# The Persistence, Transport and Metabolism of Fenitrothion in Conifers

by

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Candidate

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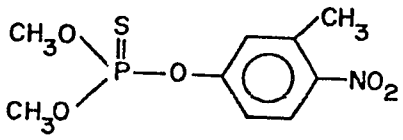

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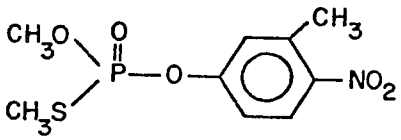

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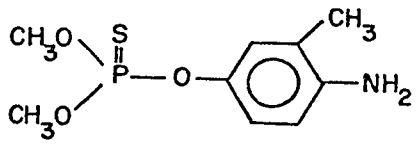

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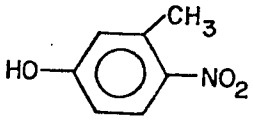

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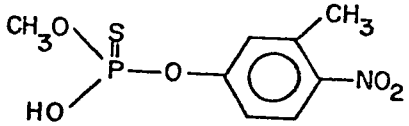

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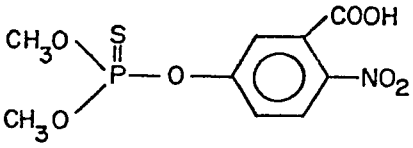

CARBOXY FENITROTHION

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Acknowledgements

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This thesis is dedicated to my friend Miss B. McDowell, who left me alone long enough to prepare this manuscript.

## ABSTRACT

An investigation was carried out to determine the fate and persistence of fenitrothion (0,0-dimethyl-0-(3-methyl-4-nitrophenyl)-phosphorothioate), applied to four year old seedlings of balsam fir, white spruce, and Jack pine under greenhouse conditions. The insecticide disappeared rapidly from the surface of conifer tissue while the absorbed residues were more persistent. Greater absorption of the pesticide was coupled with increasing persistence in pine, spruce and fir. An *in vitro* study carried out on glass surfaces demonstrated that rapid disappearance of the pesticide was primarily due to volatilization. TLC analysis of the conifer extracts was consistent with this dissipation mechanism since $^{14}C$-ring degradation products were present for the most part only in trace amounts.

Autoradiographic tracing studies demonstrated the ability of $^{14}C$-fenitrothion to be transported acropetally into the young foliage of fir, and to a lesser extent in spruce. That this took place *via* the xylem (apoplastic transport) was confirmed by histoautoradiography. These results were taken as evidence for the systemic potential of fenitrothion for budworm control.

## RESUME

On a fait une étude du devenir et de la rémanence du fénitrothion (0,0-dimethyl-0-(3-methyl-4-nitrophenyl)-phosphorothioate), appliqué en serre à des semis de sapins baumiers, d'épinettes blanches et de pins gris de 4 ans. L'insecticide a disparu rapidement de la surface du tissu des conifères alors que les résidus absorbés ont été plus rémanents. Une plus grande absorption du pesticide est reliée à la rémanence et les deux étaient plus élevées pour le pin, l'épinette et le sapin selon un ordre croissant. Une étude in vitro effectuée sur des surfaces de verre a démontré que la disparition rapide du pesticide était probablement due à la volatilisation. La chromatographie sur couche mince des produits d'extraction des conifères a confirmé ce mécanisme de dissipation puisque les métabolites cycliques marqués étaient présents pour la plupart en quantités infimes (traces).

Le tracage autoradiographique a révélé la tendance du fénitrothion $C^{14}$ à se transporter de facon acropète dans le feuillage du sapin et, à un degré moindre, de l'épinette. L'histoautoradiographie a confirmé que ce phénomène se produisait par les vaisseaux du xylème. On a conclu que ces résultats confirmaient l'aptitude du fénitrothion a servir d'insecticide endothérapique contre la tordeuse des bourgeons de l'épinette.

# I - Introduction

## (1) Literature Review

Increasing concern over environmental contamination with pesticides has resulted in curtailment of the use of several persistent organochlorine insecticides and their replacement with more selective and short-lived organophosphorus compounds.

Fenitrothion (0,0-dimethyl-0-(3-methyl-4-nitrophenyl)-phosphorothioate) has been used since 1969 to replace DDT for operational control of lepidopterous defoliators in Canadian Forests (Fettes, 1968). By 1975, some 52 million acres of forest land had been sprayed with the pesticide at an average rate of 4 oz/acre, in an attempt to control the spruce budworm, Choristoneura fumiferana (Clemens) (N.R.C.C., No. 14104, 1975).

Fenitrothion was introduced in 1959 as an experimental insecticide by the Sumitomo Chemical Co., Japan, under the trade name Sumithion and independently by Bayer Leverbusen (Folithion) and by the American Cyanamid Co. (Accothion). It is synthesized by the reaction of 0,0-dimethyl phosphorochloridothioate with an alkali salt of 3-methyl-4-nitrophenol (Nishizawa et al, 1961).

This pesticide has a low mammalian toxicity; the acute oral $LD_{50}$ of the technical product for mice is 870 mg/kg and 250 mg/kg for rats (Schrader, 1961). The lowering of mammalian toxicity by the introduction of a methyl group in the o-position to the nitro group was discussed by Schrader, (1961) and also by Drabek and Pelikan, (1956). They showed that the mammalian toxicity of fenitrothion is much lower than that of many similar organophosphorus insecticides such as methyl

parathion and parathion, and it is for this reason that it was selected for use in Canada to control forest insect pests. Fenitrothion has been used throughout Europe, East Pakistan, East Africa, the United Arab Republic, Japan, the Republic of China, New Zealand and Brazil.

In Canada the pesticide is registered for forest use only against spruce budworm, hemlock looper (Lambelink fiscellaria Guenée) and sawfly species. The applied dosage may not exceed 6 oz./acre of active ingredient (Fettes, 1968). All forest spraying is performed by aerial application because of the vast regions involved. The optimum rate of application was shown to be 2-4 oz./acre when distributed by aircraft equipped with Tee-Jet nozzles manufactured to produce an average spray droplet of 100μ for the fenitrothion formulation (Randall, 1974). Field studies (Armstrong and Randall, 1969) have shown that the degree of temperature inversion in the zone above the trees is the major factor affecting the amount of insecticide reaching the forest canopy. The drift of the spray cloud from the target area is affected by the strength of the temperature inversion present at the time, and also by the amount of cross-wind. Field observations during aerial spray applications have shown that anywhere from 15-75% of the pesticide emitted from the aircraft will reach the forest canopy when applied during a temperature inversion condition and in some instances during unstable conditions, 'on target' deposit was less than 2% of the total emitted material. Fenitrothion is presently applied in Canadian aerial spray operations in a 10% (v/v) formulation of the active ingredient in water, with 1% Aerotex 3470 and 1% Atlox 3409 or Toximul MP-8 (adjuvants) added to emulsify the pesticide

(Safe et al, 1977). These adjuvants are currently under suspicion of being related to the high incidence of Reye's Syndrome in the maritime provinces (Crocker et al 1974, 1976). For this reason, in the future, the use of water-based formulations may become unacceptable necessitating a return to the costlier, oil-based formulation (10% a.i. in fuel oil #2,4 or 6) (N.R.C.C., No. 14104, 1975).

Since 1969, an average of 617,000 Kg of fenitrothion have been applied on an annual basis to New Brunswick forests (Symons, 1977). In 1972, Yule and Duffy investigated the persistence of fenitrothion on balsam fir and mixed spruce foliage, in the New Brunswick forests following aerial application of the pesticide at an average rate of 4oz./acre. They reported that 50% of the initial deposit was lost by the foliage within four days and 70-85% disappeared within two weeks. However, 10% persisted for at least ten months. The rapid disappearance of fenitrothion from various crop plants has been reported in the literature (Miyamoto and Sato, 1965; Bowman and Beroza, 1969; Leuck and Bowman, 1969). It was suggested by Yule (1974) that the relatively high persistence of fenitrothion in conifers is due to solubilization of this lipophilic compound in the heavy cuticles characteristic of conifer foliage. Recent evidence indicates that this persistent residue may accumulate in balsam fir foliage proportionately to the number of annual applications and the total dosage of fenitrothion applied (Yule, 1974). McLeod (1975) has also reported a possible residual effect of fenitrothion on Swaine Jack pine sawfly (Neodiprion swainei Midd.), 42 days after aerial application of the pesticide to Quebec forests. These studies constituted the only residue data of fenitrothion on conifers that was reported in

the literature at the time the present study was initiated.

(a) Biophysical degradation and relation to toxicity:

The basic mechanism involved with the toxicity of organophosphorus insecticides is the inhibition of the acetylcholinesterase of the nervous system with consequent accumulation of acetylcholine at the nerve synapses. The phosphorothioates, such as fenitrothion are only weak inhibitors of cholinesterase and activation takes place by conversion to their oxygen analogues. The normal route of formation of the oxygen analogue of fenitrothion (fenitro-oxon) is *via* oxidative desulfuration of the molecule (Miyamoto *et al*, 1964). Fenitro-oxon was found in the leaf blade and sheath of rice plants 24 hours after treatment (Miyamoto and Sato, 1965). Kovaciova *et al* (1973) reported that an S-methyl isomer was present in commercial preparations of fenitrothion and that this isomer had an *in vitro* anticholinesterase activity that was two to three orders of magnitude greater than the parent compound. Greenhalgh *et al* (1975) also showed that some commercial preparations of fenitrothion contained up to 4.43% S-methyl fenitrothion and detected it in field samples after spraying.

The major detoxification mechanisms for fenitrothion are well documented in the literature (see N.R.C.C. No. 14104, 1975). A brief summary is included here. The primary mechanism in plants, mammals, and insects is *via* enzymatic hydrolysis. This involves cleavage of the P-O-aryl bond to give water soluble degradation products including dimethyl phosphorothioic acid and 3-methyl-4-nitrophenol. A major route of detoxification in mammals occurs by dealkylation of the O-methyl group to form demethyl-fenitrothion (Hollingworth 1969, 1973). Ruminant animals and some anaerobic bacteria have a further detoxification

mechanism *via* reduction of the nitro group to form amino-fenitrothion (Yasumo *et al*, 1965; Bull, 1972).

Another mechanism of physico-chemical degradation has been reported recently that can account for the formation of degradation products *via* photo-oxidation of fenitrothion. Okawa *et al* (1974) noted rapid photodecomposition on irradiation of fenitrothion with UV light or sunlight yielding carboxyfenitrothion and traces of fenitro-oxon, carboxyfenitro-oxon, 3-methyl-4-nitrophenol, and S-methyl fenitrothion.

(b) Analytical methodology:

Analytical procedures for fenitrothion are documented in the literature for a wide variety of crop plants and are based on spectroscopy, thin-layer chromatography and gas chromatography. Fenitrothion levels can be quantified using the method of Averell and Norris (1948), which involves reduction of the nitro group to a primary amine, diazotisation and coupling with N-(1-naphthyl) ethylenediamine to give an azo dye which can be measured spectrophotometrically (Dawson *et al*, 1964). Another colorimetric method involves determination of the 3-methyl-4-nitrophenol after hydrolysis of the pesticide with alkali. The limits of detection of these colorimetric methods are about 0.05 ppm (relative to the plant tissue). Thin-layer chromatography (TLC) has been used for qualitative analysis of fenitrothion and its degradation products. A relatively sensitive TLC method (limit of detection 0.1-0.01 ppm) relies upon cholinesterase inhibition for development of TLC plates (Mendoza *et al*, 1968).

The most reliable and highly sensitive analytical methods utilize gas chromatography with either the flame-photometric (Bowman and Beroza, 1969) or the thermionic detector (Miyamoto *et al*, 1967; Sato

et al, 1968). These detectors are highly specific for the phosphorus in the fenitrothion molecule. An alkali flame ionization detector (AFID) fitted with a cesium bromide annulus has recently been reported to be more sensitive to fenitrothion than the other detectors previously used (least detectable amount, $1.7 \times 10^{-15}$ gm/sec) (Greenhalgh et al, 1975). This detector was employed by Hallett et al (1975) for analysis of fenitrothion in extracts of white pine seeds, and was also used in the present study. Although the detector is highly specific and sensitive to P, it is necessary to clean-up extracts prior to analysis, with the further possibility of loss of some of the degradation products during clean-up. To avoid this possibility, the present study took advantage of basic radio-tracer methodology which did not require sample clean-up prior to analysis. This methodology as well as other improvisations made in the extraction and analytical methodology are discussed in the "Materials and Methods" section of this report.

(c) General definitions:

A number of chemicals of diverse characteristics have been arbitrarily classed together as insecticides on the basis of their use. The chemical and physical properties of an insecticide and interacting environmental factors determine its behavior. The behavior dictates the ultimate fate of the insecticide (Van Middelem, 1966). The behaviour of a chemical is its characteristic movement, persistence, and fate in the environment and determines both its field effectiveness as well as its residue characteristics. The present study is concerned in part with the absorption of fenitrothion by conifer foliage. Absorption will be defined as the movement of

chemicals from the surface as extracuticular residues to a region at some subcuticular location. The surface of conifer foliage is covered by a cuticle, which as in other plants, is continuous through the stomatal chambers (Essau, 1962). The cuticle is characteristically thick in conifers, one of several adaptations made by this group of plants to withstand xeric conditions. The cuticle is composed of plates and protuberances of waxes embedded in various layers of cutin which is a mixture of polymers of dicarboxylic and hydroxycarboxylic acid esters. The properties of the cuticle vary with environmental conditions and position on the plant but generally the polarity of the cuticle increases towards the interior (Franke, 1967; Schieferstein and Loomis, 1958; Van Overbeek, 1956). The external cuticle of conifers contains much wax and is highly oxidized and polymerized, whereas the interior cuticle has less wax and more pectinaceous material. The cutin has some affinity for water and under conditions which favour hydration may swell and force the plates of wax apart (Hammerton, 1967).

The cuticle is the first barrier to pesticide entry into the plant system. The initial contact of the spray deposit is with the waxy projections of the cuticle and these may prevent good contact with the foliage surface. However, the emulsifying agents (Aerotex & Atlox) added to the spray formulation of fenitrothion have a surfactant effect and would lower the surface tension of the spray emulsion so that a better contact would be established. The chemical nature of the cuticle is of primary importance in determining the successful uptake of the pesticide. Fenitrothion is relatively non-polar and the outer portion of the lipophilic cuticle would favour

its entry. However, as the polarity increases from the waxy leaf surface towards pectins between the cell walls and to the aqueous environment of the cell, the affinity of fenitrothion for the lipid portions of the cuticle would retard its entry into the aqueous phase of the plant. If retarded sufficiently, the pesticide would remain adsorbed to the foliar surface and would not be available for transport.

Transport, which is the movement of chemicals within the plant, may occur acropetally (towards the shoot apex) in both the xylem (water transport tissue) and phloem (photosynthate transport tissue). Basipetal translocation (towards the roots) occurs only in the phloem (McCready, 1966; Crafts, 1956). If a compound can be absorbed by the plant and translocated in sufficient quantities to make the paths of transport, as well as the sites of accumulation biologically toxic with respect to an insect predator, it is said to be a systemic compound (Crisp, 1972). The systemic action of a pesticide then, is dependent on absorption, transport and persistence, and the present study was conducted to investigate these processes as well as their interactions.

Parallel studies were also conducted to determine the fate of the pesticide that was not absorbed by the conifer tissue. In the natural environment, these extracuticular residues would be exposed to weathering influences which are thought to be responsible for the rapid loss of fenitrothion from conifers following field applications of the pesticide (Yule and Duffy, 1972). The term, "weathering influences" is used here to describe the combined impact of solar radiation, wind, and precipitation that could respectively account for

photodegradation, volatilization, and leaching of the applied deposit (Ohkawa et al, 1974; Yule and Duffy, 1972).

(2) Rationale of Present Study:

The present study was conducted to determine the metabolic fate, persistence and movement of fenitrothion in balsam fir (Abies balsamea (L.) Mill), white spruce (Picea glauca (Moench Voss)), and Jack pine (Pinus banksiana (Lamb)) under greenhouse conditions comparable to the field situation, to test the hypothesis that fenitrothion has a systemic potential in conifers.

(a) Preliminary studies:

Initial studies were conducted to determine the most suitable method of applying the pesticide. A simulated field formulation of the pesticide was applied in two concentrations (20 & 200 ppm), either by painting or spraying the conifer branches. Samples were taken after 1 and 7 days to obtain a suitable sampling schedule for future studies. The samples were analyzed by gas-liquid chromatography (GLC) and thin-layer chromatography (TLC). An in vitro study was also conducted on glass surfaces under light and dark conditions to determine the effect of abiotic factors (photodegradation, volatilization etc.).

(b) Radiolabel studies:

After completion of these preliminary studies, further investigation was conducted with $^{14}C$-ring labelled fenitrothion employing basic radiotracer methodology (liquid scintillation counting (LSC), Radio-TLC, and autoradiography) to back-up and expand upon the preliminary data, and to investigate the movement of the pesticide within the plant system. The in vitro, glass surface study was repeated with the radiolabel to confirm the presence or absence of degradation

products.

## II MATERIALS AND METHODS

### (1) Culture of Tree Seedlings:

Four year old seedlings of balsam fir, white spruce, and Jack pine were obtained from the Ontario Ministry of Natural Resources nursery at Kemptville, Ontario. These were held in the greenhouse for approximately two months until the current buds had flushed out. Healthy specimens of each of the three species 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 was supplied by fluorescent "daylight" tubes (≈5,000 lux)[1] (U.S.D.A., 1976).

### (2) Chemicals and Solvents

Purified samples of fenitrothion and its degradation products were obtained from Agriculture Canada. Purity was confirmed by GLC and TLC. Detailed methods for purification of fenitrothion (Kovacicova et al, 1971) and synthesis of degradation products have recently been described by Hallett et al (1974). A water emulsion of the insecticide was made up as a simulated field formulation of 10% fenitrothion (cold or $^{14}$C-ring-labelled), 1% Arotex 3470, 1% Atlox 3409, and 88% distilled water v/v. $^{14}$C-ring-labelled fenitrothion (specific activity 10 mCi/mmol) was supplied by Dr. R. Duffy (University of Prince Edward Island). Purity was confirmed by GLC, TLC and liquid scintillation counting (LSC). The solvents used for extraction were glass distilled and were obtained from Caledon Laboratories,

[1]The artificial light source supplemented the solar radiation in the greenhouse.

Toronto. The radiolabel formulation was diluted prior to application so that the final activity would be 7.6 µCi/ml, (≈200 ppm).

(3) Method of Treatment

(a) In vivo plant study:

A 10% aqueous emulsion of fenitrothion is emitted during aerial spraying at the average rate of 4 oz/acre (N.R.C.C. No. 14104, 1975). To simulate the dilutory effect of air dispersal, the emulsion was diluted in the present greenhouse study so that the final application would approximate the deposit recovered from field samples (1-4 µg/gm fresh wt. of foliage 1 day post-spray, Yule and Duffy, 1972). Two concentrations of the emulsion, 20 and 200 ppm, were applied to the branches either by painting or spraying. Three replicates of each of two conifer species (balsam fir and white spruce) were employed for each of the four treatment methods (Paint 20 ppm; Paint 200 ppm; Spray 20 ppm; Spray 200 ppm).

