Environment Canada | Environnement Canada
Forestry Service | Service des Forêts

UPTAKE, TRANSLOCATION AND METABOLISM OF C-14 ACEPHATE IN SPRUCE TREES

by K.M.S. Sundaram, W.W. Hopewell and G. Lafrance

CHEMICAL CONTROL RESEARCH INSTITUTE
OTTAWA, ONTARIO
REPORT CC-X-139

JANUARY 1977

UPTAKE, TRANSLOCATION AND METABOLISM OF C-14 ACEPHATE IN SPRUCE TREES

by

K.M.S. SUNDARAM, W.W. HOPEWELL AND G. LAFRANCE

Chemical Control Research Institute
Canadian Forestry Service - Environment Canada
25 Pickering Place, Ottawa, Ont., Canada, K1A 0W3

REPORT CC-X-139

January, 1977

TABLE OF CONTENTS

Uptake, Translocation and Metabolism of C-14 Acephate in Spruce Trees

Abstract

The uptake, translocation and metabolism of C-14 Acephate (0,S-dimethyl acetylphosphoramidothioate) was investigated in white spruce, *Picea glauca* (Moench) under normal weathering conditions of a forest environment. The insecticide was administered by three techniques, *viz.*, trunk implantation treatment (TIT), basal bark painting (BBP) and foliar painting (FP), following which foliar residue levels were determined at intervals by gas-liquid chromatography (GLC), liquid scintillation counting (LSC) and autoradiographic techniques. The insecticide penetrated the treated region and translocated apoplastically *via* xylem vessels (transpiration stream) in different tree parts depending upon the mode of application. Uptake and foliar accumulation through xylem transport were high in TIT, considerably less in BBP and least in FP. Autoradiographic studies confirmed the acropetal translocation of the radiolabel *via* the transpiration stream into the young and growing spruce foliage. The acephate residues in foliage by TIT gradually decreased through metabolic and chemical processes forming methamidophos (0, S-dimethyl phosphoramidothioate) and possibly other polar conjugates which were not identified and quantitated. Since the residue levels of methamidophos in foliage was low (< 10%) compared to acephate, the primary detoxification process was through the rupture of P-N, P-0 and P-S bonds instead of the N-C bond in the molecule forming derivatives of phosphoric acid. The insecticide present on the foliage in FP was rapidly lost due to various physical processes whereas the adsorbed residues were persistent due to their inclusion in the

lipophylic material of the cuticle. Under the experimental conditions acephate was found to be systemic to some extent, and this potential is evaluated for budworm control.

RESUME

L'absorption, le déplacement et le métabolisme de l'acéphate (O,S-diméthyl N-acétylthiophosphoramide) marqué au ^{14}C ont été étudiés chez l'épinette blanche (*Picea glauca* (Moench)) dans des conditions météorologiques normales en forêt. L'insecticide a été administré selon trois techniques, soit par injection dans le tronc, par badigeonnage de l'écorce à la base, et par badigeonnage des aiguilles, et les teneurs des aiguilles en résidus ont été analysées après certains intervalles par chromatographie de partage gaz-liquide, par comptage par scintillation liquide et par autoradiographie. L'insecticide pénétrait dans la région traitée et se déplacait de façon apoplastique par les vaisseaux du xylème (flux de la transpiration) vers différentes parties de l'arbre selon le mode d'administration. L'absorption et l'accumulation dans les aiguilles par le transport dans le xylème étaient élevées avec la technique d'injection dans le tronc, beaucoup plus faibles avec celle du badigeonnage de l'écorce à la base et encore plus avec celle du badigeonnage des aiguilles. Les études autoradiographiques ont confirmé le déplacement acropète du marqueur dans le flux de la transpiration jusque dans les aiguilles des épinettes jeunes et en croissance.

Les résidus dans les aiguilles d'acéphate injecté dans le tronc ont diminué graduellement à cause des processus métaboliques et chimiques

qui ont entraîné la formation de méthamidophos (0, S-diméthylester-amide thiophosphorique) et peut-être d'autres dérivés polaires qui n'ont pas été analysés qualitativement ni quantitativement. Comme les teneurs des aiguilles en méthamidophos (10%) étaient faibles par comparaison à l'acéphate, on en déduit que le processus primaire de détoxication était la rupture des liaisons P-N, P-O et P-S plutôt que N-C dans la molécule, pour former des dérivés de l'acide phosphorique. L'insecticide badigeonné sur les aiguilles est rapidement disparu à cause de divers processus physiques contrairement aux résidus adsorbés qui ont persisté à cause de leur inclusion dans la matière lipophylique du cuticule. Dans les conditions expérimentales, l'acéphate s'est révélé endothérapique jusqu'à un certain point, et cette propriété est évaluée pour la lutte contre la tordeuse de bourgeons de l'épinette.

INTRODUCTION

Acephate (0,S-dimethyl acetylphosphoramidothioate) is a new commercial insecticide introduced by the Chevron Chemical Company in California and marketed under the trade name Orthene®. Recent studies (Armstrong and Nigam 1975, Sundaram and Hopewell 1976) have shown that the chemical is particularly effective against forest insect pests such as spruce budworm, *Choristoneura fumiferana* (Clemens). Experiments by Lyon (1973) indicated the systemic nature of the insecticide and offered some clues to its fate in plants. If this property could be confirmed in conifers under normal weathering conditions, it would be useful in protecting especially the individual, high-value trees against the insect pest. This study was undertaken to investigate the uptake, translocation and metabolism of acephate in white spruce, *Picea glauca* (Moench) when the C14-labelled chemical is applied in different ways to different regions of the tree, *viz.*, painting the basal bark, painting the needles of the tree and injecting the material into holes bored in the stem. Such an indepth study to evaluate the fate, persistence and systemic properties of acephate and its active metabolite methamidophos (0,S-dimethyl phosphoramidothioate) (trade name: Ortho 9006® by Chevron) in conifers has not been described before. Such knowledge will undoubtedly assist in understanding the environmental dynamics of this insecticide.

MATERIALS AND METHODS

Chemicals and Solvents - A preparation of C-14 acephate labelled in the S-methyl carbon [specific activity (S.A.) 4.77 millicurie (mCi)/millimole (mmole)] was supplied by the Chevron Chemical Company, San Francisco, California. The radiopurity of the tracer was shown to be greater than 98% by liquid scintillation counting (LSC) and gas-liquid chromatography (GLC). Nonlabelled acephate standards and the metabolite, methamidophos were also supplied by the Chevron Chemical Co. Their identity and purity were confirmed by GLC and thin layer chromatography (TLC).

All the solvents used in the study were either pesticide grade or freshly distilled in glass. Anhydrous sodium sulphate (Fisher) used for dehydration purposes was of reagent grade heated at 200°C overnight and stored in an airtight glass bottle. Silica gel used in the column cleanup was from E. Merck, Darmstadt, Germany (Cat. No. 7734) with particle size 0.063 - 0.200 mm (70 - 230 mesh ASTM) and used as received. Dimethyl sulphoxide (DMSO) used in the preparation of insecticide solutions was of analytical grade. It served not only as a good solvent for acephate but also acted as a stimulant to enhance the uptake of the material by the treated tree.

