THE EFFECTS OF ALDICARB, A NEMATICIDAL CARBAMOYLOXIME, AND

ITS OXYGEN ANALOGUES ON SOIL AND PLANT PARASITIC NEMATODES.

A thesis submitted for the degree of

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by

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Sunninghill,

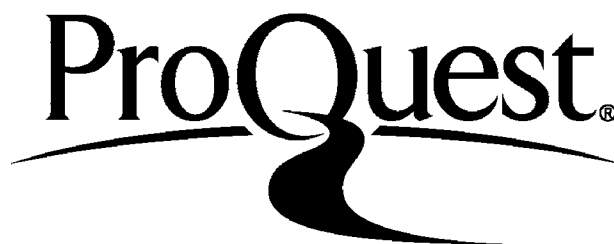Ascot, Berkshire.   September 1971

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To My   Wife

Riki

for her patience and understanding

AUTHOR'S NOTE

ABSTRACT

The effects of aldicarb and its two major toxic breakdown products , aldicarb sulphoxide and sulphone have been investigated using several species of nematode.

Of the three compounds , aldicarb was the most effective , reducing nematode hatch in water and soils , their dispersal on treated agar and locomotory activity. Meloidogyne incognita larvae accumulated in treated pot soils and their invasion of host plant roots was delayed.

In soils the contact of nematodes with a paralysing dose of the non-volatile nematicide or its sulphoxide is of fundamental importance in nematode control.

In tomato roots the reduction of Meloidogyne invasion can only be attributed to the persistence of minor amounts of the sulphone derivative. Control of invasion by the retention of toxic residues in roots is less effective since aldicarb and its breakdown products accumulate in the foliage.

The movement patterns of second stage Meloidogyne and Heterodera larvae altered in the presence of these carbamoyloximes from open to closed spiralling, the tracks becoming increasingly tortious with exposure. Contracted paralysis of nematodes resulted , especially with aldicarb and its sulphoxide , the nematodes becoming almost motionless.

When the nematicide treatments were discontinued , nematodes regained their activity. Recovery was generally more rapid after sulphoxide or sulphone treatment.

Of the three compounds , aldicarb had the greatest affect on

stylet movement , stimulating abnormal protraction except in Xiph-

inema.   Maximium stylet protraction corresponded with maximium

body inactivity.

The permeation of two nematode species by ^{14}C - labelled aldicarb

was investigated.   These were permeable to aldicarb which accumul-

ated within them.   An equilibrium was reached between the nematicide

inside the nematode and that in the exposure solution.   After pene-

treation the parent compound was rapidly converted to its sulphoxide

and subsequently to the corresponding oxime and nitrile.   The egress

of labelled sulphoxide from treated Panagrellus was slow.

The results obtained in these investigations are compared with

relevant published data.

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INTRODUCTION

A salient feature of normal cultivated soils is the presence of mixed populations of phytophagous and saprophagous nematodes (Jenkins , Taylor and Rhode , 1956 ;   Kleyburg and Oostenbrink , 1959). The presence of nematodes in soils and their role as causal agents has been the subject of considerable importance .   Their relation to the symptoms of plant attack and poor growth   have been investigated (Goodey , 1935 ;   Flegg , 1965 ;   Mountain , 1965).   The crop loss due to nematode attack is often difficult to estimate but is considered to be in excess   of 10%.

Economic crop losses are of two types :   a direct loss caused by a reduction in the crop yield and secondly indirectly by obliging the grower to use nematicides or to plant less profitable crops. Whether plant damage will occur or not depends to a large extent on the external conditions during the critical , initial growth of the crop.   The amount of damage normally depends on the density of nematodes in the soil at the time of sowing or planting the susceptable crop.

Several methods by which the soil nematode population could be effectively reduced or the crop yields increased have been investigated.   Sequential crop rotation produced encouraging results (Oostenbrink , 1961) , the addition of manure or tillage decreased nematode populations (Oostenbrink , 1960).   Other methods have included soil disinfection with volatile organohalide fumigants (Carter , 1943 ; McBeth and Bergeson , 1955) and also with less volatile organophosphate (Sasser , 1952 ; Bergeson , 1955) and carbamate nematic-

ides (Brodie , 1968 ; Birchfield , 1968).