For the painting technique, four branches (per tree) were selected and 0.5 ml of emulsion was carefully applied to the foliage and bark located basipetally within 50 cm of the shoot apex. A small 1/8" brush (Grumbacher # 4116) was used in an attempt to obtain uniform coverage. Spraying was accomplished by employing a "Wet-Pak" spray gun to deliver 10 ml of emulsion to each tree while it was being rotated on a turntable (30 rpm).

Three replicates of each of the three conifer species were employed for LSC determination of persistence in a subsequent experiment employing $^{14}$C-labelled fenitrothion. Four branches (per tree) were selected, and 0.5 ml of the $^{14}$C-fenitrothion formulation (7.6 µCi/ml) was carefully applied by the painting technique described pre-

viously. Three replicates of each species 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 2.5 to 5 cm (1"-2") sections of either the newly flushed foliage, the older foliage or the bark tissue. This AR study was initiated to determine whether pesticide was translocated and if so to identify the major direction of transport (acropetal or basipetal). Plastic sheeting was used to cover the soil in the pots to prevent contamination.

(b) In vitro glass surface study:

The in vitro fate of fenitrothion was investigated in a separate study to determine the effect of purely physical processes. Five ml of the aqueous formulation of fenitrothion (200 ppm) were pipetted onto glass petri dishes (i.d. 9 cm) which were left uncovered, either exposed to the greenhouse conditions, or in a dark growth chamber set at 22 ± 1°C and 50% relative humidity.

This experiment was repeated with $^{14}$C-fenitrothion of which 50 µl in acetone solution (≈5 X $10^4$ dpm) were applied to each of 30 circular glass cover slips (Corning # 1, 18 mm) held in scintillation vial caps. Half of the caps were placed in a light-proof cardboard box. The remainder were placed on top of the box which was left in the greenhouse, exposed to the same environmental conditions previously specified.

(4) Sampling and Extraction Procedure:

(a) Cold (unlabelled) fenitrothion study:

For the preliminary screening study with 'cold' fenitrothion, separate samples of newly flushed foliage of the terminal buds (N.F.), old (current) foliage (O.F.), and stem (woody) tissue (S T.), were

individually weighed, placed in plastic 'roll-top' bags and frozen in liquid $N_2$ before being stored at -70°C. An extraction method was devised which gave a high recovery of the pesticide from spiked samples and that yielded extracts sufficiently clean for GLC analysis. This method had to be developed and tested prior to initiation of the present study. The method described by Hallett et al (1975) was modified in that each conifer tissue sample (1-3 gm) was initially placed in a sintered glass funnel and was washed under vacuum with 200 ml ethyl acetate. The solvent was concentrated to near dryness in a rotary type flash evaporator (35°C), and then brought up to 10 ml with acetone for analysis. This provided a surface wash of the tissue. The tissue was then extracted twice with ethyl acetate (100ml each time) in a Polytron sonicator (Model # PP1020) and the extracts were filtered through Celite 545, and concentrated to approximately 25 ml. This provided a tissue extract separate from the initial surface wash[1]. A modification of the clean-up procedure employed by Yule and Duffy (1972) for large field samples was used for charcoal column preparation. Since in the present study, the sample weight was smaller, the amount of column packing and the charcoal to Celite ratio were altered to provide more rapid sample processing. Briefly, a column (i.d. 22 mm) fitted with a sintered glass disc was dry packed successively under vacuum with 1" Celite 545, 7.0 gm of a charcoal (Nuchar C-190N) and Celite mixture, and 1" of anhydrous sodium sulphate. Various proportions of charcoal and Celite were tested initially. The mixture employed (1:6; charcoal: Celite) gave 100% recovery of a sample spiked at the 200 ppm treatment level (≈100 µg/25 ml

[1]The 'surface wash' method was tested by washing samples taken one day post treatment with $^{14}$C-fenitrothion (1-3 ppm), three times before extraction. 79% of the total activity was in the first wash, 5% in the second, 2% in the third, and 14% was in the final tissue extract.

ethyl acetate) with fenitrothion, fenitro-oxon, and S-methyl fenitrothion. The packed column was prerinsed under vacuum with 100 ml hexane and then topped with the 25 ml tissue extract. Elution was carried out successively with 100 ml, 25% ethyl acetate in benzene, and 100 ml benzene. The eluants were concentrated to near dryness and made up to 10 ml with acetone for analysis.

The glass petri dishes were sampled after 1, 3, 6 and 11 days. At each sampling time, two replicates from each of the dark and light conditions were washed with identical volumes (50 ml each) of ethyl acetate. These washings were separately concentrated to near dryness and brought up to 10 ml acetone for analysis.

(b) $^{14}$C-labelled fenitrothion study:

In the radiolabel study, 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$^{o}$C. Apart from the charcoal cleanup procedure which was omitted, the extraction procedure followed that described previously.

The trees treated for autoradiography and untreated controls were sampled after 7, 14 and 21 days. The entire branches were individually bagged and refrigerated at -70$^{o}$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.

The glass cover slips treated with $^{14}$C-fenitrothion were sampled at 1, 3, 7, 14, and 21 days. Three replicates were taken on each sampling date from both the light and the dark conditions.

(5) Analytical Methodology

(a) Gas chromatography (GC):

Samples were analysed with a Pye model 104 gas chromatograph equipped with an alkali flame ionization detector (AFID). The glass column (1.8 m x 4mm (i.d.)) used, contained 3% SE-30 Ultraphase on Chromosorb W (H.P.); 80-100 mesh. Column temperature was 200°C, nitrogen 40 ml/min, air flow 500 ml/min, and hydrogen flow 35 ml/min. Peak areas of duplicate sample injections were compared with intermittent duplicate fenitrothion standard injections to calculate the amount of pesticide (ppm) in the tissue (i.e. ng/mg tissue fresh wt.). The fenitrothion standards used for GC quantitation were either kept refrigerated with the conifer extracts or were exposed to the laboratory conditions while the samples were being analysed. This greatly decreased the possibility of reporting the presence of fenitrothion degradation products in the extracts, that had formed during sample storage.

(b) Thin-layer chromatography (TLC):

The enzyme inhibition technique described by Mendoza et al (1968) using extract of steer liver homogenate as the spray reagent, was used to visualize silica gel TLC plates (Fisher Rediplates) which were developed in a solvent system of ethyl acetate and cylcohexane (1:3). The $^{14}C$ - plant extracts were chromatographed as above, and the TLC plates were exposed to Kodak NO-Screen X-ray film for 2 weeks at -4°C.

(c) Liquid scintillation counting (LSC):

All samples from the radiolabel study 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. Sample counts were corrected for quenching with the aid of a quench curve constructed by counting a set of $^{14}C$-fenitrothion standards to which had been added increasingly greater amounts of conifer 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 for GC analysis of conifer extracts.

The glass cover slips from the in vitro radiolabel experiment were counted immediately after sampling simply by inverting the plastic vial caps over scintillation vials filled with 10 ml of cocktail. The caps were screwed on and the vials were shaken vigorously prior to LSC analysis.

(d) 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 at $-4^{o}C$. The conifer branches were cut into sections (Fig. 5; i-xviii) prior to pressing to prevent artificial translocation of the $^{14}C$-label during subsequent sample processing. Histoautoradiography was performed following the method of Prasad and Moody (1974), and involved cryostat sectioning of the tissue followed by a 2 to 3 week exposure to Kodak NTB2 liquid emulsion.

(e) Scanning electron microscopy (SEM):

Balsam fir foliage was gold coated under vacuum and photographs were taken of the tissue surface in an AMR-1000 scanning electron microscope (Howden and Ling, 1973).

## III RESULTS

### (1) In Vivo Plant Study

(a) Cold (unlabelled) fenitrothion study:

Table 1 lists the concentration of fenitrothion in each of the samples analysed by gas chromatography. Each tissue type (N.F., O.F., ST.) has a value reported 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 absorbed (subcuticular) residues present. The percent absorbed (%ABS) was calculated as being equal to $\frac{\text{ppm (E)}}{\text{ppm (W) + ppm (E)}} \times 100$ and the total % ABS is plotted against time in Fig. 1 (i). Fig. 1 (ii) plots the total residual fenitrothion (total net ppm) against time and demonstrates the persistence of the pesticide under the described conditions. The percent recovery (% recov.) of the initial deposit is reported for the 1st and 7th day samples. The values given for the spray application were calculated on the assumption that there was 100% hit (i.e. all of the spray was deposited on the conifer tissues).

Overall, the results of the 'cold' fenitrothion study demonstrated rapid loss of the insecticide from fir and spruce within 7 days following treatment (Fig. 1 (ii)). For example, only 4% of the initial deposit was recovered from the spruce tissue sampled 7 days

after spraying with fenitrothion (200 ppm) (Table 1 (ii)). In all cases the amount (%) of fenitrothion absorbed by the tissue increased from 1 to 7 days (Fig. 1 (i)). Furthermore, increased absorption by one species relative to the other was matched by a relative increased persistence of the pesticide. To clarify this last point, each of the plots in Fig. 1 (i) is a mirror image of each corresponding plot in Fig. 1 (ii). Analysis of the data obtained for each of the separate tissue types (N.F., O.F. ST.: Table 1) demonstrated that the greater relative absorption and persistence of fenitrothion in fir noted in Fig. 1 (except for the painted 200 ppm treatment), could be partially explained by the rapid accumulation and persistence of fenitrothion in the new foliage of this species. Furthermore, fenitrothion was more persistent in tissues extracted by homogenization ((E): Table 1) than those extracted initially by the surface washing technique ((w): Table 1). For example, levels decreased from 4.92 ppm after 1 day to 0.85 ppm after 7 days in the new foliage wash of fir sprayed with fenitrothion (200 ppm) (S-4, N.F. (W): Table 1 (ii)) but the levels increased from 1.29 to 1.99 ppm in the corresponding tissue extracts. An exhaustive investigation by gas chromatography did not demonstrate the presence of any fenitrothion degradation products in conifer samples analysed during this study.

This data indicated that fenitrothion was being absorbed by conifer tissue and that once absorbed, the residues were more persistent. This was especially notable in balsam fir and the possibility was raised that the residues were being translocated acropetally into the new foliage of this species.

(b) $^{14}C$-labelled fenitrothion study:

Table 3 lists the concentration of radiolabel 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 S.A. (10 mCi/mmol) of the compound. As in the previous study, each tissue type (N.F., O.F., ST.) 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 described previously, and the total % ABS is plotted against time for each of the three species in Fig. 4 (i). The total residual radiolabel (total ppm) was converted to percent recovery values which are plotted against time in Fig. 4 (ii) to illustrate a persistence curve for the pesticide through the 21 day sampling period. An autoradiograph of a TLC plate spotted with a balsam fir extract is shown in Fig. 8.

Overall, the results were consistent with the previous 'cold' fenitrothion study. More than 50% of the radiolabel had been lost by the conifer tissue of all three species by one day after treatment (Fig. 4 (ii)). In all species, the amount (%) of radiolabel absorbed increased rapidly from 1 to 7 days and thereafter increased gradually (Fig. 4 (i)). Consistent with the 'cold' study, increasing absorption was matched by increasing persistence of the pesticide, and the residues were more persistent in the tissue extracts (E) than in the surface washes (w) (Table 3). The radiolabel was more persistent in fir than in spruce or pine, and this was partially explicable by accumulation and persistence of the radiolabel in the

new fir foliage. For example by 21 days the highest levels (1.55 ppm) were found in the new foliage extracts of fir (N.F. (E); Table 3).

Radio-TLC (Table 3 and Fig. 8) demonstrated that in most of the conifer extracts analysed, more than 99% of the radiolabel was fenitrothion. Low levels (2-5% of the residual activity; Table 3) of a $^{14}C$-degradation product were detected in the new foliage extracts of fir and spruce in the 3, 7, and 21 day samples. This ring-bearing product was more polar than fenitrothion since its relative $R_f$ value was 0.21 in the TLC system previously described.

This data indicated that the absorbed residues were more persistent and supported the hypothesis made during analysis of the results of the 'cold' study, that fenitrothion was being transported acropetally into the new foliage of balsam fir.

(2) In Vitro study

The results of the in vitro (glass surface) study are given in Table 2, and Fig. 2 plots the percent residual fenitrothion (% of original deposit remaining) against time for both the light and dark conditions. The results of the in vitro radiolabel study conducted in the greenhouse are plotted in Fig. 3.

(3) Transport and Microscopy Studies

The 'cold' and the radiolabel studies demonstrated that fenitrothion was rapidly lost from the glass surfaces under both light and dark conditions. In both studies a slightly greater rate of dissipation was observed in the light than in the dark (Fig. 2 and 3). More than 99% of the persisting residue was present as fenitro-

thion (Table 2). Trace amounts (less than 0.1% of the initial deposit) of fenitro-oxon were detected in the "light" samples and traces of S-methyl fenitrothion were detected in the "dark".

This data demonstrated that the rapid dissipation of the pesticide observed during the in vivo conifer studies, could be explained solely by abiotic factors.

The results of the gross autoradiography are given in Fig. 5 (i-xviii). They demonstrate that the radiolabel was transported acropetally and that this movement was most apparent in balsam fir. The predominant direction of transport was acropetal, this being irrespective of the application site (i.e. to the new foliage, old foliage, or stem) for balsam fir, however, transport was most apparent if the radiolabel was applied to the foliage (Fig. 5 (i) and (v)). Transport in fir was evident by 7 days after treatment (Fig. 5 (i)) however, this was not apparent in spruce after 7 days (Fig. 5 (vii)-(ix)) but was noted after 21 days (Fig. 5 (xi)). Transport in jack pine was not evident in either the 7 or the 21 day samples (Fig. 5 (xiii)-(xviii)).

The results of the histoautoradiography are given in Fig. 6 (i-xviii). Only minimal quantities of the radiolabel were observed in the phloem tissue, whereas strong activity was found localized in the xylem of the conifer needles and stems. Strong $^{14}C$-activity in spruce was also localized in the fascicular tissue present at the needle bases where they attached to the stem (Fig. 6 (xvii)). This contrasted with the 'blank' obtained from the comparably exposed untreated control tissue.

The thick layer of epicuticular wax covering the conifer foliage

is shown by scanning electron micrographs in Fig. 7 (i-iv). This layer was thought to be dissolved by the surface wash with ethyl acetate since a waxy precipitate was observed after refrigeration of these extracts.

These results indicated that the transport of fenitrothion into the new foliage of balsam fir was apoplastic.

TABLE 1 (i)

G.C. Analysis of Fenitrothion (FT) Treated Conifer Tissue* (20 ppm)

| SAMPLE | | PPM | | NET PPM | | % ABS (average) | | TISSUE WT. (gm) | | FT (µg) | | % RECOV. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DAY | | 1 | 7 | 1 | 7 | 1 | 7 | 1 | 7 | 1 | 7 | 1 | 7 |
| P-1 | N.F. (W) | 0.43 | 0.15 | 0.65 | 0.21 | | | 2.14 | 2.16 | 1.39 | 0.45 | | |
| | N.F. (E) | 0.22 | 0.06 | | | 33.9 | 28.6 | | | | | | |
| | O.F. (W) | 1.21 | 0.36 | 1.60 | 0.47 | | | 1.04 | 1.39 | 1.66 | 0.65 | | |
| | O.F. (E) | 0.39 | 0.11 | | | 24.4 | 23.4 | | | | | | |
| | ST. (W) | 1.58 | 0.44 | 1.94 | 0.67 | | | 1.80 | 2.16 | 3.49 | 1.45 | | |
| | ST. (E) | 0.36 | 0.23 | | | 18.6 | 52.3 | | | | | | |
| | TOTAL: | | | 4.19 | 1.35 | 25.6 | 34.8 | | | 6.54 | 2.55 | 44 | 17 |
| P-2 | N.F. (W) | 1.10 | 0.20 | 2.05 | 0.62 | | | 1.97 | 2.38 | 4.04 | 1.48 | | |
| | N.F. (E) | 0.95 | 0.42 | | | 46.3 | 67.7 | | | | | | |
| | O.F. (W) | 0.76 | 0.19 | 1.64 | 0.58 | | | 1.70 | 1.95 | 2.79 | 1.13 | | |
| | O.F. (E) | 0.88 | 0.39 | | | 53.7 | 67.2 | | | | | | |
| | ST. (W) | 0.94 | 0.58 | 1.18 | 0.83 | | | 1.55 | 1.77 | 1.83 | 1.47 | | |
| | ST. (E) | 0.24 | 0.25 | | | 20.3 | 30.1 | | | | | | |
| | TOTAL: | | | 4.87 | 2.03 | 40.1 | 55.0 | | | 8.66 | 4.08 | 58 | 27 |
| S-1 | N.F. (W) | 0.50 | 0.09 | 0.84 | 0.39 | | | | | | | | |
| | N.F. (E) | 0.34 | 0.30 | | | 40.5 | 76.9 | | | | | | |
| | O.F. (W) | 2.18 | 0.39 | 2.40 | 0.54 | | | | | | | | |
| | O.F. (E) | 0.22 | 0.15 | | | 9.7 | 27.8 | | | | | | |
| | ST. (W) | 0.58 | 0.41 | 0.67 | 0.55 | | | | | | | | |
| | ST. (E) | 0.09 | 0.14 | | | 13.4 | 25.5 | | | | | | |
| | TOTAL: | | | 3.91 | 1.48 | 21.2 | 43.4 | | | | | 65 | 25 |
| S-2 | N.F. (W) | 1.04 | 0.42 | 1.62 | 1.47 | | | | | | | | |
| | N.F. (E) | 0.58 | 1.05 | | | 35.8 | 71.4 | | | | | | |
| | O.F. (W) | 0.96 | 0.22 | 1.32 | 0.53 | | | | | | | | |
| | O.F. (E) | 0.36 | 0.31 | | | 27.3 | 58.8 | | | | | | |
| | ST. (W) | 0.89 | 0.78 | 1.19 | 1.04 | | | | | | | | |
| | ST. (E) | 0.30 | 0.26 | | | 25.2 | 25.0 | | | | | | |
| | TOTAL: | | | 4.13 | 3.04 | 29.4 | 51.7 | | | | | 69 | 51 |

P - PAINTED
S - SPRAYED
1 - WHITE SPRUCE; 20 ppm
2 - BALSAM FIR; 20 ppm

(W) - SURFACE WASH
(E) - EXTRACT
N.F. - NEW FOLIAGE
O.F. - OLD FOLIAGE
ST. - STEM TISSUE

* Standard deviation of replicate sample injections was less than 10%.

