STUDIES OF FOLIAR PENETRATION, MOVEMENT AND PERSISTENCE OF C-14 LABELLED FENITROTHION IN SPRUCE AND FIR TREES

By

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INTRODUCTION

The organophosphorus insecticide, fenitrothion [O,O-dimethyl O-(4-nitro-m-tolyl) phosphorothioate] has been widely used in Canada for forest insect control at an operational dosage of 2 to 4 oz. A.I./acre since 1968. The widespread use of this chemical is of environmental concern and research has been initiated on its persistence, distribution and mode of dissipation in the forest ecosystem. Recent studies (Yule and Duffy 1972, Yule 1974, Sundaram 1974a, 1974b) showed that aerially-sprayed fenitrothion, although short-lived in many environmental components such as soil, water and hardwood foliage, persisted in small amounts for a considerable length of time in coniferous foliage. These investigators proposed mechanisms such as partial penetration and protection of fenitrothion residues in cuticular waxes. The present study was undertaken to determine specifically the mode of penetration, translocation and fate of ring-labelled C-14 fenitrothion applied to the foliage of young (3-year seedlings) white spruce [Picea glauca (Moench) Voss] and balsam fir [Abies balsamea (L.) Mill] trees under greenhouse conditions. Although field persistence might not be represented by greenhouse conditions, intrinsic mechanisms involved in the foliar penetration and movement of fenitrothion in coniferous trees might be demonstrated under such controlled conditions using radioactive tracer technique as well as absolute chemical analysis.

MATERIALS AND METHODS

Culture of Seedlings

Healthy 3-year-old spruce and balsam fir seedlings transplanted from nursery conditions into pots and allowed to grow outdoors in chill weather in order to break dormancy. They were then transferred to a greenhouse set at 16 hr photoperiod, 72 $\pm$ 2^{o} F and 50 $\pm$ 20% R.H. The pots were treated regularly with a nutrient (Hoagland) solution and after 3 months, new buds broke dormancy and needle growth was quite vigorous.

Chemicals and solvents

Ring labelled C-14 (methyl C-14) fenitrothion of 99% purity and specific activity 10 mc/mM was supplied by Dr. J.R. Duffy, University of P.E.I. Before use, the chemical and radio-chemical purities of both the samples were checked and analysed. All solvents used were either pesticide grade or redistilled in glass.

Insecticide application

Four experiments, 1, 2A, 2B and 2C were conducted in duplicate. Experimental conditions are summarized in Table 1. Variables such as tree species, age of foliage, fenitrothion formulation (technical, emulsion and oil solution as used in field spraying), radioactive dose and method of application were combined in various ways in the 4 experiments. In experiments 1 and 2B, water emulsions of fenitrothion (% composition by volume : fenitrothion 10, Arotex-3470 1, Atlox-3409 1 and distilled water 88) containing 10 uc/ml of active material were

used for dipping the balsam fir and spruce seedlings. In experiment 1, a similar formulation containing 1 uc/ml was also used to compare dose-effects. In experiments 2A, an oil solution of fenitrothion (10% V/V) in Arotex (90% V/V) was used for painting the fir foliage. Technical fenitrothion (ca 95% purity) was used in experiment 2C for dipping the fir and spruce seedlings. Four sets of branches per tree containing newly formed needles were either dipped in the formulations contained in a 2.5 x 10 cm test tube or painted carefully using a brush in an attempt to obtain uniform coverage. Contamination due to run-off and drainage was avoided by snugly fitting a serum cap to the lower branch. A plastic sheet was used to cover the soil in the pot and the untreated parts of the tree to prevent any contamination. Samples of foliage were taken at intervals of time for residue analysis by gas-liquid chromatography (GLC), radiochemical and histoautoradiographic techniques. An appropriate control tree was included in each test.

