PHOSPHAMIDON ISOMERS IN CONIFEROUS FOLIAGE

by

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INTRODUCTION

Phosphamidon, [2-chloro-N,N-diethyl-3-hydroxycrotonamide, dimethylphosphate], is a systemic organophosphorus insecticide effective against coniferous defoliators. It has been used on an increasingly large scale especially in New Brunswick since 1962 for budworm control [*Choristoneura fumiferana* (Clemens)] by the Canadian Forestry Service (Macdonald *et al* 1968) primarily due to its rapid degradation and low mammalian toxicity (Dever and Davis 1960). Preliminary field trials in 1961-63 in New Brunswick showed that the compound was apparently harmless to fish and other aquatic organisms and provided effective budworm control when applied by aircraft at the dosage of 0.55 kg A.I./ha (0.5 lb A.I./acre) in 2.82 l (0.75 U.S. gal) of water, (Fettes 1961, Fowle 1965). The material is however very toxic to birds (Fowle 1965).

In July, 1974, aerial application trials were designed to determine the effect of phosphamidon as an adulticide to budworm moths at a low dosage level of 0.07 kg A.I./ha (1 oz A.I./acre) in northern New Brunswick. The present report summarizes the chemical aspects of that spray operation dealing especially with residue levels of phosphamidon isomers found and its observed distribution patterns in samples of conifer foliage, especially white spruce, *Picea glauca* (Moench) Voss, and balsam fir, *Abie balsamea* (L) Mill, collected from the insecticide sprayed forests.

MATERIAL AND METHODS

Spray Area

The spray area was located in the counties of Gloucester and Northumberland of northern New Brunswick. The plot layout was designed and prepared by the Forest Protection Limited in Fredericton, N.B. and is shown in Fig. 1. Plots 1 to 5 and 26 to 31 were about 29,150 ha (72,000 acres) and plots 6 to 25 were approximately 15,215 ha (37,580 acres) in area. Each plot consisted of a mixed stand of deciduous and coniferous trees of varying height representing a typical temperate forest environment.

Application of Phosphamidon

Phosphamidon was formulated as 0.0311 kg (29.6 ml) A.I. in 177.6 ml of water (1 oz A.I. in 6 oz. water) and applied twice at the rate of 207 ml of the aqueous formulation per acre using DC-6 and TBM Grumman Avenger aircrafts especially rigged for aerial spray application and fitted with boom and nozzle spray units distributing the aqueous emulsion of the insecticide while flying ca 91 m (300 ft.) above tree tops i.e., approximately 106 m (345 ft.) above the ground level. The first application was started on the morning of July 13 and the second on July 21, 1974. The meteorological conditions assessed subjectively by a spotter pilot prior to the time of spray applications were suitable for aerial spraying. The full schedule of spray application to each plot in the spray area is given in Table 2.

Sampling

Sampling of foliage etc. was done by Dr. Buckner's research

group according to the specifications of the author. Upper crown foliage of spruce and fir was collected from the four quadrants of four randomly chosen tree species from preselected locations scattered throughout the treatment area (Table 2, Fig. 1). The foliage species from each location was removed by clipping, pooled, mixed, weighed and an aliquot was stored in 200 ml of pesticide grade ethyl acetate contained in quart mason jars provided with aluminum foil lined screwcap lids. Soil from the TBM plane crash site* (Table 3) was excavated to 10.16 cm (4") depth by an auger from four different locations, pooled, placed on a plastic sheet, sorted by hand to remove debris, stone, etc. and an aliquot was stored in ethyl acetate as outlined above. Two mid-surface water samples (200 ml each) were collected from a pond near the crash site, combined into a single composite sample, extracted with 3 x 100 ml volumes of toluene, dried by passing through Na_2SO_4 columns and stored in tightly sealed amber coloured bottles. All the samples were placed in a Coleman cooler filled with dry ice and shipped to the Institute's analytical laboratory in Ottawa for analaysis.

Analytical Procedures

Analytical procedures used for foliage, soil and water samples were basically the same as those used by Sundaram and Davis (1974). Foliage and soil samples were ground for 5 min. in a Sorvall Omni-Mixer at maximum speed with 2 x 200 ml of ethyl acetate, filtered under suction using "S & S Sharkskin" filter paper, dried by passing through a column of Na_2SO_4 and concentrated under vacuum

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* Sevagle, N.B. (See Table 3 and Appendix I on page 27 for more details).

to 100 ml. The concentrated extract corresponding to 20 g of foliage or soil was dissolved in 50 ml of CH_3CN, partitioned with 2 x 25 ml of hexane; the polar phase was flash evaporated to ca 10 ml and cleaned using a charcoal-Celite column. The ethyl acetate-benzene eluate (200 ml) was gently flash evaporated to a small volume (5 ml) and stored in graduated centrifuge tubes fitted with teflon-lined screw caps. Further concentration for gas chromatographic (GC) analysis was performed, when necessary, by heating the eluate in a water bath with an airstream filtered through a Drierite filter.

The toluene extracts of water samples received were flash evaporated to small volumes and concentrated further by stream of air. The phosphamidon isomers were estimated by GC without any cleanup due to the absence of noticeable background interferences.