Experimental Site - This investigation was carried out during the summer of 1976 on a tree farm near Shawville, Quebec. Four spruce trees (S1 .. .. S4), separated by a distance of *ca* 25 m were selected at random. They were of near uniform size and shape (*ca* 4 m in height and 9 cm in diameter at the base of the trunk) with abundant foliage. Three trees S1, S2 and S3 were selected for the insecticide treatment and one tree (S4) served as the untreated check.

Insecticide solution - Non-labelled ("cold") analytical grade acephate (99.5% purity), acting as carrier was mixed with the C-14 labelled active material (S.A. 4.77 mCi/mmole) in the proportion 345 mg of inactive (1.89 mole) to 115 mg (0.63 mole) of active material and made up to 15 ml in DMSO. The solution was divided into three equal parts for application to the three trees S1, S2 and S3. Each tree received 153 mg (0.84 mmole) of acephate with specific activity 1.18 mCi/mmole and containing 38 mg (0.99 mCi) (0.21 mmole) of the active and 115 mg (0.63 mmole) of the non-active insecticide components in 5 ml of DMSO.

Application of the Insecticide - Three types of application were employed. The tree S1 was treated by the trunk implantation technique (TIT), S2 by basal bark painting (BBP) and S3 by foliar painting (FP). All the treatments were applied on May 26, 1976 from 0940 to 1120 hours. The trunk implantation treatment was done by boring four downward sloping holes 4 cm deep and 0.75 cm in diameter, with a hand drill in each of the four quadrants of the tree trunk 25 cm above ground level and injecting the insecticide solution into the holes with a hypodermic syringe. The openings were then sealed with plasticine to prevent loss of the injected solution by leakage or evaporation. For the basal bark painting the insecticide solution was painted on the bark using a small brush in a 10 cm band around the trunk 25 cm above ground level. Care was taken not to allow the liquid to run down the trunk below the band region. For foliar painting, four branches, one in each quadrant of the tree just below the midcrown were selected to permit uniform translocation to all branches around the circumference, and the acephate solution was carefully applied to the needles with a small brush to obtain uniform coverage. A rough estimation of the weight of the needles that was painted with the 5 ml of DMSO

solution was found to be 300 g giving an initial concentration of acephate on the needles as 510 ppm.

Sampling - Foliage samples were collected randomly from the mid-crown branches of the four quadrants of each treated and check tree before the insecticide application and subsequently at 1, 3, 5, 8, 12, 16, 21, 27, 34, 41, 50, 64 and 89 days. A sample from each tree was a composite of clipped twigs from each quadrant to give *ca* 50 g foliage, sealed in a plastic bag and taken immediately in polystyrene coolers to the pesticide laboratory at C.C.R.I. where it was stored in deep freeze until time for radioassay and GLC analysis. All three treated trees appeared as healthy as the check tree on the day of the final assessment, *i.e.* 89 days after application. Pieces of bark, stem, newly flushed and old foliage, and root samples were also collected from different parts of the trees at 21 and 89 days post treatment to evaluate the distribution pattern of the insecticide and its principle metabolite.

Extraction and GLC Analysis - The foliage samples were finely chopped in a Hobart grinder, mixed well to achieve uniformity and 20 g aliquots extracted with ethyl acetate using a Sorvall Omni-Mixer. Stem and root samples were processed similarly and extracted. The extracts were partitioned and cleaned up as described in detail by Sundaram and Hopewell (1976).

Flame photometric GLC was used for the analysis of acephate and metamidophos residues present in the concentrates of various plant extracts and the instrument parameters used were similar to those described by Sundaram and Hopewell (*loc cit*).

Carbon-14 Counting - A Beckman LS-100C liquid scintillation counter was used to measure the radioactivity of the raw and cleanedup extracts. Aliquots of extracts (50 - 100 µl) were added to Beckman plastic scintillation vials containing 10 ml of scintillation fluid containing 0.50% PPO (2,5-diphenyloxazole) in toluene. Each sample was counted thrice to obtain the average radioactivity. Quench corrections were made for the raw extracts using a quench curve prepared by counting a series of C14-acephate standard solutions containing progressively increasing amounts of spruce extract and plotting the observed percent counting efficiency of the instrument against the external standard ratio. All counts were corrected for the background. The average counts finally obtained for each sample were converted to total disintegrations per minute (dpm) per gram of fresh weight and the specific activities are expressed in nCi/g (2.2×10^3 dpm/g) of samples. The average counting efficiency during the study, in comparison to an external C-14 standard, was 90 ± 2%.

Autoradiography - Samples (foliage, stem, roots etc.) selected for autoradiography were numbered for later comparison of similar ones from different treatments. The samples were dried immediately after harvesting between folds of blotting paper mounted in a press for three days. Further preparation of the materials for autoradiography was similar to that described by Yamaguchi and Crafts (1958). The X-ray film was exposed to the plant samples for 3 weeks.

Table 1

Concentration and Radioactivity of Acephate and Methamidophos in Spruce Foliage After Adminstration By Trunk Injection

Days After Application *	Concentration (ppm) Acephate	Methamidophos	Sp. Activity (nCi/g) Raw Extract	Cleanedup Extract
1	0.42	0.05	3.41	3.22
3	0.98	0.11	6.20	5.87
5	2.05	0.21	12.06	11.72
8	2.80	0.35	19.66	17.95
12	3.22	0.37	23.41	21.99
16	3.20	0.40	24.22	22.07
21	2.90	0.33	23.71	20.92
27	2.27	0.36	18.42	15.33
34	1.52	0.30	14.64	10.61
41	1.02	0.19	9.18	5.42
50	0.28	0.08	6.41	2.50
64	0.14	0.03	4.68	0.96
89	0.03	N.D.	3.86	0.46

N.D. Not detected.

Minimum detection limit (MDL): Acephate 0.02 ppm; Methamidophos 0.03 ppm.

Traces (T) represent below these detection limits.

* Date of application: May 26, 1976.

Table 2

Concentration and Radioactivity of Acephate and Methamidophos in Spruce Foliage After BBP Treatment

Days After Application	Concentration (ppm)		Sp. Activity (nCi/g)	
	Acephate	Methamidophos	Raw Extract	Cleanedup Extract
1	N.D.	N.D.	0.07	0.03
3	0.07	N.D.	0.41	0.31
5	0.07	N.D.	0.65	0.51
8	0.11	N.D.	0.89	0.70
12	0.12	0.03	0.99	0.72
16	0.14	0.02	1.30	1.02
21	0.18	0.07	1.39	0.92
27	0.36	0.15	2.80	1.04
34	0.32	0.09	2.40	0.69
41	0.08	0.05	1.20	0.76
50	0.02	0.03	0.64	0.35
64	N.D.	N.D.	0.32	0.12
89	N.D.	N.D.	0.28	0.12

See the footnotes in Table 1.

