.7
Icteridae	0	0	0	0	0	0.0	0	2	0	0	2	0	0.7
Fingillidae	5	18	6	12	13	10.8	5	10	24	2	9	9	9.8
TOTAL	67	83	54	77	88	73.8	94	103	111	63	84	69	87.3

Table 33

Forest Bird Population Census
Nonyl phenol treatment plot
Dubreuilville, Ontario
24 May – 3 June 1979

Family	Species	Prespray May 24 −5	May 25 −4	May 26 −3	May 27 −2	May 28 −1	May 29 −0	Daily ave.	Postspray May 29 +0	May 30 +1	May 31 +2	June 1 +3	June 2 +4	June 3 +5	Daily ave.
Tetraonidae	Spruce Grouse	0	0	1	0	0	0	0.2	0	0	0	0	1	1	0.3
Alcedinidae	Belted Kingfisher	1	0	0	0	1	0	0.3	0	1	1	0	1	0	0.5
Picidae	Common Flicker	0	0	3	0	0	0	0.5	0	0	0	1	0	0	0.2
	Yellow-bellied Sapsucker	1	3	0	4	0	0	1.3	0	0	0	1	0	0	0.2
Tyrannidae	Great Crested Flycatcher	0	0	0	2	0	0	0.3	0	0	2	0	0	0	0.3
	Least Flycatcher	0	0	0	0	0	0	0.0	0	2	2	0	2	2	1.3
	Olive-sided Flycatcher	0	0	0	0	0	0	0.0	2	0	0	0	2	0	0.7
Corvidae	Gray Jay	0	0	1	0	1	0	0.3	0	0	0	0	0	0	0.0
	Blue Jay	2	2	1	1	1	0	1.2	0	0	0	0	0	0	0.0
	Raven	2	0	0	1	0	0	0.5	0	1	0	0	3	0	0.7
Paridae	Black-capped Chickadee	0	0	1	0	0	0	0.2	0	0	0	0	0	0	0.0
	Boreal Chickadee	0	0	1	1	0	0	0.3	0	3	1	0	0	2	1.0
Sittidae	Red-breasted Nuthatch	2	0	0	0	0	0	0.3	0	0	0	0	0	0	0.0
Troglodytidae	Winter Wren	2	0	0	2	2	2	1.3	0	0	0	0	0	2	0.3
Turdidae	American Robin	0	1	0	0	0	0	0.2	0	0	0	0	0	2	0.3
	Hermit Thrush	1	2	3	0	9	6	3.5	2	7	6	8	6	7	6.0
	Veery	0	0	0	0	0	0	0.0	0	1	0	0	0	2	0.5
Sylviidae	Ruby-crowned Kinglet	6	8	6	8	4	6	6.3	6	8	4	2	2	2	4.0
Vireonidae	Solitary Vireo	2	0	0	0	0	2	0.7	0	0	2	0	0	0	0.3
	Red-eyed Vireo	0	0	0	4	0	0	0.7	0	2	0	0	0	0	0.3
Parulidae	Black and White Warbler	0	1	0	1	3	0	0.8	0	0	0	0	0	0	0.0
	Tennessee Warbler	2	0	2	2	9	6	3.5	8	12	10	8	6	8	8.7
	Nashville Warbler	6	10	6	6	7	10	7.5	3	8	2	2	2	2	3.2
	Magnolia Warbler	8	15	4	5	6	6	7.3	8	6	8	9	8	12	8.5
	Cape May Warbler	6	8	2	10	4	2	5.3	7	6	4	4	8	4	5.5
	Yellow-rumped Warbler	13	13	8	9	0	7	8.3	4	2	0	2	4	6	3.0
	Black-throated Green Warbler	0	0	0	0	0	0	0.0	0	4	0	0	0	2	1.0
	Chestnut-sided Warbler	0	0	0	1	0	0	0.2	4	2	0	2	4	0	2.0
	Bay-breasted Warbler	2	2	0	0	2	0	1.0	0	4	0	6	2	4	2.7
	Ovenbird	0	0	0	0	4	0	0.7	0	0	0	0	0	2	0.3
	Northern Waterthrush	2	2	2	4	4	2	2.7	4	6	6	4	4	2	4.3
	Wilson's Warbler	2	0	0	0	2	0	0.7	0	0	0	0	0	0	0.0
	Canada Warbler	0	0	0	0	2	0	0.3	2	0	2	0	0	0	0.7
	American Redstart	0	0	0	1	4	0	0.8	0	0	0	0	2	0	0.3
Icteridae	Brown-headed Cowbird	0	0	2	0	0	0	0.3	0	0	0	0	0	0	0.0
Fringillidae	Rose-breasted Grosbeak	0	0	2	0	0	0	0.3	2	4	0	0	4	0	1.6
	Evening Grosbeak	1	1	9	0	0	0	1.8	1	3	0	0	1	0	0.8
	Purple Finch	0	0	0	0	0	0	0.0	0	0	2	0	0	0	0.3
	Dark-eyed Junco	0	0	1	0	0	0	0.2	0	2	1	4	4	2	2.2
	Chipping Sparrow	0	4	0	0	2	2	1.3	0	2	0	0	0	0	0.3
	White-throated Sparrow	16	18	18	9	16	19	16.0	7	4	19	15	10	9	10.7
TOTALS:		77	90	73	71	83	70	77.3	60	90	72	68	76	73	73.2