Four microliter portions of the final extracts of foliage, soil and water samples were injected into a Hewlett-Packard (H.P.) 7610 gas chromatograph. Instrument parameters were:

Column: U-shaped glass 1.22 m (48") by 0.635 mm (0.25") (I.D.) packed with 5% OV 1 on Chromosorb W. H.P. 80-100 mesh

Detector: Tracor flame photometric fitted with a 526 mu optical filter for detecting P

Temp: (^{o}C): Injector 235
Oven 185
Detector 195

Flow rate of gases (ml/min): Nitrogen 75 (3.5 on rotometer)

	Air	50
	Oxygen	18
	Hydrogen	150
Electrometer settings:	Range	10^3
	Attenuation	32
Recorder:	H.P. Model 7128A	
	Span	1 mv
	Chart speed (inch/min)	0.5
Retention times (min):	*Trans* or α-phosphamidon	2.67
	Cis or β-phosphamidon	3.31
Relative retention times:	*Trans* - isomer	0.81
	Cis - isomer	1.00

Calibration Curves: Separate GC calibration curves for *cis* - and *trans* - phosphamidons were made using a single analytical standard of 98% purity supplied by CIBA Limited, Basle, Switzerland, containing the *cis* - and *trans* - isomers in the proportion 73:27.

Technical phosphamidon: GC analysis of the technical material (Sundaram 1975)* used in preparing the aqueous spray mixture contained 92.2% (W/V) of the active ingredients (*cis*- and *trans*- isomers)

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* See Inf. Rept. CC-X-95

and the formulation used in spraying contained 13.4% (W/V) of the insecticide

Sensitivity: The level of sensitivity with 20 g samples of soil and foliage was 0.01 ppm and for water 0.01 ppb. Recovery using this procedure averaged 101% for water, 94% for foliage and 98% for soil (Table 1) when fortified samples were run through the processing and analytical procedures outlined above. Results presented in Tables 2 and 3 have not been corrected for percent recovery.

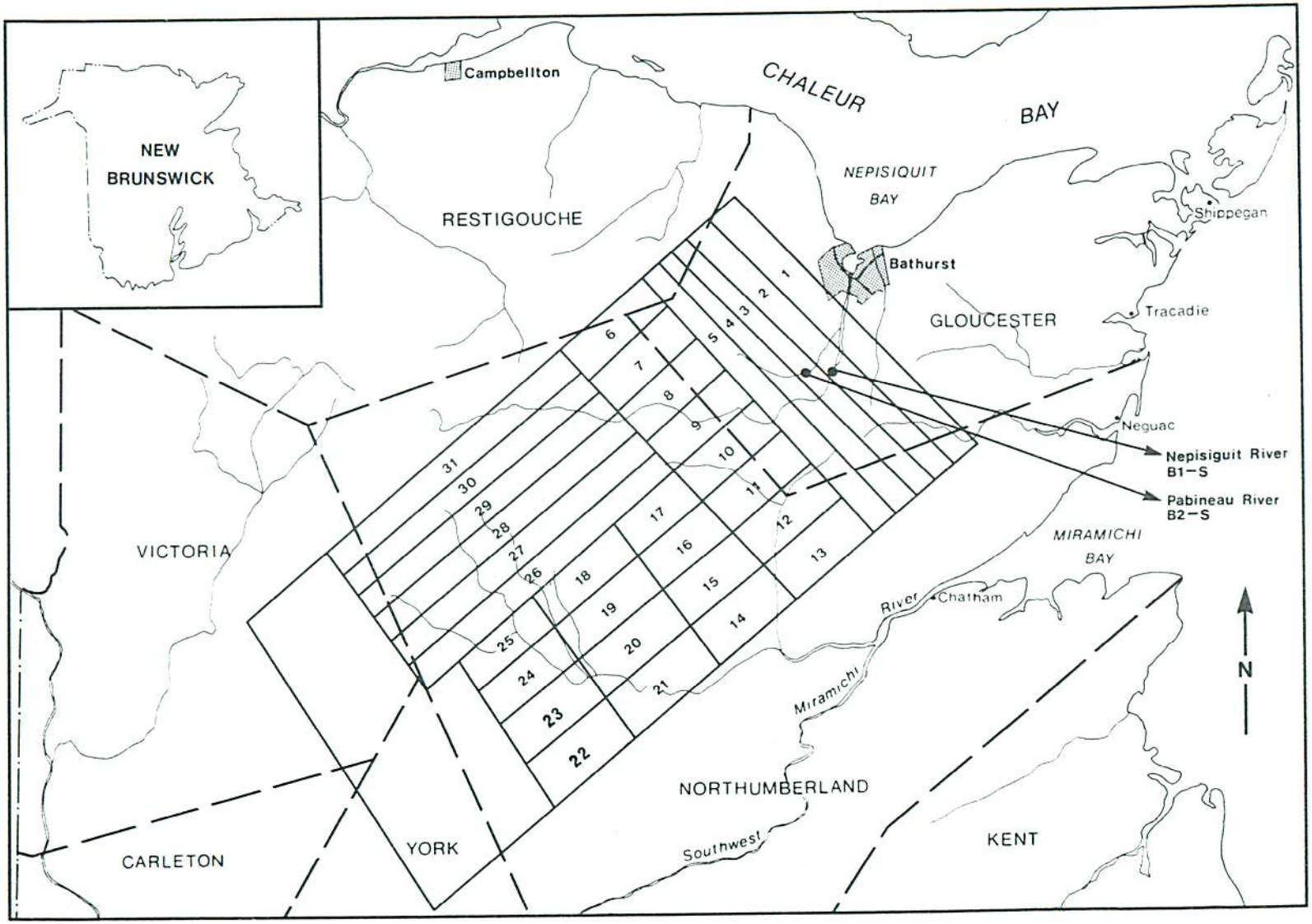

Fig. 1. Plot Design for Phosphamidon Spraying to Control Budworm Moths in Northern New Brunswick during the Summer of 1974.