Soil disinfection with organohalide nematicides are thought to kill nematodes outright (Moje , 1959 ; Castro , 1964) whereas the organophosphate and carbamate compounds are not thought to be so lethal , acting as nematostats , inactivating treated nematodes (Motsinger , 1961 ; Nelmes , 1970 , Kondrollochis , Pain and Hague , 1970). Such nematodes are then thought to recover when treatments were discontinued. The organophosphate and carbamate nematicides have supplemented or replaced the organohalides which have been found to be phytotoxic and also on certain soils very ineffective.

Jones (1969) considered that unless the nematicide treatment was unusally effective , it only served to remove nematodes which were surplus to the carrying capacity of the root system and in so doing increased root growth and therefore the numbers of nematodes the root could support. Although it has been reported that there were initial decreases in the initial nematode population after nematicide treatment the final population of cyst nematodes (Pain and Hague, 1971) and other species were unaffected (Bird , McCarter and Roncadori , 1971).

The direct contact of nematodes with the organophosphate and carbamate nematicides or their toxic breakdown products in soils or in plant tissues which nematodes invade , may reduce the activity , penetration , feeding or development of the nematode. Many assay procedures have been devised to investigate the action of nematicides (Feldmesser and Feder , 1955), but with many of these techniques it has been difficult to establish a death point.

The reduction of nematode activity has been used as a criteria of
"Mortality" even though the nematodes were not dead (Tarjan , 1953).
The inability of nematodes to migrate through a series of filters
after periods of exposure to nematicides did not asertain whether
the test organisms were dead (Moje , 1959 ; Purnell , 1964 ; Oliff ,
1965). Chitwood (1952) however examined treated nematodes for
changes in their physical appearance and observed both changes in
body shape and colouration.

Erroneous conclusions were drawn from results of some
experiments. It was reported that <u>Aphelenchus avenae</u> were only
affected by Thionazin^R when the nematicide was ingested by the
nematode when it fed on treated fungal cultures (Oliff , 1965 ; Hague
and Kondrollochis , 1969ab). The deleterious symptoms caused by this
compound have been found to be due to contact rather than ingestion of
toxic material from fungal cultures (Kondrollochis et al . 1970).

It was found that certain nematicides were taken into plant
roots (Bijloo , 1965) and such plants could be protected against nema-
tode attack (Welle and Bijloo , 1965). This simple technique has been
widely used to assess the systemic activity of potential nematicides.

There is a paucity of information about the uptake and
breakdown of systemic nematicides in plants and their affect upon
nematode invasion. The organophosphate Thionazin , was found to
protect dipped bulbs against nematode attack for several months
(Hesling , 1967). Little experimental data has been assembled to
compare the control of root invasion with the nature and quantity of
nematicide remaining in the plant tissues.

Structural manipulation of compounds of known

biological activity has created a variety of compounds, some of

which have been found to have biocidal properties. The oxime carba-

mates whose biological significance was revealed by Kilsheimer

and Hanning (1962) are a group of particular interest. A member of

this group with potentially useful nematicidal qualities is aldicarb

(Temik[R], UC 21149, 2 methyl-2-(methylthio) propionaldehyde O(methyl

carbamoyl) oxime, Union Carbide, N.Y., U.S.A).

This compound has been the subject of considerable inves-

tigation. It breakdown has been studied in soils (Bull, 1968;

Hendrickson and Meagher, 1968; Coppedge, Linquist, Bull and Dorough,

1967; in insects (Metcalf, Fukuto, Collins, Borck and Burk, 1966;

Bull, Linquist and Coppedge, 1967), in plants (Bull, 1968; Bartley,

Andrawes, Chancey, Bagley and Spurr, 1970; Skrentny and Ellis, 1970),

in higher animals (Dorough and Ivie, 1968; Knaak, Tallant and

Sullivan, 1966) and in microsomal fractions of tissues (Oonnithan

and Casida, 1968).

The nematicidal qualities of aldicarb were first

reported by Spurr and Sousa (1966) and it has been shown to be effect-

ive against the potato cyst nematode, delaying nematode invasion of

roots and increasing crop yields (den Ouden, 1968; Hague and Pain,

1970). While aldicarb has not been considered a persistent

compound in soils (Bull, 1968), on alkaline sandy soils it has been

shown to control nematode populations for two seasons (Whitehead,

Tite and Fraser, 1970).

