FATE OF FENITROTHION IN FOREST TREES

VI. SOME FACTORS AFFECTING RATE OF DISSIPATION FROM BALSAM FIR AND WHITE SPRUCE

by

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RÉSUMÉ

On a entrepris une investigation afin de déterminer le destin et la rémanence du fénitrothion appliqué, en serre, sur des semis d'épinette blanche et de sapin baumier âgés de trois ans. L'insecticide a rapidement disparu de la surface des tissus du conifère, alors que les résidus absorbés se sont révélés plus rémanents. Une étude effectuée, in vitro, sur des surfaces de verre a montré que la volatilisation était probablement le principal phénoène expliquant la rapide disparition du pesticide. On conclu que les résidus absorbes étaient plus rémanents en raison de leur faible volatilité.

On a pensé que la plus grande capacité d'absorption du pesticide dont fait preuve le sapin explique que le fenitrothion pulvérisé sur ce dernier soit plus rémanent que chez l'épinette. L'insecticide s'est accumulé dans les nouvelles aiguilles des sapins, et ceci a conduit à l'hypothése selon laquelle la plus grande protection contre la todeuse des bourgeons de l'épinette, dont jouit le sapin traité sur le terrain même par la pulvérisation de fénitrothion, provient de l'action endothérapique de ce dernier.

INTRODUCTION

Increasing concern over environmental contamination with pesticides resulted in curtailment of the use of several persistent chlorinated insecticides and their replacement with more selective and short-lived organophosphorous chemicals. Fenitrothion, (0,0-dimethyl-0- (3 methyl-4-nitrophenyl)-phosphorothioate) has been used since 1967, in place of DDT for operational control of lepidopterous defoliators in Canadian forests (Fettes, 1968). By the end of 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) (Roberts, 1975). With this large scale spray program still continuing it is important to determine the ultimate fate and environmental impact of this pesticide.

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 a rate of approximately 4 oz/acre. They reported that 50% of the initial dose was lost by foliage within 4 days; 70-85% within two weeks, and 10% persisted for at least ten months. The rapid disappearance of fenitrothion from various crop plants has been reported by other workers (Myamoto and Sato, 1965; Bowman and Beroza, 1969, Leuck and Bowman, 1969). Sundaram *et al* (1975) did not observe rapid loss of fenitrothion from fir or spruce foliage in the greenhouse, however, this finding may not be applicable to the field situation since the amount of pesticide applied was far greater than that normally deposited in the field.

The present study was undertaken to determine specifically the mode of penetration, translocation, and fate of fenitrothion applied to young (4 year old) seedlings of white spruce (*Picea glauca* (Moench) Voss) and balsam fir (*Abies balsamea* (L.) Mill) under greenhouse conditions. In order that results obtained would be relevant, precautions were taken to approximate as closely as possible those conditions present in the field situation.

The in vitro fate of fenitrothion was investigated in a separate study to determine the importance of purely physical processes. This was conducted employing glass petri dishes as recipients of the fenitrothion formulation.

MATERIALS AND METHODS

(1) Tree Seedlings and Culture Conditions

Four year old seedlings of white spruce and balsam fir were collected in the fall of 1974 from the Kemptville and Petawawa nurseries. These were held in the greenhouse for approximately two months until both species had flushed out. Twelve healthy specimens of each of the two 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 with fluorescent "daylight" tubes supplying an illumination of about 200 lux.

(2) Chemicals and Solvents

Purified samples of fenitrothion and its various metabolites were obtained from Agriculture Canada.
Purity was confirmed by gas liquid (GLC) and thin layer chromatography (TLC). Detailed methods for purification of fenitrothion and

synthesis of metabolites have recently been described by Hallett *et al* (1974). The solvents used for extraction purposes were glass distilled. A water emulsion of the insecticide was made up as a field formulation of 10% fenitrothion, 1% Arotex 3470, 1% Atlox 3409, and 88% distilled water v/v.

(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 (Roberts, 1975). To account for the dilutory effect of air dispersal, the emulsion was diluted 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[1], were applied to the branches either by painting or spraying technique. Three replicates of each of the two conifer species 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 with the aid of a small 1/8" brush (Grumbacher # 4116) 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). To prevent contamination, plastic sheeting was used to cover the soil in the pots.

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[1] Subsequent analysis determined that the higher concentration employed was actually 168 ppm. This value was used for calculation purposes (See Page 8).

(b) In vitro Glass Surface Study

For the glass surface study, 5 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°C and 50% relative humidity.