Table 3

Concentration and Radioactivity of Acephate and Methamidophos in Spruce Foliage After FP Treatment

Days After Application	Concentration (ppm)		(Sp. Activity (nCi/g)	
	Acephate	Methamidophos	Raw Extract	Cleanedup Extract
1	0.02	N.D.	0.08	0.05
3	0.03	N.D.	0.18	0.12
5	0.06	T	0.64	0.48
8	0.21	0.03	1.10	0.75
12	0.18	T	1.22	0.80
16	0.16	N.D.	0.96	0.60
21	0.08	T	0.36	0.24
27	0.02	T	0.26	0.08
34	N.D.	N.D.	0.17	0.04
41	N.D.	N.D.	0.10	0.02
50	N.D.	N.D.	0.10	0.02
64	N.D.	N.D.	0.05	0.02
89	N.D.	N.D.	0.04	0.02

See the footnotes in Table 1.

Table 4

Distribution of Acephate, Methamidophos and Radioactivity in Spruce Trees 21 and 89 Days Post Trunk Injection Treatment †

Sample	21 Days			89 Days		
	Acephate (ppm)	Methamidophos (ppm)	Sp. Activity (nCi/g) of Raw Extract	Acephate (ppm)	Methamidophos (ppm)	Sp. Activity (nCi/g) of Raw Extract
New Foliage	3.25	0.41	25.71	0.07	N.D.	1.10
Old Foliage	2.19	0.26	13.46	T	N.D.	0.32
Branch*	2.82	0.22	17.92	0.11	T	4.01
Roots	T	N.D.	0.20	N.D.	N.D.	0.04
Budworms **	0.04	T	0.42	T	N.D.	0.08

† Results were not significant for BBP and FP treatments.

* 75 cm samples from midcrown excluding foliage.

See the footnotes in Table 1.

** Collected from midcrown twigs. Average of 2 analyses.

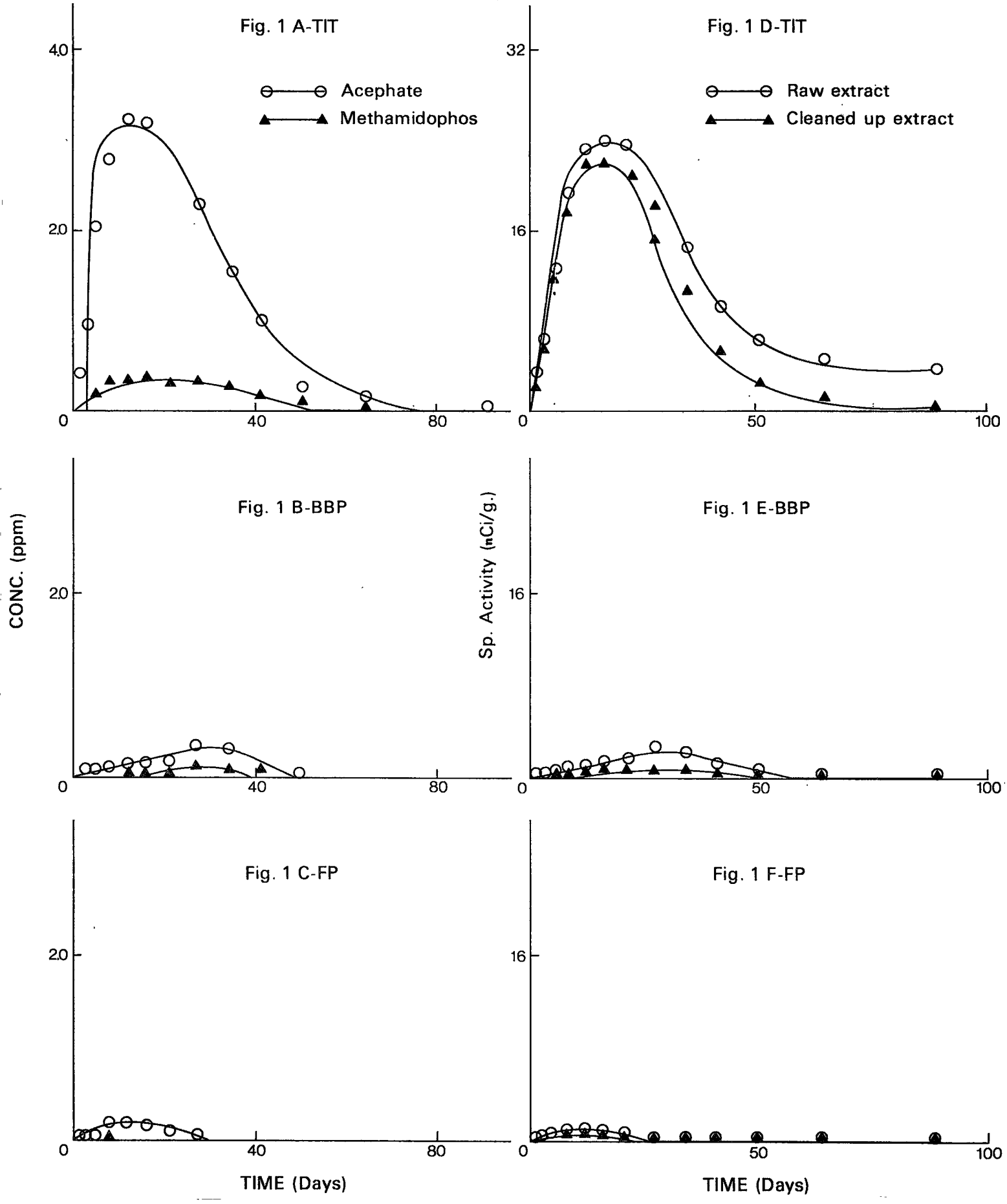

Fig. 1. Distribution of acephate, methamidophos (Figs. A to C) and radioactivity (Figs. D to F) in spruce foliage after administering the chemical by three different techniques. Trunk injection A and D; Basal bark painting B and E; Foliar painting C and F.