An attempt has been made in this thesis to explore the

action and metabolism of aldicarb in nematodes and plants. The

nematicide has been of particular interest for it is converted into

compounds of both increased and decreased toxicity. The effect of

the parent compound on nematodes would be incomplete without an

investigation of the action of the sulphoxide and sulphone analogues

which are likely to form the major toxic residues in soils and plants

during the crop growing season.

The root-knot nematode Meloidogyne incognita and the

potato cyst nematode Heterodera rostochiensis were chosen in view

of their economic importance as agricultural pests. Comparative

investigation were made on the effects of these three carbamoyloximes

on the hatch , mobility and infectivity of these nematodes. Xiphi -

nema diversicaudatum was also chosen because they were sufficiently

large to allow ease of handling in autoradiographic studies with ^{14}C

labelled aldicarb.

Some nematodes have been shown to be selectively

permeable to a wide range of non-polar compounds (Brown and Dunn , 1965;

Marks , Thomason and Castro , 1968 ;_ Castro , Thomason and Beld , 1970).

The organohalide nematicides ethylene dibromide (EDB) and dibromochloro-

propane (DBCP) penetrate nematodes and reach an equilibrium distribut-

ion between the nematode and the treatment solution (Marks et al ,

1968). The permeation characteristics of nematodes towards

different type of molecules , while possibly related to the physical

characteristic of the substance can in some instances be controlled by

the nematodes.

Some exogenous applied compounds were found not to

remain unchanged within nematodes but were transformed to oxidative

derivatives (Knowles and Casida , 1966) while other compounds like

EDB were not broken down by the nematode but were released from

them (Marks et al , 1968).

The uptake of ^{14}C labelled aldicarb was investigated

to determine whether nematodes were permeable to this compound and

once in nematodes whether it could be broken down. This study was

restricted to Panagrellus redivivus which has been extensively used

as a test organism with nematicides (Tarjan ,1955 ; Pen Cheo and Tar-

jan , 1955 ; Santmyer , 1956 ; Kampfe , 1964 ; Spurr , 1965) and Aphelen-

chus avenae , as both species were easily cultured in large numbers

that were required for the detection of aldicarb and its break-down

products.

The recovery of nematodes treated with aldicarb and its

oxygen analogues was investigated by several methods so that results

obtained by these procedures could be compared.

MATERIALS AND METHODS

1. Source and Culture of Test Organisms.

Aphelenchus avenae Bastian 1865 , a pathogenic myco-
phagous nematode was reared from a single young female obtained

from soil on Fusarium oxysporum lycopersici (Sacc.) Snyder and

Hansen , on potato-dextrose agar (PDA) at 25°C (Townshend , 1964)

in petri dishes. When large numbers of nematodes were required

they were cultured by inoculating sterilized wheat seedlings with

Fusarium and surface sterilized nematodes (Evans , 1970). The

cultures were maintained at 25°C and harvested after 6 weeks.

Ditylenchus myceliophagus Goodey J.B. 1958 , was

obtained from Dr. Hooper at Rothamsted Experimental Station. It

was cultured on Botrytis cinerea on PDA in petri dishes at 25°C.

Panagrellus redivivus (Linn ., 1767) Goodey T. 1945

were cultured in a brown flour paste medium at 20°C and harvested

after 1 month.

Second stage larvae of Meloidogyne incognita (Kofoid

and White , 1919) Chitwood 1949 , were obtained by inoculating pot

soils containing tomato seedling Lycospericum esculentum Mill ., cv

MoneyMaker , with eggs or egg masses. The cultures were maintained

in the greenhouse at 25°C. Newly developed egg masses were

collected by hand from washed tomato roots and were placed on a Cold

streem^R milk filter over a 90 μ mesh nylon seive. The larvae which had

migrated through the seive were collected.

Second stage larvae of Heterodera rostochiensis

Woll ., 1923 were obtained from cysts which had been extracted from

infested soil at Churchfield , Ascot , by means of a Fenwick Can.

The cysts which were then separated from the remaining soil detritus

were soaked in tomato or potato root diffusate at 25°C (Fenwick ,

1949 ; Moriority , 1963) and the hatched larvae were collected.

Xiphinema diversicaudatum (Micol , 1927)

Thorne 1939 were extracted from fresh soil samples taken from plots

at Ashurst Lodge , Ascot , and extracted using Flegg's Method (Flegg ,

1960).   The nematodes were hand picked from the soil and detritus

by means of a mounted hair.

