FATE OF FENITROTHION IN FOREST TREES VIII. PERSISTENCE, TRANSLOCATION AND METABOLISM OF C^{14}-FENITROTHION IN JACK PINE (*Pinus banksiana* Lamb.) IN RELATION TO SAWFLY MORTALITY

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RÉSUMÉ

Une étude de l'absorption, du transport, du métabolisme et de la rémanence du fénitrothion-C^{14} dans les tissus des feuilles et du tronc du pin gris a été réalisée à l'aide des techniques de scintillation liquide, d'histo-autoradiographie et de chromatographie sur couche mince. Aucun transport significatif du composé ne s'est produit en 21 jours, et aucun métabolite ne s'est formé dans les canaux résinifères; la molécule a plutôt semblé persister dans la cuticule et certaines parties des résaux vasculaires (xylem). On croit que la mort des tenthrèdes serait due à la sensibilité extrême de l'insecte à de faibles doses du pesticide.

INTRODUCTION

Fenitrothion [(0,0-dimethyl-0-(4-nitro-m-tolyl) - phosphorothioate)] has been used since 1967 in place of DDT for operational control of lepidopterous defoliators in Canadian forests (Roberts, 1975). In 1972, Yule and Duffy investigated the persistence of fenitrothion on balsam fir and mixed spruce foliage following aerial application of the pesticide at a rate of 0.280 kg/ha (4 oz./acre). They reported that 50% of the initial dose was lost by foliage within four days, 70-85% within two weeks, and 10% persisted for at least ten months. McLeod (1975) suspected the presence of toxic substances on fenitrothion-treated pine foliage that remained in sufficient concentration to kill emerging sawfly larvae (*Neodiprion swainei* Midd.), at least 40 days after application. He contemplated fenitrothion to be selectively stored in the resin ducts of the needles upon which the sawfly feeds and the mortality to result from excessive feeding on contents of the ducts (Eisner et al, 1974). The present study was undertaken to determine the metabolic fate, persistence, and translocation of fenitrothion in Jack pine (*Pinus banksiana* Lamb.). This investigation took advantage of basic radiotracer methodology (liquid scintillation counting and autoradiography) to establish whether fenitrothion or any toxic metabolites persisted on the foliage at levels sufficiently high to explain the sawfly mortality reported by McLeod (1975).

MATERIALS AND METHODS

(1) Culture of Tree Seedlings

Four year old seedlings of Jack pine were collected in the autumn of 1975 from the Kemptville tree nursery of the Ontario Ministry of Natural Resources. These were held in the greenhouse for approximately two months until the current buds had flushed out. Six healthy specimens were selected and set up in an area enclosed by plastic sheeting. Environmental conditions were adjusted to 22°C, 50% relative humidity, and a 12 hour photoperiod supplied by fluorescent "daylight" tubes (≈ 200 lux).

(2) Chemicals and Solvents

C^{14}-ring labelled fenitrothion (specific activity 10 mCi/m mol) was supplied by Dr. R. Duffy (University of Prince Edward Island. Purity was confirmed by liquid scintillation counting (LSC) and thin layer chromatography (TLC). The solvents used for extraction were glass distilled. A simulated field formulation was prepared with 10% C^{14}-fenitrothion, 1% Atlox 3409, 1% Arotex 3470, and 88% distilled water (v/v). This emulsion was diluted prior to application so that the final activity would be 7.6 μC/ml, the optimal concentration (≈ 200 ppm) established by our previous investigation (Moody et al, 1975).

(3) Method of Treatment

(a) In Vivo Plant Study

Three replicate trees were employed for LSC determination of persistence. Four branches (per tree) were selected, and 0.5 ml of the C^{14}-fenitrothion formulation (7.6 Ci/ml) was

carefully applied with the aid of a small 3.18 mm (1/8") brush (Grumbacher #4116) in an attempt to obtain uniform coverage. Three replicate trees were also used for autoradiographic (AR) detection of the labelled pesticide. For this purpose, three branches (per tree) were selected and the formulation (7.6 μCi/mℓ) was applied by painting 25-50 mm (1"-2") long sections of either the newly flushed foliage, the older foliage or the bark tissue. This AR study was set up to determine whether the major direction of pesticide translocation was acropetal or basipetal. Plastic sheeting was used to cover the soil in the pots to prevent contamination.

(4) Sampling and Extraction Procedure

Separate samples of newly flushed foliage (N.F.), old foliage (O.F.) and stem tissue (S.T.), were taken at 1,3, 7 and 21 day intervals for LSC analysis. These were individually weighed, placed in plastic "roll-top" bags and frozen in liquid nitrogen prior to being stored at -70°C. The extraction procedure followed that used previously (Moody et al 1975) except that the cleanup procedure was omitted. Each conifer tissue sample (1-5 g) was placed in a sintered glass funnel and was washed under vacuum with 200 ml of ethyl acetate.

The solvent was concentrated to near dryness in a rotary evaporator (35°C) and then brought up to 10 ml acetone for analysis. The tissue was then extracted twice with ethyl acetate (100 ml each time) in a polytron sonicator (model # 1020) and the combined extracts were filtered through pre-rinsed Celite 545,

concentrated, and brought up to 10 ml acetone prior to analysis.

The trees treated for AR were sampled after 7, 14 and 21 days. The entire branches were individually bagged and refrigerated at -70°C. The branches taken at 7 and 21 days after treatment were used for gross autoradiography while the 14 day samples were used for histoautoradiography.

(5) Analytical Procedure

(a) Liquid Scintillation Counting (LSC)

All samples were analysed with a Beckman LS-100C liquid scintillation counter. Fifty µℓ aliquots of the plant extracts were dispensed into Beckman plastic scintillation vials, each holding 10 ml of Fisher scintiverse cocktail. All sample counts were corrected for quenching with the aid of a "quench curve" constructed by counting a set of C^{14}-fenitrothion standards to which had been added, increasingly greater amounts of coniferous extracts. The percent counting efficiency was plotted against an external standard ratio and the resulting "quench curve" was used to correct for any loss in sample counting efficiency. This simple construction permitted omission of the time-consuming "clean-up" procedure that was necessary in our previous study for GC analysis of conifer extracts.

(b) Autoradiography (AR)

Gross autoradiography was conducted following the basic procedure outlined by Yamaguchi and Crafts (1958). Briefly, this involved pressing blotter-paper mounted conifer branches for 2

days followed by exposure to Kodak No-Screen X-ray film for 6 weeks. Histoautoradiography was performed following the method of Prasad and Moody (1974), and involved cryostat sectioning of the untreated tissue followed by 2-3 week exposure to Kodak NTB2 liquid emulsion.

(c) Thin Layer Chromatography (TLC)

The C^{14} plant extracts were run on silica gel TLC plates (Fisher Rediplates) in a solvent system of 1:3, ethyl acetate to cyclohexane. The plates were then exposed for 2 weeks to X-ray film for C^{14} detecting anticholinesterase activity (Mendoza et al, 1968).