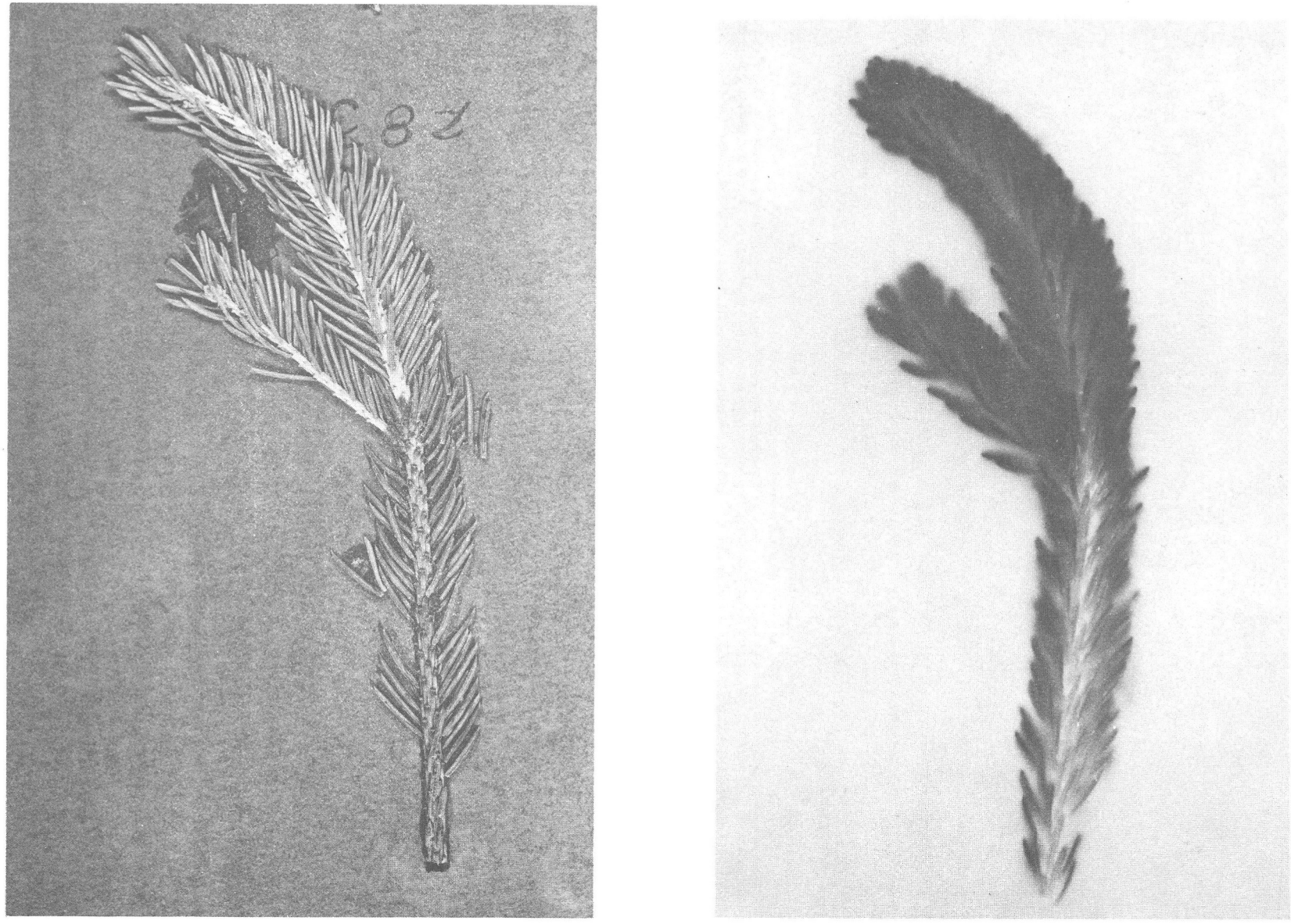

Fig. 2. Autoradiograph of the foliage of spruce sampled 21-days after treatment with C-14 Acephate by trunk implantation technique. Pressed foliage on the left and its autoradiograph on the right. Note the evidence of acropetal translocation to the flushed foliage.

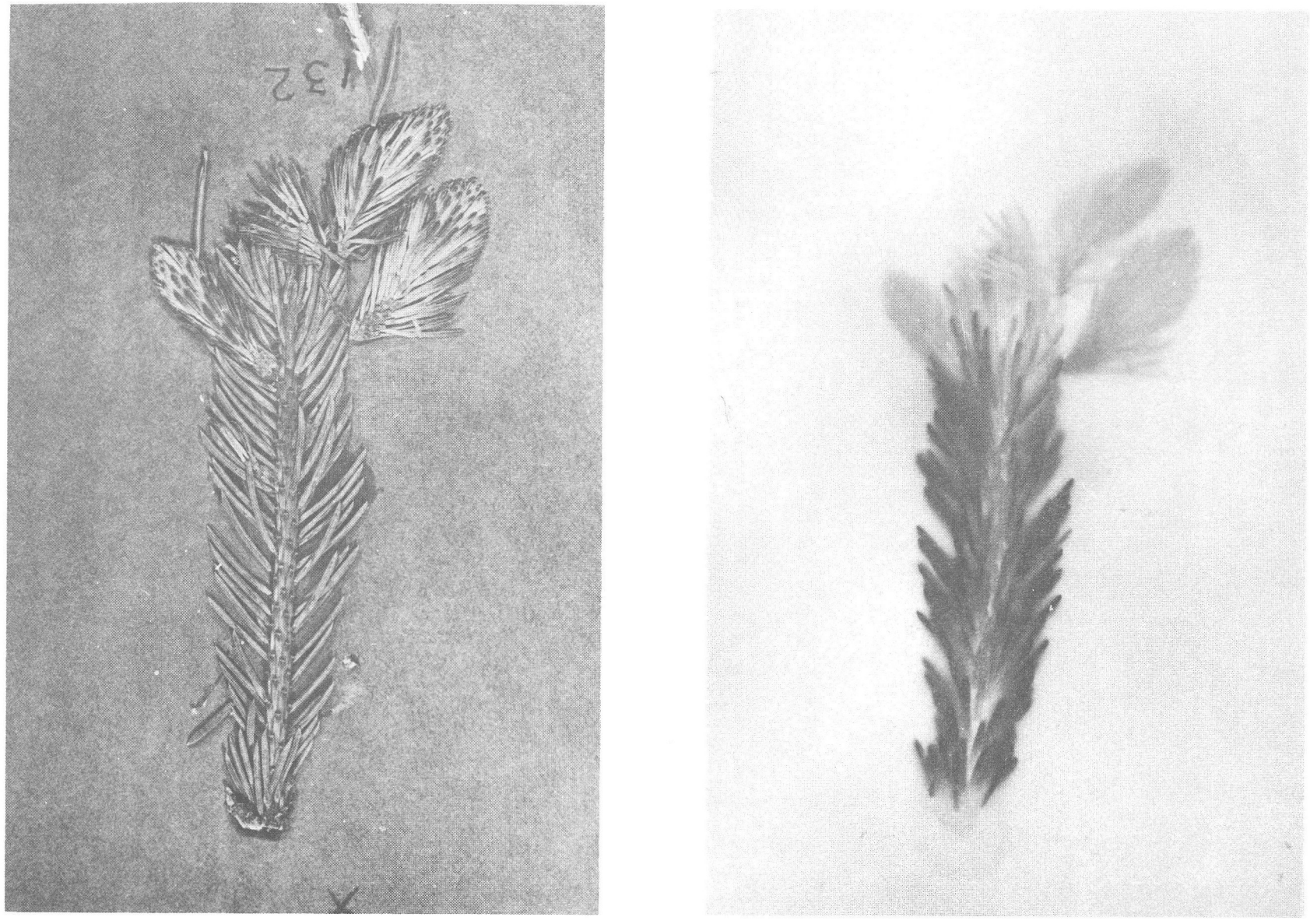

Fig. 3. Above left, pressed spruce foliage sampled 21-days after treatment with C-14 acephate by BBP technique and above right its autoradiograph. Note the decreased acropetal translocation in the new foliage.