2.   Cleaning and Sterilizing Nematodes.

Extracted nematodes were cleaned by centrifugal flotation

(Caveness and Jenson , 1955) with 1M sucrose and then rewashed several

times with water.   Water and remaining sucrose were removed by applying

a vacuum to a glass sinter suspended just above the bottom of a round

bottomed glass vessel containing the nematodes.

Nematodes cultured axenically were surface sterilized

with dihydrostreptomycin sulphate (1% solution) and washed several

times in sterile distilled water (Barker , 1963) before inoculation.

Some nematodes have shown deleterious symptoms after

the use of antibiotics (Chen , 1964 ; Zuckerman and Brzeski , 1965) , so

to eliminate   any unnecessary symptoms nematodes used for behaviour-

al assays were not treated prior to use.

3.   Nematicides Investigated.

a) Non-Labelled Compounds

Aldicarb [2-methyl-2-(methylthio) propionaldehyde

O(methylcarbamoyl) oxime = Temik] ,   aldicarb sulphoxide [2-methyl

-2-(methylsulphinyl) propionaldehyde O(methylcarbamoyl) oxime =

Temik Sulphoxide] ,   and aldicarb sulphone [2-methyl-2-(methyl-

sulphonyl) propionaldehyde O(methylcarbamoyl) oxime = Temik sulphone].

$$CH_3SC - CH = NOCO.NHCH_3$$

with CH_3 above and CH_3 below attached to the central C.
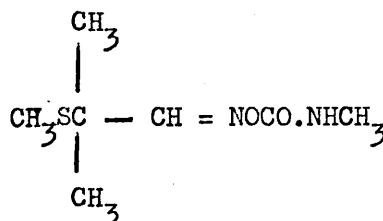
Aldicarb Molecular weight 190.3

b) Labelled Compounds

Studies on the uptake of aldicarb and its break-down in

nematides were made with ^{14}C labelled nematicide [2-methyl-2-(methyl-C[14]

thio) propionaldehyde O (methylcarbamoyl) oxime] (sp. activity 2.03

mCi/mmole).   This compound was obtained from Messers Mallinckrodt

Ltd , St Louis , U.S.A.

4.   Quantitative Estimation of Aldicarb and its Breakdown Products.

a)   Extraction of Non-Labelled Nematicide

All treated material was frozen at -18°C for at least
24 hr before analysis.   The procedure used was that of Skrentny
and Ellis (1970).   Weighed amounts of the frozen material were
homgenised in vortex flasks using an M.S.E Homogeniser for 10 minutes.
Sixty millilitres of acetone and chloroform (1:1 v/v) were used as
a solvent to separate pesticide residues from plant , fungal and
agar material and a weight of anhydrous sodium sulphate equal to
the material was added prior to homogenising.   After filtration
by suction the resulting cake was washed and again homogenised in
the same solvents for a further 5 minutes.   The combined filtrates
were concentrated in vacuo   at 40°C to 50 ml by means of a rotary
film evaporator to give crude extracts. ·   These were then cleansed
for thin-layer chromatography (TLC) by the addition of decolourising
charcoal (Norit NK) 0.25 g/g of extracted material.   The mix-
ture was evaporated to dryness under a stream of nitrogen gas over a
water bath at 40°C , and taken up in 50 ml hexane.   This was add-
ed to the top of a glass chromatographic column (i.d 18 mm) packed
with 1g of anhydrous sodium sulphate and 5 g florisil (60 - 100 mesh).
The hexane eluate was dicarded , but the addition of 100 ml acetone to
the top of the column was then collected.   This eluate was then con-
centrated to 5 ml under vacuum at 40°C.   This was quantitatively
transferred to a 10 ml centrifuge tube and further concentrated under
a stream of nitrogen gas to 20 -30 μl.

Thin layer Chromatography.

The concentrated samples were analysed by spotting on
20 X 20 mm silica gel G (E. Merck , Germany) 0.25 mm thickness on glass plates.
Non-labelled compounds were developed in a solution of ethyl acetate
and acetone 25:4 .   The plate was sprayed with two chromogenic reagents
as suggested by Abdel-Wahab , Kuhr and Casida (1967).   Firstly a
10% aqueous sodium hydroxide solution was sprayed , the plate heated at
70°C for 3 minutes , cooled , and oversprayed with 2% ninhydrin in 65%
ethanol.   The plate was reheated for 30 minutes at 70°C to develop
the parent compound and its two oxygen analogues.