RESULTS

Table 1 lists the concentration of fenitrothion in each of the samples analysed. The ppm values were calculated by simple conversions from the dpm (disintegration per minute) recorded by LSC, using the known specific activity (10 mCi/m mol) for the compound. Each tissue type (N.F., O.F., S.T.) has a value recorded for both the surface wash (W) and the tissue extract (E) which enabled a rough estimation to be made of the levels of surface (cuticular) and subsurface (subcuticular) residues present. The percent absorbed (% ABS.) was calculated as being equal to $\frac{\text{ppm (E)}}{\text{ppm(W)} + \text{ppm(E)}} \times 100$ and the total % ABS. is plotted against time in Fig. 1 (i). The total residual fenitrothion (total ppm) was converted to precent recovery values which are plotted against time in Fig. 1 (ii) to illustrate a persistence curve for the pesticide through the 21 day sampling period. The results of the TLC

analysis are also included in Table 1. The results of the gross autoradiography are presented in Fig. 2 (i - vi) and are followed by photomicrographs of the histoautoradiography (Fig. 3 (i - viii)).

Table 1

Persistance, Translocation and Metabolism of Fenitrothion-C^{14} by Jack pine

L.S.C. and T.L.C. analyses of treated tissues.

Sample		Time (Day) 1* ppm	1* % ABS	3** ppm	3** % ABS	7 ppm	7 %ABS	21 ppm	21 % ABS
Pine	N.F. (W)	2.09		0.65		0.26		0.26	
	N.F. (E)	0.58	21.7	0.50	43.5	0.42	61.8	0.24	48.0
	O.F. (W)	0.53		0.29		0.13		0.08	
	O.F. (E)	0.12	18.5	0.10	25.6	0.06	31.6	0.06	42.9
	ST. (W)	2.05		1.19		0.47		0.25	
	ST. (E)	1.06	34.1	0.72	37.7	0.46	49.5	0.54	68.4
	Average	2.14	24.8	1.15	35.6	0.60	47.6	0.48	53.1

* All extracts gave a spot (Rf; 0.52) on the TLC autoradiographs which corresponded to that obtained for the standard C^{14}-fenitrothion. This spot exhibited anitcholinesterase activity after spraying with the liver spray homogenate described by Mendoza (1972).

** All extracts exclusive of the 1 day samples (i.e. 3, 7 and 21 day samples) gave a faint spot at the origin (Rf; 0) which was not toxic to the liver homogenate.