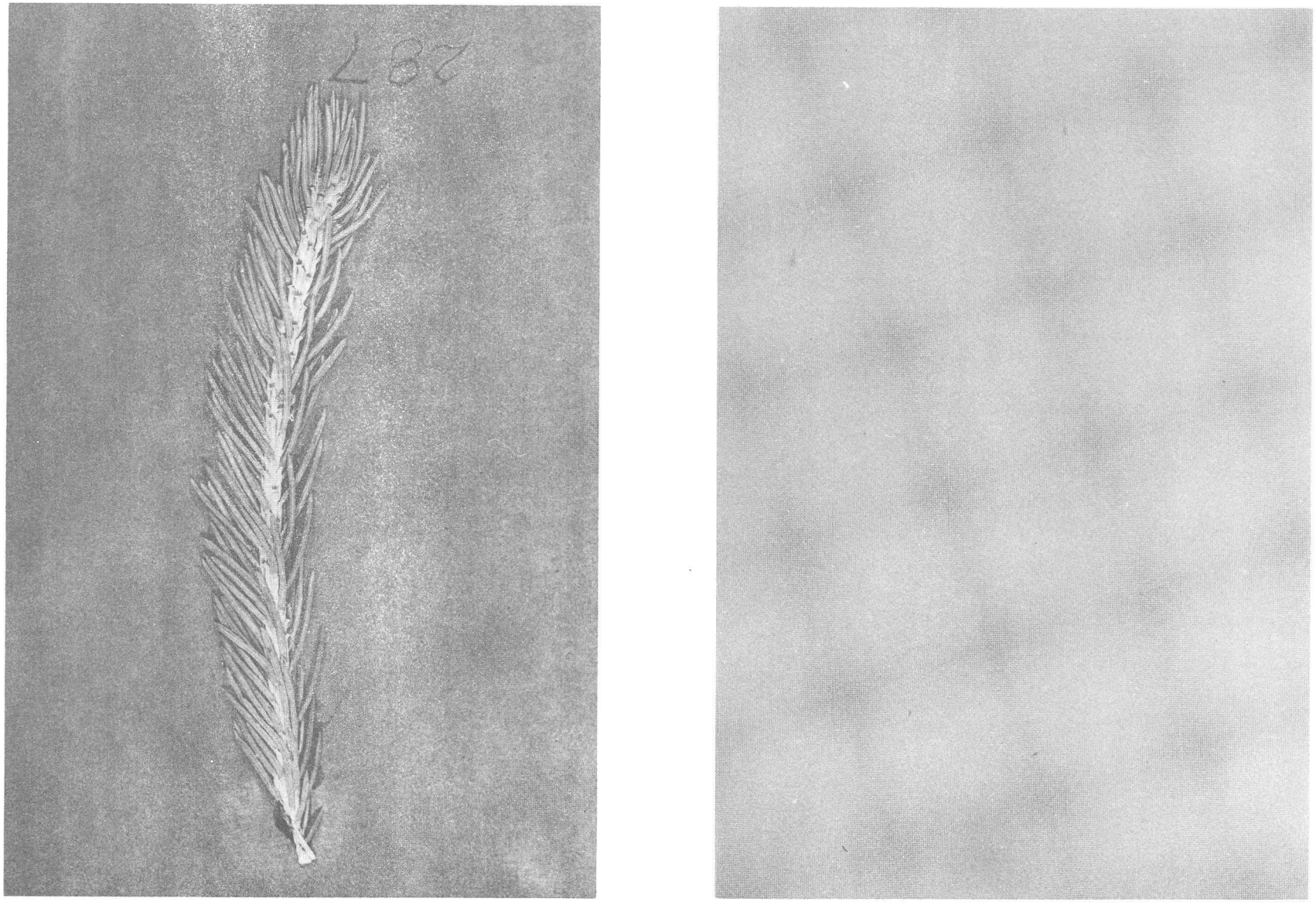

Fig. 4. Autoradiograph of 21-day foliage of spruce treated with C-14 acephate by FP technique. Pressed foliage on the left and its autoradiograph on the right. Although feeble, acropetal translocation is evident.

