PERSISTENCE STUDIES OF INSECTICIDES:

IV. ROOT PENETRATION, TRANSLOCATION AND METABOLISM OF C-14 LABELLED FENITROTHION IN YOUNG SPRUCE TREES.

by

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INTRODUCTION

Fenitrothion [0,0-dimethyl 0-(4-nitro-m-tolyl) phosphorothioate] has been used since 1969 in place of DDT for operational control of lepidopterous defoliators in Canadian forests. Its fate and persistence in conifer foliage, soil and water samples collected from sprayed forest areas have been studied extensively by Yule and Duffy (1972) and Sundaram (1974a, 1974b). Although much as been learned of the characteristics of this insecticide in various components of the forest environment, so far no study has been reported on the uptake of the toxicant through the roots of young conifer trees and its translocation to other vegetative parts of the tree, especially under controlled laboratory conditions. Such a study is useful for a more complete understanding of degradational and metabolic processes as well as of residue data of the insecticide in a wider and complete forest environment. The present investigation was undertaken with that objective in mind. The preliminary observations made on the uptake and translocation patterns in young white spruce [Picea glauca (Moench) Voss] trees are summarized in this report.

MATERIALS AND METHODS

Chemicals and solvents

Uniformly labelled methoxy C-14 fenitrothion (200 mg, 0.722 mM, sp. act. 10 mC/mM) was supplied by Dr. J.R. Duffy, University of P.E.I., Charlottetown. The material contained more than 99% fenitrothion, as indicated by gas-liquid chromatography (GLC) and liquid scintillation spectroscopy.

The unlabelled fenitrothion used was of analytical grade supplied by Sumitomo Chemical Co. of Japan.

All solvents used were either pesticide grade or freshly redistilled using all glass apparatus.

Anhydrous sodium sulphate (Fisher) was of reagent grade heated at 150° C overnight and stored in a glass-stoppered bottle. Charcoal and Celite used in the adsorption chromatography were similar to those used by Sundaram (1974a). Dimethyl sulphoxide (DMSO) used for stimulating the insecticide uptake was of analytical grade. Hoagland nutrient solution was freshly prepared prior to the experiment according to the methods described by Hoagland and Arnon (1950).

Plant material

Ten 18-month old uniform size spruce trees of ca 23 cm (9") high with abundant foliage and weighing about 65 g were collected from the Kemptville nursery. The roots were rinsed thoroughly with distilled water to remove adhering soil particles and placed in 175 ml (6 oz) Mason type jars and maintained in a growth chamber* (12-hr day and 12-hr night, ambient temperature of 26 ± 0.5° C and RH of 70 ± 3%.) Two trees served as control and the other eight were used for fenitrothion uptake studies.

Insecticide treatment

The roots of each control tree were placed separately and grown in a solution containing 99 ml of 0.5 Hoagland solution (pH 6.5) and 1 ml of DMSO. The other eight plants were treated separately with similar quantities of the nutrient solution and DMSO mixed with 1 µl of C-14 fenitrothion (2.7×10^{-3} mM) and 10 µl of unlabelled fenitrothion (27×10^{-3} mM) to make a final insecticide concentration of 0.011 ml (29.7×10^{-3} mM) in 100 ml or 110 ppm.

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* Controlled Environments, Inc., 601 Stutsman Street, Pembina, N.D. 58271, U.S.A.

Analytical procedures

At the end of 4 and 14 days, one control and four treated plants were removed from the nutrient solution, roots were rinsed well with water; roots, stem and foliage were separated using a hand clipper, weighed and stored in plastic bags at -20° C until extracted. Each nutrient solution along with the rinse was extracted with chloroform as described by Sundaram (1974a) and the organic and the aqueous phases analysed by liquid scintillation counting (LSC) and GLC methods.

The roots, stem and foliage of the four treated plants collected after 4 days were pooled individually and ground in a Hobart chopper. Duplicate 20 g aliquots of the ground material were extracted as described by Sundaram (loc. cit.) in a Sorvall homogenizer using 100 ml of chloroform-methanol (9:1, v/v) mixture as solvent. The extract was partitioned thrice with 100 ml of water. The radioactivity levels of the aqueous and organic phases were determined by counting aliquots in a scintillation counter, using dioxane and toluene based scintillation mixtures respectively for the two phases, according to the procedure described by Sundaram et al (1975.) The chloroform layer was further petitioned with hexane, cleaned by Charcoal-Celite column adsorption chromatography and eluted with benzene-ethyl acetate mixture and analysed by flame photometric GLC for fenitrothion and its oxygen analog as described by Sundaram (loc. cit.) using similar operating parameters. The fourteen day samples and the controls were analysed similarly except that in the latter case only half the quantities were used for extraction.

