PENETRATION, TRANSLOCATION AND FATE OF C-14 AMINOCARB IN SPRUCE TREES

by

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TABLE OF CONTENTS

ABSTRACT

Gas-liquid chromatographic (GLC), liquid scintillation and autoradiographic studies on the translocation, persistence and fate of carbonyl C-14 aminocarb (4-dimethylamino-*m*-tolyl methylcarbamate) in white spruce [*Picea glauca* (Moench) Voss] trees under normal weathering conditions showed that the chemical was apoplastically accumulated by xylem transport in branches and foliage when applied by trunk implantation treatment (TIT). The major route of translocation of the radiolabel is acropetal, from the old to the newly developing foliage. The residue levels were low when applied by foliar painting (FP) and insignificant in basal bark painting (BBP). The absorbed aminocarb in TIT was gradually lost after 64 days probably due to hydroxylation forming water soluble metabolites some of which became persistent due to their incorporation into cellular structure of the foliage. The mechanism of dissipation of the insecticide from the treated surfaces in FP and probably in BBP techniques appeared primarily due to physical rather than metabolic processes. It appears that the material is weakly systemic and labile but more research is required to elucidate its mechanism of dissipation and metabolism in conifers under forest environmental conditions.

RESUME

Des études par chromatographie de partage gaz-liquide, par scintillation liquide et par autoradiographie du déplacement, de la rémanence et du devenir de l'aminocarbe (N-méthylcarbamate de dimethylamino-4 méthyl-3 phényle), marqué au ^{14}C dans son groupement carbonyle, chez l'épinette blanche (*Picea glauca* (Moench) Voss) dans des conditions météorologiques normales ont démontré que ce composé s'est accumulé de façon apoplastique dans les branches par transport dans le xylème après son injection dans le tronc. Le déplacement était acropétal, c'est-à-dire des vieilles aiguilles aux nouvelles. La concentration de résidu était faible lorsque l'application se faisait par badigeonnage des aiguilles et infime, lorsqu'elle se faisait par badigeonnage de l'écorce à la base. L'amicocarb absorbé après injection dans le tronc avait disparu graduellement au bout de 64 jours probablement par hydroxylation pour former des métabolites solubles dans l'eau dont certains sont devenus rémanents à cause de leur incorporation dans la structure cellulaire des aiguilles. Dans le cas du badigeonnage des aiguilles et probablement dans celui du badigeonnage de l'écorce à la base, la disparition de l'insecticide des surfaces traitées a senbké être davantage attribuable à des processus physiques plutôt que métaboliques. Il semble que l'aminocarb soit faiblement endothérapique et labile, mais il faudra étudier davantage son mécanisme de disparition et son métabolisme dans les conifères en milieu forestier.

INTRODUCTION

Aminocarb (4-dimethylamino-*m*-tolyl methylcarbamate) has been used in increasing amounts since 1972 for the operational control of lepidopterous defoliators in Canadian forests. It is effective against a number of insect pests, notably spruce budworm, *Choristoneura fumiferana* (Clemens) and to date *ca* 3.5 million hectares (ha) of Canadian forest has been treated with aminocarb by aircraft at an average dosage of 0.07 kg AI/ha. Initial field investigations (Sundaram *et al* 1976) have shown rapid dissipation of this carbamate in different components of the forest environment but information on its residual nature and metabolic fate in a variety of target and nontarget forest substrate species is still unavailable.

Current studies (Sundaram *et al* 1977) at this Institute have indicated that the uptake, distribution, persistence and ultimate fate of insecticides in conifers depended to some extent on their mode of application. The present study was designed to confirm this observation as well as to determine and compare the translocation patterns and metabolic fate of aminocarb in white spruce, *Picea glauca* (Moench), under normal weathering conditions of a forest environment by applying a solution of aminocarb in DMSO (dimethyl sulphoxide) to the trees in three different ways, *i.e.*, (1) by direct injection into the tree trunk, (2) by painting on the basal bark and (3) by direct application to the needle surface. Using the gas-liquid chromatographic (GLC) technique to quantitate intact aminocarb molecules and established radiotracer methods such as liquid scintillation counting (LSC) and autoradiography, to locate the radiolabels, an attempt was made in this study to elucidate the mechanism of penetration, translocation and stability of aminocarb in white spruce.

MATERIALS AND METHODS

Chemicals and Solvents

Analytical grade carbonyl C-14 labelled aminocarb with specific activity (S.A.) 14.35 millicurie (mCi) per millimole (mmole) and nonlabelled aminocarb standard were supplied by courtesy of the Chemagro Chemical Company, Kansas City, Missouri. Purity was confirmed by liquid scintillating counting (LSC) and gas-liquid chromatography (GLC). All solvents used were either pesticide grade or freshly distilled in glass. The purity of the chemicals used in the study were in accordance with the American Chemical Society specifications. Anhydrous sodium sulphate used was of reagent grade heated at 200°C overnight and stored in airtight glass bottles.