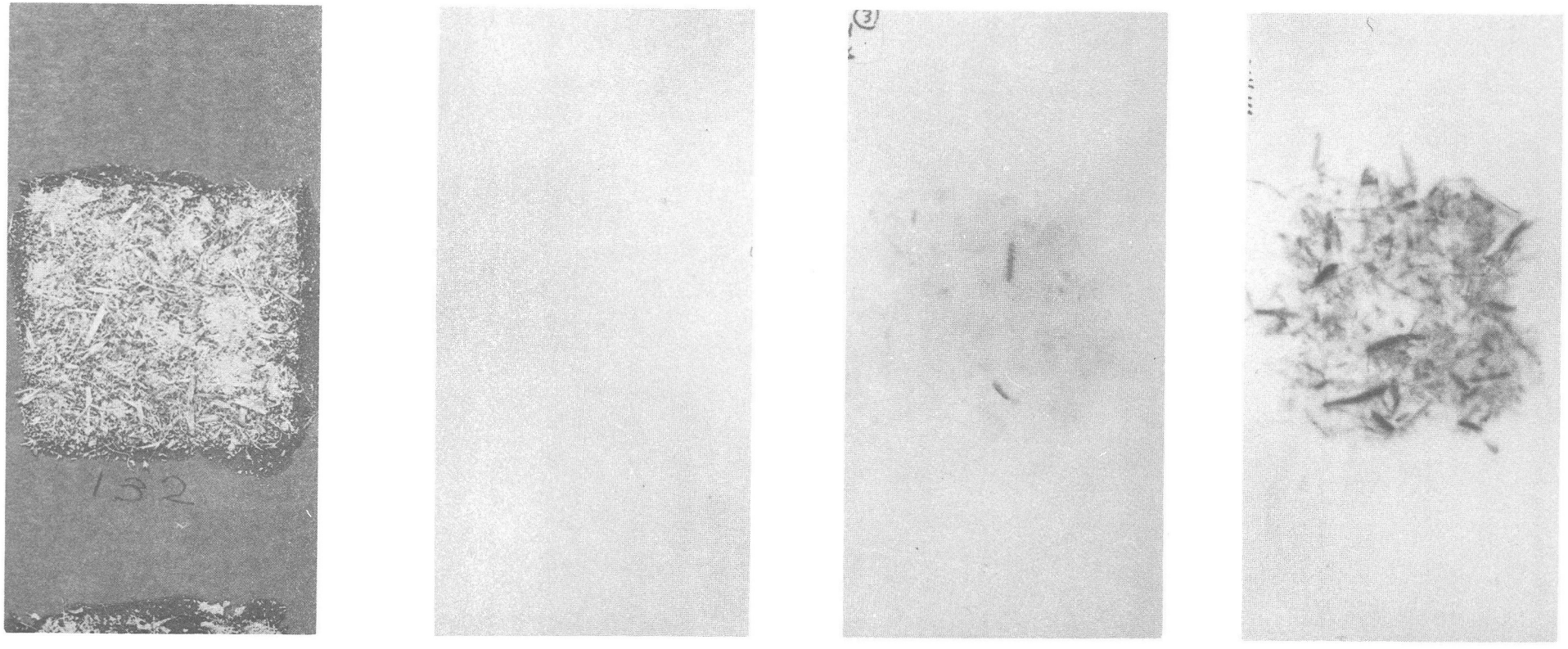

Fig. 5. Autoradiograph of foliar residues by TIT. (a) Pressed residue, (b) 21-Day sample, (c) 34-Day sample and (d) 50-Day sample. Note the increasing amounts of nonextractable radiolabel in the residue with time probably due to the incorporation of the active moieties into cellular structure.

RESULTS AND DISCUSSION

Gas Chromatographic Studies

Amounts of acephate and methamidophos in ppm found in the fresh foliage of spruce trees treated with the insecticide in three different ways (TIT, BBP and FP) are recorded in Tables 1 to 3. The check and prespray foliage samples did not contain any detectable levels of the insecticide residues and are not recorded in this publication.

As can be seen in Table 1 and Fig. 1A, the rate of uptake and foliar translocation of acephate by the TIT is much higher than in the other two sets of experiments (BBP and FP), with the maximum accumulation of 3.22 ppm occurring 12 days after treatment thus showing an incrase of 2.80 ppm from the initial concentration (0.42 ppm) observed one day after treatment. In the case of BBP, the rate of uptake was relatively small (*ca* 11% of TIT) and slow, showing a maximum of 0.36 ppm (Table 2, Fig. 1B) at an interval of 27 days post treatment probably due to the immobility of the insecticide molecules through thick bark and inclusion of the chemical in the lipophylic material of the phloem tissues retarding translocation. With FP, however, the uptake is the smallest with only half the maximum amount observed in BBP (0.18 ppm) after 12 days but the rate of absorption is relatively higher than BBP suggesting, as observed earlier, the inclusion of acephate molecules in the lipophylic resinous wax of the cuticle. The little that is translocated in the foliage (Fig. 1C) *i.e.* 0.04% of the chemical painted on zero day, dissipated quickly (27 days) compared to TIT (> 89 days) and BBP (50 days) treatments. The residue data obtained by GLC studies show at least qualitatively the relationship existing between the maximum amount of pesticide translocated systemically and the

mode of application. Taking the maximum concentration of acephate (3.22 ppm) observed on Day 16 in TIT as 100%, the percent ratio of the insecticide maximum in BBP and FP treatments are respectively 11 (Day 27) and 6 (Day 12).

Liquid Scintillation Counting

Measurements of the radioactivity in the raw and cleaned up foliar extracts are recorded in Table 1 to 3. The radiocarbon assays confirmed the observations made in GLC studies that all the spruce trees took up the applied radiolabel and translocated the toxicant moieties into foliar parts. Degree of uptake and foliar accumulation and accompanying dissipation of radioactivity (Fig. 1 - D, E, and F) in relation to the age of application, corresponded closely with the GLC studies and exhibited similar maxima approximately on the same days as observed in gas chromatographic evaluation (Fig. 1 - A, B and C). On converting the recorded activity of raw extract in nanocuries (*n*Ci) to dpm (1 *n*Ci = 2.2×10^3 dpm) and calculating the ppm of radiolabels in terms of total acephate and methamidophos, using the specific activity of the former, the concentration levels obtained in ppm units by both techniques (GLC and LSC), agreed reasonably well in earlier stages. Considering the limitations of the LSC technique; with increase of time after application, deviations between these techniques occurred, probably due to the formation of metabolites with C-14 moieties which are not detected by the GLC technique used. Consequently the quantities of radioactivity recorded during the later part of this study did not represent the

actual amounts of C-14 acephate and methamidophos accumulated in the foliage but also included some chemical and/or metabolic conversion products containing C-14 moieties. However, their exact chemical structures await final identification followed by quantitation. It is also interesting to note that the cleaned up extract in all three experiments showed progressively lower activity with passage of time compared to the crude homogenate; the loss of activity is probably due to the retention of some highly polar C-14 labelled moieties formed from the parent material, on the silica gel used in the column cleanup. No attempt was made to characterize these polar conjugates although subsequent thin layer chromatographic (TLC) studies are planned for a later date.

As can be read from Fig. 1 - C to E, the least amount of radiolabel is absorbed (1.22 *n*Ci/g) by spruce when the FP was employed. In fact, the amount absorbed (24.22 *n*Ci/g) with TIT is about 20 times greater than with FP. With BBP, however, the uptake (2.80 *n*Ci/g) is only about 2.3 times greater than with FP. These observations are in agreement with the GLC studies. The radiolabel in all the foliar extracts decreased concomitantly from the observed maximum levels (Tables 1-3) yielding probably polar metabolites with activites at the final sampling of, 3.86, 0.28 and 0.04 *n*Ci/g for TIT, BBP and FP treatments respectively. Only the extract from TIT showed any detectable levels of acephate and methamidophos by GLC quantitation on the last day of sampling (89 days).

Autoradiography

Autoradiography of the spruce foliage (Figs. 2 to 4) also showed that the C-14 acephate was taken up and translocated acropetally into the growing and new foliage. The figures (especially Fig. 2) show that the label was not

distributed uniformly throughout the foliage at 21 days post-treatment. At this time the label was more concentrated in the apical region consisting of actively growing buds and newly flushed needles that had grown since the treatment period than the older needles confirming that the major route of translocation is acropetal from the old to the newly developing foliage. The concentration of the radiolabel in the 21-day foliar samples as illustrated in Figs. 2 to 4, depended upon the mode of application. Foliage samples from the TIT was heavily (Fig. 2) labelled due to the high levels of acephate moieties present in them, whereas the samples from the FP treatment was much lighter (Fig. 4) because of little translocation of the toxicant and its active metabolites. The foliage samples from the apical regions of a check tree was autoradiographed under similar experimental conditions and no positive response was observed.