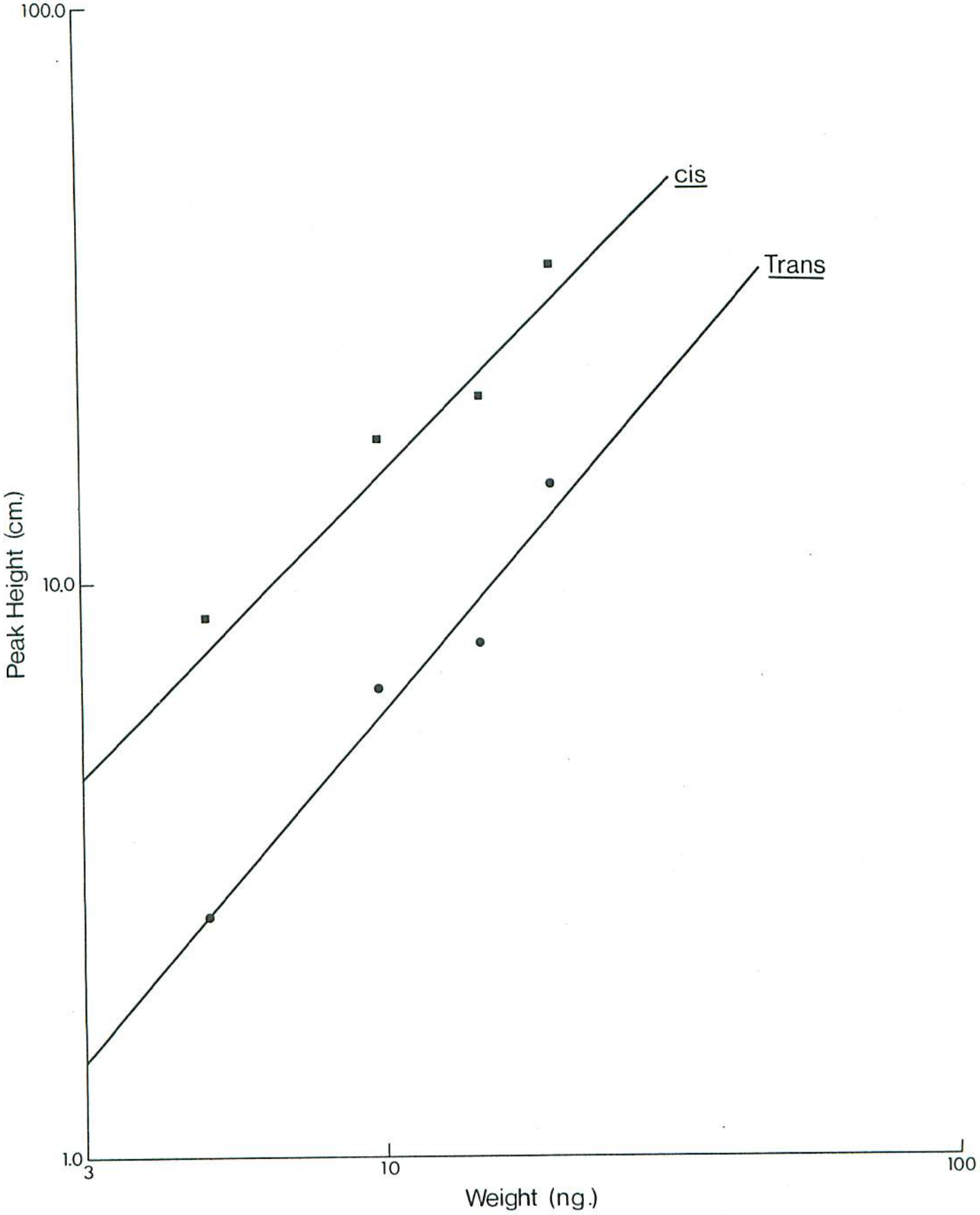

Fig. 2. Gas Chromatographic Calibration Curves for Phosphamidon Isomers.