Experimental Site

The study was carried out during the early part of the summer in 1976 on a tree farm near Shawville, P.Q. Four spruce trees (A1 ... A4) of uniform size and shape (*ca* 4 m in height and 9 cm in diameter at base) with abundant foliage were selected at random *ca* 25 m apart. Trees A1, A2 and A3 were treated with the insecticide and tree A4 served as the untreated check.

Insecticide Treatment and Sampling

A mixture of pure nonlabelled and C14-labelled aminocarb was dissolved in DMSO for application. Solutions were prepared so that 15 ml contained 9.3 mg (0.045 mmole) of the labelled [specific activity (S.A.) 14.35 millicuries (mCi) per millimole (mmole)] and 450.7 mg (2.167 mmole) of the nonlabelled aminocarb insecticide in DMSO solvent. Each of the three trees received 5 ml of DMSO solution containing 0.736 mmole of aminocarb with S.A. 0.291 mCi/mmole.

Three types of application were employed, *viz.*, trunk implantation technique (TIT, tree A1), basal bark painting (BBP, tree A2) and foliar painting (FP, tree A3). Detailed descriptions of the three techniques used in the present study are given in a recent publication (Sundaram *et al* 1977) and for the sake of brevity, it is not repeated here.

Thirteen 50 g foliage samples were collected for residue analysis from the four trees at various intervals from 1 day post-treatment up to 89 days as described elsewhere (Sundaram *et al loc. cit.*). Pieces of bark, branch stems, new and old foliage and root samples were also collected from different parts of the trees at 21 and 89 days post-treatment to assess the distribution pattern of aminocarb in them.

Extraction and GLC Analysis

The foliage samples were ground in a Hobart machine, mixed thoroughly and 20 g aliquots were used in the residue analysis using the methodology reported by Sundaram *et al* (1976) except that the derivatization procedure was omitted. The analytical steps included solvent extraction, partitioning, cleanup, and final quantitation using a Tracor 550 gas chromatograph fitted with a Hall detector (Model 310). The complete procedure is given as an outline in Fig. 1.

The gas chromatographic method of determining aminocarb using a Hall detector in nitrogen mode has been developed recently at this laboratory. The operating parameters are given in Table 1.

The gas chromatograph was standardized on the same day as the foliar extracts were analysed by injecting freshly prepared aliquots (2 - 5 ul) of aminocarb standards in benzene, measuring the peak heights and constructing a calibration curve by plotting peak heights (cm) against the nanograms of aminocarb injected. Quantitative results of the extracted samples were obtained by measuring the peak height after injection (2 - 4 ul), under the same operating conditions, and reading the concentration from the prepared calibration curve. The average recovery of aminocarb from 20 g aliquots of spiked spruce foliage at 1.0 to 2.0 ppm levels were 85% with a coefficient of variation 5. The GLC method is reasonably sensitive with minimum interference and is useful for the analysis of aminocarb in conifers at a minimum 0.2 ppm concentration level.

Liquid Scintillation Counting

A Beckman LS-100 liquid scintillation counter was used. Aqueous samples were counted in 10 ml of cocktail D [0.5% diphenyloxazole (PPO) and 10% naphtalene in dioxane]. Nonaqueous samples were assayed in 10 ml toluene containing 0.5% PPO. Quench corrections were made as described by Sundaram *et al* (1977) and all counts were corrected for the background.

Autoradiography

The plant materials (foliage, branch, stem, roots, etc.) were dried in a press mounted with absorbent paper. Further preparation 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

Gas Chromatographic Parameters

GC	:	Tracer (Model 550)
Detector	:	Hall (Model 310) (Nitrogen mode)
Column	:	1.83 m x 6 mm O.D. Pyrex glass packed with 6% SE 30 on Chromosorb W, H.P., mesh 80/100
Solvent	:	15% Isopropanol in distilled water
Rate	:	1 mℓ/min
Temp (^{o}C)		
Oven	:	210
Outlet	:	280
Inlet	:	215
Carrier gas (mℓ/min)	:	He, 100
Gas flow (mℓ/min)	:	H_2, 150
Attenuation	:	2
Range	:	1
Recorder	:	Linear instruments, 1 mV
Chart speed (cm/hr)	:	76
Retention time (min)	:	3.25

Table II

Concentration and Radioactivity of Aminocarb in Spruce Foliage After Trunk Injection Treatment

Days After Application*	Aminocarb Concn. (ppm)	Specific Activity (*n*Ci/g)			
		CH_3CN Extract	Ether/$CHCl_3$ Extract	Aqueous Phase	Benzene Extract
1	0.05	0.23	0.02	0.07	0.15
3	0.72	0.91	0.06	0.18	0.66
5	1.26	1.62	0.04	0.30	1.40
8	1.49	2.29	0.12	0.45	1.52
12	1.63	2.76	0.33	0.40	1.85
16	2.05	2.91	0.29	0.59	2.20
21	2.28	2.78	0.36	0.72	2.15
27	2.16	3.36	0.41	0.68	2.20
34	2.25	2.99	0.39	0.76	2.14
41	1.39	2.64	0.22	1.02	1.65
50	0.89	2.71	0.29	1.42	1.05
64	0.42	1.89	0.14	1.02	0.72
89	N.D.	1.41	0.19	0.78	0.49

N.D. Not detected

Minimum detection limit (MDL) 0.20 ppm. Traces (T) represent below this limit.