Distribution of Acephate

The longitudinal distribution of acephate and radiolabel in various parts of the spruce tree (foliage, stem and roots) by trunk implantation technique after 21 and 89 day intervals is given in Table 4. It is evident that the chemical penetrates the treated region and is translocated primarily in the foliage and branch stem and is preferentially accumulated in the most active growth region, *i.e.*, the new foliage due to upward movement of the chemical *via* the transpiration stream. A direct transfer of the residues from the phloem to the xylem cells is indicated, as occurs with some organic substances (Stout and Hoagland 1939), pesticides (Sundaram and Sundaram 1967) and herbicides

(Crafts and Yamaguchi 1958, Radwan *et al* 1960, Sundaram 1965, Sundaram and Sundaram 1970). It is apparent that in the TIT, the toxicant being hydrophilic and mobile, migrates from the assimilate stream (phloem) to the xylem and translocated in an acropetal direction with the transpiration stream and concentrated in the aerial tree parts especially in the newly flushed foliage (Table 4). The average insecticide concentration and the radioactivity in the foliage decreased concomitantly from 2.72 ppm [(3.25 + 2.19)/2] and 19.59 *n*Ci/g [(25.71 + 13.46)/2] on the 21st day to near trace levels (*ca* 0.04 ppm) and 0.71 *n*Ci/g at the final sampling on 89th day. A similar trend is also observed in the branch samples. The observed decline in acephate by TIT may be ascribed to chemical and/or metabolic conversions although a minor contribution may be attributed to dilution from the tree growth.

Results for the BBP and FP treatments, although indicating similar distribution patters (*ca* 2% of the residue and activity levels observed in TIT) on the two sampling periods, were too low to permit quantitation or to draw any definite conclusions. The present study shows that the intensities of accumulation of acephate in different parts of the tree depends primarily upon the mode of treatment; the maximum concentration translocated is observed in TIT and only detectable levels with the other two methods. In BBP and FP treatments the bulk of the applied chemical, being moderately lipophilic, is probably adsorbed and trapped as a solid solution in the epicuticular waxes at the treated areas (bark and needles respectively) and only fractional amounts migrate to subcuticular levels which are then transported apoplastically, *i.e. via* the xylem vessels (transpiration stream) and translocated systemically to detectable levels in the acropetal direction into the primary needles

(Tables 2 and 3).

The residue levels of acephate found in samples of spruce budworm collected while feeding on untreated conifer needles from a tree 21 days after TIT, demonstrates that the chemical is translocated from the foliar tissues to the target insect in a biologically toxic state while feeding but whether the concentration is high enough to protect the tree against this insect pest is still obscure and necessitates further investigation. Under the present experimental conditions, no translocation of the intact chemical at detectable levels had occurred in the FP treatment even though *ca* 510 ppm of acephate in solution has been painted on the needles.

Metabolism of Acephate in Spruce Trees

The experiments discussed so far provided conclusive evidence of the uptake and translocation of acephate in spruce trees under normal weathering conditions existing in a forest environment; however they did not give much information about its metabolism. GLC studies of the foliar extracts from TIT showed that methamidophos was formed as a metabolite up to *ca* 10% of the observed acephate levels up to the end of 12-day post treatment period (Table 1), later on it showed a build-up in concentration up to 41 days due to the breakdown of the parent material, and then disappeared rapidly showing that the moiety was labile. A somewhat low but similar pattern was observed in BBP, whereas the concentrations of methamidophos found in FP treatment were too low to permit GLC quantitation (Table 3) or to draw meaningful correlations. The results are in good agreement with Magee (1974) who also reported that the formation of methamidophos in bean plants treated by FP were low

and less than 5% of acephate applied. Likewise, the kinetic studies of the parent material (Anonymous, 1971) show that only about 5 - 10% of the acephate degrades *via* methamidophos; the remainder degrades directly to innocuous salts. From these observations, it is evident that the probable breakdown products formed are through the splitting of the P-N, P-O and/or P-S bonds of acephate yielding P-OH acids instead of the N-C bond to form the toxic metabolite, methamidophos which would be eventually detected by GLC. According to Heath (1961), conversion of any of these bonds to the P-OH group is sufficient to detoxify the compound completely. No attempts were made in this study to characterize these conjugates and metabolites of phosphate acids except that an autoradiographic study of the residues of 21-day foliage from the TIT, after extraction, exhibited some activity (Fig. 5) increasing with time, showing that some of these polar moieties are strongly adsorbed onto/or absorbed into cellular structures of the needles and are unextractable by normal extraction procedures.

The acephate painted foliage samples were harvested 89 days after the experiment was initiated and the residue levels and activity in the extract were measured in the usual way. The amounts of acephate, methamidophos and radiolabel were respectively 86.04 ppm, 2.10 ppm and 695 *n*Ci/g. Apparently acephate disappeared rapidly in foliage from 520 ppm to 86.04 ppm (84%) in the 89 day interval. Evidently metabolism and translocation appear to be minor factors in the loss because of the low residue levels found in various tree parts (Table 4) and the major route for the dissipation of the insecticide appears to be *via* physical processes such as volatilization, photolysis and weathering as observed in other

organophosphates (Yule and Duffy 1972, Sundaram 1974, Varty and Yule 1976). A similar explanation could be offered for the loss of acephate by BBP treatment but the observed loss of the insecticide with time in TIT is probably through internal metabolic degradation precluding the large scale formation of methamidophos by the rupture of N-C bond in the acephate molecule, since such a formation could be detected and confirmed by the GLC technique used.

The persistent acephate molecules found in the spruce foliage on the final day of the experiment, being lipophilic were probably absorbed, transported and subsequently stored in cuticular waxes of spruce foliage resisting leaching, volatilization, photo and biodegradations since solubilization in plant waxes and oils would retard the above physiochemical processes (Spencer *et al* 1973). The permeation of acephate through the foliar tissues is probably achieved by cuticular pores, the lipoidal nature of the cuticle enabling the polar toxicant molecules to the cutin layer for storage (Linskens *et al* 1965). Extending this hypothesis to this study, it is possible to speculate that the acephate molecules translocated systemically in the growing foliar parts of spruce by the three modes of treatment, are also preferentially accumulated at some subcuticular levels probably between the lipophilic boundary formed between the layers of cutin and epidermis. There, it could not only be protected, at least for a shortwhile, against degradation, and dissipation, because of adsorption and dissolution in foliar lipid resins, but could also attain concentration levels, high enough to protect the spruce trees against various lepidopterous defoliators. Further indepth research is necessary to confirm some of these hypotheses.

Conclusion

The results presented in this report clearly demonstrate the acropetal translocation of acephate in spruce under the normal weathering conditions existing in a forest environment when applied at higher concentration levels as described under the described experimental conditions and substantiate the observations made earlier by Lyon (1973). The chemical appears to be a promising systemic insecticide provided ways, *i.e.*, dosage, formulation, time, mode and frequency of application, etc. could be found to allow transport and rentention of relatively high concentrations of this material in newly flushed foliage, *i.e.*, high enough to be biologically toxic to spruce budworm. However, its usefulness may be limited due to its short residual activity and low persistence (Sundaram and Hopewell 1976) under the current dosage levels (0.21 kg AI/ha) recommended (Armstrong and Nigam 1975) in spray operations. Apoplastic transportation of acephate under these operating conditions and concentration levels, would be minimal, if any, to exhibit the systemic and toxic potentials of the chemical to be effective enough to protect the tree against various lepidopterous defoliators.