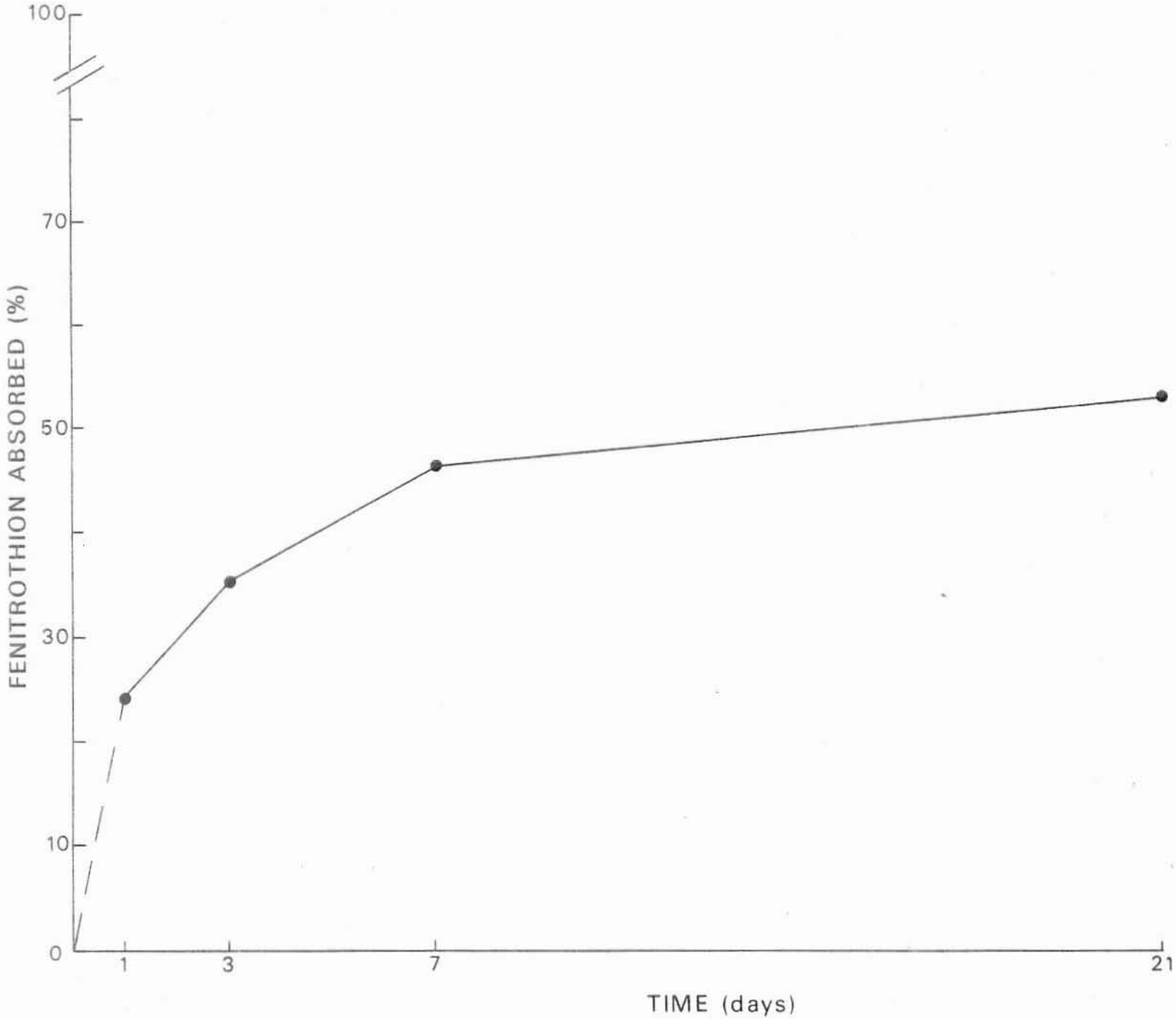


Fig. 1. (i) Absorption of Fenitrothion by Jack Pine

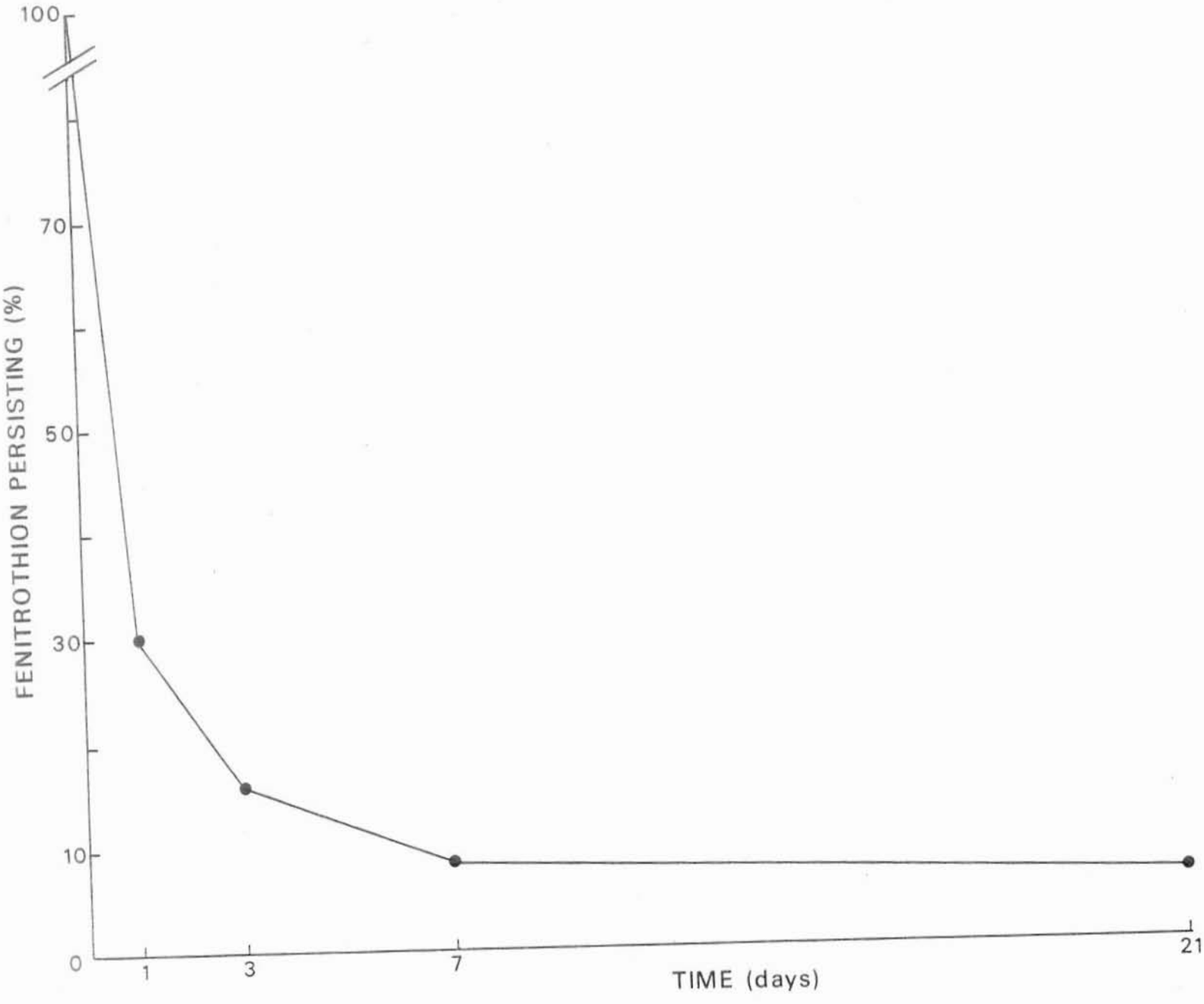


Fig. 1. (ii) Persistence of Fenitrothion on Jack Pine

Fig. 2 Persistence and translocation of fenitrothion-C^{14} from young foliage of Jack pine.
(i) Autoradiograph (right) of pine sampled 7 days after treatment. Note absence of any major translocation.

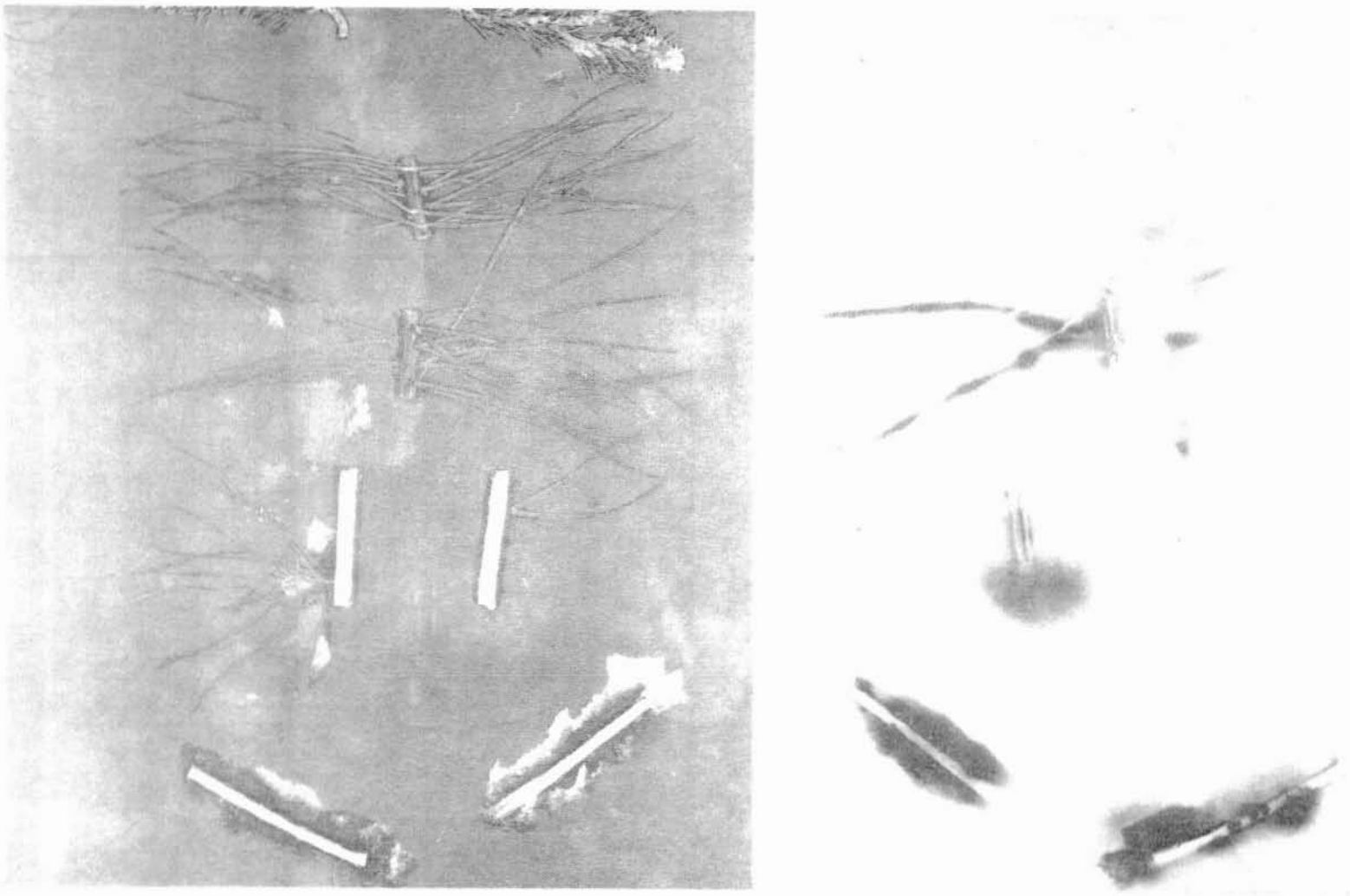


Fig. 2 Persistence and translocation of fenitrothion-C^{14} from old foliage of Jack pine.
(ii) Autoradiograph (right) of pine sampled 7 days after treatment. Note absence of any major translocation.