Aldicarb , its sulphoxide and sulphone developed as
red spots at R_f 0.61, 0.06 and 0.39 respectively (Skrentny and Ellis ,
1970).   According to Skrentny the recovery efficiency by this
method was approximately 90%.   However losses of 13 to 15% were
found when ^{14}C labelled aldicarb was similarly cleaned-up and spotted.

Estimates of the amounts of compounds located were made
by comparing the size and colour intensity of the spots with those of
known standards.   The errors associated with visual quantitation were
about 10% , the lowest detection limits with non-labelled standards was
approximately 0.25 μg for aldicarb sulphoxide and 0.50 μg for aldicarb
and its sulphone.

b)   Extraction of Labelled Nematicide

Labelled aldicarb was purified prior to use by preparat-
ive TLC.   The metabolism of aldicarb may yield initially organo-
soluble products which are transient in nature (Bull,1968 ;   Coppedge
et al, 1967 ;   Bartley et al , 1970) which are eventually converted
into non organo-soluble residues.   These have been described as
'water'-soluble products.   Their isolation and identification was
not attempted in this study , since it was assumed that few water sol-
uble products would be formed during short exposure intervals.

Labelled aldicarb was used to study uptake and break-
down in nematodes.   Treated and washed nematodes were suspended in
5 ml acetone and disintegrated with a M.S.E Ultrasonic Homogeniser for
10 minutes at $4^{\circ}C$.   The supernatant liquid was collected by cent-
rifugation and the precipitate washed three times with small   volumes
of acetone.   The combined washings and the supernatant were
concentrated to 5 ml in vacuo and further concentrated in 10 ml
centrifuge tubes so they they could be spotted on TLC plates.

The nematode precipitate was stored at $-4^{\circ}C$ in glass
phials until such time as quantitative estimation of the radioactiv -
ity were made.

The aqueous treatment solution was shaken with three
50 ml volumes of chloroform to partition the organo-soluble fraction
into the solvent system.   The solvent was concentrated to dry -
ness in charcoal and taken up in 50 ml hexane and eluted through a
chromatography column as described in 4 a).   The eluate was con -

centrated to 10 ml and volumes of this final solution which were
equivalent to 10 and 90 μg of the original concentration of aldicarb
were spotted on TLC plates.

Bartley et al,(1970) reported that a loss of 5% of the
compound could be expected when the solvents were concentrated under an
inert gas.

Thin layer Chromatography.

The total radioactivity in the nematode supernatant
and precipitate fractions and in the original treatment solution were
located by two - dimensional TLC using 2:1 ether - hexane plus 20%
acetone and secondly 3:1 methylene chloride - acetonitrile (Andrawes ,
Dorough and Linquist , 1967 ; Dorough and Ivie , 1968 ; Bartley et al ,
1970).

Radioactive spots were located by means of autoradio -
graphy utilizing Kodarex Estar[R] medical X - ray film which was exposed
for 20 days before developing in Phen-X[R] (Ilford Ltd) and fixed in
Amfix[R] (Ilford Ltd) (Figure 1).

The radioactive spots were also detected with the
chromogenic agents described in 4 a) and then oversprayed with 1%
potassium permanganate solution which turned all spots on the plate
yellow. Authentic non-labelled standards were mixed with the
concentrated fractions prior to spotting so that the position of un-
known compounds detected on the silica plates could be identified
against the R_f values of the known standards shown in Table 1.

<u>Fig 1.</u>   Two-dimensional Thin Layer Chromatography of ^{14}C

labelled aldicarb and its organo-extractable metabolites.