TABLE 1 (ii)

G.C. Analysis of Fenitrothion (FT) Treated Conifer Tissue* (200μgm)

| SAMPLE | | PPM | | NET | PIM | % ABS (average) | | TISSUE WT. (gm) | | FT (μg) | | % RECOV. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DAY | | 1 | 7 | 1 | 7 | 1 | 7 | 1 | 7 | 1 | 7 | 1 | 7 |
| P-3 | N.F. (W) | 1.08 | 0.08 | 1.97 | 0.59 | | | 1.98 | 2.31 | 3.90 | 1.36 | | |
| | N.F. (E) | 0.89 | 0.51 | | | 45.2 | 86.4 | | | | | | |
| | O.F. (W) | 2.91 | 1.75 | 3.70 | 2.87 | | | 2.22 | 1.53 | 8.21 | 4.39 | | |
| | O.F. (E) | 0.79 | 1.12 | | | 21.4 | 39.0 | | | | | | |
| | ST. (W) | 1.59 | 1.33 | 2.10 | 1.89 | | | 1.41 | 1.54 | 2.96 | 2.91 | | |
| | ST. (E) | 0.51 | 0.56 | | | 24.3 | 29.6 | | | | | | |
| | TOTAL: | | | 7.77 | 5.35 | 30.3 | 51.7 | | | 15.1 | 8.66 | 24 | 14 |
| P-4 | N.F. (W) | 1.88 | 0.41 | 2.60 | 1.26 | | | 1.76 | 1.40 | 4.58 | 1.76 | | |
| | N.F. (E) | 0.72 | 0.85 | | | 27.7 | 67.5 | | | | | | |
| | O.F. (W) | 1.65 | 0.51 | 2.19 | 1.08 | | | 1.96 | 1.69 | 4.29 | 1.83 | | |
| | O.F. (E) | 0.54 | 0.57 | | | 24.7 | 52.8 | | | | | | |
| | ST. (W) | 2.81 | 1.63 | 3.20 | 2.27 | | | 1.79 | 1.70 | 5.73 | 3.86 | | |
| | ST. (E) | 0.39 | 0.64 | | | 12.2 | 28.2 | | | | | | |
| | TOTAL: | | | 7.99 | 4.61 | 21.5 | 49.5 | | | 14.6 | 7.45 | 23 | 12 |
| S-3 | N.F. (W) | 1.55 | 0.22 | 2.30 | 0.50 | | | | | | | | |
| | N.F. (E) | 0.75 | 0.28 | | | 32.6 | 56.0 | | | | | | |
| | O.F. (W) | 1.80 | 0.39 | 2.07 | 0.54 | | | | | | | | |
| | O.F. (E) | 0.27 | 0.15 | | | 13.0 | 27.8 | | | | | | |
| | ST. (W) | 0.79 | 0.54 | 1.07 | 0.84 | | | | | | | | |
| | ST. (E) | 0.28 | 0.30 | | | 26.2 | 35.7 | | | | | | |
| | TOTAL: | | | 5.44 | 1.88 | 23.9 | 39.8 | | | | | 11 | 4 |
| S-4 | N.F. (W) | 4.92 | 0.85 | 6.21 | 2.84 | | | | | | | | |
| | N.F. (E) | 1.29 | 1.99 | | | 20.8 | 70.1 | | | | | | |
| | O.F. (W) | 4.09 | 1.01 | 4.99 | 2.10 | | | | | | | | |
| | O.F. (E) | 0.90 | 1.09 | | | 18.0 | 51.9 | | | | | | |
| | ST. (W) | 4.08 | 1.93 | 6.12 | 2.90 | | | | | | | | |
| | ST. (E) | 2.04 | 0.97 | | | 33.3 | 33.5 | | | | | | |
| | TOTAL: | | | 17.3 | 7.84 | 24.0 | 51.8 | | | | | 34 | 16 |

P - PAINTED
S - SPRAYED

3 - SPRUCE; 200 ppm
4 - FIR; 200 ppm

(W) - SURFACE WASH
(E) - EXTRACT
N.F. - NEW FOLIAGE
O.F. - OLD FOLIAGE
ST. - STEM TISSUE

* Standard deviation of replicate sample injections was less than 10%

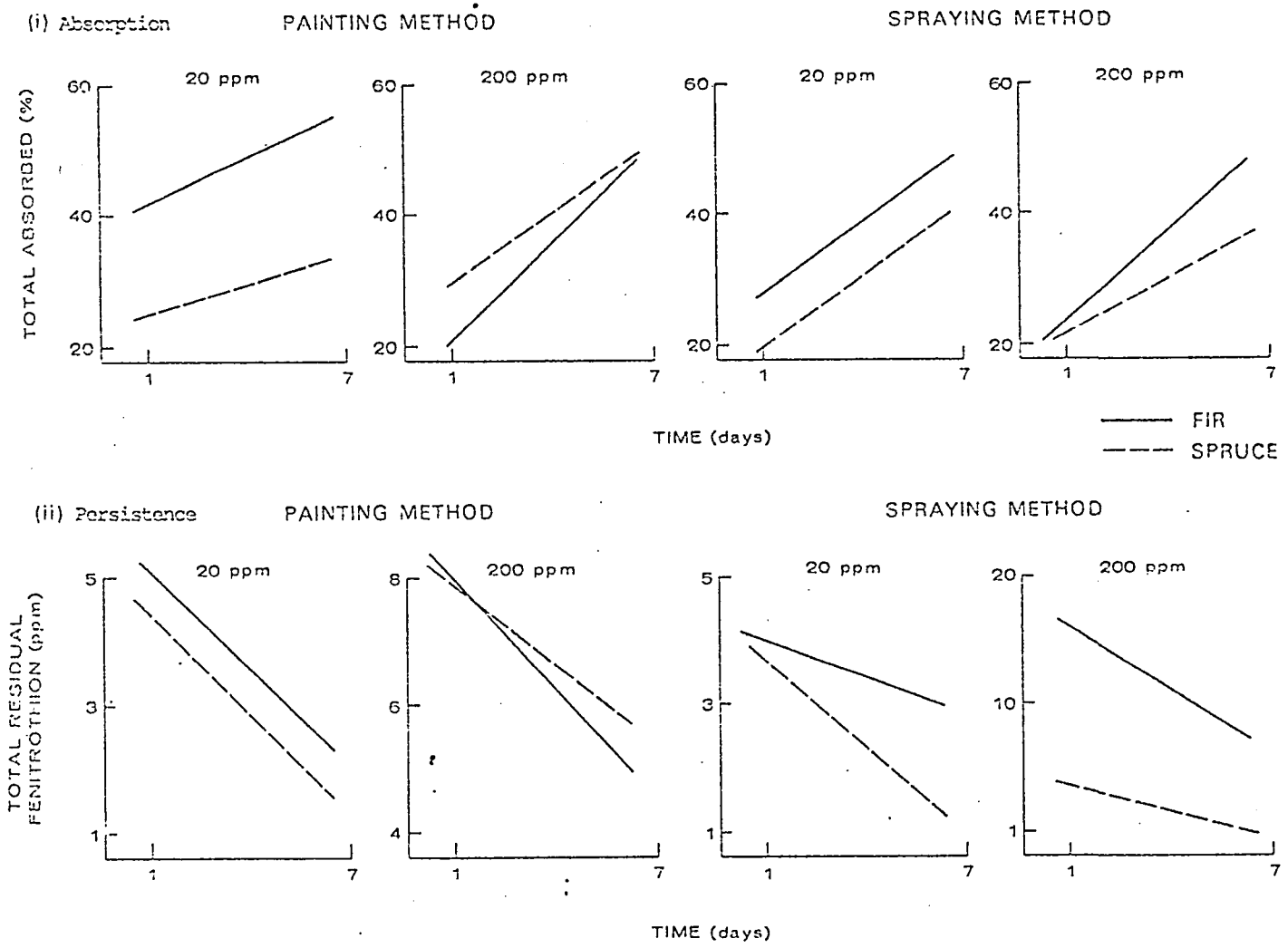



FIG. 1. Absorption and Persistence of Fenitrothion in Conifers (Samples taken after 1 and 7 days)

TABLE 2

Rf Values for Thin Layer Chromatography of Fenitrothion and its Metabolites from the Glass Surface Experiment.

| | R F | R R F |
|---|---|---|
| Fenitrothion | 0.52 | 1 |
| Fenitro-oxon | 0.09 | 0.17 |
| S-methyl Fenitrothion | 0.15 | 0.29 |
| Light 1 day | 0.52; 0.09 | 1; 0.17 (3) [1] |
| Light 3 day | 0.52; 0.09 | 1; 0.17 (2) |
| Light 6 day | 0.52; 0.09 | 1; 0.17 (1) |
| Light 11 day | 0.52 | 1 |
| Dark 1 day | 0.52; 0.15 | 1; 0.29 (1) |
| Dark 3 day | 0.52; 0.15 | 1; 0.29 (2) |
| Dark 6 day | 0.52; 0.15 | 1; 0.29 (3) |
| Dark 11 day | 0.52; 0.15 | 1; 0.29 (3) |

[1] The numbers in brackets give an estimate of the relative quantity of the metabolite present in each sample based on spot diameter and degree of enzyme inhibition. The maximum quantity of either metabolite present never exceeded 0.1% of the original fenitrothion applicant.

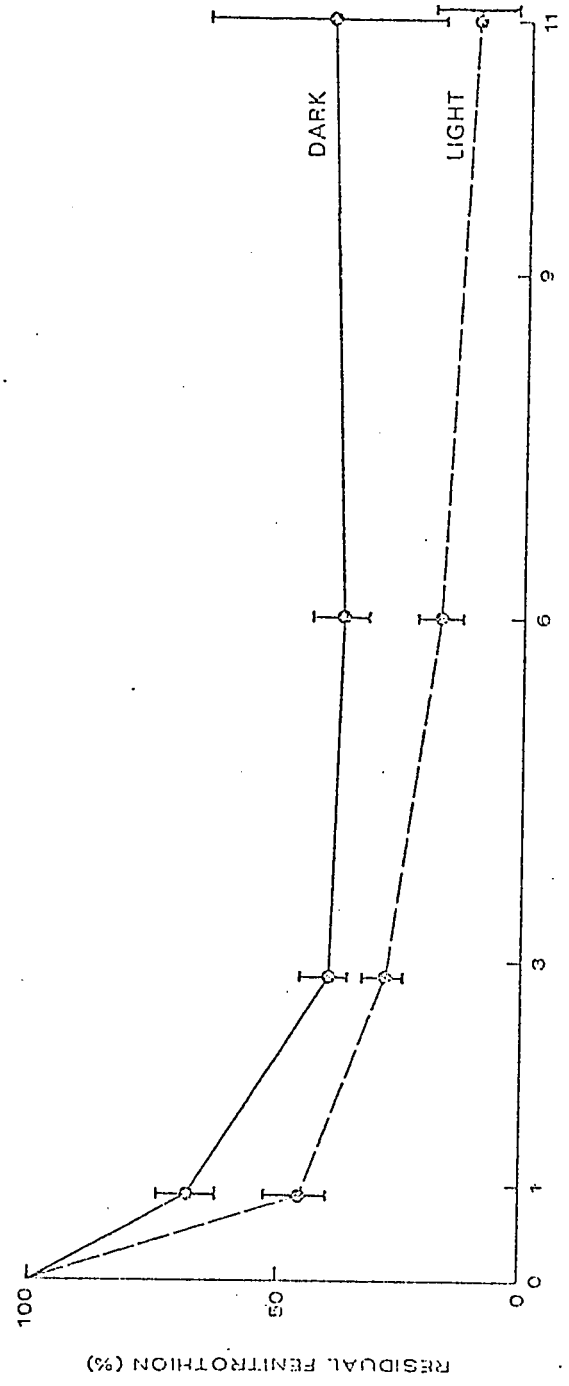



FIG. 2. Dissipation of Fenitrothion from Glass Surfaces under Conditions of Light and Dark in the greenhouse.

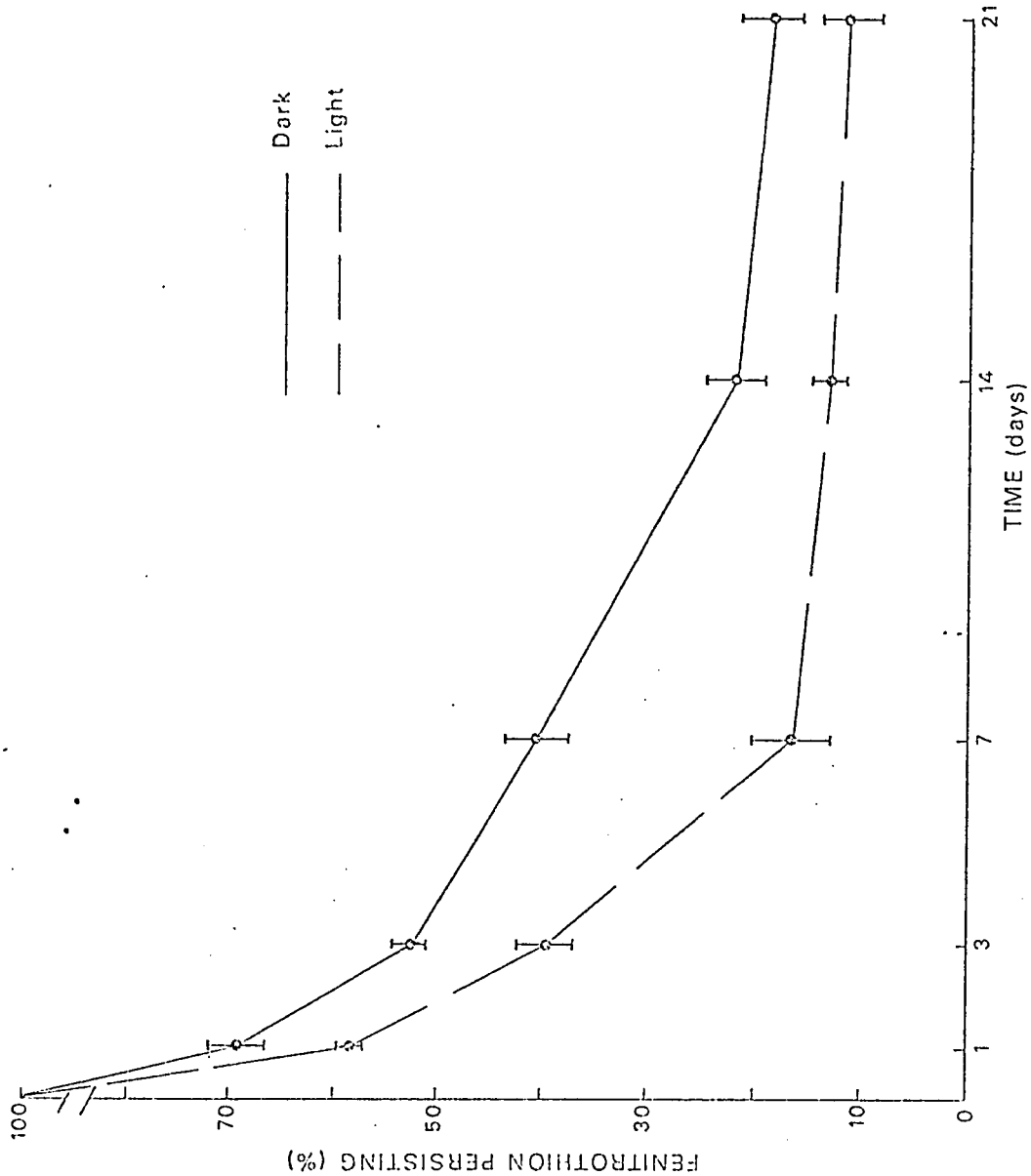



Fig. 3 Persistence of $C^{14}$-Fenitrothion on Glass Surfaces.

Table 3

LSC and TLC Analysis of $^{14}C$-Fenitrothion Treated Conifer Tissues

| Sample | | Time (Day) 1* ppm | 1* % ABS | 3** ppm | 3** % ABS | 7 ppm | 7 % ABS | 21 ppm | 21 % ABS |
|---|---|---|---|---|---|---|---|---|---|
| Fir | N.F. (W)[1] | 1.42 | | 0.38 | | 0.31 | | 0.21 | |
| | N.F. (E) | 1.17 | 45.2 | 0.99(A)*** | 72.3 | 2.03(A) | 86.8 | 1.55(A) | 88.1 |
| | O.F. (W) | 2.10 | | 2.22 | | 0.55 | | 0.25 | |
| | O.F. (E) | 0.44 | 17.3 | 0.82 | 27.0 | 1.37 | 71.4 | 0.67 | 72.8 |
| | ST. (W) | 2.82 | | 1.40 | | 1.24 | | 0.95 | |
| | ST. (E) | 0.77 | 21.5 | 0.83 | 37.2 | 0.75 | 37.7 | 0.95 | 52.8 |
| | Average | 2.91 | 28.0 | 2.21 | 45.5 | 2.08 | 65.3 | 1.49 | 71.2 |
| Spruce | N.F. (W) | 1.16 | | 0.48 | | 0.24 | | 0.05 | |
| | N.F. (E) | 0.25 | 17.7 | 0.63(A) | 56.8 | 0.47(A) | 66.2 | 0.32(A) | 86.5 |
| | O.F. (W) | 3.06 | | 1.70 | | 0.40 | | 0.22 | |
| | O.F. (E) | 0.52 | 14.5 | 0.66 | 28.0 | 0.86 | 68.3 | 0.37 | 62.7 |
| | ST. (W) | 1.47 | | 1.15 | | 0.78 | | 0.52 | |
| | ST. (E) | 0.22 | 13.0 | 0.72 | 38.5 | 0.88 | 53.0 | 0.86 | 62.3 |
| | Average | 2.23 | 15.1 | 1.78 | 41.1 | 1.21 | 62.5 | 0.78 | 70.5 |
| 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 $^{14}C$-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. (levels of this metabolite were less than 0.1% of the residual $^{14}C$-activity).

*** A non-toxic, $^{14}C$-metabolite (2-5% of the residual $^{14}C$-activity) was detected by TLC in extracts denoted by (A) which had an Rf of 0.21.

1 Refer to Table 1 for legend. Standard deviation of replicate sample counts was less than 5.0%.

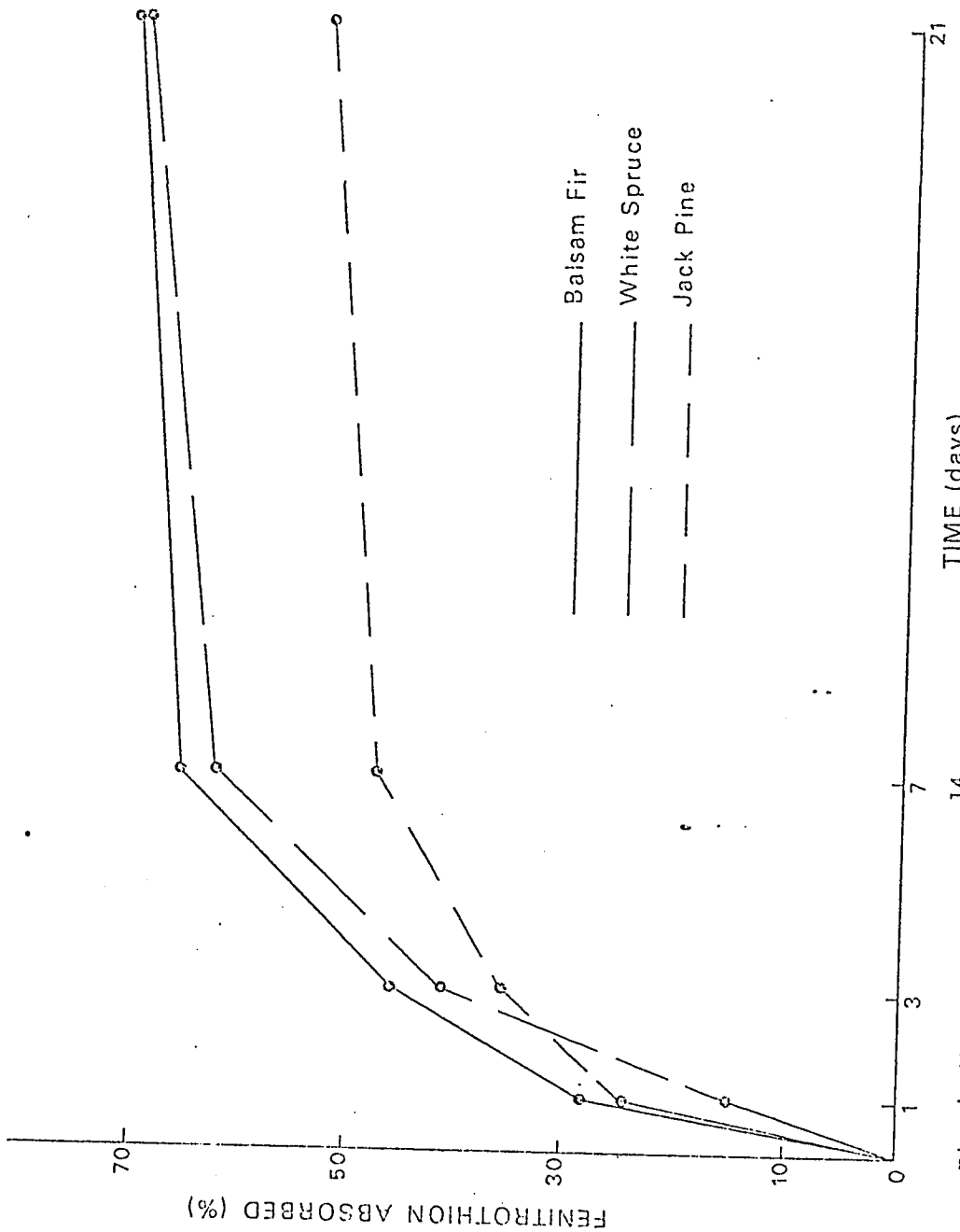



Fig. 4 (i) Absorption of $^{14}$C-fenitrothion by Fir, Spruce and Pine.