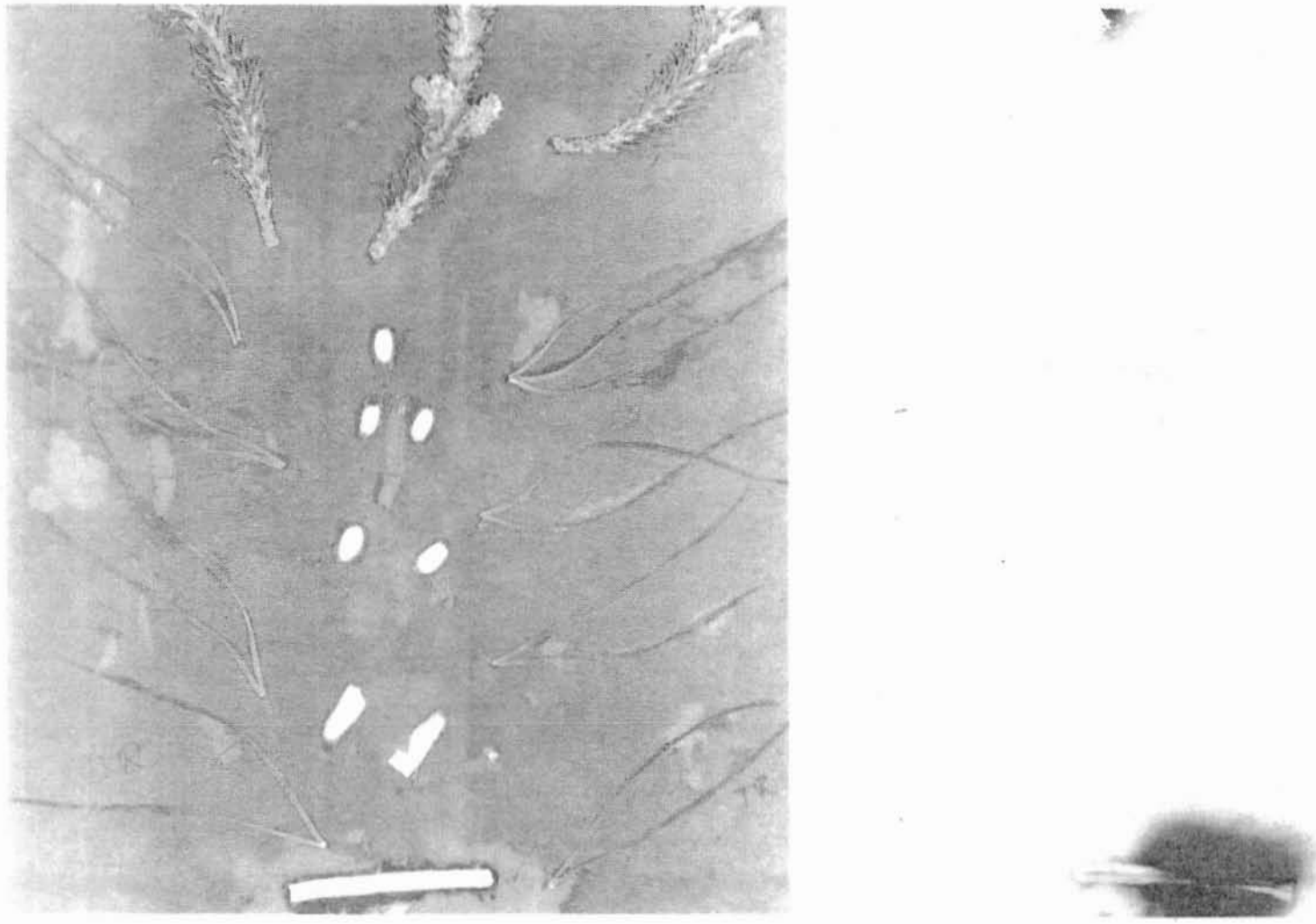


Fig. 2 Persistence and translocation of fenitrothion by stem of Jack pine.
(iii) Autoradiograph (right) of pine sampled 7 days after treatment. Note absence of any major translocation.