SUMMARY

The uptake, translocation and metabolism of C-14 acephate insecticide (0,S-dimethyl N-acetylphosphoramidothioate) (labelled in the S-methy group) was studied in white spruce [*Picea glauca* (Moench) Voss] trees under normal weathering conditions. Three types of applications *viz.*, trunk implantation treatment (TIT), basal bark painting (BBP) and foliar painting (FP) were employed. Foliage samples were collected from the trees at various times for residue analysis by gas-liquid chromatography (GLC), liquid scintillation counting (LSC) and gross autoradiography. The insecticide was taken up and translocated apoplastically in different parts of the spruce tree and the degree of accumulation depended upon the mode of application. The residue levels and activity showed that the uptake and movement through xylem vessels were high in TIT but progressively decreased in BBP and comparatively negligible in the FP treatment. The major route of translocation of the radiolabel is acropetal, from the old to the newly developing foliage, is confirmed by autoradiography. The absorbed acephate residue in TIT was gradually lost through metabolic and chemical degradation forming small amounts (*ca* 10%) of methamidophos (0,S-dimethyl phosphoramidothioate) and other polar conjugates of the parent phosphoric acid indicating that the primary detoxification process in spruce is not through the rupture of N-C bond of the insecticide molecule. The mechanism of dissipation of the insecticide from the treated surfaces in FP and probably in BBP techniques appeared to be due to various physical factors (volatilization, weathering, photolysis etc.) rather than metabolic process. Under the experimental conditions, the material was found to be systemic but whether this potential will be exhibited in a normal operational spray program for controlling forest pests, is questionable.

ACKNOWLEDGEMENTS

The research program reported in this publication was undertaken in association with the Chevron Chemical Company to examine the persistence and systemic properties of the insecticide acephate in conifer trees for budworm control. The authors wish to express their appreciation and gratitude to Chevron Chemical Company as represented by Drs. J.A. Oakley and A.V. Dearman for supplying the C-14 acephate and providing necessary support for this research.

The authors wish to thank Mr. P. E. LeCompte for carrying out the GLC quantitation, Dr. R. Prasad and Mr. D. Travnick for their assistance during the initial stages of LSC and autoradiographic measurements, and Drs. J.A. Armstrong, C.H. Buckner, P.C. Nigam and R. Prasad for their constructive comments on the manuscript.

In conclusion, grateful appreciation is extended to Mr. G. Eades for granting permission to carry out these studies at his tree farm near Shawville, P.Q.

LITERATURE CITED

ANONYMOUS. 1971. General Data Pertinent to the Environmental Impact of Orthene®. *Chevron Chem. Co. Rept.*, p.2.

ARMSTRONG, J.A. and P.C. NIGAM. 1975. The Effectiveness of the Aerial Application of Orthene® Against Spruce Budworm at Petawawa Forest Experiment Station During 1974. *Env. Canada For. Serv. Chem. Cont. Res. Inst. Rept.* CC-X-82, 29 pp.

CRAFTS, A.S. and S. YAMAGUCHI. 1958. Comparative Tests on the Uptake and Distribution of Labelled Herbicides by *Zebrina Pendula* and *Tradesscantia Fluminesis. Hild.* 27(16): 421-454.

HEATH, D.F. 1961. "*Organophosphorus Poisons*", Pergamon Press, p. 5.

LINSKENS, H. F., W. HEINEN, and A.L. STOFFERS. 1965. Cuticula of Leaves and the Residue Problems. *Res. Rev.* 8: 136-178.

LYON, R.L. 1973. Reports from the Insecticide Evaluation Project PSW-2203. *U.S.D.A. For. Serv. Pacific South West For. Range Expt. Stn.*

MAGEE, P.S. 1974. New Aspects of Organophosphorus Pesticides. II. Structure and Bioactivity of Orthene® Insecticide Analogs. *Res. Rev. 53*: 3-18.

RADWAN, M.A., C.R. STOCKING and H. B. CURRIER. 1960. Histoautoradiographic Studies of Herbicidal Translocation. *Weeds.* 8: 657-665.

SPENCER, W.F., W.J. FARMER and M.M. CLIATH. 1973. Pesticide Volatilization. *Res. Rev.* 49: 1.

STOUT, P.R. and D.R. HOAGLAND. 1939. Upward and Lateral Movement of Salt in Certain Plants as Indicated by Radioactive Isotopes of Potassium, Sodium and Phosphorus Absorbed by Roots. *Am. J. Bot.* 25: 320-324.

SUNDARAM, A. 1965. A Preliminary Investigation of the Penetration and Translocation of 2,4,5-T in some Tropical Trees. *Weed Res.* 5: 213-225.

SUNDARAM, A. and K.M.S. SUNDARAM. 1967. Penetration, Translocation and Persistence of the Insecticide 1-Naphthyl N-Methyl Carbamate (Sevin®) and 0,0-Dimethyl-0-(3-methyl-4-Nitrophenyl) Phosphorothioate (Sumithion®) in the Coca Seedlings of *Theobroma Cacao L. Ghana Journ. Sci.* 7: 1-17.

SUNDARAM, A. and K.M.S. SUNDARAM. 1969. Studies on the Translocation Pattern, Persistence Characteristics and Metabolic Pathway of Sumithion® [0,0-Dimethyl-0-(3-Methyl-4-Nitrophenyl) Phosphorothioate] in the Cocoa Tree *Theobroma cacao L. Ghana Journ. Sci.* 9 (2): 96.

SUNDARAM, A. and K.M.S. SUNDARAM. 1970. Penetration, Translocation and Metabolism of 2, 4, 5-T in *Combretum Ghasalense. Ghana Journ. Sci.* 10 (2): 121-132.

SUNDARAM, K.M.S. 1974. Distribution and Persistence of Fenitorthion Residues in Foliage, Soil and Water in Larose Forest. *Env. Canada For. Serv. Chem. Cont. Res. Inst. Inf. Rept.* CC-X-64, 43 pp.

SUNDARAM, K.M.S. and W.W. HOPEWELL. 1976. Distribution, Persistence and Translocation of Orthene® in Spruce Trees After Simulated Aerial Spray Application. *Env. Canada For. Serv. Chem. Cont. Res. Inst. Rept.* CC-X-121, 25 pp.

VARTY, I. W. and W.N. YULE. 1976. The Persistence and Fate of Phosphamidon in a Forest Environment. *Bull. Env. Contam. and Toxicol.* 15 (3): 257.

YAMAGUCHI, S and A.S. CRAFTS. 1958. Autoradiographic Method for Studying Absorption and Translocation of Herbicides Using C-14 Labelled Compounds. *Hild.* 28: 161.

YULE, W.N. and J.R. DUFFY. 1972. The Persistence and Fate of Fenitrothion Insecticides in a Forest Environment. *Bull. Env. Contam. and Toxicol.* 8 (1): 10.