* Date of application: May 26, 1976.

Table III

Concentration and Radioactivity of Aminocarb in Spruce Foliage After Basal Bark Painting Treatment

Days After Application	Aminocarb Concn. (ppm)	Specific Activity (*n*Ci/g)	
		CH_3CN Extract	Benzene Extract
1	N.D.	0.07	0.02
3	N.D.	0.10	0.04
5	T	0.18	0.10
8	T	0.20	0.10
12	T	0.20	0.13
16	T	0.19	0.09
21	T	0.21	0.08
27	N.D.	0.22	0.07
34	N.D.	0.13	0.04
41	N.D.	0.14	0.05
50	N.D.	0.09	0.04
64	N.D.	0.09	0.03
89	N.D.	0.09	0.03

See the footnotes in Table 1

Table IV

Concentration and Radioactivity of Aminocarb in Untreated Spruce Foliage After Foliar Painting Treatment

Days After Application	Aminocarb Concn. (ppm)	Specific Activity (nCi/g)	
		CH_3CN Extract	Benzene Extract
1	T	0.18	0.10
3	0.20	0.25	0.12
5	0.23	0.28	0.11
8	0.20	0.26	0.16
12	0.20	0.27	0.09
16	T	0.29	0.14
21	T	0.20	0.15
27	N.D.	0.12	0.08
34	N.D.	0.14	0.04
41	N.D.	0.07	0.02
50	N.D.	0.07	0.03
64	N.D.	0.07	0.02
89	N.D.	0.05	0.02

See the footnotes in Table 1.

Table V

Concentration and Radioactivity of Aminocarb in Spruce Foliage After Trunk Injection Treatment

Days After Application	Aminocarb Concn. (ppm)	Specific Activity (*n*Ci/g) in terms of ppm of Aminocarb			
		CH_3CN Extract	Ether/$CHCl_3$ Extract	Aqueous Phase	Benzene Extract
1	0.05	0.16	0.01	0.05	0.11
3	0.72	0.65	0.04	0.13	0.40
5	1.26	1.16	0.03	0.21	1.00
8	1.49	1.64	0.09	0.32	1.09
12	1.63	1.97	0.24	0.29	1.32
16	2.05	2.08	0.21	0.42	1.57
21	2.28	1.99	0.26	0.51	1.54
27	2.16	2.40	0.29	0.49	1.57
34	2.25	2.14	0.28	0.54	1.53
41	1.39	1.89	0.16	0.73	1.18
50	0.89	1.94	0.21	1.01	0.75
64	0.42	1.35	0.10	0.73	0.51
89	N.D.	1.01	0.14	0.56	0.35

See the footnotes in Table II.

Table VI

Longitudinal Translocation of Aminocarb in Spruce Tree*

After 21-Day Trunk Injection Treatment

Sample	Aminocarb Concn. (ppm)	Specific Activity (*n*Ci/g) of CH_3CN Fraction
New Foliage	2.49	3.18
Old Foliage	1.97	2.79
Branch**	2.15	2.88
Roots	T	0.11
Budworms†	N.D.	-

See the footnotes in Table II

* The data represent the average of two replication. Variability of the values did not exceed ± 10%.

** 75 Cm samples from midcrown excluding foliage.

† Collected from midcrown twigs.