Autoradiography

For preparation of autoradiographs, the control and 4 day samples were freeze-dried and then exposed to x-ray film (non-screen) for 2-3 weeks, developed and processed according to the standard procedures (Prasad 1972).

TABLE 1

Percent Radioactivity Observed in Different Parts of Spruce Trees

After Root Exposure to C-14 Fenitrothion

Days After Treatment	Percent Radioactivity observed*							
	Foliage		Stem		Roots		Nutrient Solution	
	H_2O phase	$CHCl_3$ phase	H_2O phase	$CHCl_3$ phase	H_2O phase	$CHCl_3$ phase	Aqueous phase	$CHCl_3$ phase
4	0.5	4.5	0.2	2.1	4.1	21.0	15.2	52.4
14	5.2	15.8	1.2	8.7	16.6	6.6	32.6	13.3

*The water and chloroform phases of the control samples contained less than 0.01% of the observed radioactivity.

TABLE 2

Residues of Fenitrothion and Fenitrooxon Found in Spruce Trees After GLC Analysis

Days After Treatment	Residue concentration (ppm) as sampled							
	Foliage		Stem		Roots		Nutrient Solution	
	Fen	Fenox	Fen	Fenox	Fen	Fenox	Fen	Fenox
4	2.42	0.11	0.81	T	6.45	0.75	80.5	N.D.
14	3.95	0.09	0.48	0.08	4.95	0.49	24.9	N.D.

Control samples did not contain any detectable amount of fenitrothion or its oxon.

T = Traces (< 0.01 ppm)

N.D. = Not detectable

Fen = Fenitrothion

Fenox = Fenitrooxon

Fig. 1. Autoradiograph of the spruce seedling fed with nutrient solution containing C-14 fenitrothion for 4 days. Plant material is on the left-hand and its autoradiograph is on the right-hand side.