Extraction procedure

Foliage samples were clipped from treated and untreated parts of the young spruce and fir trees at varying intervals of time, cut into small pieces and extracted with ethyl acetate using a Sorvall Omni-Mixer. Stem and root samples collected on the last days of the experiments were processed similarly and extracted. The extracts were partitioned and cleaned up as described by Yule and Duffy (1972).

Gas-liquid chromatography (GLC)

Flame photometric GLC was used for the analysis of fenitrothion residues present in the concentrates of various plant extracts and the

analytical procedure used was identical to the one used by Yule and Duffy (loc cit).

Radioactive measurements

The radioactivity in cleaned-up plant extracts (foliage, stem and roots) was measured by counting replicates of 100 ul sample mixtures in toluene cocktail (5 g PPO and 0.3 g POPOP/liter) [15 ml] in a Picker Nuclear (Ansitron II) liquid scintillation counter with external standardization. Each treated and untreated sample was extracted thrice and counted. The average percent extraction efficiency for the three extractions of treated samples in the whole series, assuming total recovery, were ca 82, 12 and 6 respectively. For the untreated samples, the values were erratic probably due to the low number of counts recorded compared to background levels. The total radioactivity, obtained by adding the counts for the three extractions of each sample, was corrected for background and counter efficiency. Quenching corrections were also introduced by counting blanks of untreated inactive extracts prepared in the same manner as the active ones. The counts of replicates were averaged for each sample and converted to total disintegrations per minute per gram (DPM/g) and expressed as percent radioactivity observed in different parts of the tree species. The average counting efficiency during the whole experiment was compared to an external C-14 standard and the overall counting efficiency was ca 83%. Whenever practical, the samples were counted with less than 5% counting error at the 95% confidence level.

Histoautoradiography (HAR)

The persistence and localization of residues at the cellular level was also investigated and for this the HAR of treated foliar and stem segments was carried out according to the method recently developed by Prasad and Moody (1974). Briefly, this involved the use of the standard cryostatic procedure with some modifications during the pretreatment of the coniferous tissue with a 10% solution of glycerol.

RESULTS AND DISCUSSION

Fenitrothion residues found by GLC analysis in different parts of the treated fir and spruce seedlings and the distribution of radioactivity in them are given in Tables II and III respectively. The insecticide concentration is expressed in units of ppm "as sampled" with 0.01 ppm as the minimum detectable limit.

The GLC and radiochemical analysis show that the residue levels of fenitrothion in the treated foliage samples harvested after 3 weeks, although variable, were still at a high level (Tables II and III), suggesting that the dissipation of the insecticide under greenhouse conditions was not rapid. This observation is contrary to the situation found in the forest environment (Yule and Duffy 1972, Sundaram, 1974a), where as pointed out by these authors, physical factors (e.g. climatic) may be important in the dissipation process. Spruce needles treated with either emulsion or technical fenitrothion retained greater amounts of the insecticide than the

Table I

Experimental Conditions

Variables	Experiment No.			
	1	2A	2B	2C
Tree species	Spruce	Fir	Fir and spruce	Fir and spruce
Formulation	Water emulsion	Oil solution	Water emulsion	Technical fenitrothion
Volume (ml)	100	5	25	5
Fenitrothion concn. (%)	10	10	10	95
Activity (µc/ml)	1 and 10	10	10	10
Mode of application	Dipping	Painting	Dipping	Dipping

Table II

Distribution of Fenitrothion Residues (expressed as ppm) in Foliage, Stem and Roots of Balsam Fir and White Spruce Following Foliar Application

Experiment No	Days after treatment	Activity uc/ml	Formulation	Balsam Fir				White Spruce			
				Treated	Untreated			Treated	Untreated		
				Foliage	Foliage	Stem	Root	Foliage	Foliage	Stem	Root
1	21	1	Emulsion					700·00	0·02	0·07	N.D.
	21	10	Emulsion					937·50	0·12	0·02	N.D.
2A	23	10	Oil	16,150·00	0·83	0·55	0·09				
2B	22	10	Emulsion	8,750·00	1·36	0·63	0·05	17,250·00	7·50	0·13	0·04
2C	27	10	Technical	330·00	24·50	0·40	0·03	350·00	9·75	0·06	0·02