Table 34

Forest Bird Population Census
Nonyl phenol untreated control plot
Dubreuilville, Ontario
24 May – 3 June 1979

Family	Species	Prespray May 24 −5	May 25 −4	May 26 −3	May 27 −2	May 28 −1	Daily ave.	Postspray May 29 +0	May 30 +1	May 31 +2	June 1 +3	June 2 +4	June 3 +5	Daily ave.
Tetraonidae	Ruffed Grouse	0	1	0	0	1	0.4	2	0	1	0	0	1	0.7
Picidae	Yellow-bellied Sapsucker	2	1	2	0	2	1.4	5	1	2	0	1	0	1.5
Tyrannidae	Least Flycatcher	0	0	0	0	0	0.0	0	2	0	0	2	2	1.0
	Olive-sided Flycatcher	2	0	0	0	0	0.4	0	2	2	0	4	2	1.7
Corvidae	Gray Jay	0	0	0	1	0	0.2	0	0	0	0	0	0	0.0
	Blue Jay	1	0	0	0	0	0.2	0	4	1	0	0	0	0.8
	Raven	1	0	0	0	0	0.2	1	0	1	0	0	0	0.3
Paridae	Black-capped Chickadee	2	0	0	0	2	0.8	0	0	0	0	0	0	0.0
	Boreal Chickadee	0	0	0	1	0	0.2	0	0	0	0	1	0	0.2
Troglodytidae	Winter Wren	2	2	4	4	2	2.8	2	0	0	2	2	2	1.3
Turdidae	American Robin	0	2	0	2	0	0.8	0	3	0	0	2	0	0.8
	Hermit Thrush	3	2	0	2	4	2.2	2	6	6	0	4	5	3.8
	Swainson's Thrush	1	0	0	2	0	0.6	0	0	0	3	0	0	0.5
Sylviidae	Golden-crowned Kinglet	0	0	0	0	2	0.4	0	0	0	0	0	0	0.0
	Ruby-crowned Kinglet	6	6	4	4	8	5.6	8	10	2	3	2	6	5.2
Vireonidae	Solitary Vireo	0	0	0	0	0	0.0	0	2	2	2	0	2	1.3
	Red-eyed Vireo	0	0	0	0	0	0.0	0	2	2	0	2	0	1.0
Parulidae	Black-and-white Warbler	4	0	2	4	2	2.4	0	0	0	1	0	0	0.1
	Tennessee Warbler	8	4	4	6	16	7.6	20	14	18	10	12	8	13.7
	Nashville Warbler	4	13	2	4	3	5.2	4	2	3	2	0	2	2.2
	Magnolia Warbler	10	6	10	6	10	8.4	6	4	6	8	4	6	5.7
	Cape May Warbler	6	4	8	4	6	5.6	10	8	6	4	8	0	6.0
	Yellow-rumped Warbler	2	6	2	11	4	5.0	6	2	8	4	4	4	4.7
	Black-throated Green Warbler	0	2	0	0	0	0.4	0	0	4	0	2	0	1.0
	Blackburnian Warbler	2	0	0	2	0	0.8	0	0	2	6	6	4	3.0
	Chestnut-sided Warbler	2	4	0	0	0	1.2	2	4	4	0	2	4	2.7
	Bay-breasted Warbler	0	8	8	6	5	5.4	10	15	4	4	4	6	7.2
	Ovenbird	4	4	2	4	6	4.0	7	4	4	4	4	2	4.2
	Northern Waterthrush	0	0	0	2	2	0.8	2	2	4	4	4	4	3.3
	Canada Warbler	0	0	0	0	0	0.0	2	4	5	4	3	0	3.0
Icteridae	Red Winged Blackbird	0	0	0	0	0	0.0	0	0	0	0	2	0	0.3
	Brown-headed Cowbird	0	0	0	0	0	0.0	0	2	0	0	0	0	0.3
Fringillidae	Rose-breasted Grosbeak	2	4	2	0	2	2.0	0	3	4	0	2	0	1.5
	Evening Grosbeak	1	1	2	1	2	1.4	1	0	2	0	2	1	1.0
	Purple Finch	2	0	0	0	0	0.4	0	4	6	0	0	2	2.0
	Pine Siskin	0	0	0	2	0	0.4	0	0	0	0	0	0	0.0
	Dark-eyed Junco	0	1	0	0	2	0.6	0	0	0	0	0	2	0.3
	Chipping Sparrow	0	0	0	1	0	0.2	2	0	0	0	0	0	0.3
	White-throated sparrow	0	12	2	8	7	5.8	2	3	12	2	5	4	4.7
TOTALS:		67	83	54	77	88	73.8	94	103	111	63	84	69	87.3