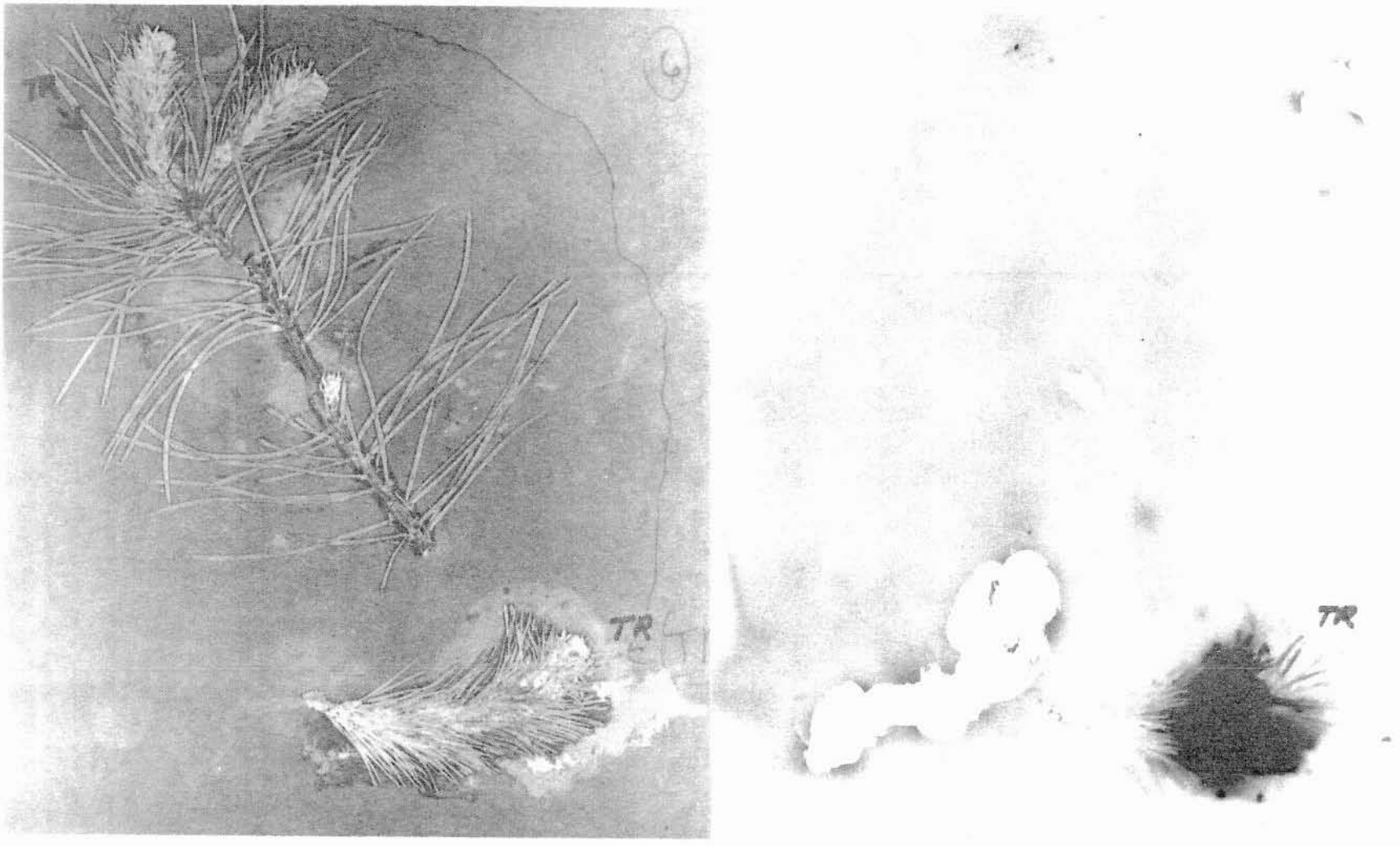


Fig. 2 Persistence and translocation of fenitrothion-C^{14} by young foliage of Jack pine after 21 days. (iv) Autoradiograph of pine sampled 21 days after treatment. Note absence of any major translocation.

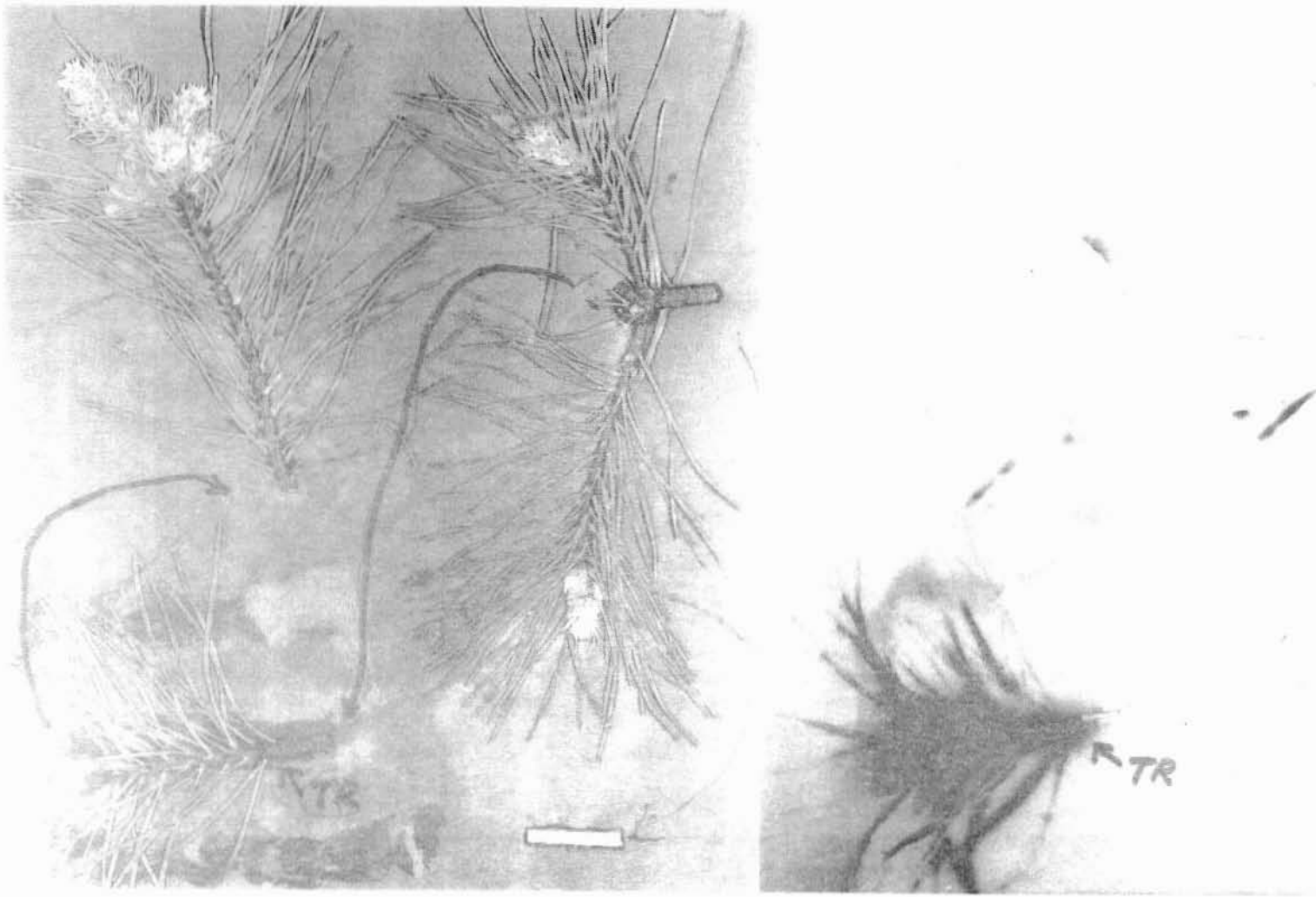


Fig. 2 Persistence of fenitrothion-C^{14} in old foliage of Jack pine.
(v) Autoradiograph of pine sampled 21 days after treatment of old foliage with C^{14}-fenitrothion. Note absence of any major translocation. Arrow denotes attachment site of treated tissue (TR).