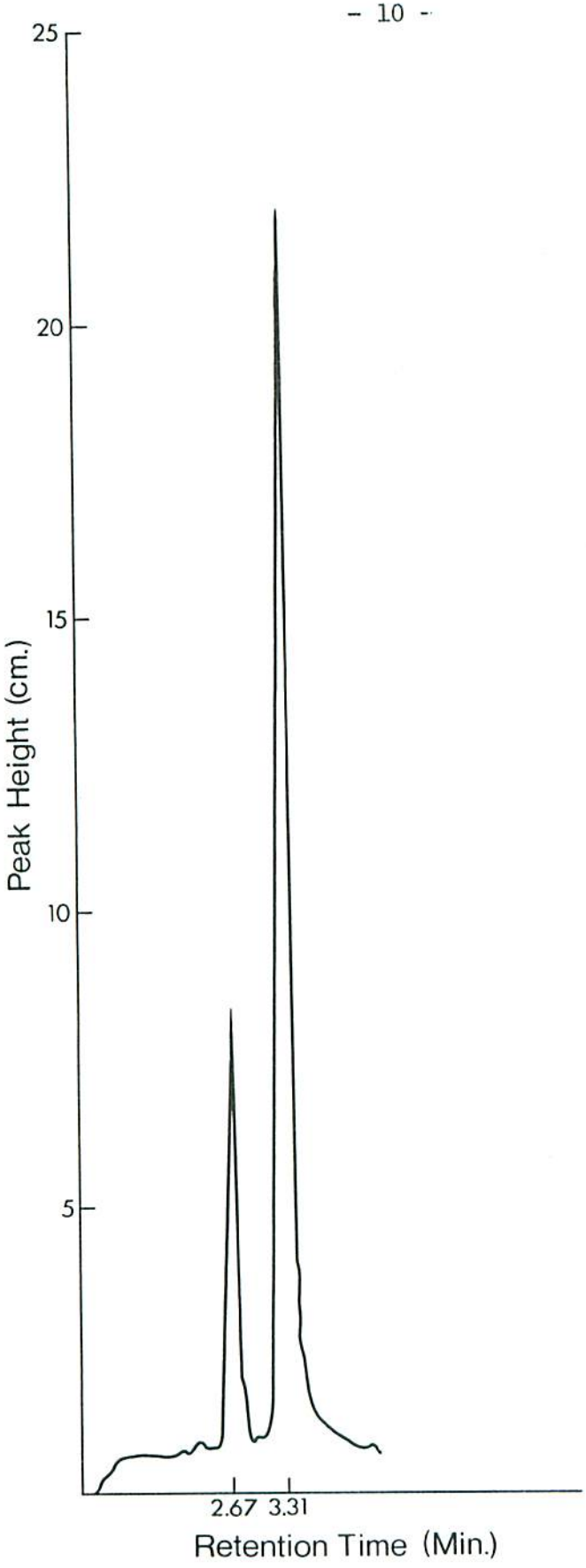

Fig. 3. Gas Chromatogram of 15 ng of Phosphamidon Standard in Benzene.

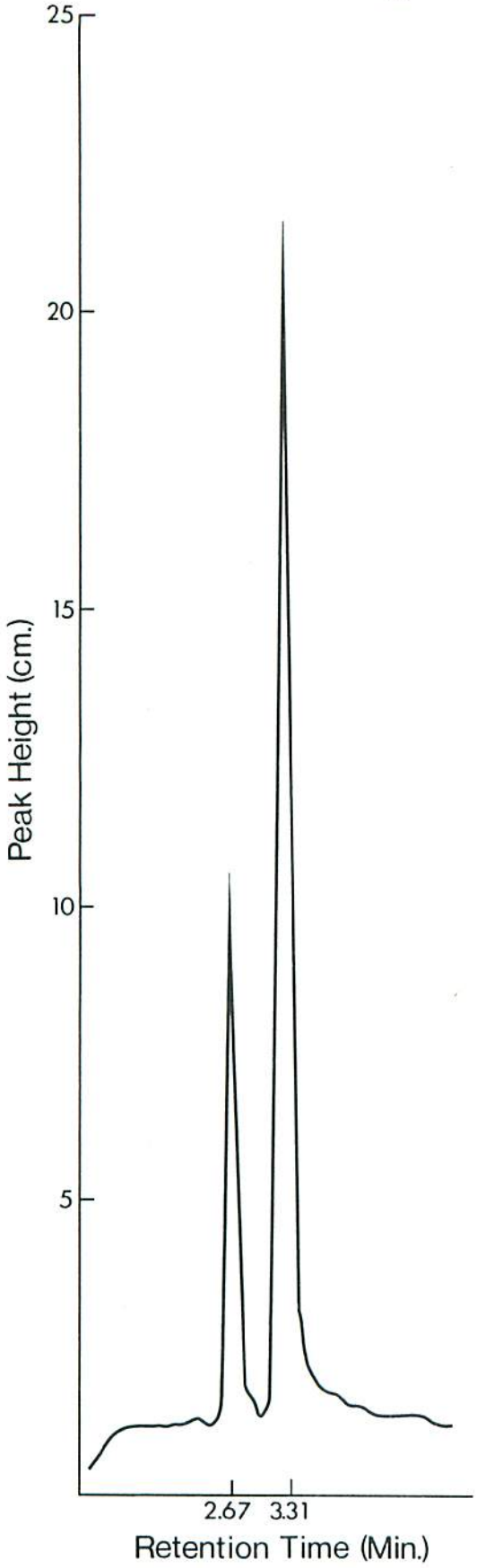

Fig. 4. Gas Chromatogram of 4 ul Shot of Spruce Extract Containing Phosphamidon.