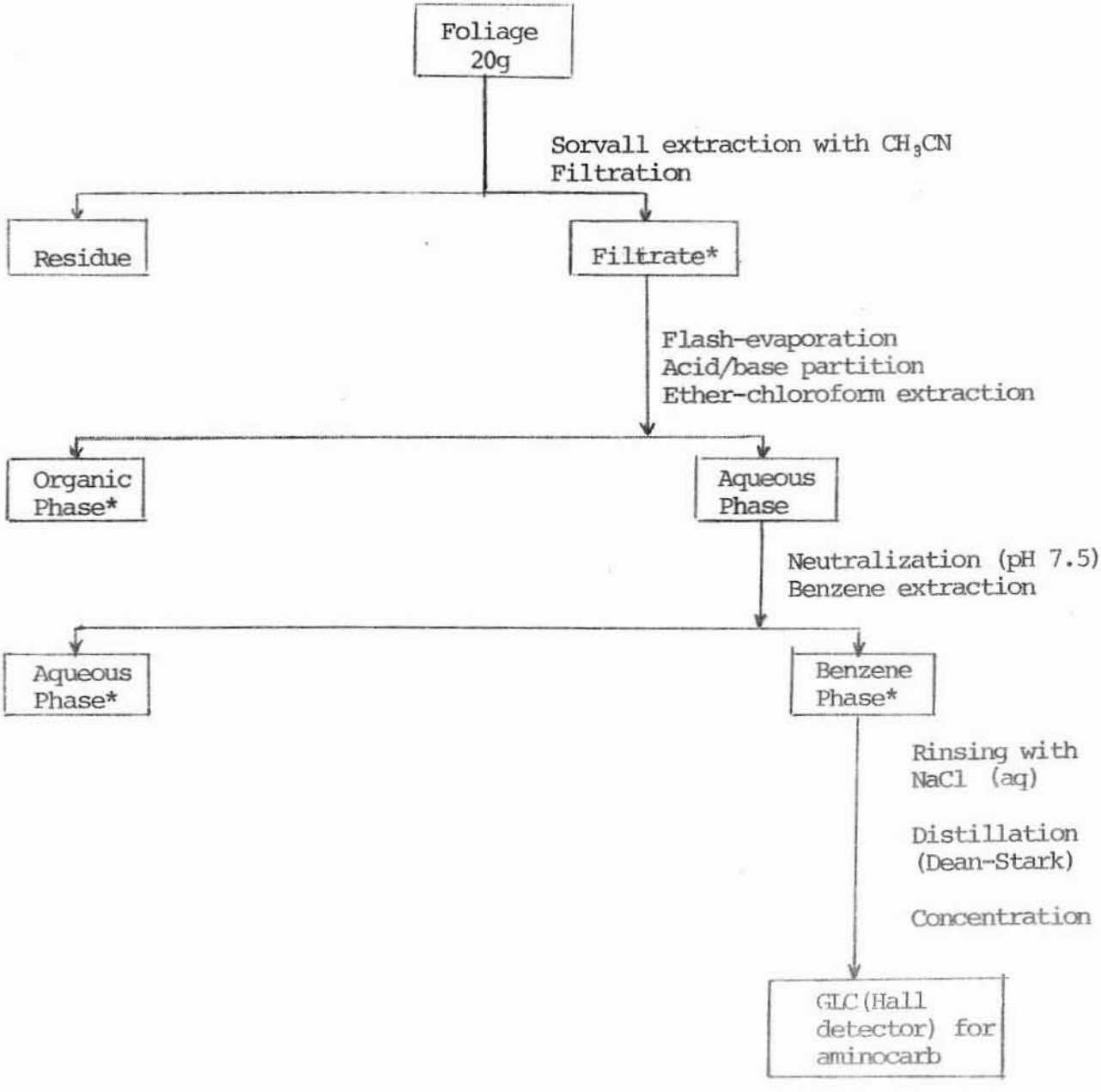

Fig. 1 Schematic representation of extraction and analysis of aminocarb in spruce foliage.

* Samples used in liquid scintillation counting. See Tables II to IV.