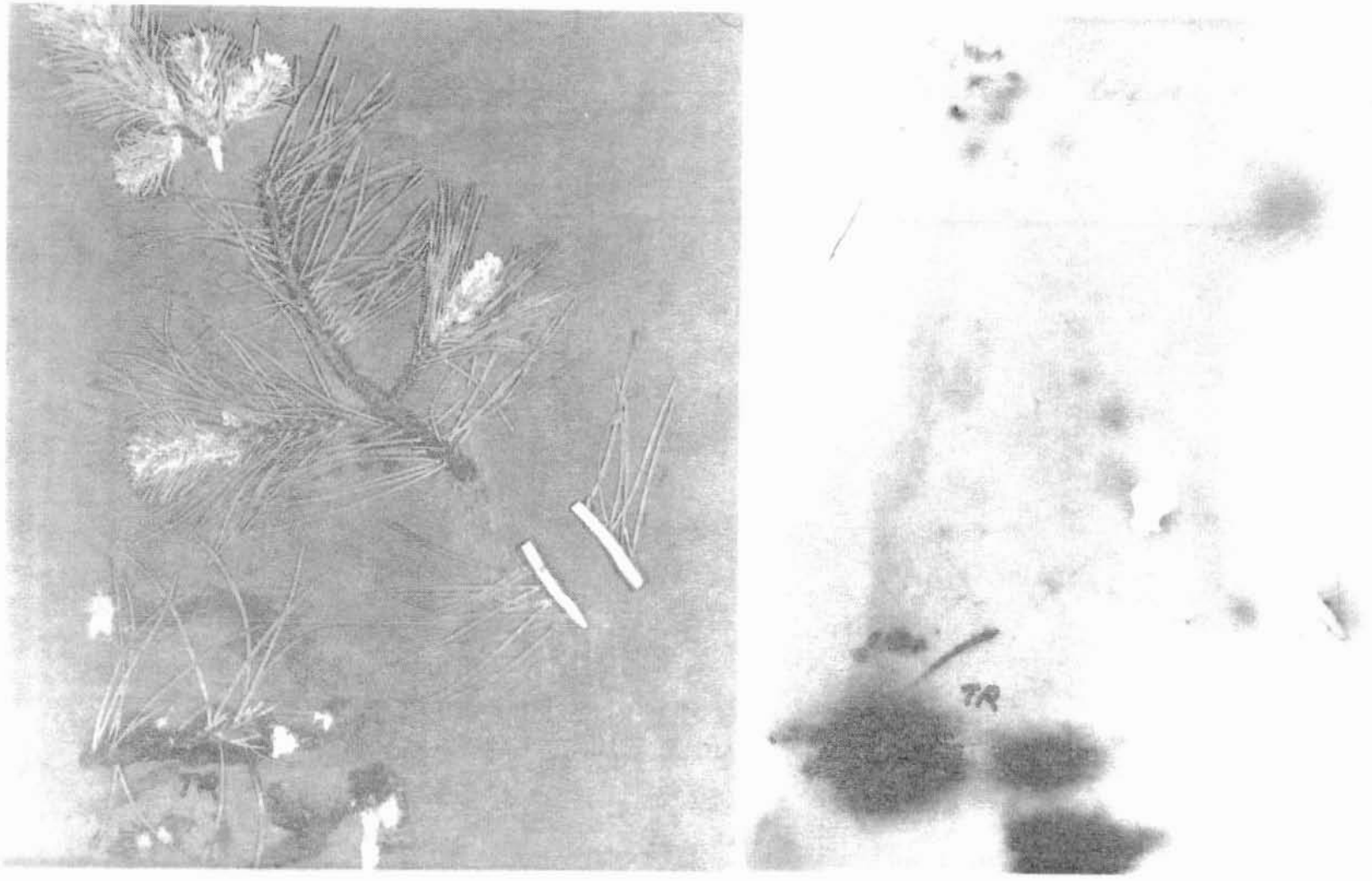


Fig. 2 Persistence of fenitrothion-C^{14} in stem of Jack pine.
(vi) Autoradiograph of pine sampled 21 days after treatment of stem with C^{14}-fenitrothion. Note absence of any major translocation.

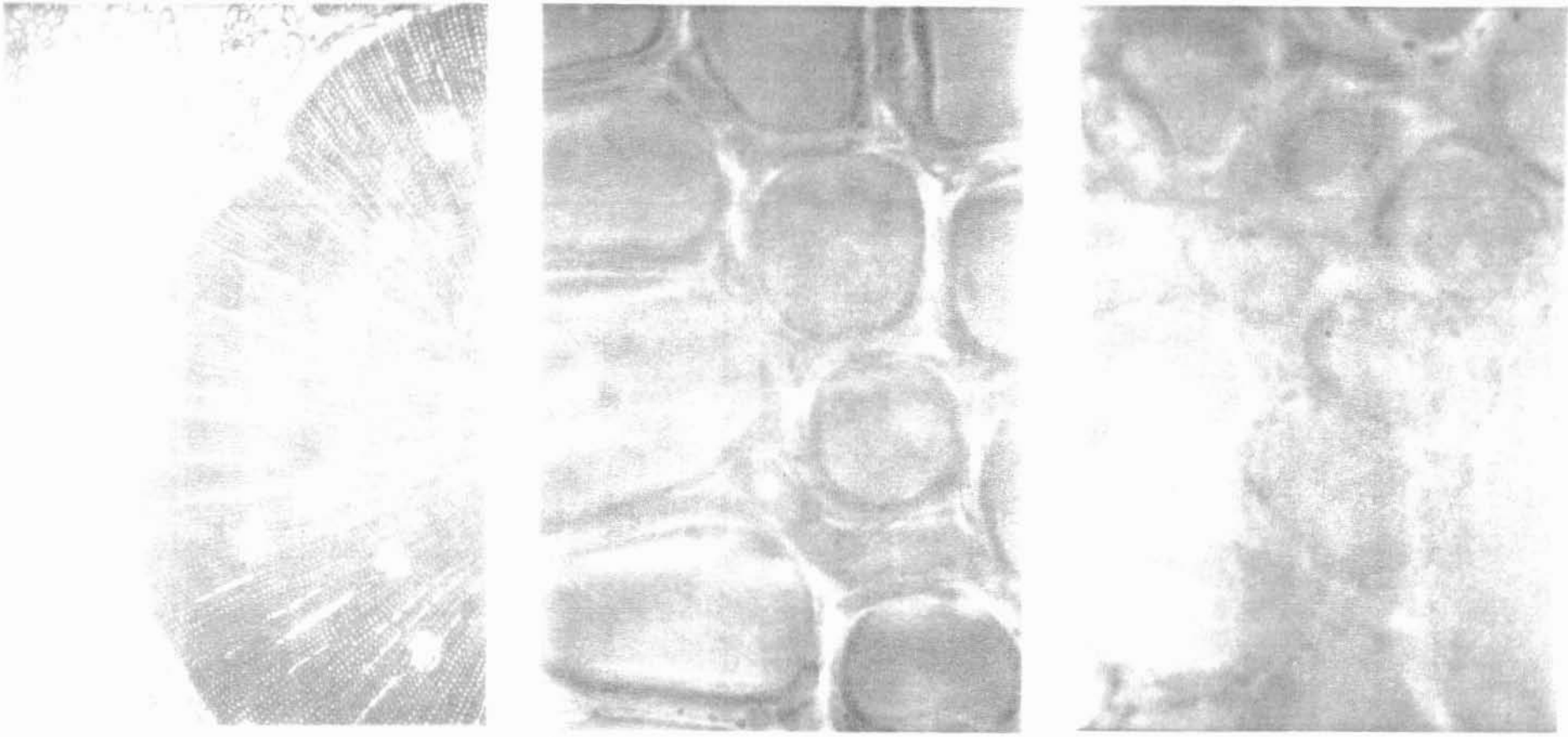
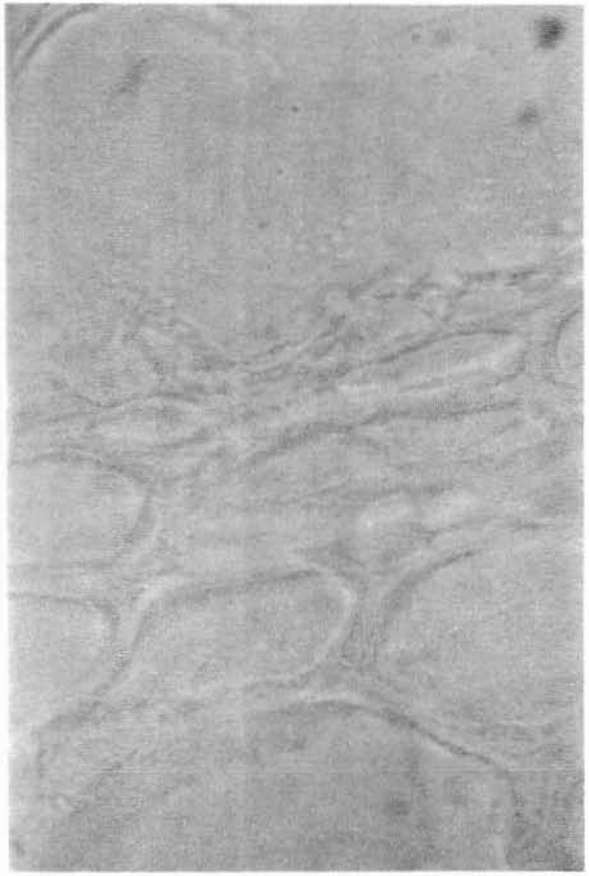