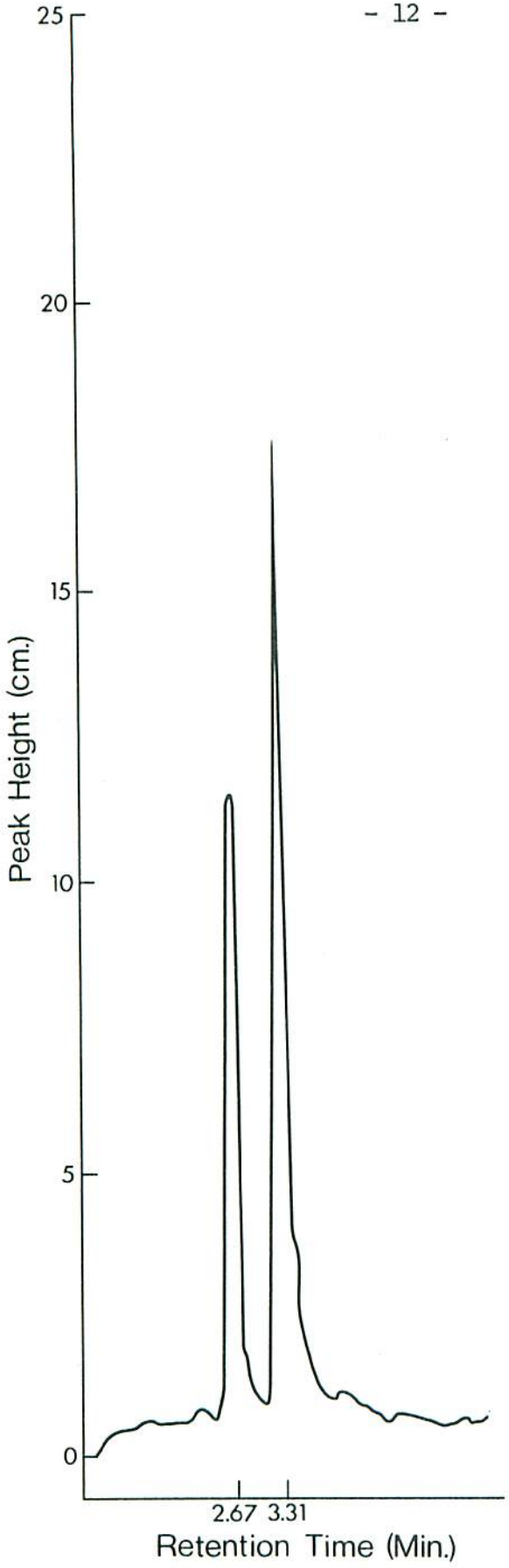

Fig. 5. Gas Chromatogram of 4 ul Shot of Soil Extract Containing Phosphamidon.

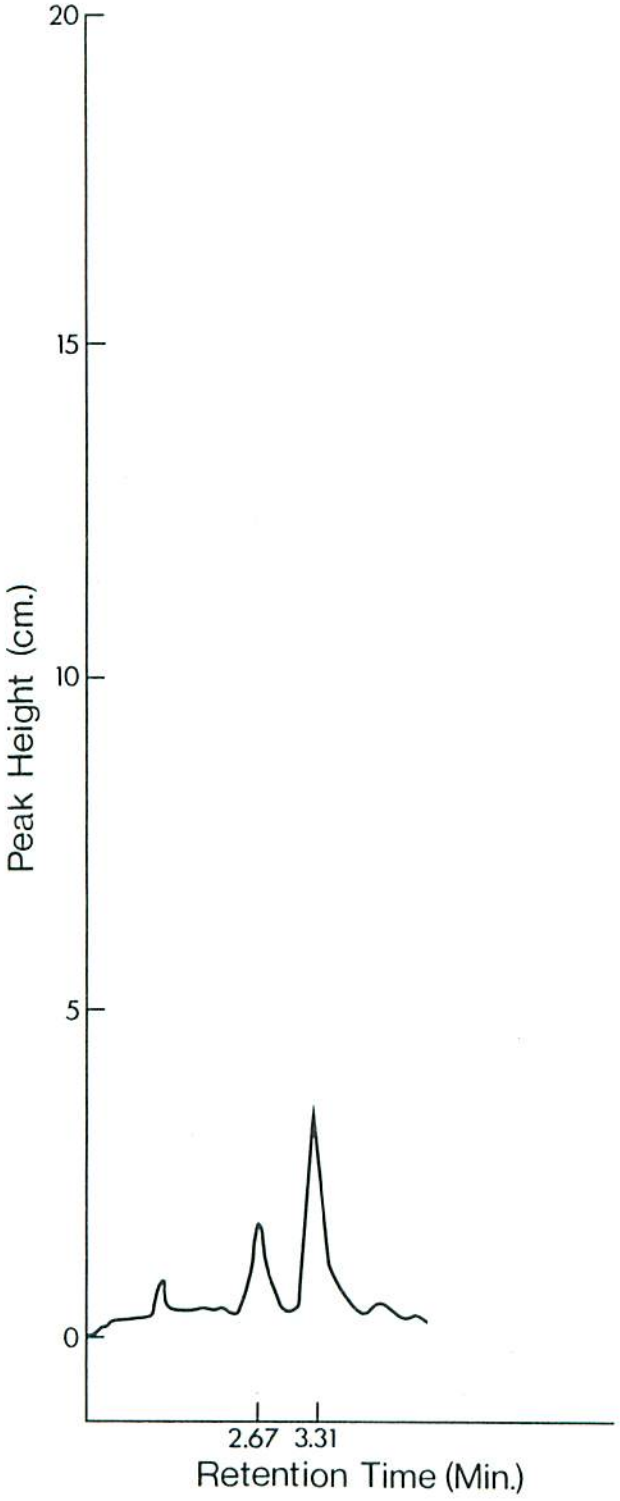

Fig. 6. Gas Chromatogram of 4 ul Shot of Water Extract Containing Phosphamidon.