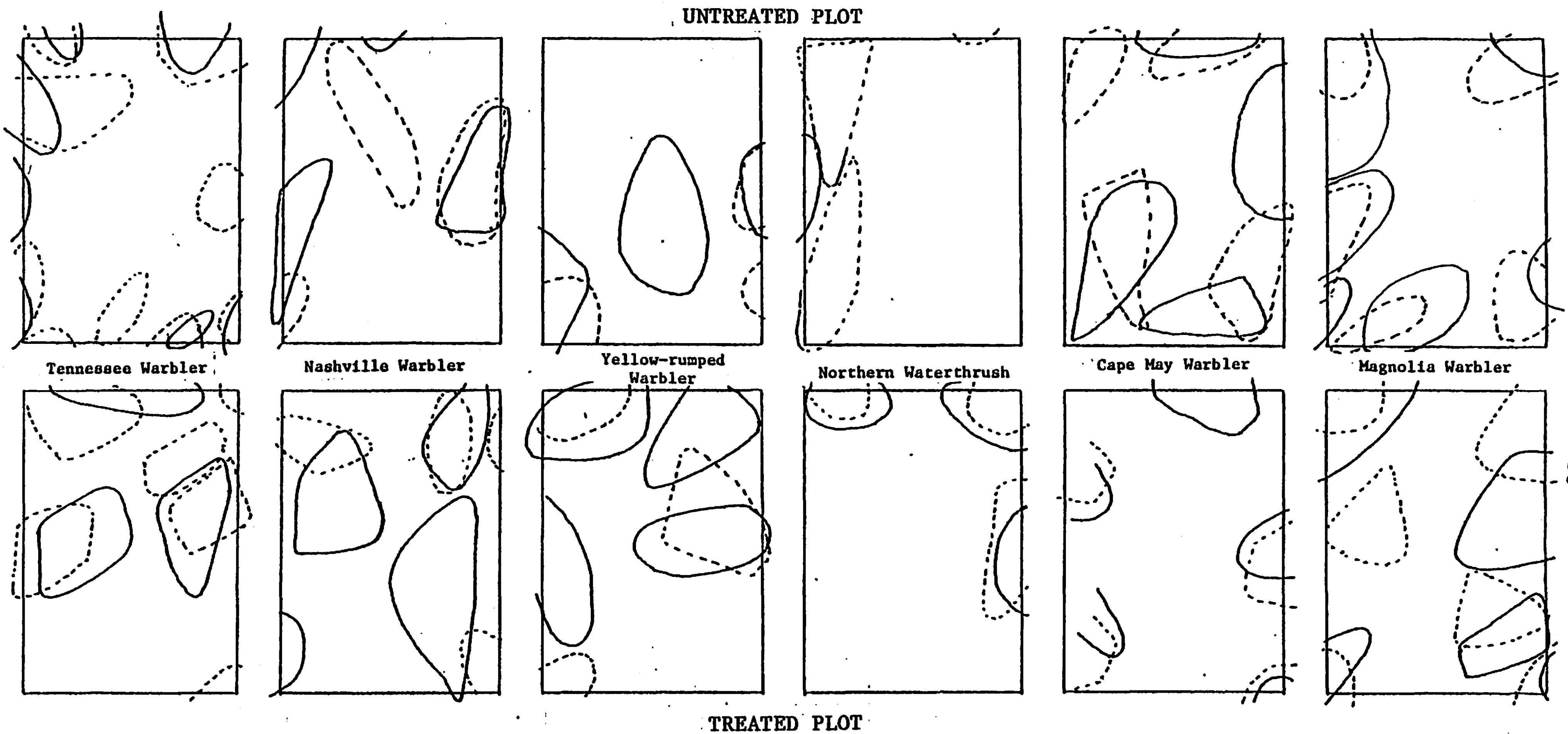


Fig. 10

Pre and post-spray territories of warblers (Parulidae) on nonyl phenol treatment and control plots, Dubreuilville, Ontario, 1979.