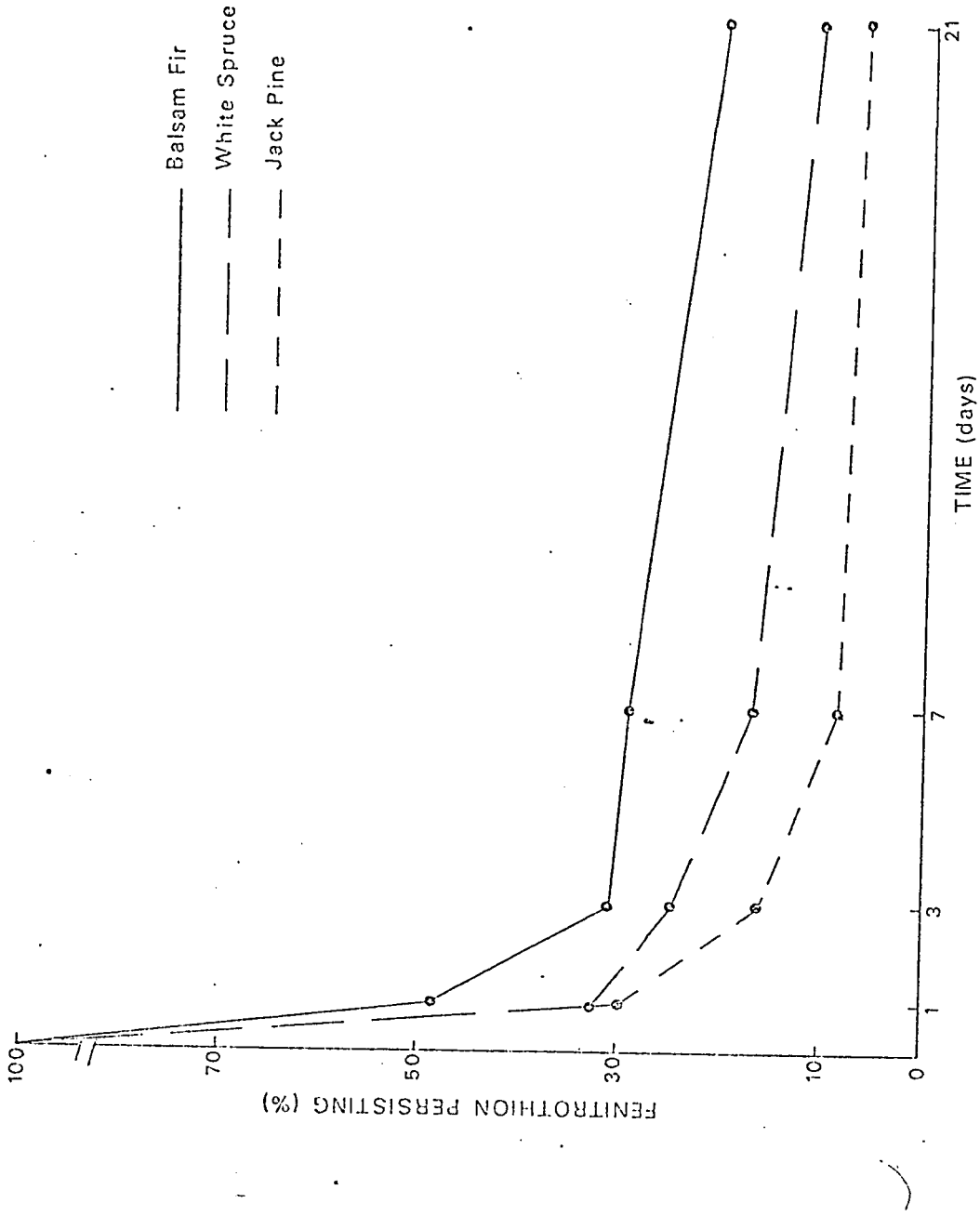



Fig. 4 (ii) Persistence of $C^{14}$-fenitrothion on Fir, Spruce and Pine.


Fig. 5 (i) Above left, pressed tissue, and above right, autoradiograph of balsam fir sampled 7 days after treatment of young foliage with $^{14}C$-fenitrothion. Note evidence of acropetal translocation to the young foliage as well as some basipetal movement. Arrow denotes attachment site of treated tissue (TR).


Fig. 5 (ii) Above left, pressed tissue, and above right autoradiograph of balsam fir sampled 7 days after treatment of old foliage with $^{14}C$-fenitrothion. Note evidence of acropetal translocation to young foliage. Arrow denotes attachment site of treated tissue (TR).



Fig. 5 (iii) Above left, pressed tissue, and above right autoradiograph of balsam fir sampled 7 days after treatment of stems with $^{14}C$-fenitrothion. Note evidence of acropetal translocation to the young foliage. Arrow denotes attachment site of treated tissue (TR).

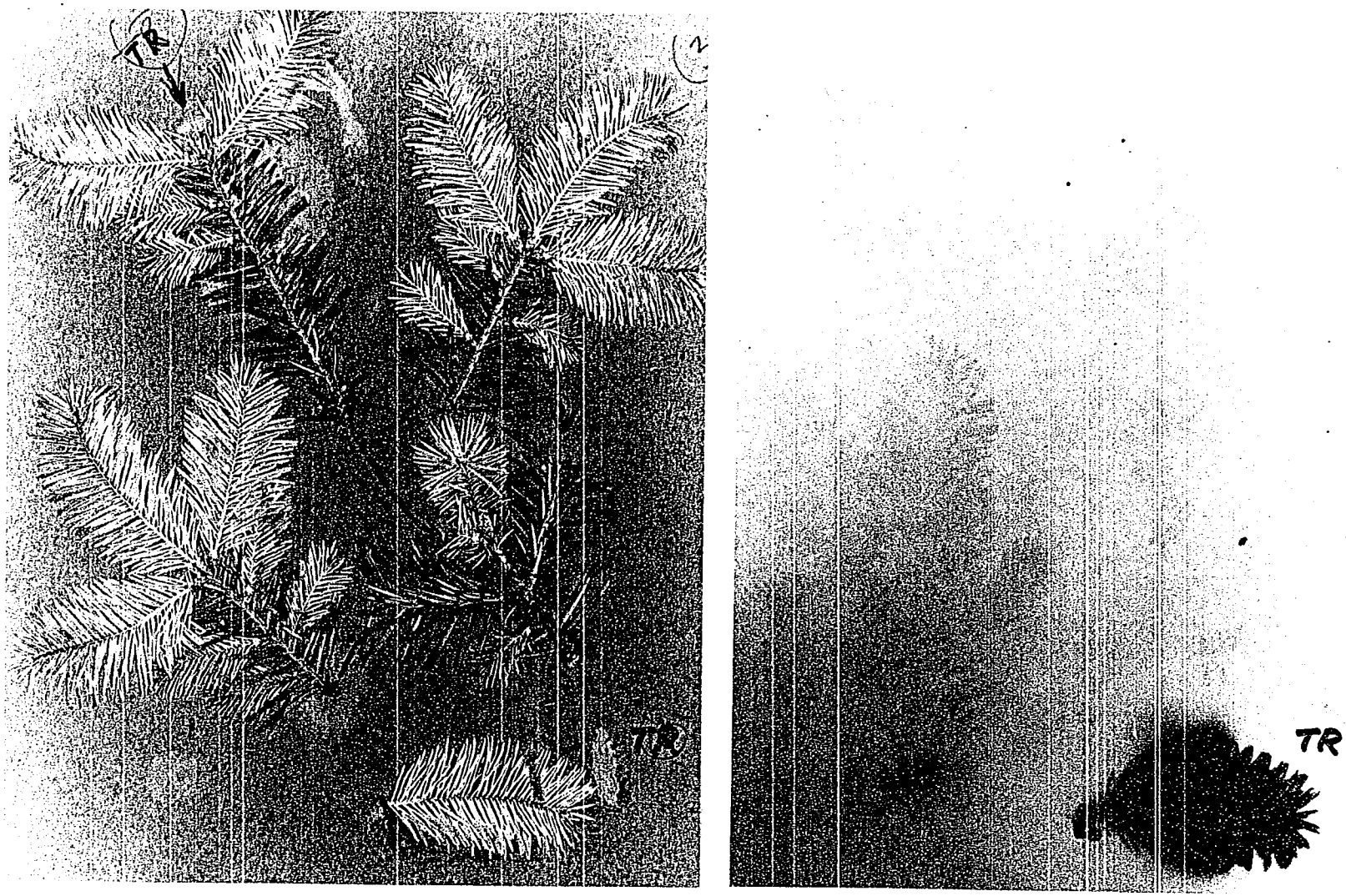



Fig. 5 (iv) Above left, pressed tissue, and above right autoradiograph of balsam fir sampled 21 days after treatment of young foliage with $^{14}C$-fenitrothion. Note evidence of basipetal translocation, with subsequent acopetal translocation and persistence in the young foliage. Arrow denotes attachment site of treated tissue (TR).


Fig. 5 (v) Above left, pressed tissue, and above right autoradiograph of balsam fir sampled 21 days after treatment of old foliage with $^{14}C$-fenitrothion. Note evidence of acropetal translocation to the young foliage. Arrow denotes attachment site of treated tissue (TR).

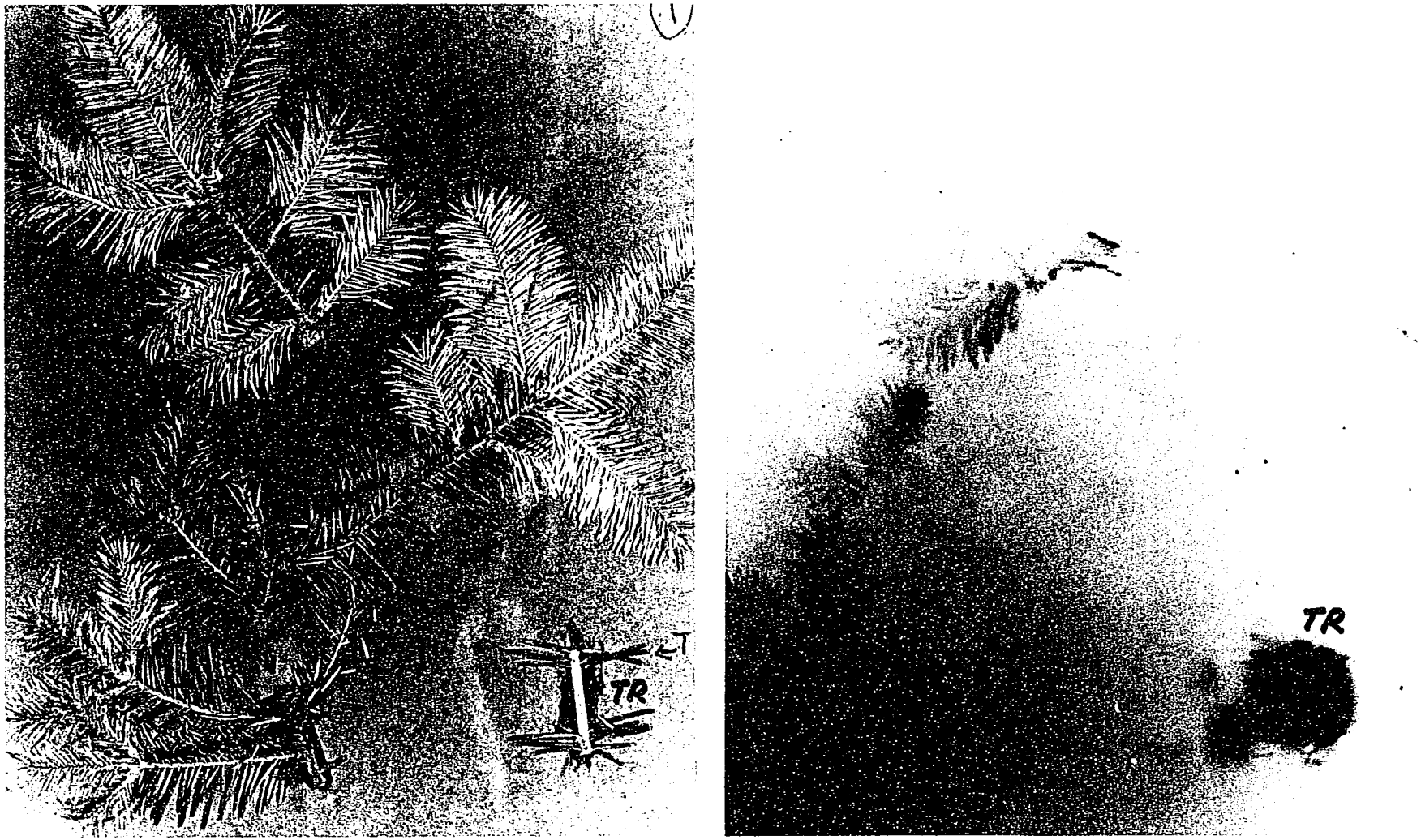



Fig. 5 (vi) Above left, pressed tissue, and above right autoradiograph of balsam fir sampled 21 days after treatment of stem with $^{14}C$-fenitrothion. Note evidence of acropetal translocation to the young foliage. Arrow denotes attachment site of treated tissue (TR).

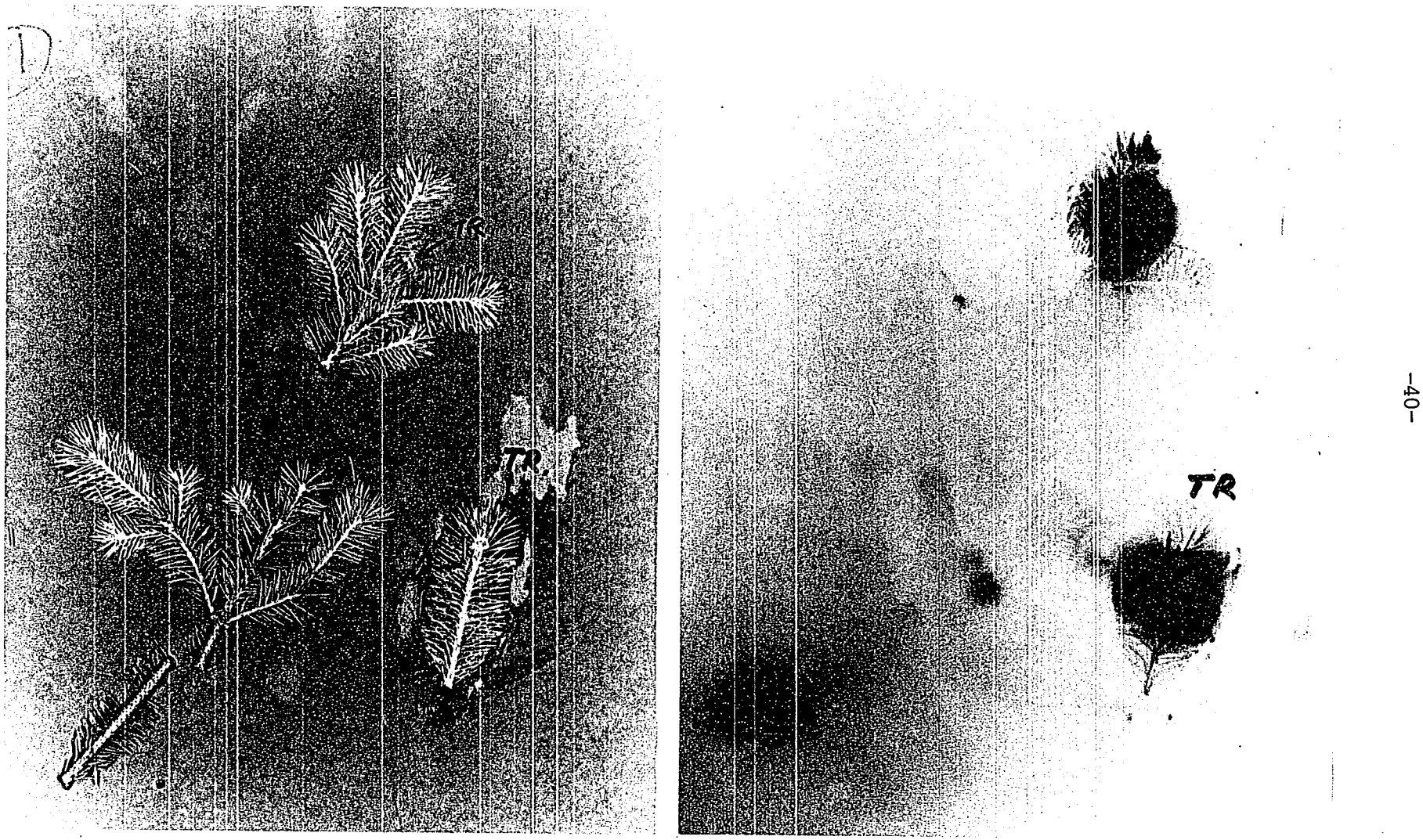



Fig. 5 (vii) Above left, pressed tissue, and above right autoradiograph of white spruce sampled 7 days after treatment of young foliage with $^{14}C$-fenitrothion. Note absence of any major translocation. Arrow denotes attachment site of treated tissue (TR).


Fig. 5 (viii) Above left, pressed tissue, and above right autoradiograph of white spruce sampled 7 days after treatment of old foliage with $^{14}C$-fenitrothion. Note evidence of only minor acropetal translocation to the young foliage. Arrow denotes attachment site of treated tissue (TR).