The plate was run in two solvent systems , 2:1 ether-hexane

+ 20% acetone and secondly 3:1 methylene chloride - aceto-

nitrile.   The labelled compounds were then located by

exposure of the plate to medical X-ray film for 20 days.
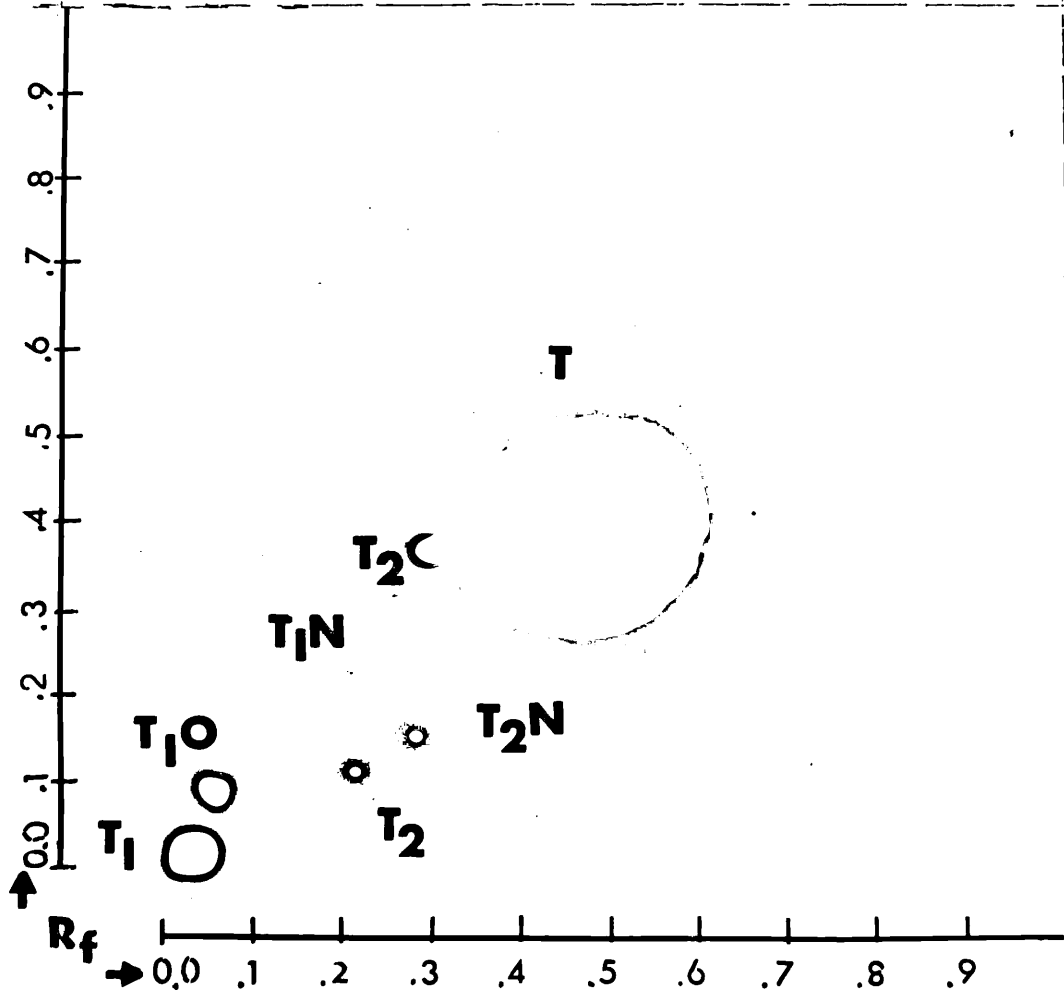
Code	Compound	R_f value in solvent systems	
		2:1 ether-hexane + 20% acetone	3:1 methylene chloride - acetonitrile
T	2-methyl-2-(methylthio) proprionaldehyde O(methylcarbamoyl) oxime (Aldicarb).	0.40 (0.61)	0.50 (0.83)
T_1	2 methyl-2-(methylsulphinyl) propionaldehyde O(methylcarbamoyl) oxime (Aldicarb sulphoxide)	0.01 (0.05)	0.03 (0.13)
T_2	2-methyl-2-(methylsulphonyl) propionaldehyde O(methylcarbamoyl) oxime (Aldicarb sulphone).	0.11 (0.10)	0.21 (0.59)
T_1^O	2-methyl-2-(methylsulphinyl) propionaldehyde oxime (Oxime sulphoxide).	0.08 (0.13)	0.05 (0.19)
T_2^O	2-methyl-2-(methylsulphonyl) propionaldehyde oxime (Oxime sulphone).	0.27 (0.61)	0.28 (0.76)
T_1^N	2-methyl-2-(methylsulphinyl) propionitrile.	0.23 (0.38)	0.20 (0.62)
T_2^N	2-methyl-2-(methylsulphonyl) propionitrile.	0.14 (0.57)	0.27 (0.69)

Table 1.   Thin Layer Chromatographic Identification of Aldicarb and Related Standards. Figures in brackets represent the R_f values reported by Andrawes et al ,(1967) Dorough and Ivie , (1968) ,Bartley et al , (1970).