Fig. 3 Cellular localization of fenitrothion-C^{14} in Jack pine stem.

(i) X.S. of Jack pine stem showing xylem (dark area) and surrounding cortex (includes two resin ducts) x 250.

(ii) Histoautoradiograph (HR of X.S. of Jack pine stem showing little C^{14}-activity (black dots) in xylem vessels. x 1,000.

(iii) H.R. of X.S. of Jack pine stem showing some C^{14} activity in the outermost xylem vessels. X 1,000.



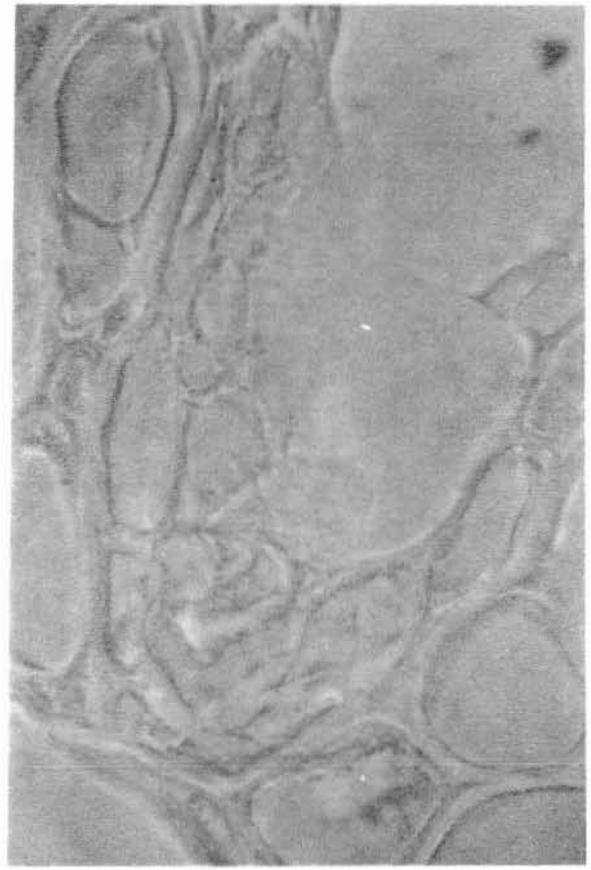
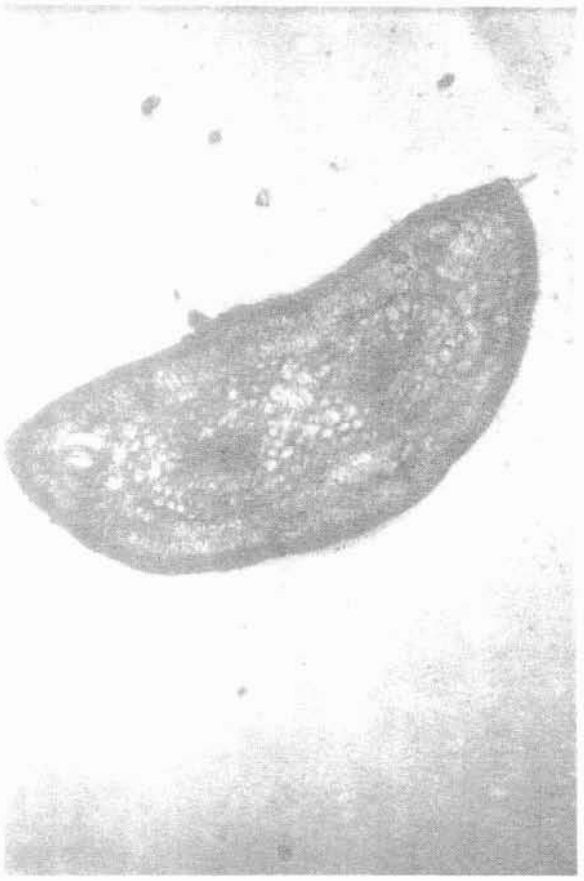
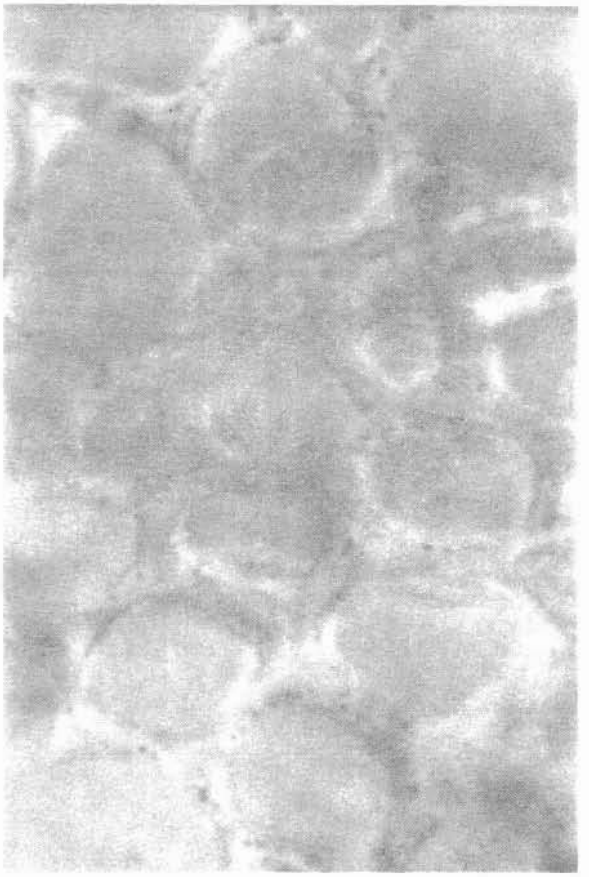


Fig. 3 Cellular localization of fenitrothion-C^{14} in Jack pine stem.

(iv) H.R. of X.S. of Jackpine stem. There is no C^{14}-activity in resin duct. x 1,000.

(v) H.R. of X.S. of Jack pine stem. There is no C^{14}-activity in resin duct. x 1,000.