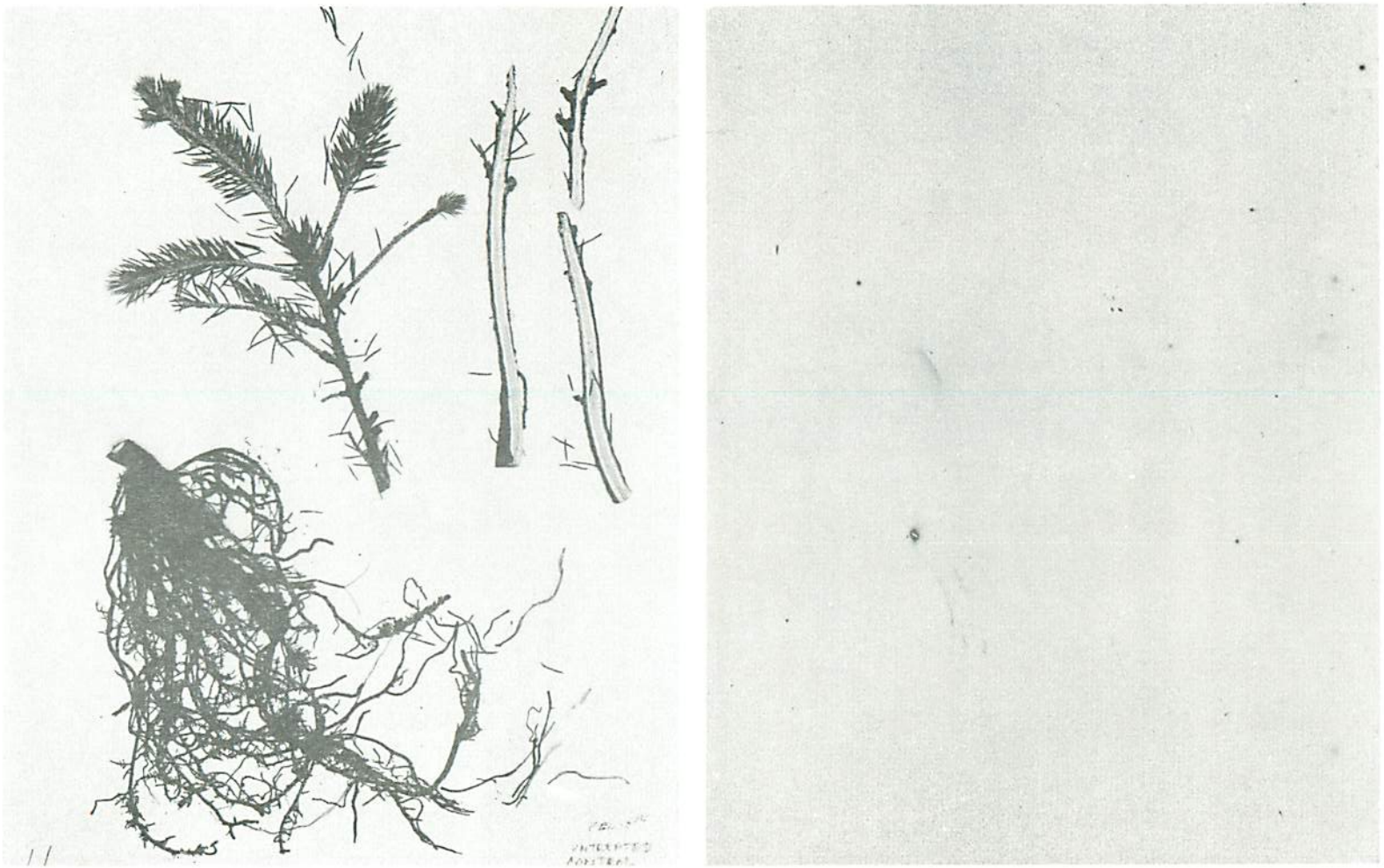

Fig. 2. Control spruce seedling exposed to x-ray film, developed and processed. Note the complete lack of radioactivity on the photograph on the right-hand side.

RESULTS AND DISCUSSION

The amount of radioactivity extracted in chloroform-water mixtures from the different parts of the treated spruce trees and from the nutrient solution is given in Table 1. The readings (DPM/g), obtained for foliage, stem, roots and the nutrient solution after each treatment period by the LSC method were totalled and expressed as percent radioactivity found in these four components. The data represent the distribution pattern of extractable C-14 on a percentage basis with time among the various parts of the plant. No attempts were made to determine the unextractable radioactivity that might be present in the plant tissues after the chloroform extraction. Consequently the total radioactivity present in the plant materials was not known.

Of the total radiocarbon extracted from the plants after four days of root exposure to C-14 fenitrothion, 77.5% was recovered from the roots (excluding the activity found in the nutrient solution - see Table 1) and only 15.4% and 7.1% from the foliage and stem respectively. After fourteen days of plant growth, however, only 42.0% of the extractable C-14 compounds were recovered from the roots, while 38.8% and 18.2% were from the foliage and stem respectively. This result indicated a movement with time of C-14 compounds into the green plant parts after their penetration into the root system probably through the water conducting channels of the xylem.

Of the extractable radiocarbon recovered from various parts of the spruce trees after growing for 4 days in nutrient solution containing C-14 fenitrothion, ignoring the activity of the solution, only 14.8% was hydrophilic metabolites associated with the water phase, this increased to 42.5% after 14 days of growing time. The rate of increase of water-soluble

metabolites in the system derived from applied fenitrothion, was comparatively high in the foliage (from 0.5% to 5.2%, tenfold increase) and low in the root samples (from 4.1% to 16.6%, fourfold increase) (Table 1). It appears that the metabolism of the insecticide into water-soluble metabolites was primarily associated with the foliage part, since it was here that the major increase (from 0.5% to 5.2% of the radiocarbon recovered) in the presence of water-soluble C-14 compounds was noticeable. This view is corroborated by Charnetski and Lichtenstein (1973) with lindane in pea plants. During that time, the chloroform-soluble C-14 compounds decreased from 21.0% to 6.6% (Table 1) in roots, whereas in stem and foliage appreciable increases were observed probably due to the upward movement of these compounds through the xylem vessels. Further cogent evidence of the apoplastic type of movement is forthcoming from the autoradiographs (Figs. 1 and 2). The concentration of the activity in needles is accomplished largely by the transpiration stream. However, a strong pool of activity still remained in the roots. The radioactivity of the organic phase of the nutrient solution decreased during the interval in accordance with the decrease of the intact insecticide molecules while a corresponding increase in activity of the aqueous phase was observed due to the formation of hydrophilic metabolites.