The lowest detection limits of all labelled compounds exposed

to X-ray film for 20 days was 0.001 μg. The radioactive zones were

scraped from the TLC plate into scintillation bottles.

Quantitation of Radioactivity

Radioactivity was determined by liquid scintillation

counting using a Tracerlab Corumatic 200. The samples were counted

in 10ml of POP : POPOP : Naphthalene (0.7: 0.08 : 1.0) in dioxane.

An internal standard channel-ratio system was utilized for quench

correction and a quench correction curve plotted (Figure 2). The

typical channel-ratio values were between 4 and 5 which from the correct-

curve gave a percentage counting efficiency of 89 and 85% respectively.
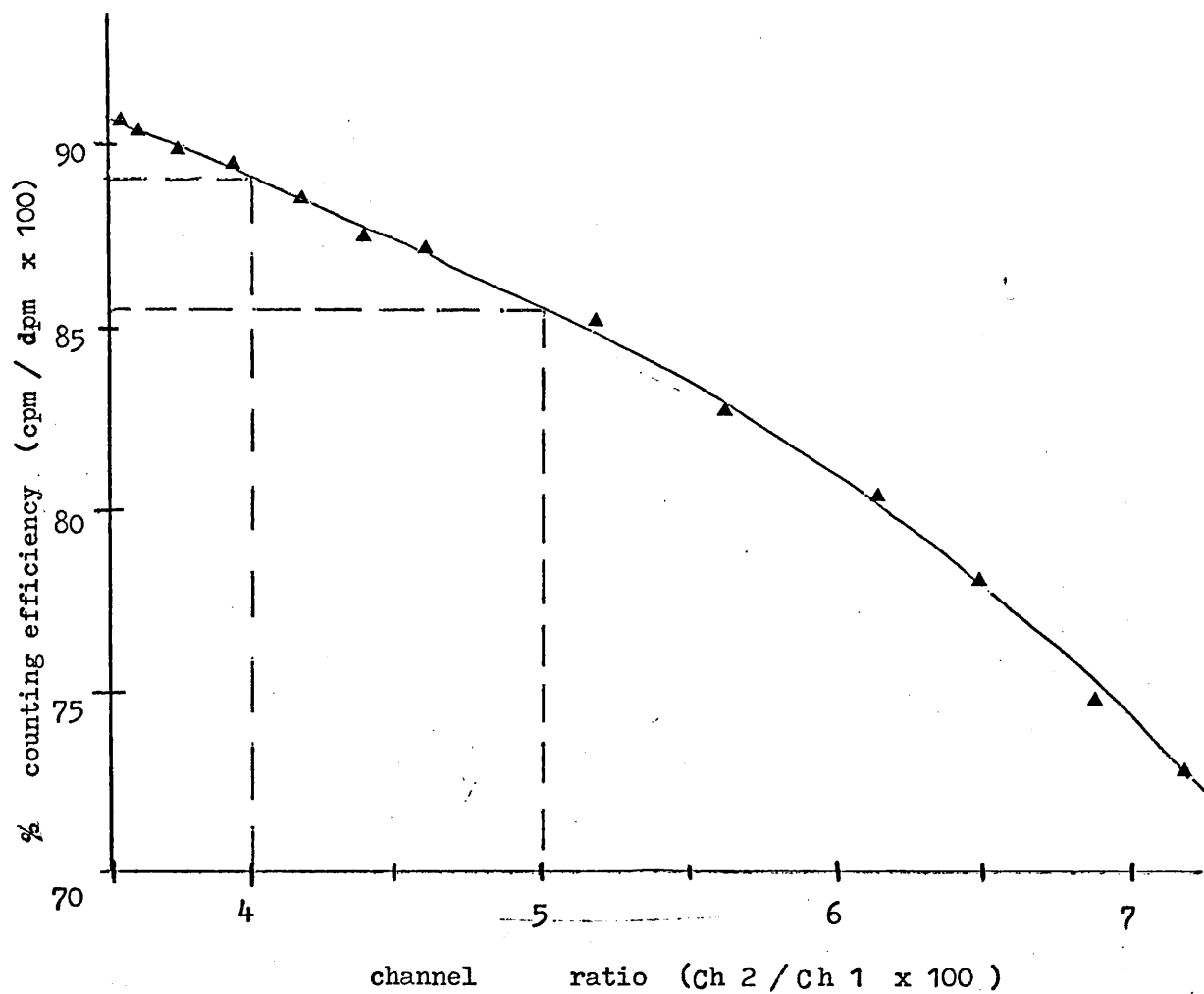
Figure 2   A quench correction curve for the ^{14}C

counting efficiency against the channel ration

The channel ratios were found at between 4

and 5 which gave a counting efficiency of

89% to 85.6% respectively.