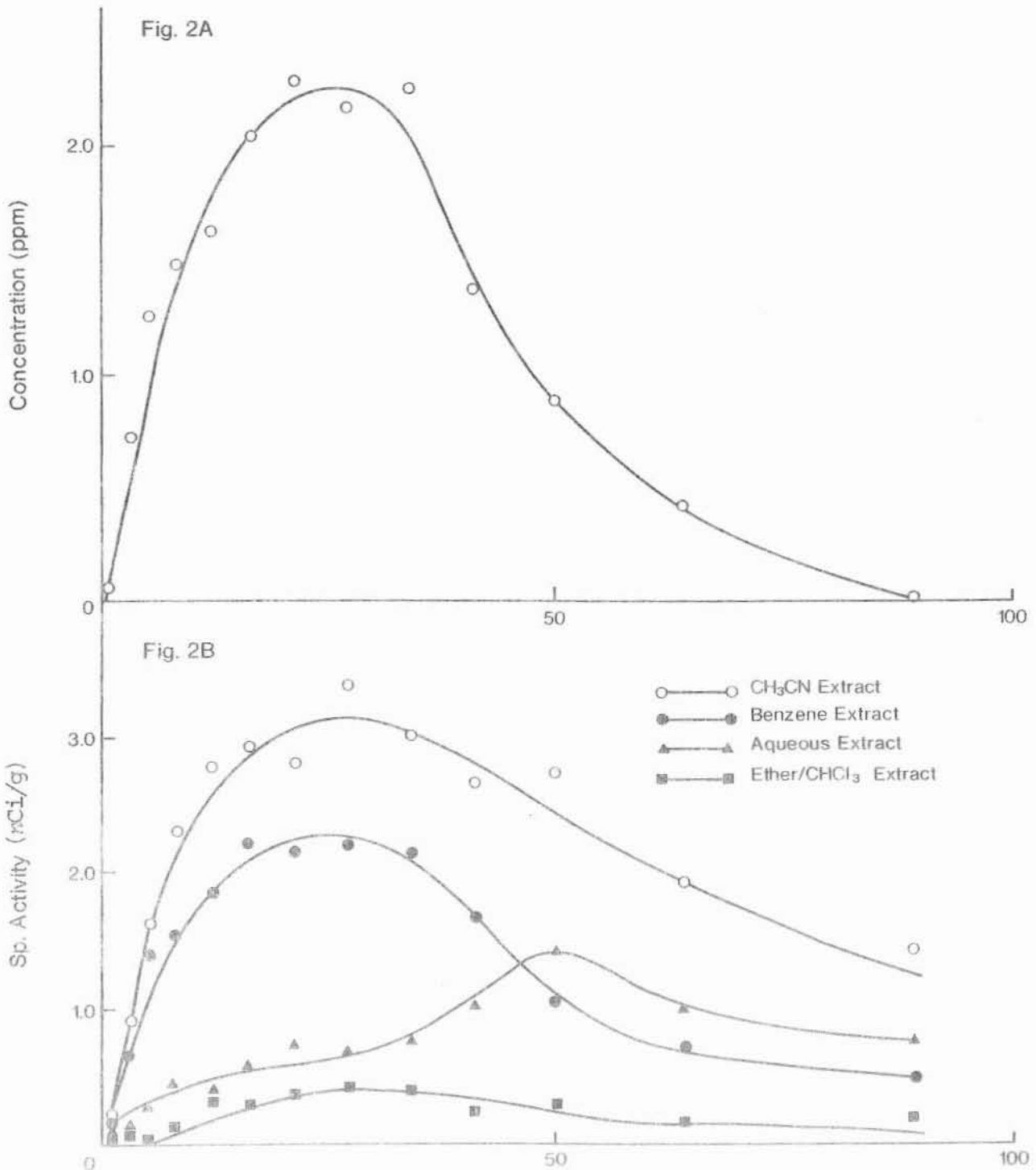

Fig. 2. Distribution of aminocarb (Fig. 2A) and radioactivity (Fig. 2B) in foliar extracts of spruce tree after TTT.

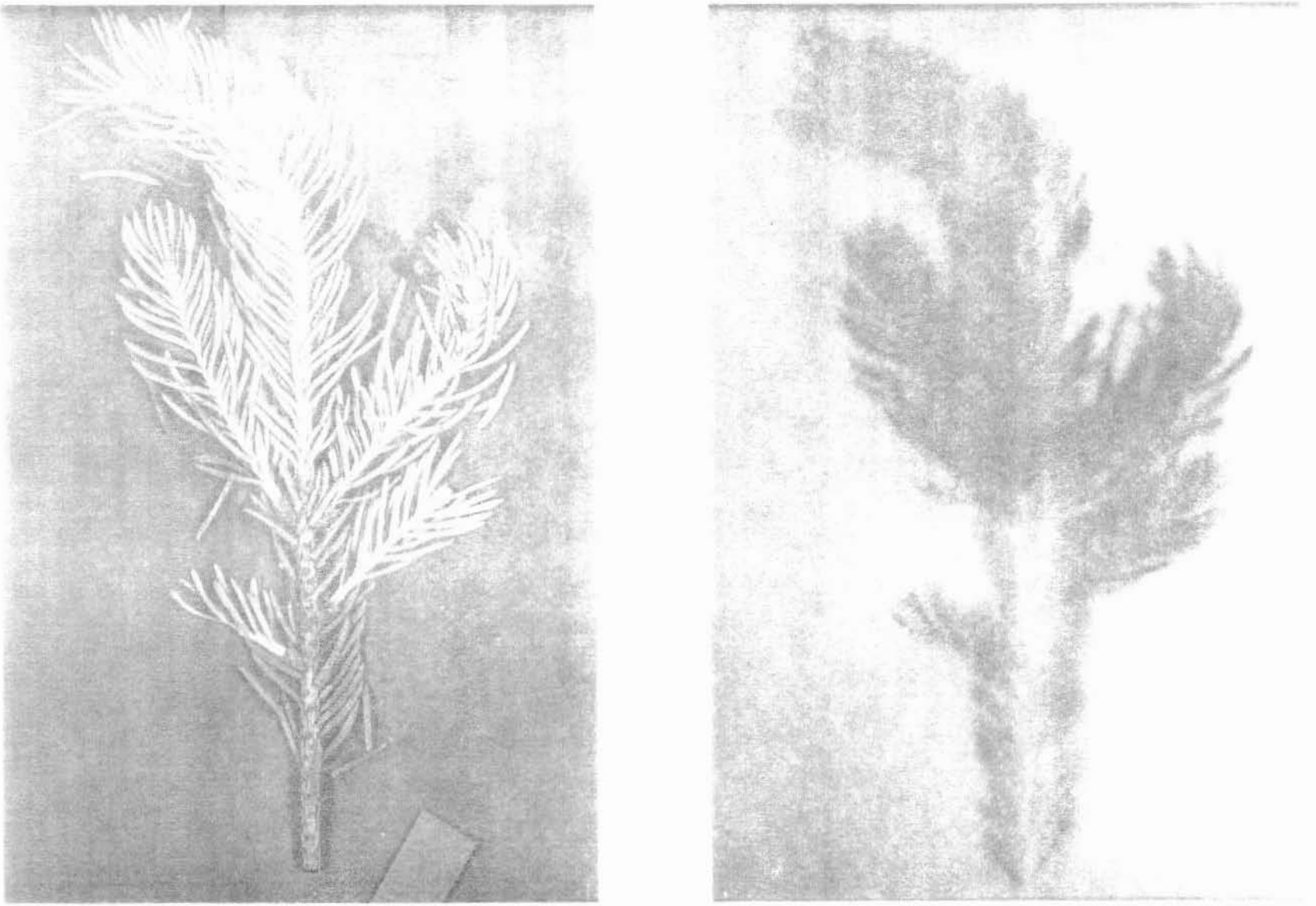

Fig. 3. Autoradiograph of spruce foliage sampled 21-days after treatment with C-14 aminocarb by TIT. Pressed foliage on the left and its autoradiograph on the right.