The radioactive measurements show that the tissues of spruce trees seem to be capable of metabolizing fenitrothion rapidly under the experimental conditions. No attempts were made during the experiment to separate and identify the water soluble metabolites of fenitrothion. The probable breakdown products formed as observed earlier in rice plants by Miyamoto and Sato (1965), are through the splitting of the P-O aryl bond to give the corresponding dimethyl esters of phosphorothioic and phosphate acids and demethylation of the parent compound to give desmethyl derivatives. Certainly

more work is necessary to confirm and identify these detoxified hydrophilic metabolites.

Results obtained by the GLC analyses of the chloroform phases of roots, stem and foliage of spruce trees and the nutrient solutions used to grow them, are given in Table 2. The concentration of fenitrothion in the nutrient solution on zero day was 110 ppm which decreased to 80.5 ppm after an interval of 4 days. During that period, 9.68 ppm of fenitrothion accumulated in the tissues of root, stem and foliage of the plant. Among the various parts of spruce tree analysed, roots contained the maximum amount, i.e. 6.45 ppm (67%), foliage samples contained 2.42 ppm (25%) and only 0.81 ppm (8%) was found in the stem. The total fenitroxon concentration found was only 0.86 ppm and the bulk (87%) of it was present in the roots (0.75 ppm). After 14 days of plant growth, although the concentration of the toxicant in the nutrient solution decreased from the initial concentration of 110 ppm to 24.9 ppm, no significant change in the total concentration level in the plants was noticed. Actually the amount absorbed decreased from 9.68 ppm to 9.38 ppm during the interval of 4 to 14 days; foliar concentration increased from 2.42 ppm to 3.95 ppm whereas a decrease of 1.50 ppm was observed in the roots. Thus the GLC studies also indicated the movement of fenitrothion molecules into various parts of the spruce plant and, as observed earlier in LSC analyses, preferentially accumulated in the foliage after their penetration into the root system under the experimental conditions studied. In spite of the high fenitrothion cocentration (110 ppm) of thenutrient solution in which the roots were immersed, there was no rapid and significant uptake and accumulation of the toxicant in various parts of the plant, probably due to the high metabolic activity found in

the plant tissues especially in the foliage. Since the oxon concentration found in the various parts of the plant are not significant, the detoxification mechanism probably did not proceed through the oxidative desulfurization and degradation routes.

No meaningful comparison could be made at this juncture between the data obtained by LSC and GLC methods because of the initial variations in concentrations used and the complex metabolic degradation route of the toxicant in conifers which is still obscure and warrants a careful re-examination. These preliminary experiments indicated that fenitrothion, its metabolites containing C-14 moieties and other water soluble conjugates of the toxicant entered through the root system and moved slowly upwards probably through the water conducting channels of the xylem and translocated in the aerial parts in varying amounts when young spruce trees were allowed to grow in the nutrient solution containing 110 ppm of fenitrothion initially for 14 days. This observation is further confirmed by the autoradiography of the spruce seedling, the roots of which had been exposed to the labelled insecticide in nutrient solution for 4 days (Fig. 1). No positive response was observed in the control sample (Fig. 2).

From the results in Table 2, it is evident that the amount of fenitrothion in the nutrient solution had decreased considerably with time due to the production of hydrophilic and possibly lipophilic metabolites and conjugates. The approximate half-life of fenitrothion in the nutrient solution with pH 6.5 was found to be 9.5 days which is nearly 4.8 times higher than the value found (Sundaram 1973) in buffered water samples at the same pH.

SUMMARY

The uptake and translocation pattern of C-14 fenitrothion in young spruce trees was investigated using LSC GLC and autoradiographic techniques by growing the plants in a nutrient solution containing the labelled insecticide. After 4 days of growing time, roots contained 77.5% of the extractable radioactivity in chloroform-methanol solvent mixture, 15.4% was found in the foliage and only 7.1% in the stem. These figures were 42, 38.8 and 18.2% respectively after 14 days of growing time. Partition of the extractant with water showed an increase in hydrophilic C-14 compounds occurring with time, indicating a metabolism of the insecticide into water-soluble compounds. The radiocarbon associated with the chloroform phase was identified by GLC and contained 9.68 ppm of fenitrothion after 4 days of root exposure; bulk of which was in roots, 25% in foliage and only 8% in stems. No significant change in the total fenitrothion concentration in the plant was observed after a growing time of 14 days. The oxon metabolite was found in small amounts in the plant tissues indicating that detoxification mechanism did not primarily proceed through oxidative desulfurization and degradation routes.

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