Table III

Percent Distribution of Radioactivity Observed in Foliage of

Fir and Spruce Trees Following Foliar Application of Fenitrothion C-14

Experiment Days After Treatment	2A		2B				2C			
	Fir		Fir		Spruce		Fir		Spruce	
	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated
1			99·997	0·003	99·976	0·024	99·986	0·014	99·969	0·031
3	99·977	0·023	99·990	0·010	99·974	0·026	99·929	0·071	100	0
9	99·969	0·004					100	0	100	0
13			99·915	0·085	99·982	0·018				
15									100	0
18			99·985	0·015	100	0				
19	99·986	0·014								
22			99·706	0·220*	99·925	0·075				
23	99·979	0·014*								
27							99·537	0·436	99·780	0·220

Untreated foliage samples in Expt. No. 1 did not contain any measurable radioactivity after 21 days at the two dosage levels applied.

*Except in two cases (*) where 0·007 - 0·074 radioactivities were found in stem sections, no activities were recorded for any of the stems and roots in this series of experiments.

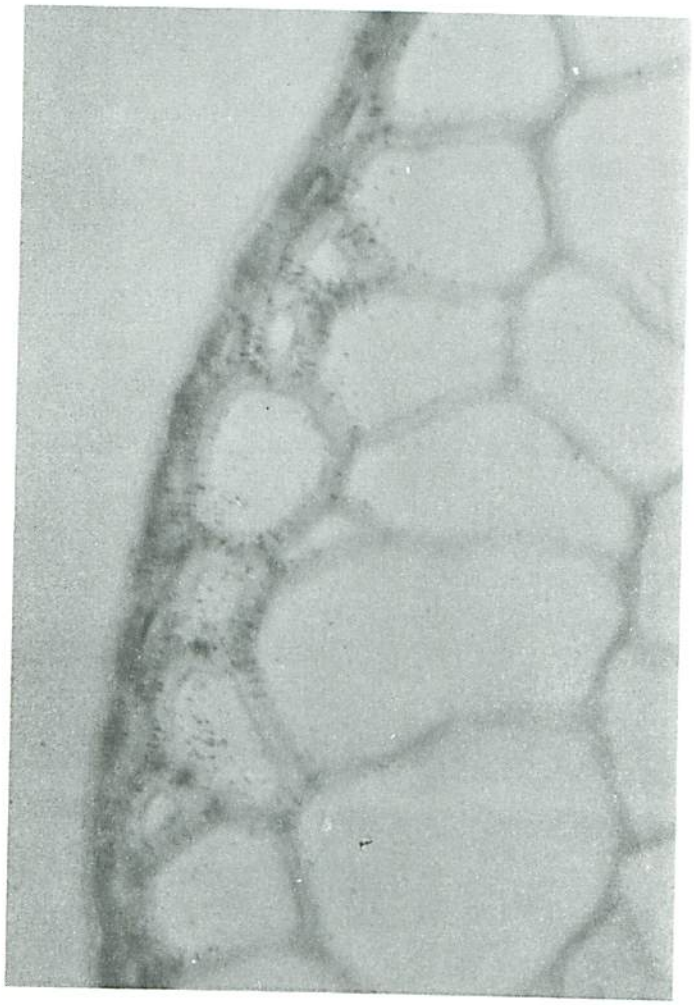

Fig. 1. Section through spruce needle sampled 21 days after treatment with fenitrothion C-14 showing the localization of activity (dark silver granules) in the cuticle, epidermis and hypodermis. Magnification 400 X.

Fig. 2 Transverse section through balsam fir needle sampled 16 days after treatment with fenitrothion C-14 showing the localization of silver grains (activity) in cuticle, epidermis and hypodermis. Magnification 400 X.