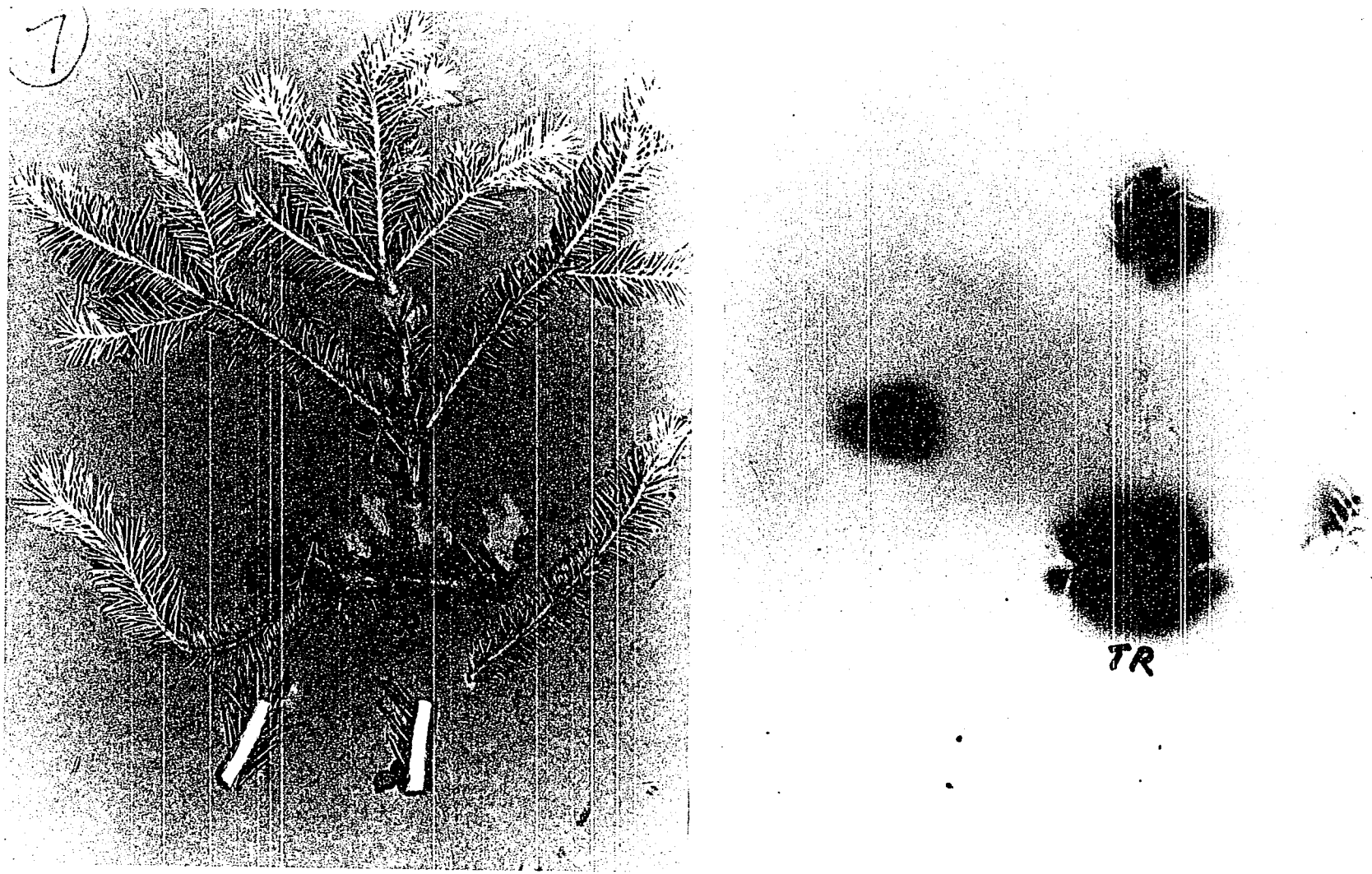



Fig. 5 (ix) Above left, pressed tissue, and above right autoradiograph of white spruce sampled 7 days after treatment of stem tissue with $^{14}C$-fenitrothion. Note absence of any major translocation. Arrow denotes attachment site of treated tissue (TR).

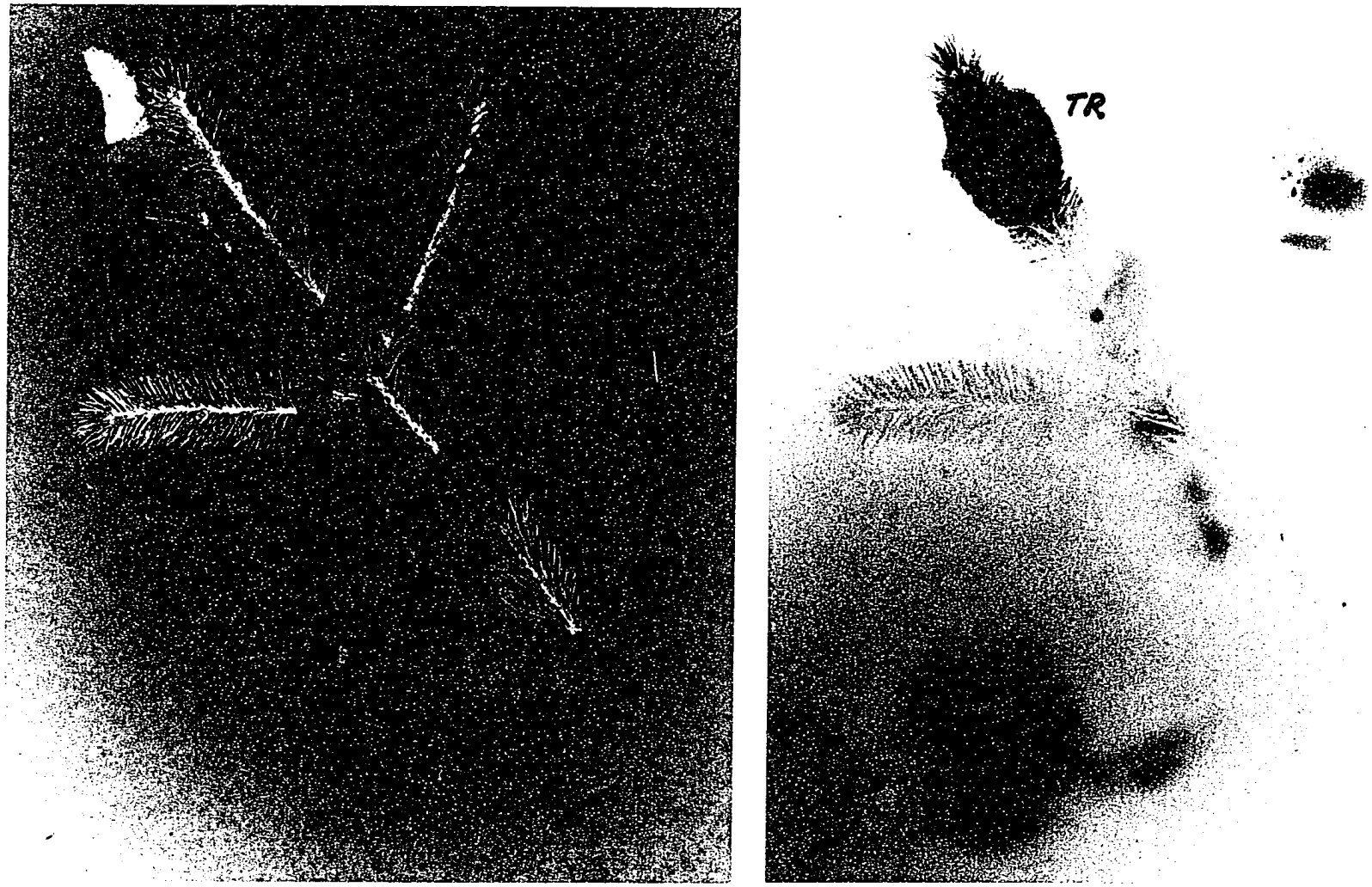
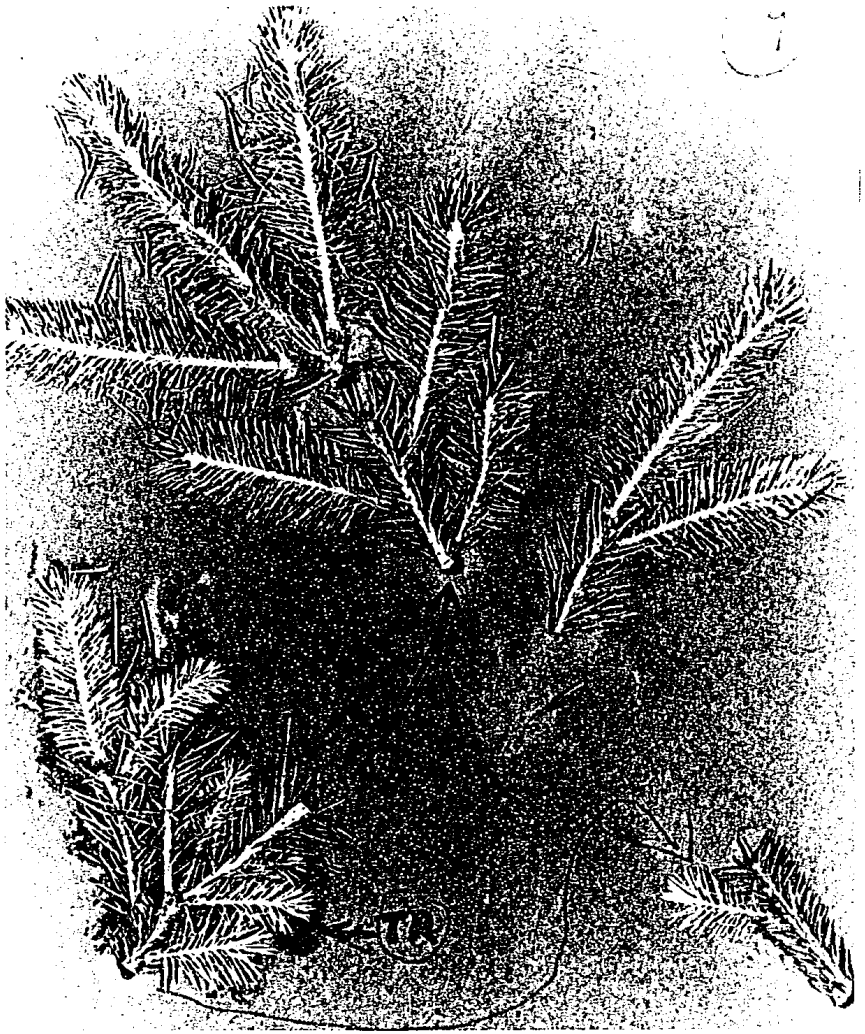



Fig. 5 (x) Above left, pressed tissue, and above right autoradiograph of white spruce sampled 21 days after treatment with $^{14}C$-fenitrothion. Note evidence of basipetal translocation. Arrow denotes attachment site of treated tissue (TR).



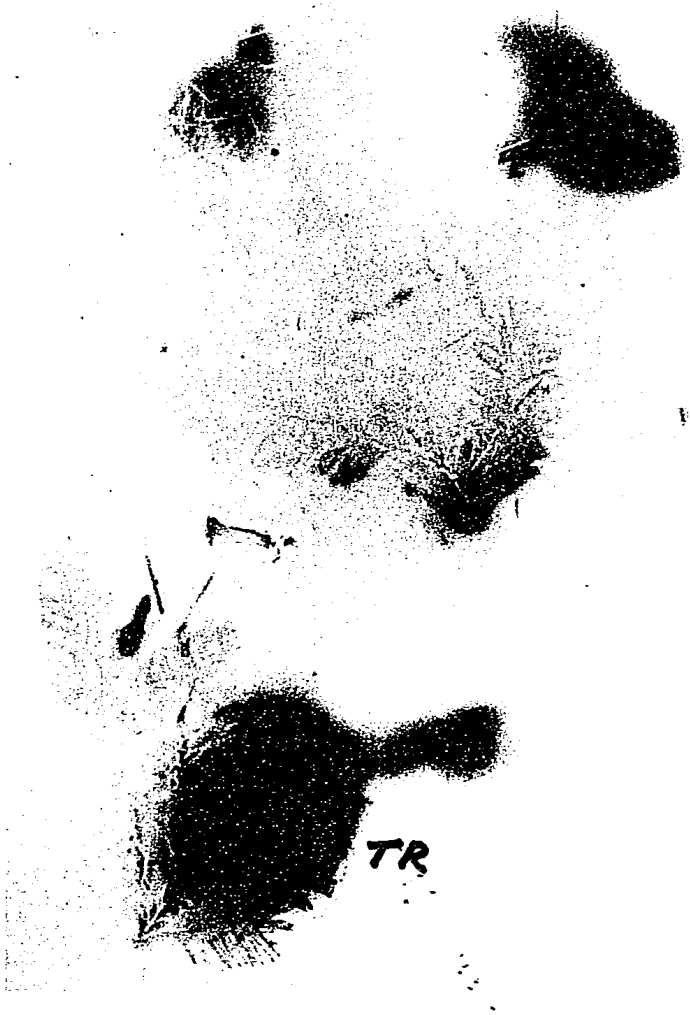




Fig. 5 (xi) Above left, pressed tissue and above right autoradiograph of white spruce sampled 21 days after treatment of old foliage with $^{14}C$-fenitrothion. Note evidence of some acropetal translocation. Arrow denotes attachment site of treated tissue (TR).

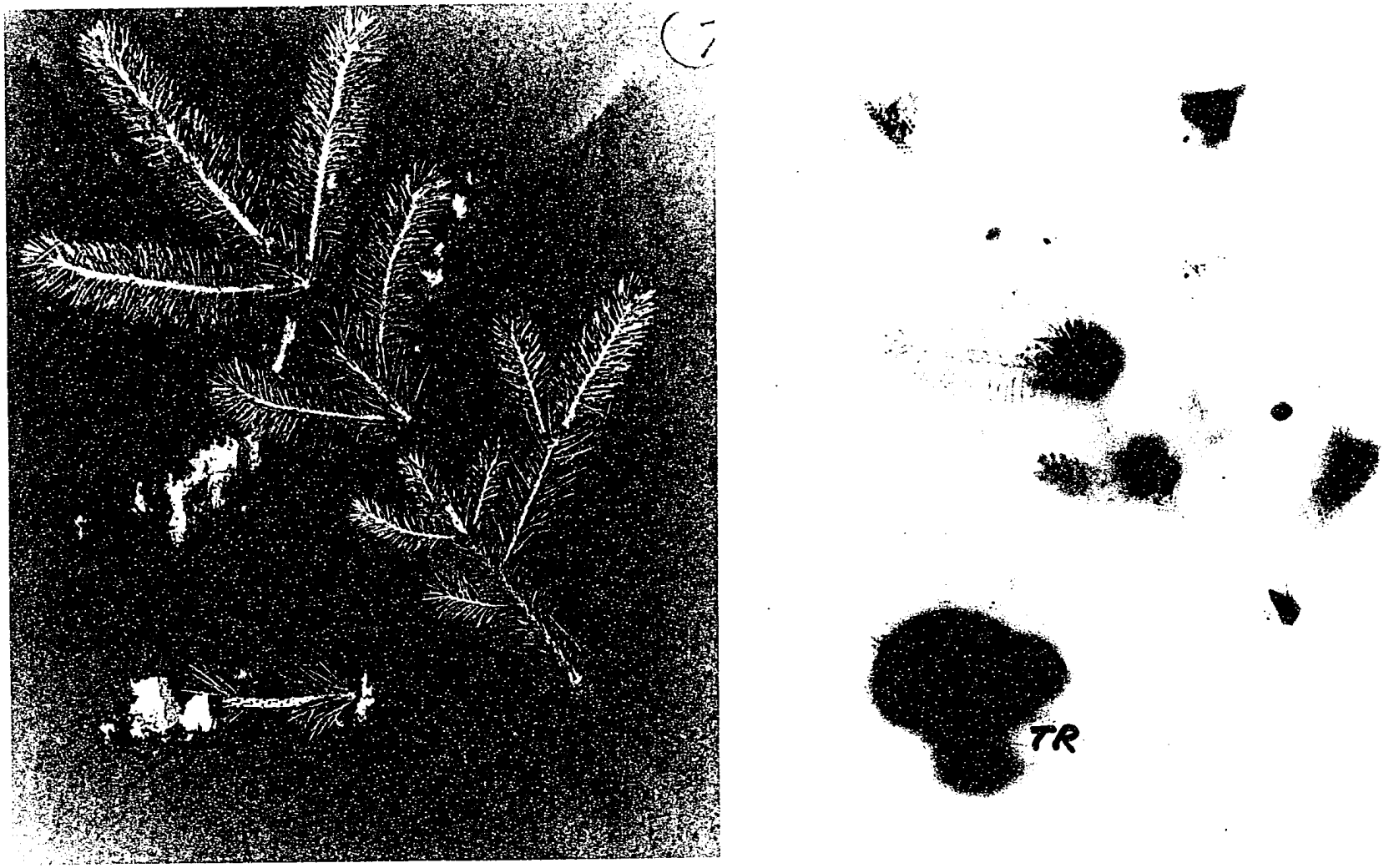



Fig. 5 (xii) Above left, pressed tissue, and above right autoradiograph of white spruce sampled 21 days after treatment of stem with $^{14}C$-fenitrothion. Note evidence of some acropetal translocation. Arrow denotes attachment site of treated tissue (TR).