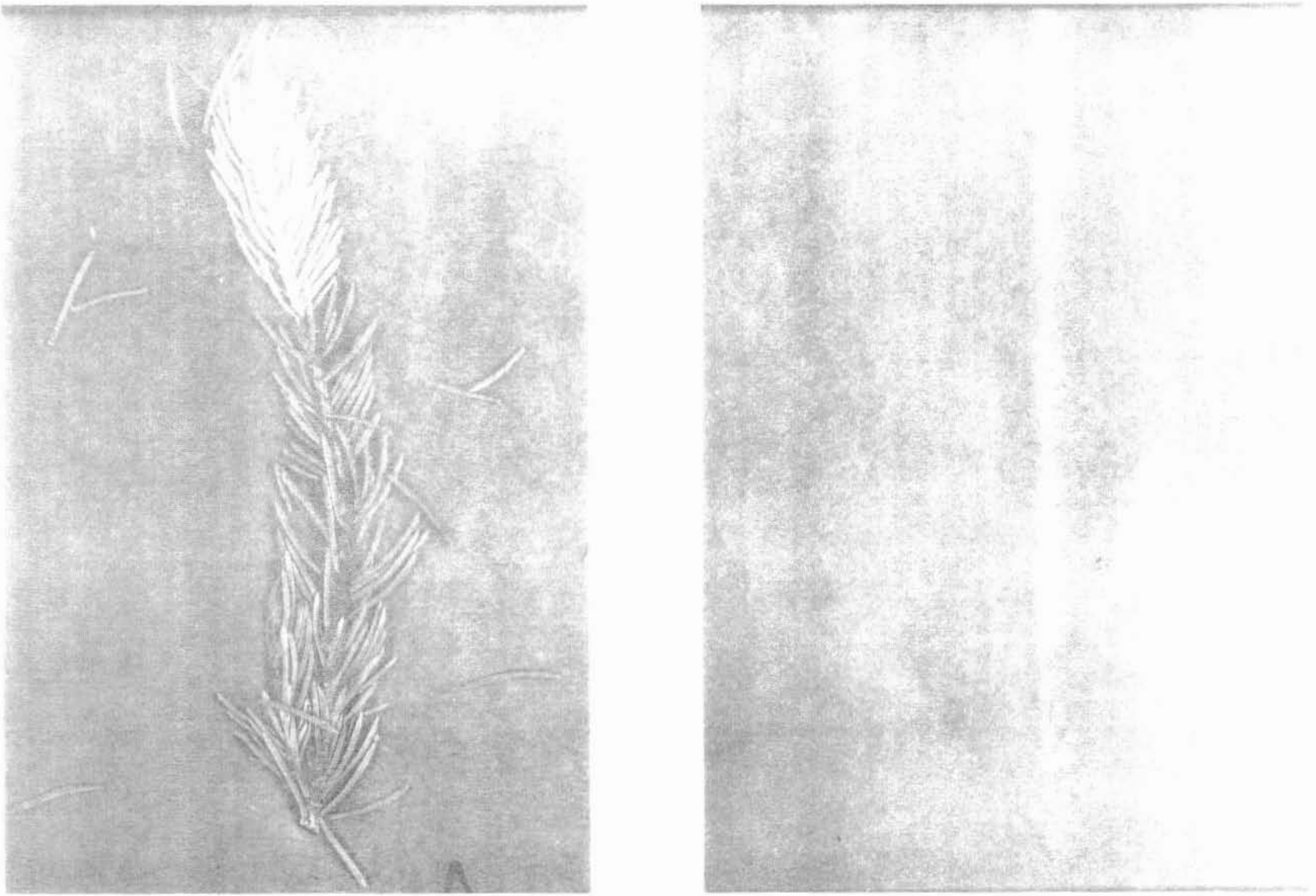

Fig. 4. Autoradiograph of 21-day untreated spruce foliage after foliar painting treatment using C-14 aminocarb.

(a) (b) (c) (d)

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.

RESULTS AND DISCUSSION

Translocation and Persistence of Aminocarb in Spruce Foliage

Tables II, III, and IV give aminocarb concentrations found in different foliar extracts of the three treated trees. The foliage samples analysed from the check tree showed no detectable level *i.e.*, 0.20 ppm, of the insecticide and are not recorded here. The insecticide concentrations are expressed in units of ppm (parts per million) for fresh weight of the foliage and the minimum detection limit that has been achieved during this study by the use of a Hall detector was 0.20 ppm.