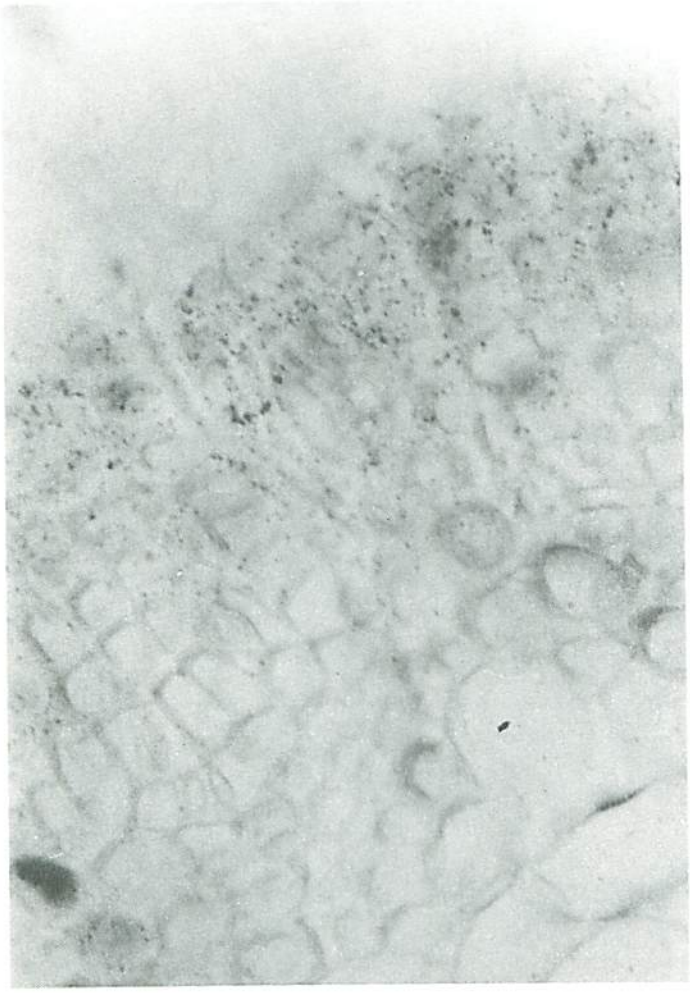

Fig. 3. Localization of activity in xylem vessels of balsam fir stem sectioned 21 days after treatment with fenitrothion C-14. Magnification 400 X.