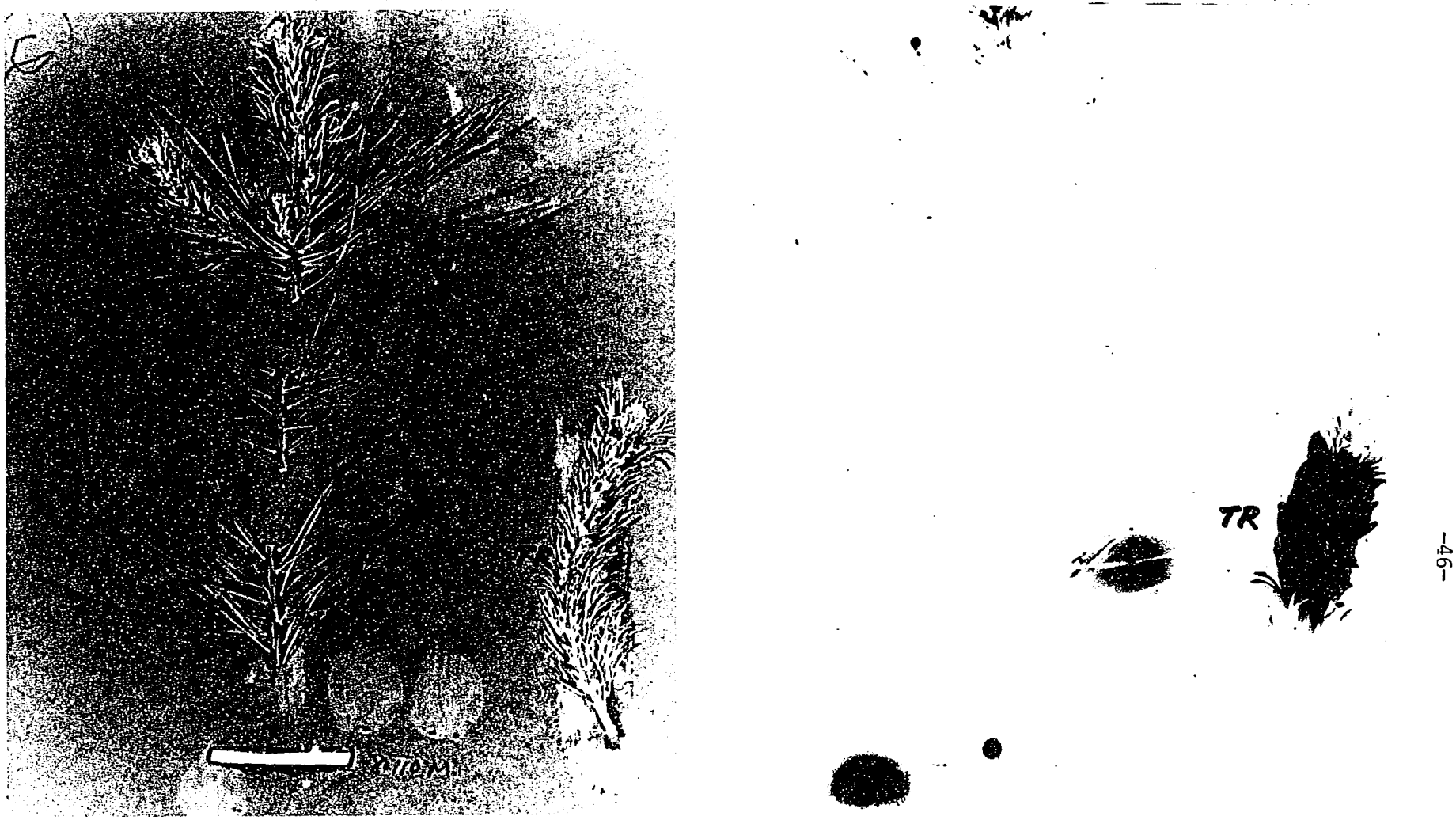



Fig. 5 (xiii) Above left, pressed tissue, and above right autoradiograph of Jack pine sampled 7 days after treatment of young foliage with $^{14}C$-fenitrothion. Note absence of any major translocation. Arrow denotes attachment site of treated tissue (TR).

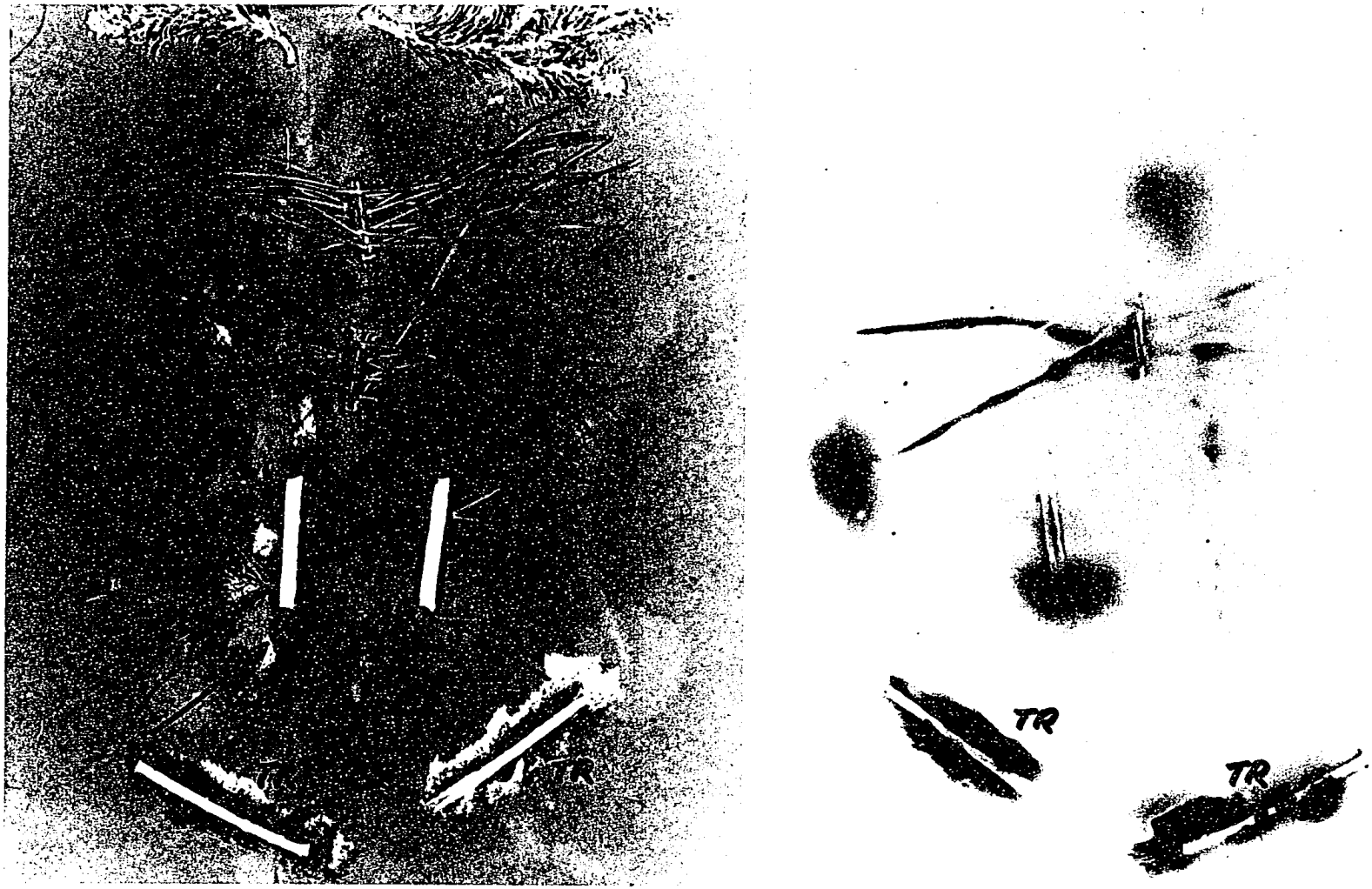



Fig. 5 (xiv) Above left, pressed tissue, and above right autoradiograph of Jack pine sampled 7 days after treatment of the old foliage with $^{14}C$-fenitrothion. Note absence of any major translocation. Arrow denotes attachment site of treated tissue (TR).

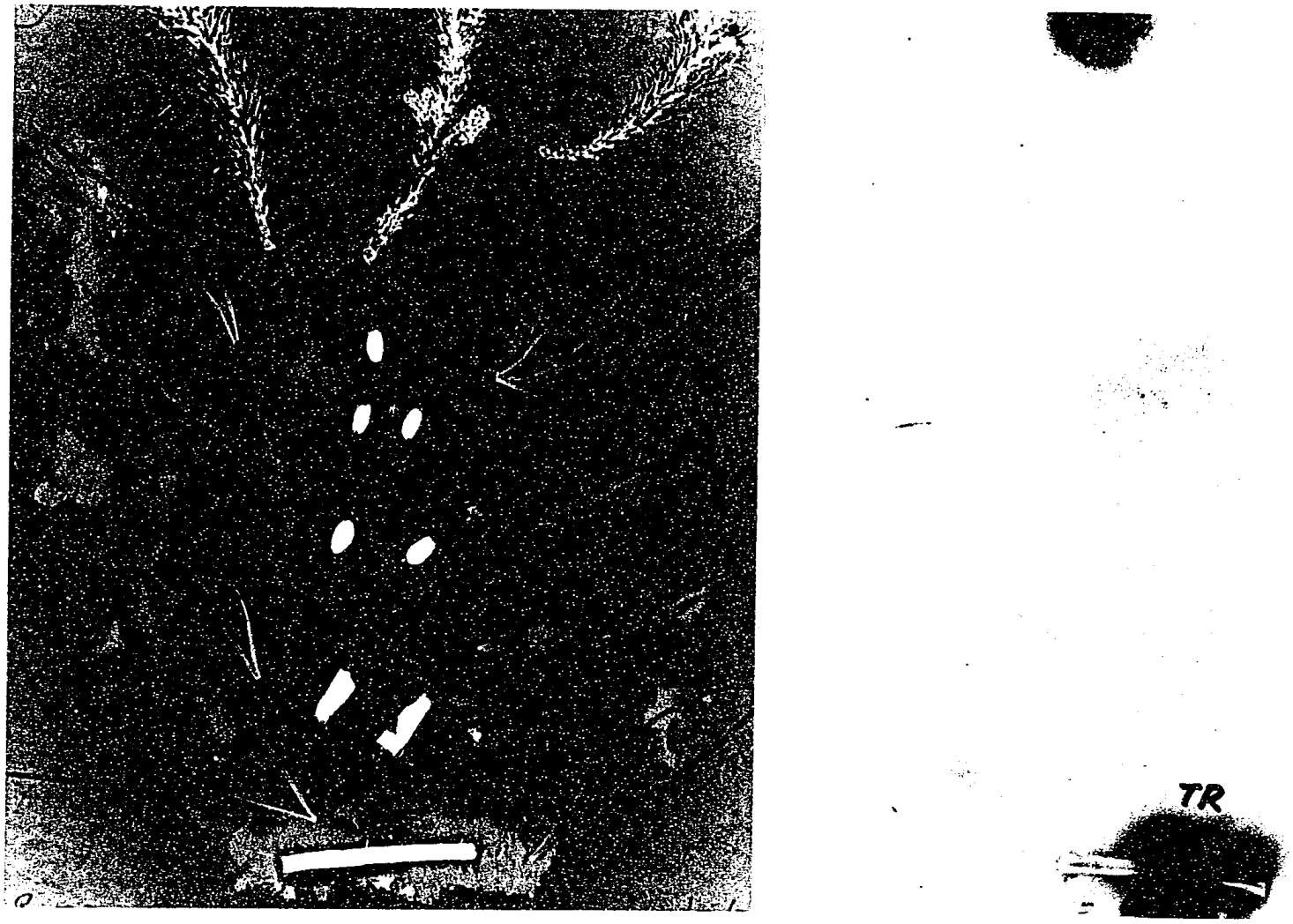



Fig. 5 (xv) Above left, pressed tissue, and above right autoradiograph of Jack pine sampled 7 days after treatment of stem with $^{14}C$-fenitrothion. Note absence of any major translocation. Arrow denotes attachment site of treated tissue (TR).



Fig. 5 (xvi) Above left, pressed tissue, and above right autoradiograph of Jack pine sampled 21 days after treatment of young foliage with $^{14}C$-fenitrothion. Note absence of any major translocation. Arrow denotes attachment site of treated tissue (TR).


Fig. 5 (xvii) Above left, pressed tissue and above right autoradiograph of Jack pine sampled 21 days after treatment of old foliage with $^{14}C$-fenitrothion. Note absence of any major translocation. Arrow denotes attachment site of treated tissue (TR).


Fig. 5 (xviii) Above left, pressed tissue, and above right autoradiograph of Jack pine sampled 21 days after treatment of stem with $^{14}C$-fenitrothion. Note absence of any major translocation. Arrow denotes attachment site of treated tissue (TR).

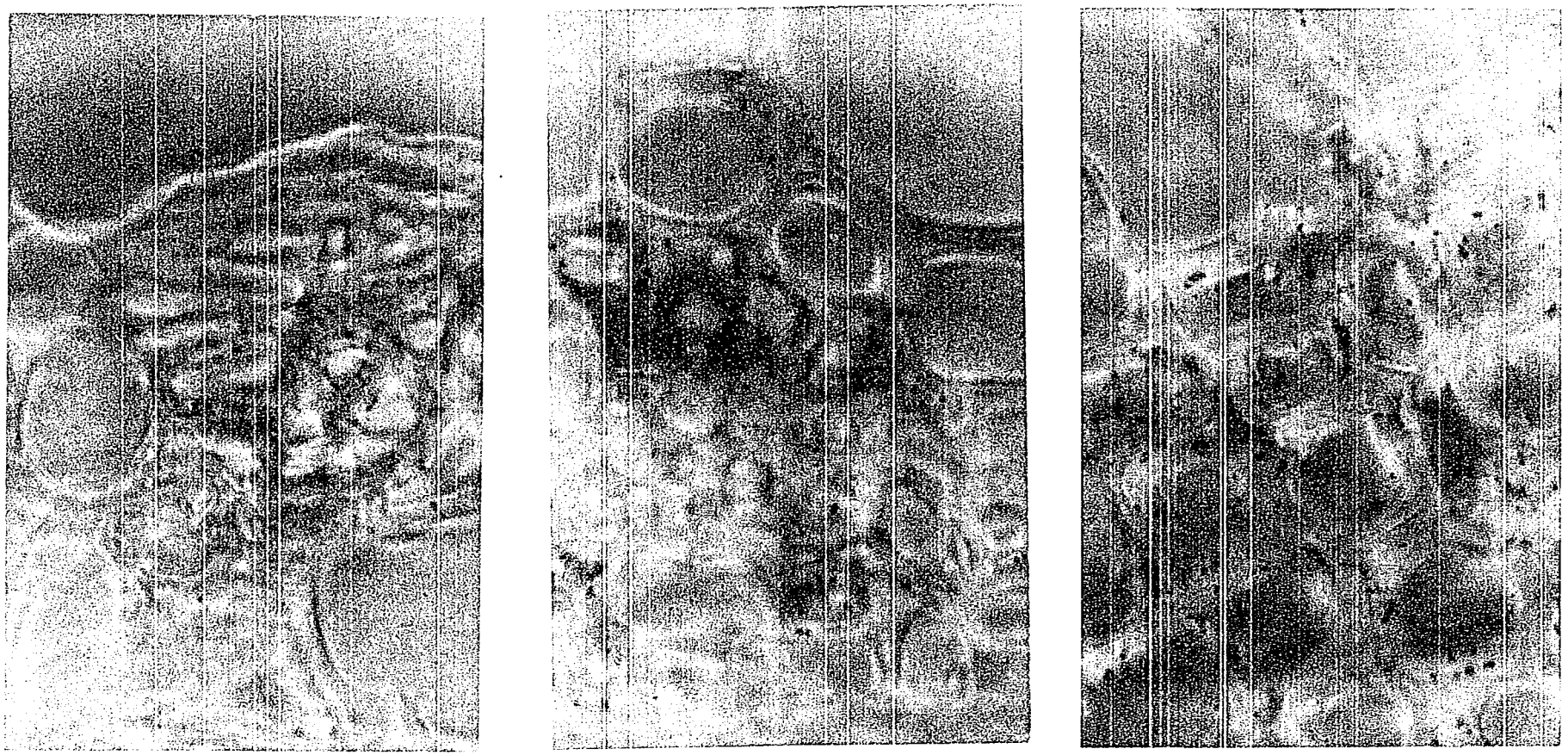


Fig. 6 Translocation of $^{14}C$-fenitrothion in balsam fir (histoautoradiography)

(i) Cross section of balsam fir young foliage showing $^{14}C$ activity in vascular system (x 1,000)

(ii) Cross section of balsam fir old foliage showing $^{14}C$ activity in vascular system. (x 1,000)

(iii) Cross section of balsam fir stem showing $^{14}C$ activity in xylem. (x 1,000)

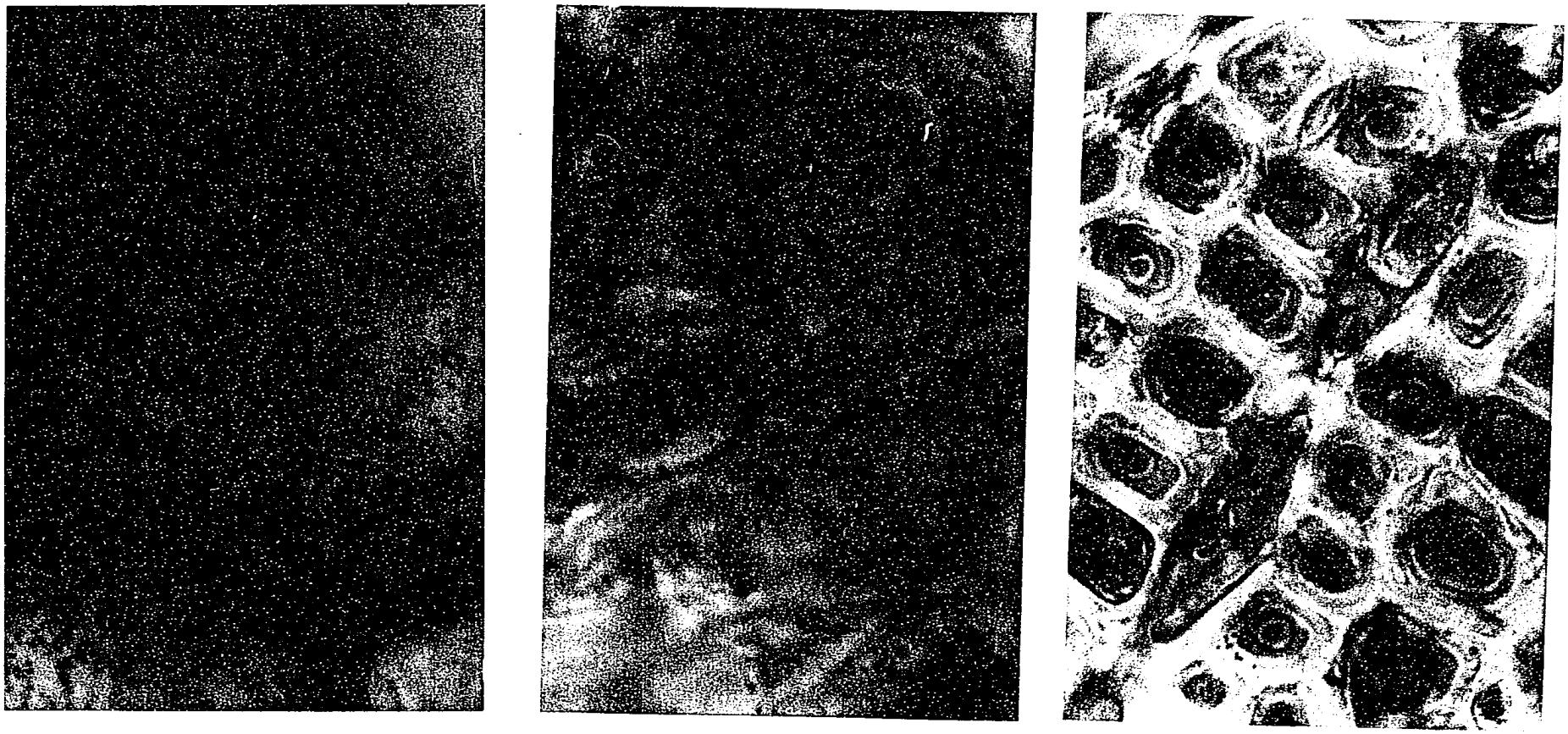


Fig. 6 Translocation of $^{14}C$-fenitrothion in white spruce (histoautoradiography)

(iv) Cross section of white spruce young foliage showing $^{14}C$ activity in mesophyll. (x 1,000)

(v) Cross section of white spruce old foliage showing $^{14}C$ activity in vascular system. (x 1,000)

(vi) Cross section of white spruce stem showing $^{14}C$ activity in xylem. (x 1,000)