(4) Sampling and Extraction Procedure

Separate samples of newly flushed foliage (N.F.), old foliage (O.F.), and stem tissue (S.T.), were individually weighed, placed in plastic "roll-top" bags and frozen in liquid N_2 before being stored in a freezer. An extraction method was employed which gave full recovery of the pesticide from "spiked" samples and that yielded extracts sufficiently "clean" for GLC analysis. Each conifer tissue sample (1-3 gm), was 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 acetone for analysis. The tissue was then extracted twice with ethyl acetate (100 ml each time) in a polytron sonicator (model # PP1020) and the extracts were filtered through Celite 545, and concentrated to approximately 25 ml. A modification of the method employed by Yule and Duffy, 1972, was used for charcoal column preparation. Briefly, a glass column (i.d. 22 mm) fitted with a sintered glass disc was dry packed successively under vacuum with 1" Celite 545, 70 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 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 eluant was concentrated to near dryness and brought up to 10 ml 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 (50ml each) of ethyl acetate. These washings were separately concentrated to near dryness and brought up to 10 ml acetone for analysis.

(5) Chromatographic Analysis

(a) GLC - Samples were analysed with a Pye model 104 gas chromatography (GC) equipped with an alkali flame ionization detector (AFID). The glass column (1.8 m x 4mm (i.d.)) used, contained 3% SE30 Ultraphase on Chromosorb W (H.P.); 80-100 mesh. Column temperature was 200°C, nitrogen flow 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 standard injections to calculate the ppm of fenitrothion in the tissue. (i.e. µg/gm tissue fresh wt.).

(b) TLC - The enzyme inhibition technique described by Mendoza (1972) using extract of steer liver homogenate as the spray reagent, was used to develop silica gel TLC plates run in a solvent system of 1:3 ethyl acetate and cyclohexane.

RESULTS

Table 1. lists the concentration of fenitrothion in each of the samples analysed. 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 $\frac{\text{ppm (E)}}{\text{ppm (W)} - \text{ppm (E)}} \times 100$ 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 tissue).

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 the dark conditions.

TABLE 1

G.C. Analysis of Fenitrothion Treated Conifer Tissue

SAMPLE		PPM		NET PPM		% ABS		TISSUE WT. (gm)		FEN'N (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

TABLE 1 Cont'd

G.C. Analysis of Fenitrothion Treated Conifer Tissue

SAMPLE		PPM		NET PPM		% ABS		TISSUE WT. (gm)		FEN'N (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 (corr'd 168 ppm)
4 - FIR; 200 ppm (168 ppm)