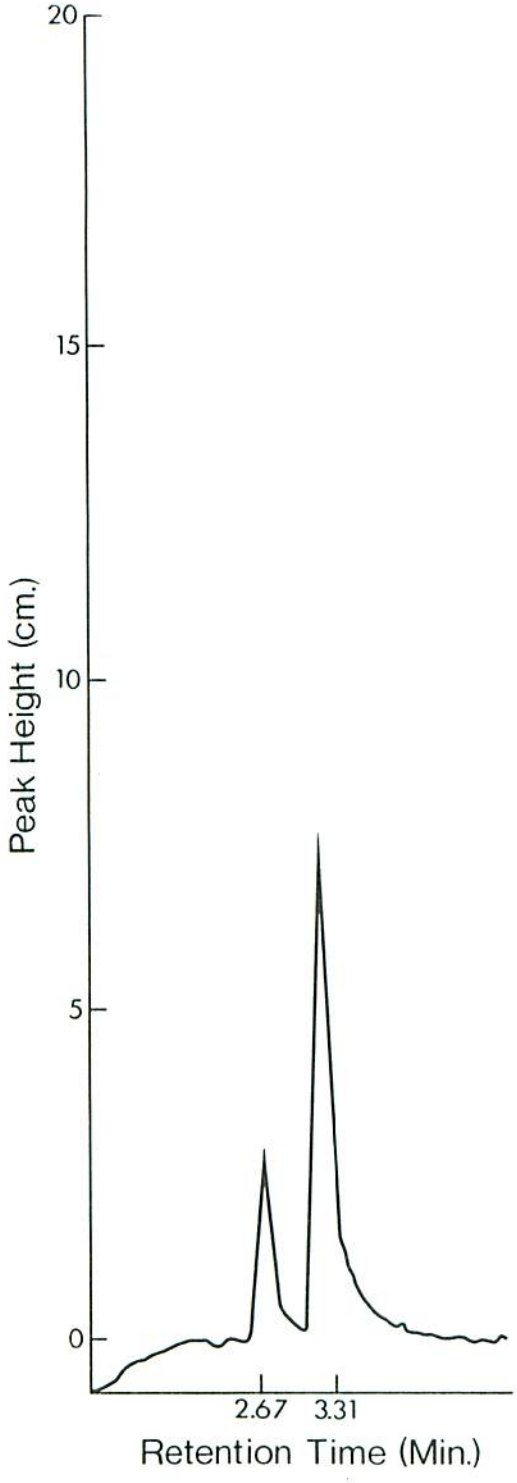

Fig. 7. Gas Chromatogram of Phosphamidon Formulation.

TABLE 1

Percent Recovery of Total Phosphamidon* From Spiked Samples of Water, Spruce Foliage and Soil

Sample	Mass (g)	Fortification Level of Phosphamidon (ug)	Average % Recovery	Coefficient of Variation (%)
Water	200	10	101	2.6
Foliage	20	20	94	6.4
Soil	20	20	98	3.0

* Each value represents the average of three analytical replicates.

TABLE 2

Concentration of Phosphamidon in Coniferous Foliage Samples Collected from Different Sprayed Areas of New Brunswick

Serial No.	Sample No.	Sample Description	Spray Date	Mass of Foliage (g)	Phosphamidon Conc. (ppm)		
					α or Trans	β or Cis	Total
1	1	B13, A1, WS	13-7-74	170	0.13	0.05	0.18
2	2	B16, A1, BF	14-7-74	140	1.59	1.41	3.00
3	3	B17, A1, WS	16-7-74	100	0.11	0.05	0.16
4	4	B10, A1, WS	16-7-74	100	1.30	0.12	1.42
5	5	B13, A2, WS	21-7-74	160	1.67	0.76	2.43
6	6	B10, A1, WS	16-7-74	130	2.81	2.44	5.25
7	7	B11, A1, BF	16-7-74	130	1.91	1.15	3.06
8	8	B11, A1, WS	16-7-74	130	2.65	1.93	4.58
9	9	B16, A2, BF	21-7-74	160	2.68	0.73	3.41
10	10	B12, A1, WS	14-7-74	130	0.75	0.18	0.93
11	11	B17, A1, WS	16-7-74	150	0.75	0.44	1.19
12	12	B17, A2, BF	22-7-74	170	6.38	4.25	10.63
13	13	B8, A2, BF	22-7-74	100	0.68	0.38	1.06