——— prespray territorial boundary

------ postspray territorial boundary

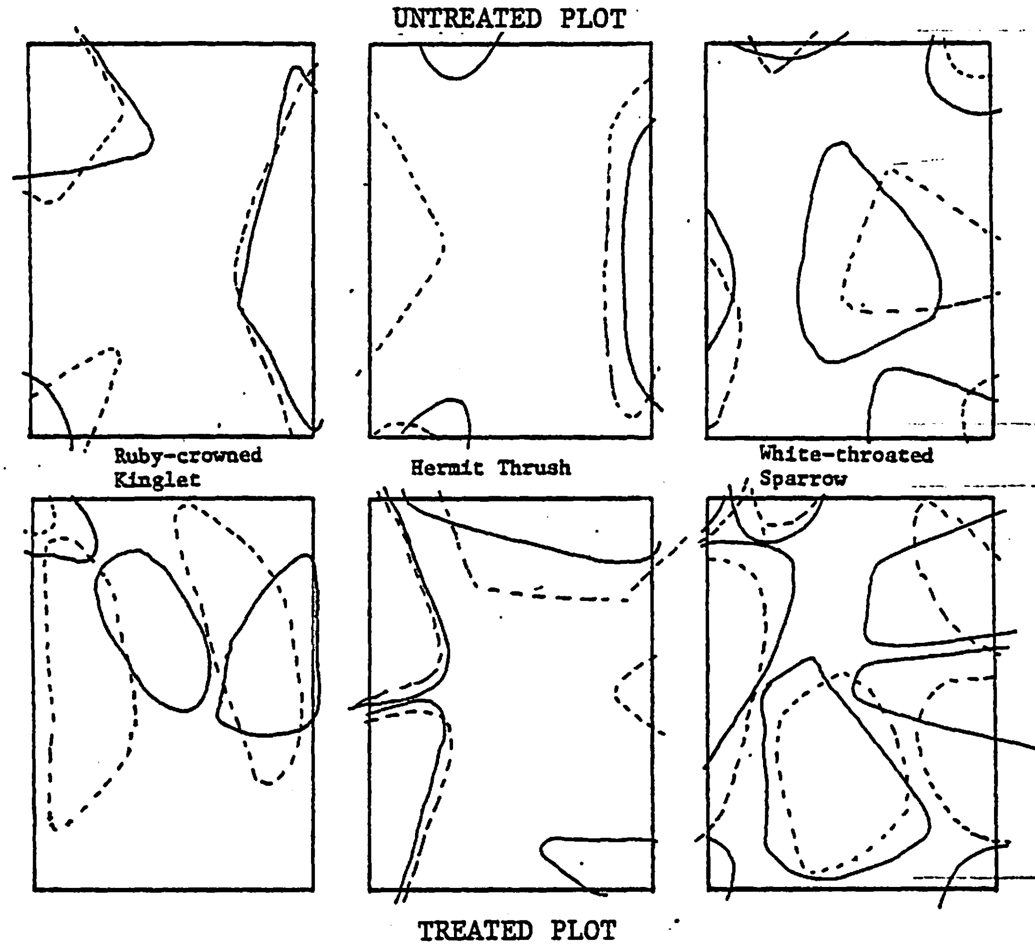


Fig. 11

Pre and post-spray territories of ruby-crowned kinglet, *Regulus calendula* (Linnaeus), hermit thrush, *Hylocichla guttata* (Pallas), and white-throated sparrow, *Zonotrichia albicollis* (Gmelin), on Nonyl phenol treatment and control plots, Dubreuilville, Ontario, 1979.

———————— prespray territorial boundaries

- - - - - - - - postspray territorial boundaries

plots are 4 ha.

DISCUSSION AND CONCLUSIONS

The experimental application of nonyl phenol was planned to ensure that environmental impact studies would be carried out under "worst case" conditions rather than typical operational spray program conditions. This objective was achieved by attaining within the study areas a relatively high measured deposit of nonyl phenol applied at a rate equivalent to the quantity of nonyl phenol applied in allowable maximum seasonal applications of Matacil® (2 to 3 1/2 times suggested single application rates).