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

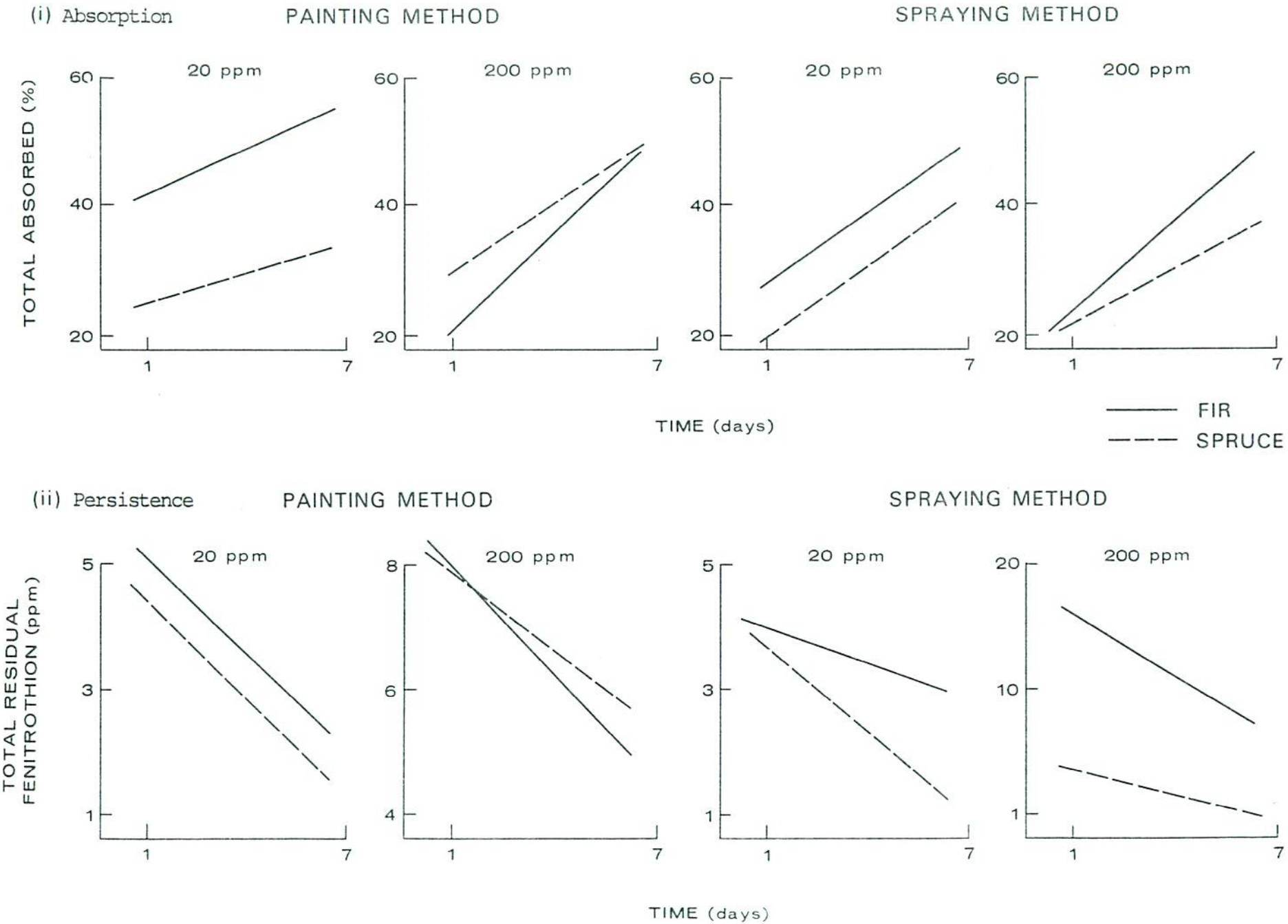

FIG. 1. Absorption and Persistence of Fenitrothion in Conifers

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.

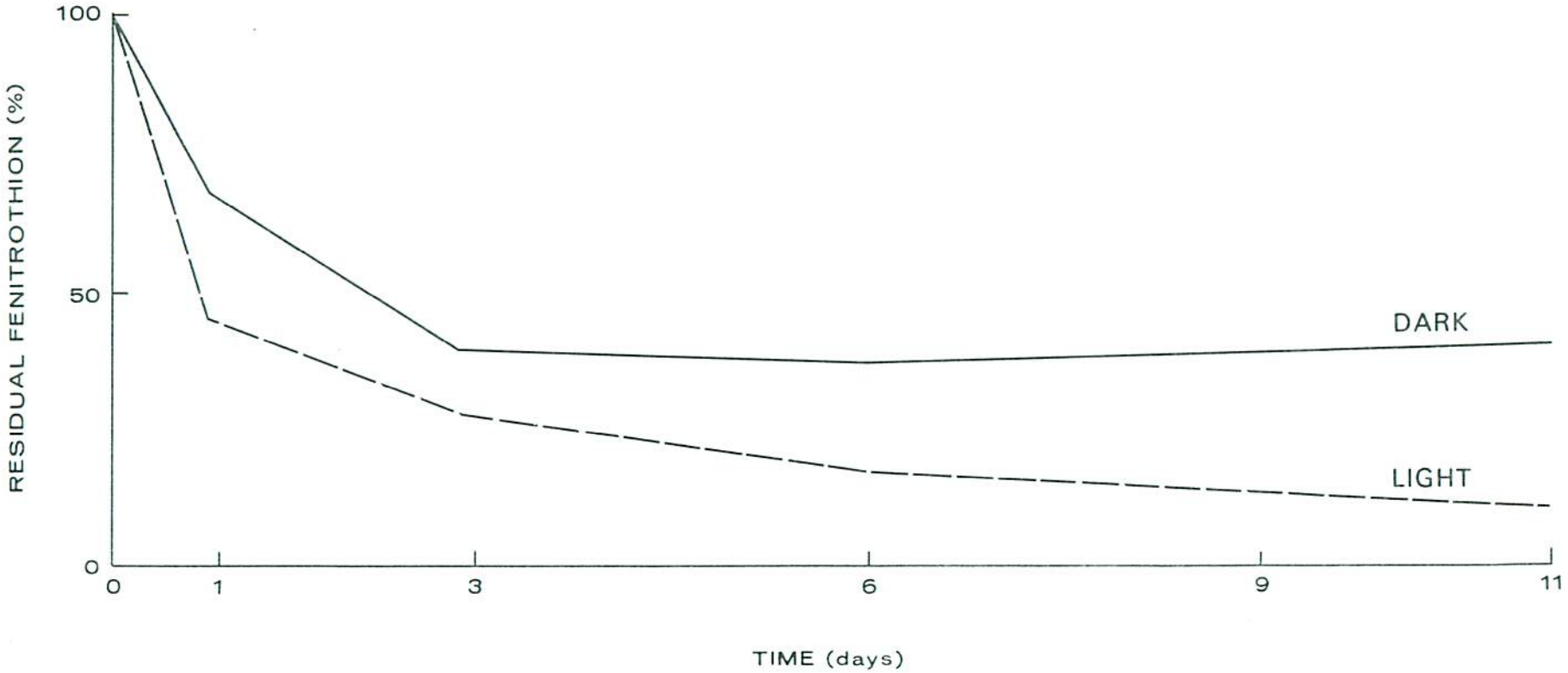

FIG. 2. Dissipation of Fenitrothion from Glass Surfaces under Conditions of Light and Dark.