14	14	B1, A1, WS	14-7-74	150	0.61	0.31	0.92
15	15	B9, A1, WS	16-7-74	170	0.81	0.54	1 35
16	16	B2, A1, BF	14-7-74	150	0.18	0.09	0.27
17	17	B2, A2, BF	21-7-74	130	3.13	2.25	5.38
18	18	B3, A1, WS	16-7-74	180	0.68	0.28	0.96
19	19	B7, A1, WS	17-7-74	100	0.39	0.21	0.60
20	20	B10, A2, WS	21-7-74	120	0.88	0.43	1.31
21	21	B8, A1, BF	16-7-74	100	0.55	0.33	0.88
22	22	B8, A1, WS	16-7-74	90	0.81	0.51	1.32
23	23	B31, A2, WS	21-7-74	140	0.14	0.07	0.21
24	24	B31, A2, BF	21-7-74	150	0.19	0.08	0.27
25	25	B8, A2, WS	21-7-74	100	2.03	1.48	3.51
26	26	B8, A2, BF	21-7-74	130	1.66	0.80	2.46
27	27	B2, A2, WS	22-7-74	120	0.97	0.53	1.50
28	28	B3, A2, WS	21-7-74	140	0.43	0.24	0.67
29	29	B5, A1, WS	17-7-74	80	0.43	0.11	0.54
30	30	B5, A2, WS	22-7-74	80	0.97	0.39	1.36
31	31	B4, A1, WS	17-7-74	80	0.48	0.21	0.69
32	32	B4, A2, WS	22-7-74	75	1.31	0.64	1.95
33	33	B1, A2, WS	21-7-74	75	0.29	0.08	0.37
34	34	B12, A2, WS	21-7-74	140	3.80	3.80	7.60

35	35	B11, A1, WS	16-7-74	140	2.35	1.48	3.83
36	36	B11, A2, WS	21-7-74	140	0.41	0.29	0.70
37*		P1 - 1973, WS	Summer 73	100	N.D.	0.35	0.35
38*		P2 - 1973, WS	"	100	0.20	0.63	0.83
38*		P3 - 1973, WS	"	100	0.22	0.69	0.91
39*		P4 - 1973, WS	"	100	0.10	0.38	0.48
40*		P5 - 1973, WS	"	100	0.06	0.36	0.42
41*		P6 - 1973, WS	"	100	N.D.	N.D.	--

Abbreviations:
- B Block
- A1 First Application
- A2 Second Application
- WS White Spruce Foliage
- BF Balsam Fir Foliage
- P Plot
- N.D. Not detected
- * Foliage samples collected during the 1973 summer spray program by D. Ray and analysed according to the method developed by Sundaram and Davis (1974).

TABLE 3

Phosphamidon Residues in Foliage, Soil and Water Supplies

Collected* From T.B.M. Crash Site

Serial No.	Sample	Mass (g)	Phosphamidon Concn. (ppm)		
			α or Trans	β or Cis	Total
42	Foliage	20	713	575	1,288
43	Soil	30	10,250	9,800	20,050
44	Water	500	1,830	7,400	9,230

* Sampled on July 25, 1974 four days after the T.B.M. crash.

RESULTS AND DISCUSSION

Percent recoveries of the phosphamidon isomers from fortified water, spruce foliage and soil samples are given in Table 1. Average recoveries of the total insecticide from these substrates were excellent with low co-efficient of variations for water (2.6) and soil (3.0) and with slightly high (6.4) but acceptable value for foliage. Spiked soil samples gave a 98% recovery showing that the modified method adopted now is more suitable for analysing soil substrates than the one reported earlier (Sundaram and Davis 1974). Calibration curves and chromatographic profiles for the three substrates including the formulation analysed are given in Figs. 2 to 7. The background interference in the chromatograms were small, showing that the extraction, separation and cleanup operations used in the analysis were adequate.

Residue levels of phosphamidon isomers found in spruce and fir foliage samples collected from the forest areas of northern New Brunswick after the two spray applications in July 1974 are listed in Tables 2 and 3. The residue levels are quite widespread ranging from 0.16 to 7.60 ppm in white spruce compared to 0.27 to 10.63 ppm in balsam fir. This wide range may be due to non-uniform spray distribution or inadequate sampling procedures.

Among the 36 samplings done during the spray program, 26 were of white spruce and the remainder were balsam fir. The average concentration of phosphamidon in white spruce was found to be 1.75 ppm compared to the 3.04 ppm in balsam fir, the ratio of

residue levels being 1: 1.7 showing that the fir foliage, due to its geometry, acts as a better receptor, i.e. *ca* 74% more, of the insecticide spray than the spruce foliage. The mean concentrations of *cis*- and *trans*-phosphamidon isomers found in spruce and fir foliages were 0.67 and 1.08 and 1.15 and 1.90 ppm respectively; the ratios observed were for spruce 1: 1.6 and for balsam 1: 1.7. This shows that the *trans*-isomer, in spite of its low concentration in the technical and spray formulations (27% *vs* 73% for *cis*-) is preferentially adsorbed and retained on the conifer foliage than the predominant *cis*-isomer. Probably the *cis*-form is more rapidly dissipated by physical and metabolic processes from the coniferous foliage than the *trans*-isomer, agreeing with the observations made earlier by Bull *et al* (1967) and Westlake *et al* (1973) in leafy crops.

Since the major isomer, *cis*-phosphamidon (76%) is considerably more active as a cholinesterase inhibitor and more toxic to mammals and insects and more unstable than the minor isomer-*trans*-phosphamidon (23%); toxicity and stability characteristics of the individual isomers would have appreciable significance in determining the ecological impact of the insecticide in forests sprayed with the toxicant.

The average phosphamidon concentration in conifer foliage observed after the second application was 2.64 ppm compared to 1.64 ppm after first forest spray. The apparent increase of 61% in residue level, after the second application, is primarily due to the

persistence of the insecticide in spite of its low half-life of 1.5 days (Gunther and Gunther 1971) and the short one week interval between the two applications.