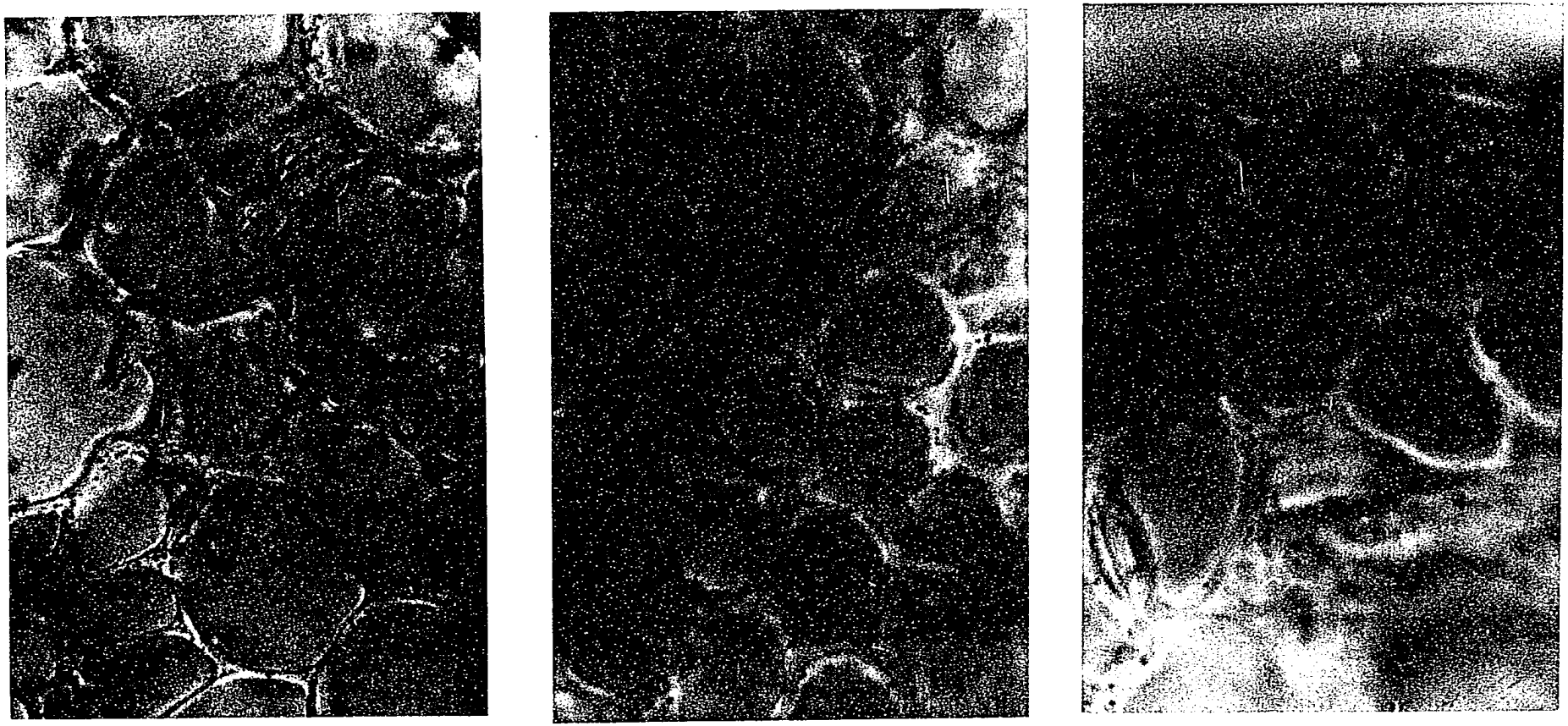


Fig. 6 Translocation of $^{14}C$ - fenitrothion in Jack pine (histoautoradiography)

(vii) Cross section of Jack pine young foliage showing $^{14}C$ activity in vascular system (x 1,000)

(viii) Cross section of Jack pine old foliage showing $^{14}C$ activity in vascular system. (x 1,000)

(ix) Cross section of Jack pine stem showing $^{14}C$ activity in outer xylem. (x 1,000)

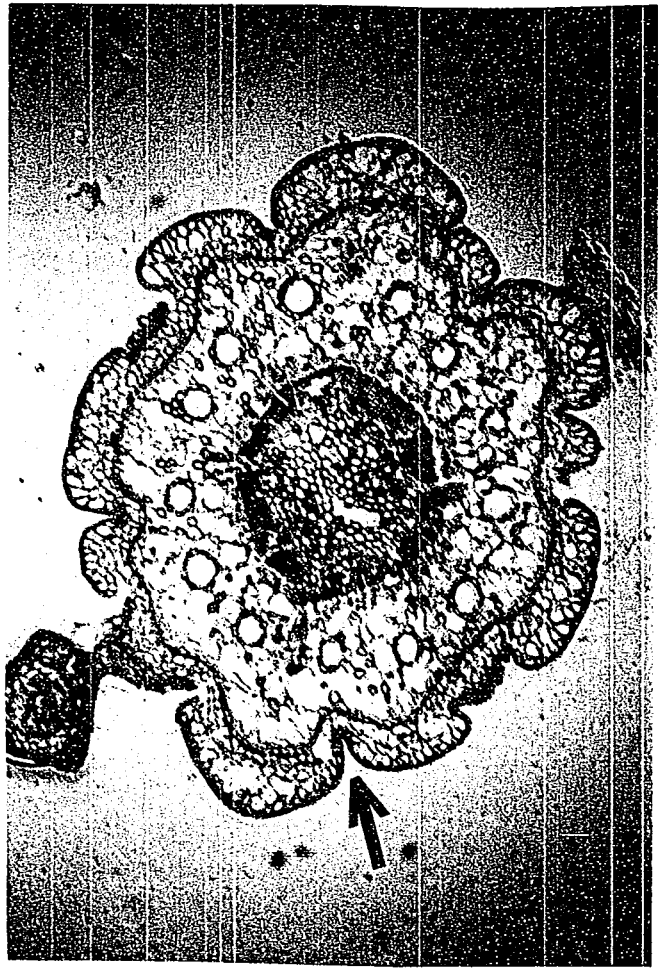


Fig. 6 (x) A cross section through the rachis of spruce. Note the arrangement of resin ducts around the secondary vascular tissues. The outermost layer is the fascicular tissue and arrow denotes the "groove" region enlarged in Fig. 6 x(v), (x40).

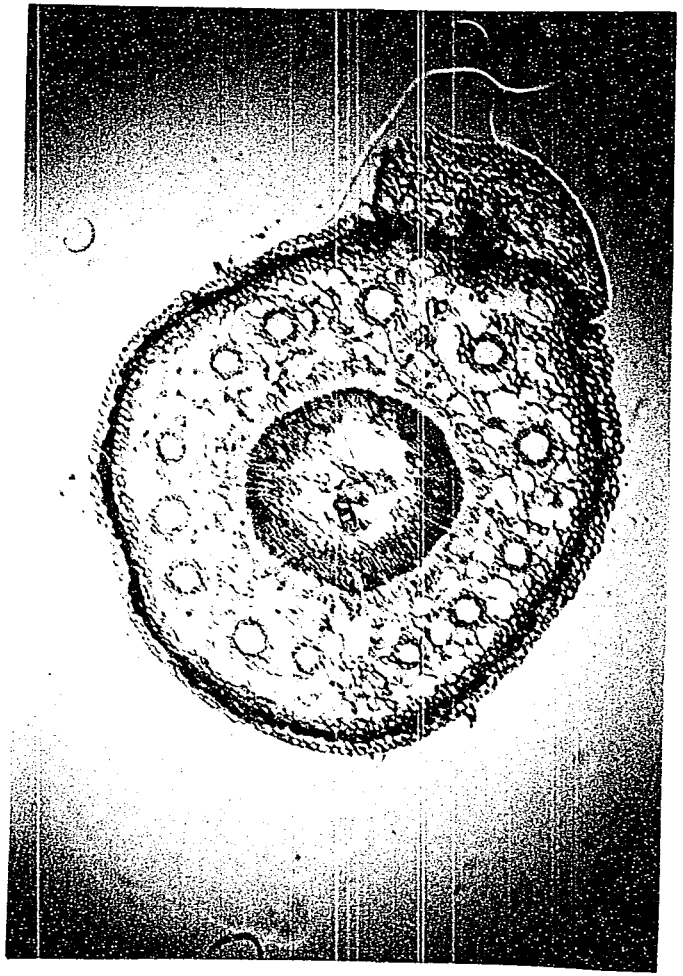
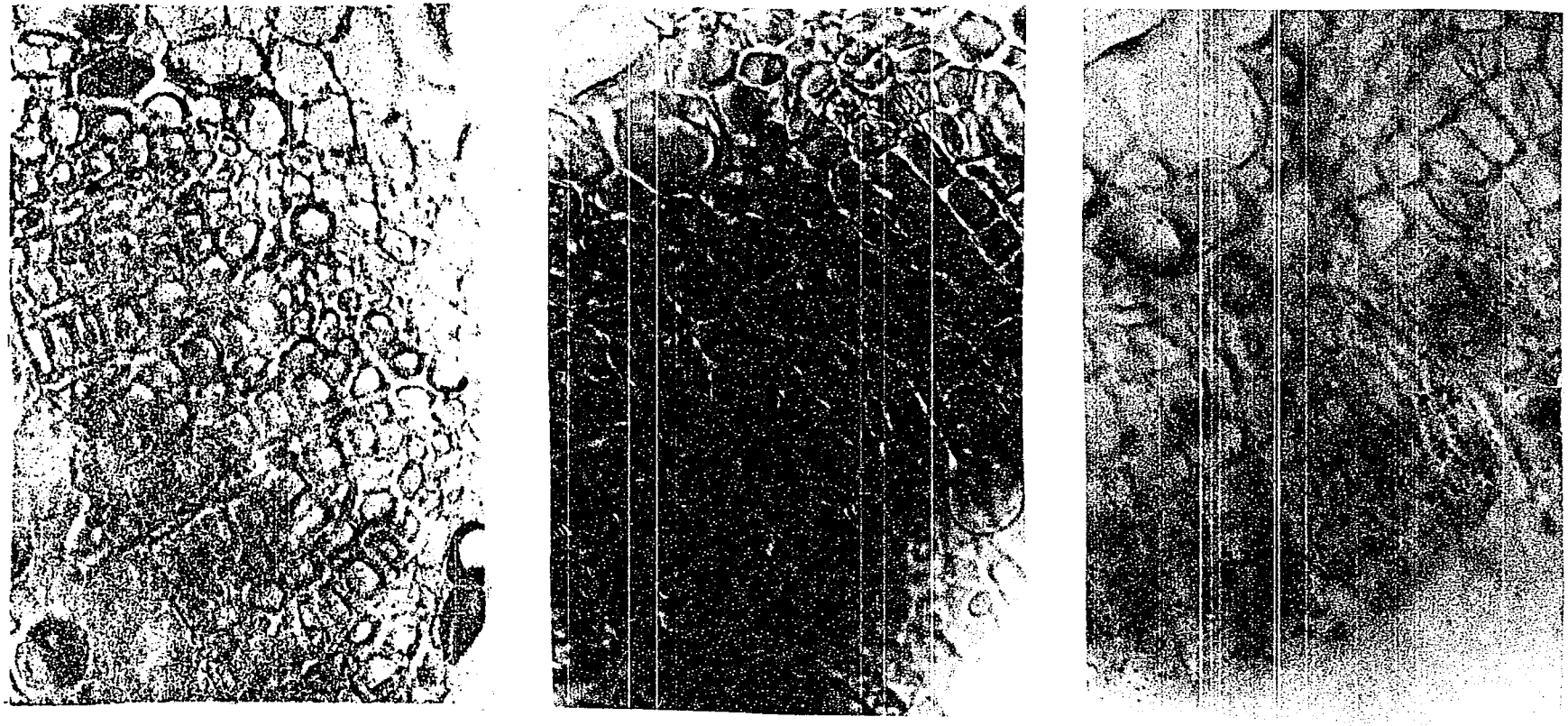
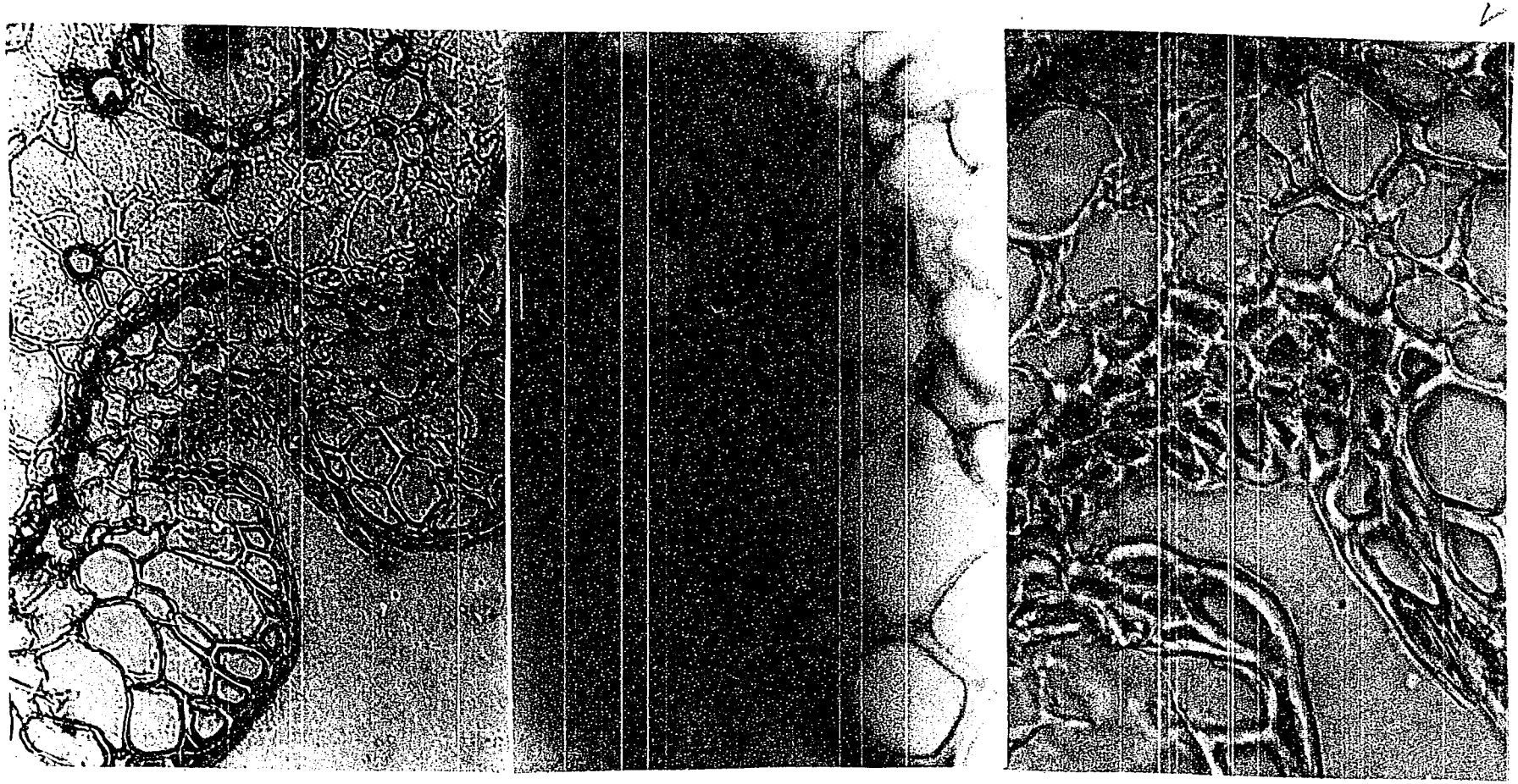


Fig. 6 (xi) A cross section through the rachis of balsam fir. (x40).



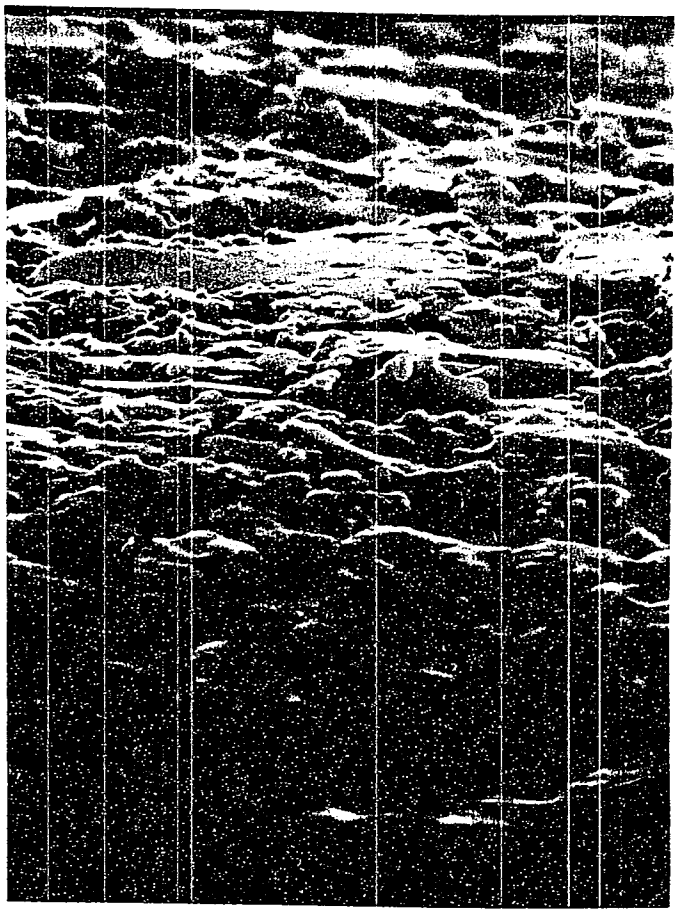
(xii) (xiii) (xiv)

Fig. 6 Localization of activity in vascular system of (xii) spruce, and (xiii) and (iv) balsam fir stem treated with fenitrothion for 21 days. Most of the silver granules are localized in xylem. (x400).



(xv) x40 (xvi) x400 (xvii) x400

Fig. 6 Accumulation of radioactivity (silver grains) in the epi- and hypodermis regions of fasicular tissue of spruce rachis. Sampled 21 days after treatment with $^{14}C$-fenitrothion. Note localization of $^{14}C$ activity in the "groove" region denoted by an arrow in Fig. 6(x).