From the results it is evident that absorption and translocation of aminocarb into the foliage is very high in TIT compared to BBP and FP treatments. A gradual increase to a maximum of 2.28 ppm in foliage occurred in TIT at 21-day post treatment. After this period, the translocated insecticide gradually decreased to below the detection limit (0.20 ppm) after 64 days. A somewhat similar but diminished translocation pattern was observed in foliar painting, showing a peak concentration of 0.23 ppm of aminocarb (10 times lower than the TIT maximum) after 5 days due to rapid cuticular penetration but decreased movement probably due to absorption and dissolution of the material in cuticular waxes of the needles. The little that is translocated to the untreated foliage dissipated rapidly below the detection limit within 16 days (Table IV). The amount translocated was much smaller (0.23 ppm) compared to the trunk injection treatment (2.28 ppm) even though *ca* 510 ppm of the insecticide was painted on the foliage (*i.e.* 153 mg of aminocarb on *ca* 300 g of needles). However this is not surprising since a considerable distance

is involved in movement of the insecticide molecules from the brushed foliage to branch stem, to trunk, and dispersed an equal distance *via* branch stem to foliage. With BBP, however, no detectable aminocarb residues were present in the foliage (Table III). Either the insecticide did not penetrate the corky outer bark layers (cortex) of the tree in sufficient quantities due to inclusion in the resinous wax of the bark, or the molecule had undergone rapid structural alterations by biochemical processes within the tree.

The recovery and distribution of aminocarb in spruce trees as determined by the GLC technique clearly demonstrate that the uptake, translocation and persistence of the material is related to the method of application. Although trunk injection and basal bark painting methods are not normal routes of entry of an insecticide into conifers under aerial spray operations, these experiments have given important information on absorption under different experimental conditions. Especially, TIT gave some indication of the persistence of aminocarb under *in vivo* conditions in spruce trees. Foliar absorption is usually considered the normal route of a systemic insecticide entry into trees under aerial spray conditions. The residue data obtained in FP treatment did not confirm the systemic action of aminocarb. Therefore it could be inferred that in aerial application of the insecticide for budworm control, especially at operational dose levels of 0.07 kg AI/ha, aminocarb is not absorbed and translocated into other foliar tissues from surface deposits but may be absorbed locally.

Distribution of Radiolabel in Spruce Foliage

Tables II and V show the distribution of radioactivities in organic and aqueous fractions obtained from spruce needles at various

intervals after TIT, BBP and FP. From the pattern of radioactivities observed, there is strong evidence for both absorption and upward translocation of aminocarb in all the three treated spruce trees but the rate and the amount of movement, as pointed out earlier, varied with the mode of application. Analyses of the acetonitrile extracts indicated that the maximum distribution of the radiolabel had occurred on day 27 for TIT and BBP and on day 16 for FP treatments since the specific activities (μCi/g) showed maxima on these days and the numerical values recorded (Tables II to IV) were respectively 3.36, 0.22 and 0.29.

In plants, two tissue systems are responsible for rapid movement of materials - the xylem and the phloem. In the xylem movement is in general upwards from roots to shoots and leaves in the transpiration stream. Phloem movement is from foliage to roots in the assimilate stream. Currently from the region of TIT, the insecticide should have entered the xylem conduits in the stem, moved upwards in the transpiration stream and translocated in the growing foliage. Probably in BBP, the chemical did not penetrate the epidermis, cortex and phloem tubes in sufficient amounts to enter the xylem vessels for upward movement thus accounting for the low radioactivity observed in the foliar extracts (Table III). Similarly with FP, movement of C-14 from the application site was relatively low. The measured activity was also low, consequently either the chemical did not penetrate the needle surface in sufficient amounts to be mobile, or it was immediately lost by various physiochemical processes including volatilization and hydrolysis.

Assuming that the bulk of the radiolabels present in the foliage are soluble in acetonitrile, the measured specific activities of this extract (Table II) would give an indication of the translocated radiolabel

in the foliage by TIT. Degree of uptake, foliar accumulation and dissipation of radioactivity (Fig. 2B) in relation to the age of application, corresponded closely with the GLC studies and exhibited similar maxima approximately on the same day (day 27) as observed in gas chromatographic evaluation (Fig. 2A). By converting the recorded radioactivity of acetonitrile extract in nanocuries (nCi) to dpm (1 nCi = 2.2×10^3 dpm) and calculating the radiolabels in terms of aminocarb by using its specific activity, the concentration levels in ppm units were obtained for the LSC measurements and are given in Table V. Comparing the concentration levels obtained from both techniques, it could be seen that there was a general agreement up to 34 days post-treatment (Table V - columns 2 and 3). After that period deviations occurred between these two techniques probably due to the formation of metabolites with C-14 moieties which were detected by the LSC but not 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 aminocarb accumulated in the foliage but also included some chemical and/or metabolic conversion products containing C-14 moieties. So far no attempt has been made to identify and quantitate these degradation products. No such comparisons could be made for the BBP and FP techniques due to negligible translocation of the insecticide in foliage observed for these two methods.