DISCUSSION

It has only recently been established that volatilization and vapour phase transport are important 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 various pesticides under field conditions closely approximated the potential volatilization rates predicted by calculations based on vapour pressure measurements. According to their estimate, parathion with a vapour pressure in the order of 10^{-5} mm Hg at 30°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 is appreciably less. Murai and Tanaka (1968) reported that 100 μ diameter droplets of fenitrothion (the average droplet diameter of field spray (Roberts, 1975)) deposited on cardboard, evaporated completely in 50 hours at a wind velocity of 7 mph.

These observations are consistent with Table 1 which shows that 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)) the levels were actually increasing. The greater persistence of fenitrothion in 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). However, the surface deposit should volatilize at a rate closely resembling that predicted in theory, since only a small fraction of the applied pesticide would be in direct contact with the tissue surface.

The hypothesis that fenitrothion dissipation is largely the result of volatilization and not some form of biodegradation, is further substantiated by the in vitro study. Fenitrothion disappeared rapidly from the glass surfaces under both light and dark conditions. The greater rate of dissipation observed in the light may be due to photodegradation (Ohkawa et al, 1974), as traces of fenitro-oxon were detected (Table 2 (ii)) in these samples. However, the importance this mechanism played in the overall dissipation of the pesticide cannot be determined from the data obtained, since the amount of fenitro-oxon lost by volatilization is not known. Traces of S-methyl fenitrothion were detected in the "dark" samples which could have been formed by thermal isomerization of the parent compound.

During this study, precautions were taken to ensure that the metabolites 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 lab 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 "quantitation" standards. As such, they were thought to have formed during storage. However, traces of fenitro-oxon and S-methyl fenitrothion 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 initial deposits of fenitrothion and also by the absence of interfering impurities.

Fig. 2 demonstrates that in three of the four treatment methods (Paint 20 ppm; Spray 20 ppm; and Spray 200 ppm) the percent fenitrothion absorbed was greater by fir than by spruce and also (Fig. 2 (ii)) that the residual levels were greater in fir than in spruce. 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 relation 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 be theoretically more resistant to volatilization and photodegradation.

If the assumption is made that the spray application under greenhouse conditions was relevant to the field situation, it follows that fenitrothion should be more persistent in fir than in spruce. Previous investigation has shown this is actually the case for field sprayed fir and spruce (Yule and Duffy, 1972). 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 Table 1 which shows that the new fir foliage was the main contributor to the differences exhibited by the two species. Fenitrothion levels have actually increased within the new fir foliage (N.F.(E); S-2 and S-4) during the 1 to 7 day sampling period. 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).

(2) Fenitrothion was being translocated acropetally to the young fir foliage, perhaps due to the ability of developing foliage to act as a photosynthate "sink" (Crisp, 1972).

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. Consequently, the greater protection conferred to fir against budworm predation may be the result of the systemic action of fenitrothion in fir. This is consistent with previous investigation (Prasad and Moody, 1975) which employed

histoautoradiography with C^{14} labelled fenitrothion to demonstrate that the systemic action of this pesticide may involve acropetal, apoplastic (xylem) translocation.

Future investigation should aim at determining the actual physiological mechanism involved in the systemic action of this pesticide. Some endeavour should also be made to determine whether modification of the spray formulation, or time and method of application could take advantage of the mechanism operating in fir, or activate the same mechanism in spruce.

SUMMARY AND CONCLUSIONS

An investigation was carried out to determine the fate and persistence of fenitrothion applied to four year old seedlings of white spruce and balsam fir held in the greenhouse. The insecticide disappeared rapidly from the surface of conifer tissue while the absorbed residues were more persistent. An in vitro study carried out on glass surfaces demonstrated that volatilization was probably the major mechanism responsible for the rapid dissipation of the pesticide from the conifer tissue. It was concluded that the absorbed residues were more persistent since they were less susceptible to volatilization.

The observation that a spray application of fenitrothion was more persistent on fir than on spruce was thought to be associated with the greater absorption of the pesticide by fir. Accumulation of the pesticide took place in the newly flushed fir foliage and this led to speculation that the greater protection against budworm predation conferred to field sprayed fir, was the result of the systemic action

of the pesticide.

Further investigations should be made to determine the actual mechanism responsible for the systemic action in fir so that better advantage can be taken of this system in future field spray programs.

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