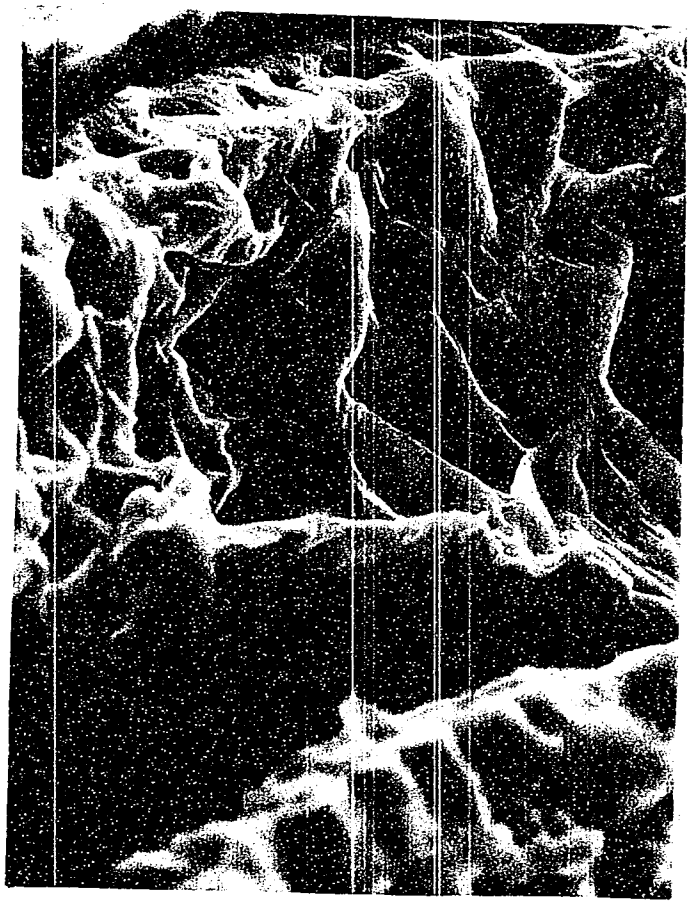


Fig. 7 Scanning electron micrographs

(i) Upper surface of a balsam fir needle (x 5,000) showing surface deposit of epicuticular wax.

(ii) Cross section of a balsam fir needle (x 1,000).

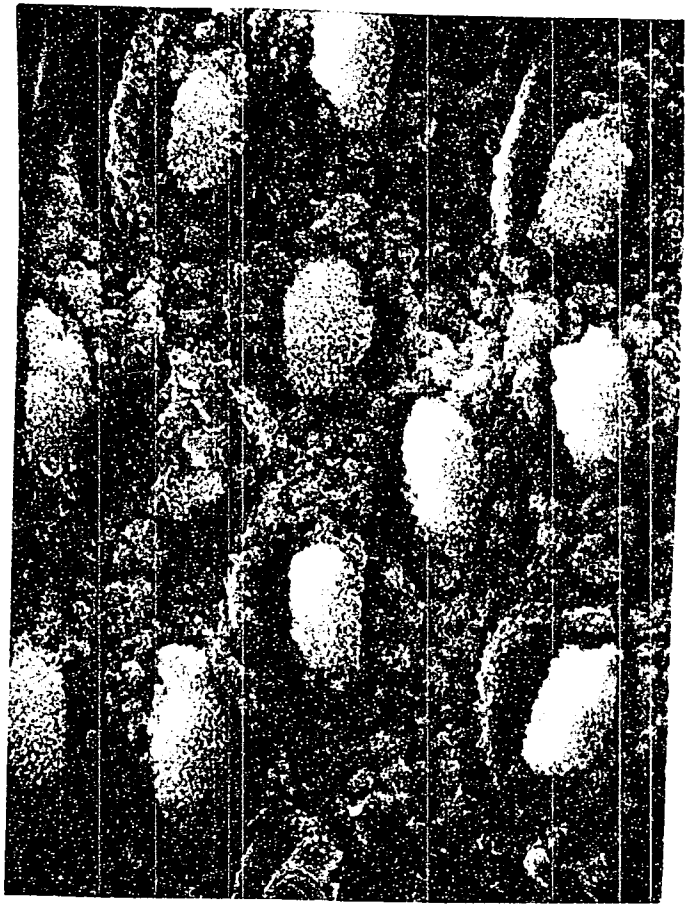


Fig. 7 Scanning electron micrographs

(iii) Lower surface of balsam fir needle (x 500) showing stomata covered by wax deposits.

(iv) Lower surface of balsam fir needle (x 2,000) showing the wax deposit covering one stoma.

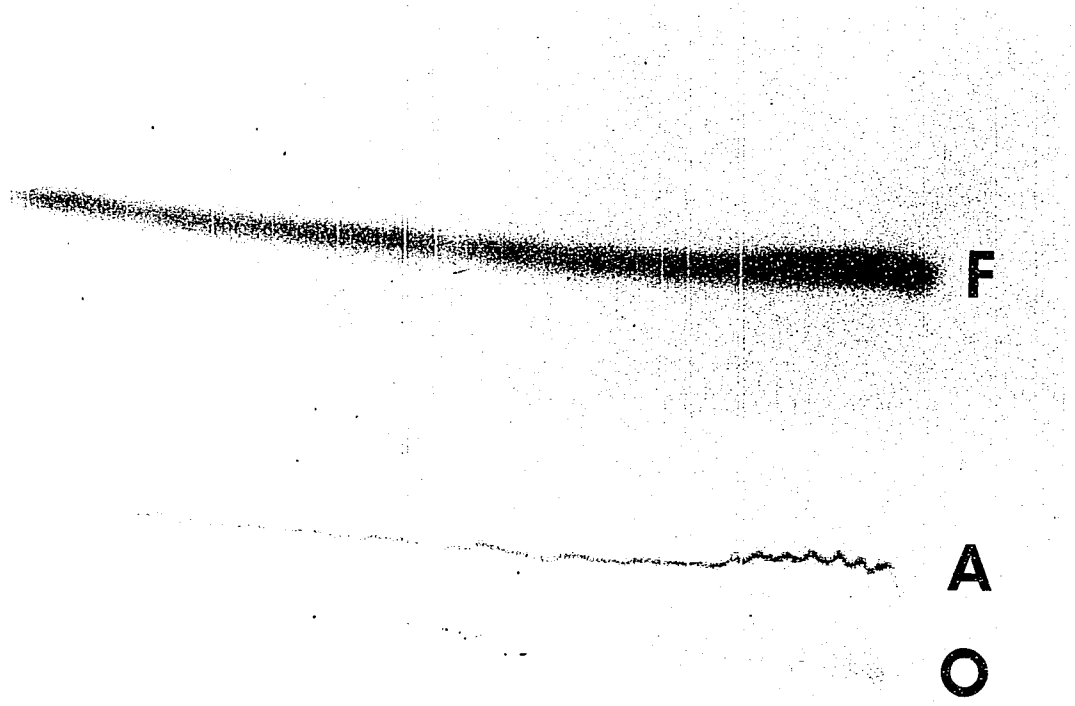



Fig. 8 Thin-layer chromatography (TLC) autoradiograph of TLC plate spotted with extract of balsam fir young foliage sampled after 21 days. F - fenitrothion; A - metabolite; O - origin

## IV DISCUSSION

Volatilization and vapour phase transport have recently been established as important factors in the dissipation of the so-called "non-volatile" pesticides such as DDT and fenitrothion. Spencer et al (1973), demonstrated that the rates of dissipation of pesticides under field conditions closely approximated the potential volatilization rates predicted by calculations based solely on vapor pressure measurements. According to their estimate, parathion with a vapor pressure in the order of $10^{-5}$ mm Hg at $30^{o}$C has a volatilization rate of 18 $\mu g/cm^2$/day. Theoretically, then, fenitrothion with a vapour pressure in the same order of magnitude could volatilize completely within one day under field conditions where the amount applied per unit area (2.6 $\mu g/cm^2$ (Randall, 1970)) is appreciably less than its calculated volatilization rate. These measurements do not take wind velocity into account. Murai and Tanaka (1968) reported that 100 $\mu$ diameter droplets of fenitrothion (the average droplet diameter of field spray (Randall, 1974)) deposited on cardboard, evaporated completely in 50 hours at a wind velocity of 7 mph.

These observations are consistent with the results of the preliminary study with conifers held in the greenhouse (Table 1) since fenitrothion levels decreased rapidly during the first seven days following treatment. The fenitrothion content of the tissue extracts, however, decreased less rapidly than in the corresponding surface washes and in some cases (notably the extracts of sprayed new fir foliage; N.F. (E), S-2 and S-4)[1] the levels were higher in the 7 than in the 1 day samples. The greater persistence of fenitrothion in

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[1] see Table 1

the conifer tissue extracts could be accounted for by penetration through the tissue surface with subsequent solubilization in plant waxes and oils. These subcuticular or absorbed residues would tend to dissipate more slowly due to their slow diffusion to the evaporating surface and to their reduced vapour density when dissolved in waxes or oils (Spencer et al, 1973). The unabsorbed surface deposit, however, should volatilize at a rate closely resembling that predicted in theory.

The hypothesis that fenitrothion dissipation from conifers is largely the result of volatilization and not some form of biodegredation, is further substantiated by the in vitro study. The insecticide was rapidly lost from the glass surfaces under both light and dark conditions. It was thoughtoriginally, that the greater rate of dissipation observed in the light (Fig. 2 and 3) was due to photodegradation (Ohkawa et al, 1974) as traces (less than 0.1% of initial $^{14}C$ deposit) of fenitro-oxon were detected (Table 2). However, photodegradation should not increase the rate of volatilization of the $^{14}C$-label, since the non-polar degradation products, such as fenitro-oxon, would have volatilities similar to fenitrothion and the polar products would be less volatile. The faster dissipation in the light was more likely due to the heating effect of the light impinging on the glass surfaces or to greater air circulation over the glass held in the light, since the 'dark' samples were held in a box to exclude light. Both of these factors would tend to increase the rate of volatilization and hence the dissipation of the insecticide. Since the data for the radio-label in vitro study (Fig. 3) were obtained by monitoring of total $^{14}C$-activity, the observed loss of activity with time would preclude the

possibility of large scale degradation of fenitrothion, since any persisting product possessing the $^{14}C$- ring moiety would still be detected. This point is significant, since the GC methodology used previously for detection of 'cold' fenitrothion (Fig. 2) was not sensitive to some of the more polar products such as desmethyl- and carboxy-fenitrothion.

During this study, precautions were taken to ensure that the products detected were not the result of degradation of the parent compound during storage. Towards this end, the fenitrothion emulsions used for treatment were extracted and analysed for purity. Furthermore, the originally 'pure' fenitrothion standards used for GC quantitation were either kept refrigerated with the conifer extracts, or were exposed to the laboratory conditions while the samples were being analysed. Although apparent traces of fenitro-oxon and S-methyl fenitrothion were sometimes detected in the conifer extracts, they never exceeded the levels found in the standards. As such, they were thought to have formed during storage. However, traces (less than 0.1% of initial $^{14}C$ deposit) of fenitro-oxon (in the light) and S-methyl fenitrothion (in the dark) were detected in the washings of the glass surfaces, but were not detected in the original fenitrothion emulsion. It is most likely that their detection in this in vitro study was facilitated by the use of relatively large deposits of fenitrothion and also by the absence of interfering impurities.

Fig. 1 (i) demonstrates that in three of the four treatment methods (Paint 20 ppm; Spray 20 ppm; and Spray 200 ppm), the percent fenitrothion absorbed by fir was greater than that absorbed by spruce and also that the residue levels were greater in fir than in spruce

(Fig. 1 (ii)). This situation was reversed for the Paint 200 ppm application where the percent absorbed and the residual fenitrothion levels were both greater for spruce than for fir. These results imply that a direct correlation existed between these two factors, i.e. that greater absorption promoted greater persistence. As discussed previously, this would be expected since the absorbed residue would theoretically be more resistant to volatilization and photodegradation.

The data from the radiolabel study also demonstrate a direct relationship between the amount of pesticide absorbed (Fig. 4 (i)) and the amount persisting (Fig. 4 (ii)) in the conifer tissue, since successively greater absorption was matched by increasing persistence in pine, spruce and fir.

Yule and Duffy (1972) reported greater persistence of fenitrothion in fir than in spruce following an operational spray application of the pesticide. The greater persistence in fir may result from greater absorption by fir tissue due to inherent species related physiological and morphological differences (degree of cutinization, needle morphology and spacial orientation, etc.). In this connection, some evidence can be gained from Tables 1 and 3 which show that the new fir foliage was the main contributor to the differences exhibited by the conifer species. Fenitrothion levels increased within the new fir foliage (N.F. (E); S-2 and S-4) during the 1 to 7 day sampling period of the GC study (Table 1). The results of the LSC study (Table 3) show that the greater persistence of fenitrothion in fir after 21 days was mainly due to the high levels present in the new foliage extracts. There could be two reasons for this: (1) The newly flushed fir foliage was exceptionally permeable to fenitrothion, perhaps due to

the chemically different nature of the cuticle of young foliage (Linskens et al, 1965), and/or (2) Fenitrothion was being transported acropetally to the young fir foliage, perhaps due to the ability of developing foliage to act as a "sink" (Crisp, 1972).

The latter explanation is consistent with the preferrential localization of the $^{14}C$-product (A) (Table 3) in the young foliage extracts of fir and spruce. Hallett et al (1974) reported that concentrations of desmethyl-fenitrothion increased rapidly between 8 and 14 days in the embryo of germinating white pine seeds. The accumulation of this water-soluble product, specifically in the seed embryo was thought to be due to increased water transport to this tissue during germination. The relative $R_f$ of 0.21 in the TLC system described for the $^{14}C$-product (A) corresponds to a value of 0.22 given for carboxy-fenitrothion under identical TLC conditions (N.R.C. Report, 1978). Carboxy-fenitrothion is relatively water-soluble compared to fenitrothion (as is the polar desmethyl product (Hallett et al, 1974)) and could therefore be transported within the aqueous environment of the vascular system. Interestingly, Crisp (1972) suggested that a -COOH functional group was necessary for an insecticide to be symplastic (phloem-mobile) since "acid activation" of the insecticide favoured penetration of the plasmalemma membrane bordering the symplast. After entering the symplast, the insecticide would translocate in a "source to sink" direction. In the present study, the young developing foliage present at the shoot tips would act as a sink for the food materials manufactured by the older foliage (source) and translocation of the insecticide would be towards the shoot tips (i.e. from source to sink). As mentioned previously, this theory was supported by the

present study since the polar $^{14}C$-product tentatively identified as carboxy-fenitrothion, was only present in the young foliage extracts of fir and spruce.

The SEM photomicrographs (Fig. 7 (i-iv)) illustrate the presence of a thick deposit of epicuticular wax present on the surface of balsam fir foliage. Yule and Duffy (1972) speculated that the unusual persistence of fenitrothion on conifer foliage could be attributed to solubilization in these waxes, where the residue would be protected from physical loss resulting from leaching and volatilization. However, Table 3 shows that the persistent residues were mainly present in the tissue extracts and not in the surface washes which contained much of the epicuticular waxes.[1] It seems more likely then, that the persistent residues were located at some subcuticular level, raising the possibility of their being transported. This is in fact the case, as shown by the gross autoradiographs given in Fig. 5 (i-xviii). Although some basipetal transport is apparent (Fig. 5 (i)) the major route of movement is acropetal, from the old to the newly developing foliage (Fig. 5 (v)), explaining the relatively high levels of absorbed residues found persisting in this tissue (Tables 1 and 3). Movement was less apparent in spruce than in fir and was practically negligible in pine. As mentioned previously, the presence of the $^{14}C$-product (A) in the young foliage extracts of fir and spruce but not in pine, may be further evidence of acropetal transport. The superior transport observed in fir is consistent with the earlier hypothesis that fenitrothion is more persistent in fir than in spruce or pine since the absorbed, potentially mobile residues, would not be

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[1] Surface washing of Sitka spruce foliage with organic solvents such as chloroform removed the epicuticular wax layer (Jeffree et al, 1971).

directly exposed to weathering influences. The histoautoradiographs (Fig. 6 (i-xvii)) reveal that the major transport was apoplastic, i.e. via the xylem explaining the predominance of acropetal transport noted in the gross autoradiography study. This is consistent with the findings of Crisp et al (1972), who reported that all of the organophosphorus insecticides investigated were apoplastic systemics. The small amount of $^{14}C$-activity located in the phloem tissue (symplast) could be due to carboxy-fenitrothion which would be expected to be symplastic as discussed previously.

The species related differences noted here are probably related to the ability on a time basis to absorb fenitrothion. The histoautoradiography (Fig. 6) demonstrated $^{14}C$-activity that had moved from the treatment site into the xylem of all three species, implying that the transport mechanism was operating in all species. However, the gross autoradiography (Fig. 5) demonstrated that movement was evident in fir 7 days after treatment but was only apparent in spruce after 21 days, at a time when most of the surface deposit had been lost. The lack of apparent transport in spruce and pine then, could be due to their slow uptake of the pesticide, coupled with the simultaneous rapid volatilization of the surface deposit.

McLeod (1975), reported a possible residual effect of fenitrothion on Swaine Jack pine sawfly, Neodiprion swainei Midd., 42 days after aerial application of the pesticide to Quebec forests. However, analysis of this conifer tissue showed only traces of fenitrothion persisting at this time. The results of the present study show that fenitrothion is less persistent and is not transported in pine to the extent observed in fir or spruce. The data (Fig. 6 (xvi and xvii))

suggest that a localized persistence of trace residues might account for the toxicity reported by McLeod (1975). The sawfly ingests the tissue present at the base of the older pine foliage (McLeod, personal communication), at the same location where the radiolabel was shown to persist on a treated spruce rachis. This type of localized persistence may also occur in Jack pine, since in this species the needles occur in pairs and the needle bases of each pair are ensheathed by a woody projection of the periderm (fascicle) which would tend to shield the residues from weathering influences (light, rain, wind, etc.). This species also has a special feature that enables it to endure drought conditions that takes advantage of the 'paired' condition of the needles. In nature, condensed water vapour (dew) is drawn by a capillary action between the needle pair towards the site of needle attachment. The tissue forming the boundary between the base of each pair is not cutinized, permitting rapid uptake of the water condensate (Linkens et al, 1965). It is quite possible then, that the fenitrothion deposit would be leached basipetally and absorbed by the tissue at the needle base, and would persist there protected by the fascicle until ingested by sawfly larvae. Howse et al (1971) reported that population reduction of the spruce budworm was significantly greater on balsam fir than on white spruce following operational spray application of fenitrothion. This field observation may be due to the greater potential of fenitrothion absorption by fir with resultant increased persistence of the pesticide. According to Miller (1975), the most critical period in the life cycle of the budworm is during June when the larvae are feeding on the newly flushed foliage. The present study has demonstrated that the

greater persistence of fenitrothion in fir was primarily due to the accumulation of the pesticide within the new foliage which resulted from acropetal, apoplastic transport in this species. Consequently, the greater protection conferred to fir against budworm predation may be the result of the systemic action of fenitrothion in fir.

## V Summary and Conclusions

The persistence and fate of fenitrothion on four year old seedlings of balsam fir, white spruce, and Jack pine were examined under greenhouse conditions. The rapid loss of the pesticide from the foliage surfaces was thought to be due to volatilization since the radiolabel disappeared rapidly from glass surfaces treated with $^{14}$C-ring-labelled fenitrothion held under light or dark conditions. Radio-TLC analysis of the conifer extracts also demonstrated that $^{14}$C-ring degradation products were present for the most part only in trace amounts with the exception of a product present in the young foliage of fir and spruce that was tentatively identified as carboxy-fenitrothion. The pesticide was absorbed by the conifer tissue, especially by balsam fir, which was thought to be the reason for localized persistence of these residues in the young foliage of this species. Once absorbed, these residues would be protected from weathering influences and would be potentially mobile, i.e. transportable throughout the plant system. Autoradioagraphy confirmed acropetal, apoplastic transport in balsam fir and this was considered to be evidence for the systemic potential of fenitrothion. The possibility of a localized systemic effect of the pesticide with respect to the pine sawfly was inferred from the histoautoradiography.

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