The concentrations of phosphamidon isomers observed in the water, foliage and soil samples collected from the crash site of TBM aircraft are extremely high (Table 3) and it is anticipated that due to its short half-life observed in various substrates (Gunther and Gunther 1971), the insecticide is expected to dissipate rapidly. Probably sampling and analysis of these substrates after a year, *i.e.* by the end of July 1975* will provide useful data on the residue levels present in them and throw some light on the persistence and half-life of the insecticide in these forest components.

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* See Appendix I on page 27.

SUMMARY

Distribution patterns and residue levels of aerially sprayed phosphamidon for adult spruce budworm control were studied in coniferous foliage samples collected in July 1974 from the northern New Brunswick forests. Foliage analyses were done by using a sensitive gas-liquid chromatographic method developed at this Institute. Initial deposits on coniferous foliage samples varied from 0.16 to 10.63 ppm. The residue levels found in balsam fir foliage were 70% more than the spruce, and the minor isomer, *trans*-phosphamidon deposited on average 62% more than the predominant and toxic *cis*-isomer. The different levels of deposition, toxicity and stability between the two isomers may bear some significance especially in determining the moth as well as larval kill on coniferous trees and in influencing the ecological impact of phosphamidon insecticide in Canadian forests. Data on initial residue levels of the insecticide found in spruce foliage, soil and water samples collected from a crash site of a spray aircraft are included and briefly discussed.

ACKNOWLEDGMENTS

The technical assistance of D. Lewis, P. LeCompte, G.G. Smith and M. Bryan is gratefully acknowledged. Analytical grade phosphamidon was provided by B.J. Watt, Ciba-Geigy Canada Limited. The article was reviewed by W.W. Hopewell, J.A. Armstrong and C.H. Buckner. Sampling of materials were done by B. McLeod, D. Ray, and P. Kingsbury.

LITERATURE CITED

BULL, D.L., D.A. LINDQUIST and R.R. GRABBE, 1967. Comparative Fate of the Geometric Isomers of Phosphamidon in Plants and Animals. *J. Econ. Entomol.* 60: 332.

DEVER, D.A. and D.L. DAVIS. Aerial Application of Phosphamidon to Forested Areas - Maine, 1960. *California Chem. Co.* (now *Chevron Chem. Co.) Ortho Division, Richmond, Calif. (U.S.A.) File Rep.* 721-110, pp 28.

FETTES, J.J. 1961. An Appraisal of the Systemic Insecticide Phosphamidon Tested Against the Spruce Budworm. *Chem. Cont. Sec., Forest Ent. and Pathol. Br., Can. Dept. Forestry. Int. Rep.* pp 5.

FOWLE, C.D. 1965. A Preliminary Report on the Effects of Phosphamidon on Bird Populations in Central New Brunswick. *Can. Wildl. Serv. Occasl. Pap.* 7.

GUNTHER, F.A. and J.D. GUNTHER (Eds.) 1971. Phosphamidon. *Residue Rev.* Vol. 37, pp 202.

MACDONALD, D.R., D.G. CAMERON and M.B. CRAIG. 1968. Studies of Aerial Spraying Against the Spruce Budworm in New Brunswick. *Can. Dept. Forestry and Rural Develop. Forest Res. Lab. Fredericton, N.B. Int. Rep.* M-25, pp 99.

SUNDARAM, K.M.S. and P.G. DAVIS. 1974. Gas Chromatographic Determination of Phosphamidon Isomers in Foliage, Soil and Water. *Env. Can. Forestry Serv. Inf. Rep.* CC-X-66, pp 23.

WESTLAKE, W.E., M. ITTIG, D.E. OTT, and F.A. GUNTHER. 1973. Persistence of Residues of the Insecticide Phosphamidon and in Oranges, Lemons and Grapefruit, and on and in Orange Leaves and in Dried Citrus Pulp Cattle Feed. *J. Agr. Food Chem.* 21 (5) : 846.

APPENDIX I

Foliage and soil samples were collected from the TBM plane crash site at Sevagle, N.B.on August 7, 1975 and analysed for phosphamidon isomers. Water samples were not available because the initial source (a trench near the crash site) became dry due to drought. The results are recorded below:

TABLE 4

Serial No.	Sample	Mass (g)	Phosphamidon conc. (ppm) trans	cis	Total	Percent loss after one year **
45	Foliage*	20	7.3 (713)	5.0 (575)	12.3 (1288)	99.0
46	Soil	20	59.3 (10250)	26.3 (9800)	85.6 (20050)	99.6

* White spruce

** 377 days to be more exact.

Initial concentrations of phosphamidon are given in parenthesis (See Table 3, p. 19).

After an interval of one year, the concentration levels of phosphamidon observed in foliage and soil samples were comparatively low confirming the anticipated rapid dissipation of the insecticide from the forest environment. A loss of 99.0% in foliage and 99.6% in soil samples have occurred compared to the initial residue levels (see Table 3, p. 19). The disappearance of the cis-isomer was comparatively higher than the trans-form in both the substrates confirming the previous observations. It is also interesting to note that the overall rate of degradation of the insecticide was nearly 2.5 times higher in soil than in foliage samples.