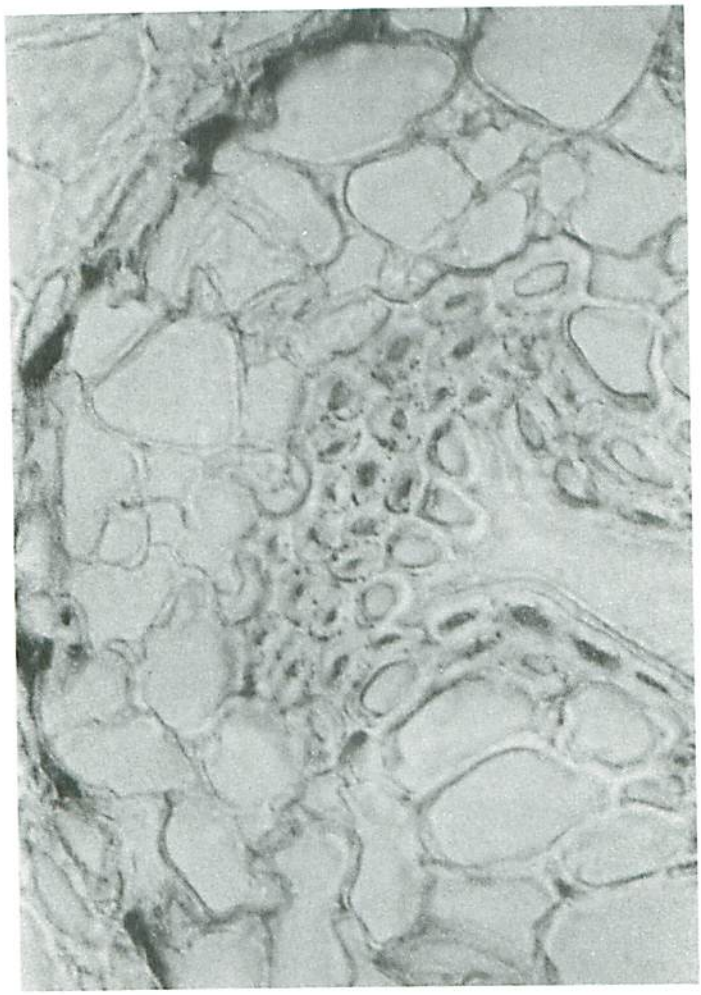

Fig. 4. Section through spruce stem sampled 21 days after treatment with C-14 labelled fenitrothion depicting the localization of activity in external fascicle tissue. Magnification 400 X.

fir foliage at the end of the final harvest period. It can also be seen from the Tables that the foliage retained more of the applied insecticide molecules present in the oil and emulsion formulations than in the technical material, probably due to the surfactant properties of the cosolvents in the formulation. No detectable fenitro-oxon was found in the foliar extracts.

When applied on the foliage, fenitrothion penetrated and translocated, after an interval of 3 weeks, to the untreated parts of the foliage to the extent of less than 0.1% of that amount found in the treated parts. Smaller amounts (Table II) were also found in the stem and root samples. In fir foliage, the amount translocated ranged from 0.83 to 24.50 ppm whereas in spruce the range was only from 0.12 to 9.75 ppm. The amount translocated in stems in general were lower than in foliage and the root samples contained only barely detectable amounts of the toxicant. The overall ratio of the insecticide translocated in conifer foliage, stem and roots, compared to the foliar concentration in the treated part, were 7.3:0.3:0.04. The data (Table II) showed that the movement of the translocated material, although very low (< 0.1%) was lateral and upwards only.

The radioactive measurements (Table III) of the untreated foliage, stem and root samples were also high, confirming a similar translocation pattern observed among the samples studied. Because the amount of translocated material is very low even when applied at high concentration, it is doubtful whether fenitrothion can effectively act as a systemic insecticide as postulated by Randall (1970), especially

in forest spray operations where the operational dosage is extremely low (2 to 4 oz A.I./acre) unless some concentration occurred in the actively growing buds in the spring. It is also apparent that further metabolic studies based on the operational dosage would be futile unless analytical techniques have been improved and refined to detect the possible breakdown products below pico (10^{-12}) gram levels.

Penetration and accumulation of the insecticide in the coniferous seedlings were found to be related to the dosage and formulation used. For example higher insecticide concentrations (ca 6 times more) were found in the spruce foliage (Table II, Expt. 1) by an application of a 10% than by a 1% emulsion. Application of the technical fenitrothion and oil formulations, instead of the emulsion gave after 27 days, appreciably increased residue levels of the insecticide (in ppm) in the untreated parts of the foliage. The percent ratio for the untreated and treated foliage of balsam and spruce were 7.4 and 2.8 respectively (Table II, Expt. 2C). These observations were also corroborated by the higher counts observed in the radioactivity measurements (Table III) of the same foliage samples. The corresponding ratio levels for the counts were 0.47 for the balsam and 0.22 for the spruce. The overall ratio for residue levels in experiments 2B and 2C for the untreated fir and spruce foliage by radioactive counting were 1.0:0.38 showing that the penetration was more rapid in fir foliage than in spruce and the process primarily depended on the dosage and formulation; higher dosage and the use of technical material favoured greater penetration and translocation of fenitrothion in coniferous trees.