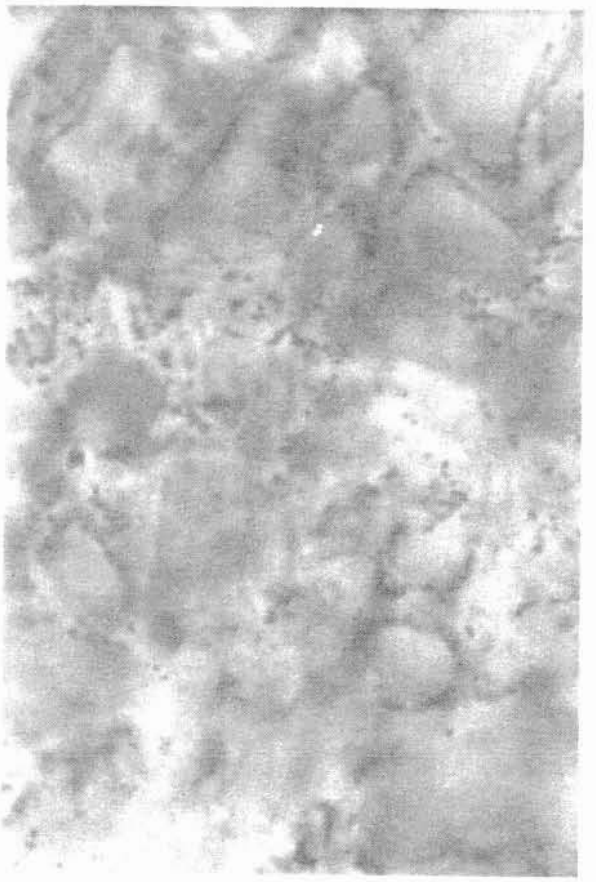


Fig. 3 Cellular localization of fenitrothion-C^{14} in old foliage of Jack pine.

(vi) X.S. of Jack pine old foliage x 250 (vii) H.R. of X.S. of Jack pine old foliage showing some C^{14}-activity in mesophyl. x 1,000. (viii) H.R. of X.S. of Jack pine old foliage showing C^{14}-activity in vascular bundle. x 1,000.

DISCUSSION

Our previous investigation (Moody et al, 1975) demonstrated rapid loss of fenitrothion from conifers held in the greenhouse. The present study was consistent with the earlier results as indicated by the percent recovery data plotted in Fig. (ii) which show that 70% of the applied fenitrothion had been lost one day after treatment. Since the present data were obtained by LSC of total C^{14} activity, the observed loss of activity with time would preclude the possibility of large scale degradation via metabolite formation, since any metabolite possessing the C^{14}-ring moiety would still be detected. Furthermore, the TLC analyses did not reveal the presence of any toxic metabolites in the conifer extracts. These observations rule out the theory that the sawfly larvae mortality observed by McLeod (1975) was due to the formation of highly toxic metabolites of fenitrothion.

The gross autoradiography demonstrated little or no translocation of the radiolabel in Jack pine and thus suggesting erroneously the possibility of no or little systemic action of the pesticide in this conifer species. The histoautoradiograph, on the other hand, demonstrated localization of the radiolabel in the xylem vessels of the stem tissue sampled 21 days after treatment. Similarly the LSC method also showed activity in the tissue, thus explaining the observation (Table 1) that the greatest amount of pesticide was present in the extracts of the stem tissue and not in the surface washes of the 21 day samples. This "absorbed" residue would be more persistent since

its solubilization in the plant waxes and oils would retard volatilization (Linskens et al, 1965) and would also be protected from loss due to the leaching effect of rainfall. The unexpectedly high mortality of sawfly larvae observed by McLeod (1975) could simply be due to the persistence of low levels of fenitrothion (0.48 ppm; 21 days) on and within the treated tissue. That fenitrothion does not selectively get in to resin ducts to affect mortality of the sawfly is amply demonstrated by histoautoradiographs.

These "low" levels may be sufficient to cause the unexpectedly high sawfly mortality reported since this insect is highly sensitive to fenitrothion (Nigam, 1970) when compared with other forest insects such as the spruce budworm (Nigam, 1969).

SUMMARY & CONCLUSIONS

Employing liquid scintillation, histoautographic and thin layer chromatographic techniques, a study of the persistence, uptake, movement and metabolism of fenitrothion-C^{14} was investigated in Jack pine foliage and stem tissues. Some movement of the material took place within 21 days and no metabolites were formed in the resin ducts; the molecule seems to persist in cuticle and some parts of the vascular systems (xylem). The mortality of sawfly is thought to result from extreme sensitivity of the pest to low levels of the pesticide.

ACKNOWLEDGEMENTS

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REFERENCES

EISNER, T., JUDITH S. JOHNESSEE, J. CARREL, 1974. Defensive Use of a Plant Resin. Science, 184: 996 - 999.

LINSKENS, H.F., W. HEINEN and A.L. STOFFERS, 1965. Cuticula of leaves and the residue problem. Res. Rev. 8: 136.

MCLEOD, J.M., 1975. Possible residual effect of fenitrothion on Swaine Jackpine sawfly following aerial application against the spruce budworm in Quebec. Ann. Soc. Ent. Quebec, 20: 82.

MENDOZA, C.E., P.J. WALES, H.A. MCLEOD and W.P. MCKINLEY, 1968. Thin layer chromatographic enzyme inhibition procedure to screen for organophosphorous pesticides in plant extracts without elaborate clean-up. Analyst 93: 173.

MOODY, R.P., R. PRASAD, R.J. GREENHALGH and P. WEINBERGER, 1975. Fate of fenitrothion in forest trees. VI. Some factors affecting rate of dissipation from balsam fir and white spruce. Environ. Can. Forest. Serv. Rept. CC-X-111. 19 pp.

NIGAM, P.C., 1969. Laboratory evaluation of insecticides against fifth instar spruce budworm larvae, *Choristoneura fumiferana* (Clem.). Environ. Can. Forest. Serv. Rept. CC-X-1.

NIGAM, P.C., 1970. Toxicity of insecticides against sawfly larvae. 1. Contact toxicity of organophosphates and carbamates to *Neodiprion pratti banksianae, N. swainei* and *Pristiphora erichsonii*. J. Econ. Entomol. 63: 620.

PRASAD, R. and R.P. MOODY, 1974. Fate of fenitrothion in forest trees. III. Development of histoautoradiographic technique for investigating foliar penetration in balsam fir and white spruce. Environ. Can. Forest. Serv. Rept. CC-X-70, 26 pp.

ROBERTS, J. R. (Editor), 1975. Fenitrothion: The effects of its use on environmental quality and its chemistry. National Research Council of Canada No. 14104, 162 pp.

YAMAGUCHI, S. and A.S. CPAFTS, 1958. Autoradiographic method for studying absorption and translocation of herbicides using C^{14}-labelled compounds. Hilgardia 28: 161.

YULE, W. N. and J. R. DUFFY, 1972. The persistence and fate of fenitrothion insecticide in a forest environment. Bull. Environ. Contam. Toxicol. 8: 10.