The evidence of rapid disappearance of nonyl phenol residues from aquatic systems and its relative low-persistence in foliage and non-persistence in soil under these "worst case" conditions indicate that environmental contamination from nonyl phenol present in the insecticide formulations used in pest control operations would be minimal. The rapid dissipation of nonyl phenol residues in water could be mainly due to the dilution by water flow. Other factors such as conjugation, hydrolysis, photolysis and microorganisms may have played an important role in the degradation of this compound. The failure to detect nonyl phenol residues in sediment samples except for trace amounts found right after treatment indicates that downward movement of this chemical in water was minimal. The relatively rapid initial decline of nonyl phenol residue in foliage may be primarily due to volatilization, but other environmental and biological factors may be important for its subsequent degradation in foliage. The failure to detect nonyl phenol in soil samples collected from an open plot well exposed to the sky suggests that this chemical disappears very rapidly from soil through physical, chemical or biological processes.

The results of the field and laboratory bioassays indicate that no significant mortality of salmonids is likely to occur at concentrations below 100 μg/ℓ in 96 hours of continuous exposure. Nonyl phenol concentrations in flowing water in the treatment stream peaked at a level below 10 μg/ℓ and fell to trace levels within 6 hours of treatment, indicating that even under the "worst case" conditions there was a wide margin of safety between actual nonyl phenol concentrations in flowing water and lethal thresholds to salmonids. Less of a safety margin was evident in standing water, where nonyl phenol residues were above 100 μg/ℓ for at least six hours after spray application, but the potential hazard of these residues was again limited by their rapid disappearance.

The lack of significant differences in mortality among brook trout caged in the treatment and control streams over the first five days after treatment confirms the absence of short term lethal effects on salmonids attributable to the residue levels measured. Higher mortality in the treatment stream eight to ten days after treatment suggests that nonyl phenol may have had a delayed lethal effect on caged fish, but the removal of half the caged fish for residue analysis five days after treatment and the high mortality (32%) among control fish by the end of the caging studies weaken the significance of the observed differences in mortality. In light of the evidence of the rapid disappearance of nonyl phenol in the treatment stream and a lack of delayed lethal effects on trout exposed to low concentrations of nonyl phenol in the laboratory for several weeks, the differences in mortality observed in the field are likely due to other factors (such as differences in water quality) than the nonyl phenol treatment.

There is no evidence from the results of drift netting, bottom fauna, terrestrial insect knockdown or honeybee studies to suggest that nonyl phenol has any significant insecticidal effects in either aquatic or terrestrial ecosystems. The lack of a reliable pre-spray population fix on both treated and untreated check blocks make it impossible to state whether there was any effect of the nonyl phenol spray on the spruce budworm population. It is also impossible to say whether the slightly less defoliation of the nonyl phenol treated trees relative to the untreated trees was due to the spray application or a lower prespray population density on the untreated trees. Another possible cause for the differences found between treated and control budworm densities and tree defoliation may have been effects of the fuel oil carrier on budworm larvae. There is no evidence from the forest song-bird census data collected to suggest any effects on avian populations or interference with normal breeding activities resulted from the nonyl phenol treatment.

In conclusion, the data collected on residue levels and persistence and environmental effects of nonyl phenol applied under "worst case" field conditions does not indicate that this material poses any significant hazard to the integrity of either aquatic or terrestrial forest ecosystems under present usage.

Appendix I

The difference in mortality between treatment and control fish is estimated by the following approximate 95 per cent confidence interval:

$$\bar{X}_1-\bar{X}_2-1.96\sqrt{\frac{\bar{X}_1\ (1-\bar{X}_1)}{N_1}+\frac{\bar{X}_2(1-\bar{X}_2)}{N_2}} < p_1-p_2 < \bar{X}_1 - \bar{X}_2 + 1.96\sqrt{\frac{\bar{X}_1\ (1-\bar{X}_1)}{N_1}+\frac{\bar{X}_2\ (1-\bar{X}_2)}{N_2}}$$

where N_1 and N_2 = number of fish in control and treatment

$\bar{X}_1$ and $\bar{X}_2$ = proportion of dead fish in control and treatment

p_1 and p_2 = mortality in control and treatment

The hypothesis H: $p_1 - p_2 = 0$ (equal mortality) is rejected if the above confidence limits for $p_1 - p_2$ do not include 0.

3 June 1979	$-0.148 < p_1 - p_2 < 0.108$	accept
6 June 1979	$-0.570 < p_1 - p_2 < -0.130$	reject
8 June 1979	$-0.815 < p_1 - p_2 < -0.425$	reject