The exact route by which fenitrothion molecules dissipate from the conifer foliage, either under greenhouse conditions or in the forest environment, has only been studied cursorily and is still obscure. The current studies indicate that penetration, translocation and possible biodegradation of the insecticide molecules are minimal and the effective mechanism in the forest environment, as pointed out earlier (Yule and Duffy 1972, Sundaram 1974a and b), seems to involve physical (climatic) and chemical factors (hydrolysis, photodegradation, volatilization, etc.) rather than internal metabolic processes. The persistent fenitrothion molecules, being lipophilic were probably absorbed, transported and stored in cuticular waxes of the coniferous foliage resisting leaching, volatilization, photo and biodegradations. The permeation of fenitrothion through the foliar tissues is probably achieved by cuticular pores, the lipoidal nature of the cuticle enabling the passage of the polar toxicant molecules to the cutin layer for storage (Linskens et al 1965, Sundaram 1974 b). This hypothesis was confirmed by supplementary investigations using HAR techniques (Prasad and Moody 1974). Figures 1 and 2 show a concentration of fenitrothion C-14 and the possible active metabolites formed in the hypodermis region of needle tissues, which probably migrated further towards the mesoderm and concentrated in xylem tissues (Figs. 3 and 4). Thus translocation of fenitrothion as measured so far by both quantitative (Tables II and III) and qualitative methods (Figs. 1-4), was found to be extremely minimal in coniferous trees and the bulk of the material applied persisted for a while in the cuticle and hypodermis of the needle and rachis sections.

SUMMARY

Foliar penetration and movement of fenitrothion in potted spruce and fir trees under greenhouse conditions were studied by painting and dipping needles with oil and emulsion formulations of the toxicant and with technical fenitrothion admixed with the methyl C-14 labelled insecticide. The direction of migration of the substance was investigated by using gas-liquid chromatography, liquid scintillation spectrometry and histoautoradiographic (HAR) techniques. After an interval of three weeks, the foliar penetration of the toxicant was found to be extremely small and the little that penetrated, translocated laterally and upward to the untreated parts of the foliage in less than 0.1% of the amount found in the treated parts. Similarly, the amounts found in stems and roots were negligible indicating that fenitrothion has no foliar systemic action. Consequences arising from the penetration and translocation behaviours are briefly discussed with regard to the foliar type, mode of application, formulation and dosage used. The dissipation of the toxicant from the foliage appeared to involve physical, primarily climatic and chemical processes rather than internal metabolic activity. The persistent fenitrothion molecules, as postulated earlier, are stored in cuticular waxes and this was confirmed by the HAR techniques.

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LITERATURE CITED

Linskens, H.F., W. Heinen and A.L. Stoffers. 1965. Cuticula of leaves and the residue problem. Residue Reviews 8: 136-178.

Prasad, R. and R.P. Moody. 1974. Fate of fenitrothion in forest trees. III - Development of a histoautoradiographic technique for investigating foliar penetration in balsam fir and white spruce. Environ. Canada, Forestry Serv. Inf. Report CC-X-70, 27 pp.

Randall, A.P. 1970. Some recent aspects of forest insect control. Rept. NE Aerial Appl. Conf. Cornell N.Y. p. 91.

Sundaram, K.M.S. 1974a. Distribution and persistence of fenitrothion residues in foliage, soil and water in LaRose Forest. Environ. Canada, Forestry Serv. Inf. Report CC-X-64, 43 pp.

Sundaram, K.M.S. 1974b. Persistence studies of insecticides: III. Accumulation of fenitrothion and its oxygen analog in foliage, soil and water in LaRose Forest. Environ. Canada, Forestry Serv. Inf. Report CC-X-65, 21 pp.

Yule, W.N. and J.R. Duffy. 1972. The persistence and fate of fenitrothion insecticide in a forest environment. Bull. Env. Contam. Toxicol. 8(1):10.

Yule, W.N. 1974. The persistence and fate of fenitrothion insecticide in a forest environment. II. Accumulation of residues in balsam fir foliage. Ibid. 12(2):249.