Longitudinal Translocation of Aminocarb in Spruce Tree

The amounts of aminocarb and radioactivity remaining in the 21-day tissues of foliar (old and new), midcrown branches and roots after TIT are given in Table VI and the data strengthen the suppositon of gradual absorption and accumulation of the chemical in the aerial parts (shoots and thin branches) of the treated tree through xylem transport. The foliar

residues obtained are consistent with the earlier evidence (Sundaram *et al* 1977 and the references cited therein) that the insecticide and C-14 moieties are preferentially translocated acropetally to newly developing needles compared to the older foliage. The newly flushed foliage up to 3 weeks after treatment contained *ca* 25% and 14% more of aminocarb and radiolabel respectively compared to the older foliage. Preferential accumulation of the toxicant was also found in the thin branches (2.15 ppm) compared to the grownup needles (1.97 ppm). On the other hand root samples analysed showed negligible concentration and activity of aminocarb and its metabolites indicating an insignificant downward translocation to the roots *via* the phloem. However, although the needles contained reasonable amounts of aminocarb, the spruce budworm collected from the foliage while feeding did not contain detectable levels of the chemical indicating that it was not translocated from the foliar tissues to the target insect in a biologically toxic form. Therefore, translocation of aminocarb and its degradation products and/or metabolites was apparently limited to the xylem which is consistent with the earlier evidence obtained (Sundaram *et al* 1977) for other insecticides at this Institute.

Autoradiography

Autoradiography also indicated that absorption and translocation of C14-aminocarb occurred reasonably fast. For example, C14-aminocarb was absorbed from the injected area of the tree trunk and translocated acropetally into the growing leaf within 21 days after treatment, and a movement of C-14 toward the flushed foliage was visible in the autoradiogram (Fig. 3). Unlike the TIT sample, the autoradiogram of the foliage from the FP treatment contained only a very low amount of translocated C-14 (Fig. 4). However the foliage from BBP did not contain significant amounts of C-14 products to respond to the X-ray film. Likewise the foliage samples from the check tree showed negative response.

Metabolism of Aminocarb

The results on distribution and degradation of aminocarb found in spruce tree after TIT did not yield much information about the metabolism of the insecticide and any such interpretation will be difficult, complex and speculative since no attempt has been made at present to isolate, identify and quantitate all possible metabolites formed. Aminocarb was gradually taken up and translocated into foliage at up to 2.28 ppm after 21 days which then degraded rather slowly, and essentially none was detected on the final day (day 89) of sampling. During these processes, the material has been degraded either chemically and/or enzymatically to form organo (CH_3CN, ether-chloroform and benzene) and water-soluble products. Figure 2B shows the distribution of radiolabels in different fractions during the experimental period. The acetonitrile-soluble C-14 content, representing the total extractable radioactivity, in the leaf tissue, increased with time and reached a peak concentration on day 27 and slowly diminished. The general discernible distribution pattern of the curve is similar to Fig. 2A in which aminocarb concentration (ppm) is plotted against time. Likewise somewhat similar variations were observed (Fig. 2B) for the benzene-soluble fraction representing at least theoretically the total intact aminocarb present, as a major component in the foliar tissues, analysed on each sampling day. However, variations did occur as could be seen between the values in columns 2 and 6 of Table V probably due to the formation of varying amounts of benzene soluble radiolabelled metabolites explaining the erratic results recorded. The ether/chloroform soluble C-14 fraction showed a slight increase and then remained almost constant whereas the water-soluble fraction increased significantly with time and with the decrease of aminocarb concentration, indicating that the carbamate

was converted in the plant tissues into water-soluble metabolites. Our observations are in good agreement with those of Kuhr and Casida (1967) and Abdel-Wahab *et al* (1966) who while studying the metabolism of injected aminocarb in bean plants also found water soluble metabolites formed by hydroxylation (chemical or enzymatic) on the N-methyl group, on the ring or on a ring substitutent with C-14 carbamate group intact followed by conjugation of the hydroxylated carbamate mainly as persistent glycosides. No attempts were made in this study to characterize these conjugates except that an autoradiographic study of the foliar residues after acetonitrile extraction, exhibited some radioactivity (Fig. 5) increasing with time, showing that some of these moieties are strongly adsorbed onto and/or absorbed and incorporated into cellular structures of the needles and are unextractable by normal extraction procedures fortifying the above findings. Further experimentation is necessary to confirm these observations.

The stability or persistence of aminocarb painted on the foliage was examined by collecting the treated foliage and analysing for the residues present in it on the last day (89 days post-treatment) of the experiment. Only about 13% of the initial deposit level (510 ppm) was found in the foliar tissues. The rapid loss of the chemical from the treated leaf surface could be due to physical processes such as volatilization, hydrolysis, weathering and photolysis. Likewise no significant change in the mechanism of loss is apparent for aminocarb painted on the basal bark of the spruce tree. The conifers, especially spruce, readily degrade aminocarb either physicochemically or enzymatically once it is injected into the tree or painted on it and the rate of dissipation and the nature of metabolites formed depend upon various environmental and biochemical factors. Certainly more research is necessary to elucidate the mechanism of dissipation and metabolism

of aminocarb from conifers under normal weathering conditions of a forest environment.

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