5. **The Effect of Aldicarb Applied as Soil Drenches on the**

Control of Meloidogyne incognita Larvae on Tomatoes.

The hatch of nematode larvae in soils has been studied indirectly by placing eggs in various soil types and later extracting hatched larvae using a Baermann funnel or some other extraction procedure (Viglierchio and Lownsberry , 1960; Miller , 1970). Hatch has also been estimated by counting galls on infected plant roots (Bloom and Couch , 1959) and by staining infected roots and counting the numbers of larvae (Peachey , 1962).

Four freshly collected egg masses of Meloidogyne were inoculated into 300 g soil (60% loam , 30% sand and 10% peat , which had been previously steam sterilized and then stored) in small pots in which grew seven week old tomato seedlings , cv MoneyMaker. The confidence limits of the total eggs /4 egg masses was 1335.8 ± 82.4 eggs. Twenty four hours prior to inoculation of the larvae the pot soils were drenched with aldicarb to give soil concentrations of 0.001 , 0.002 , 0.004 and 0.008 w/w. Six replicates of each treatment were used. The pots were watered sparingly at daily intervals and maintained at $25°C$. The total number of hatched larvae and larvae in tomato roots was determined by taking separate soil and plant samples at weekly intervals for 3 weeks.

The treatment replicates were halved. The roots of three tomato plants were carefully washed , dried and stained by plunging into boiling lactophenol & acid fuschin (Peachey , 1962). The stained roots were then homogenised in a Silverson Laboratory Blender at half speed in 60 ml of water for 45 seconds. The homogenate was

was diluted to 100 ml and after agitation three 20 ml aliquots were extracted. The larvae in these aliquots were counted under the low power of a binocular microscope.

The remaining three plants of each treatment replicate were separated carefully from the soils and then discarded. The soils were placed on Kleenex^R tissues and the larvae extracted in 36 hr by means of the modified Whitehead Tray technique (Whitehead and Heming , 1965). Those nematodes which had migrated into the collection vessel were poured into 1L bottle and allowed to stand for 12 hr. The excess water was then decanted off and the re - maining nematodes finally suspended in 10 ml water and then counted.

The total egg hatch was found by the summation of the mean total number of nematodes extracted from the soils and tomato roots. The invasion of tomato roots and of total hatch in the control groups was regarded as 100% so that the depression of total egg hatch and root invasion based on the controls could be calculated as percentages.

The lower fruit sets of tomato plants which were grown in aldicarb treated soils for 15 weeks were collected and frozen. These tissues were analysed by the method outlined in 4 a) and the total amount of nematicide was estimated in $\mu g/g$ wet weight of plant tissue .

6.   The Effect of Root Dipping Tomato Plants with Aldicarb on the

Control of Root Invasion by Meloidogyne incognita Larvae.

Seven week old tomato seedlings cv MoneyMaker were

dipped in aqueous solutions of aldicarb at 0.5 , 1.0 , 4.0 , 8.0

and 12.0 ppm for 24 hr at $20^{\circ}C$ were then washed and replanted in

300 g of sterilized soil in small pots.   The soils were inoculated

on either the second day or the ninth day after replanting , with

approximately 300 freshly hatched Meloidogyne larvae.

The plants were grown at $25^{\circ}C$ with a 16 hr photoperiod.

Estimates were made of root invasion by Meloidogyne larvae when the

tomato plants were harvested 2 weeks after the soils were inoculated.

The roots were separated from the soils by careful washing , stained

and homogenised as described in Section 5.

The reduction of larval invasion as a result of nemat-

icide treatment was calculated as a percentage of larval invasion on the

numbers   in the control groups which was regarded as 100%.

Each treatment mean was determined from 3 replicates.

The roots of tomato plants which had been dipped for

6 , 12 , and 24 hrs were washed and then frozen until such time as the

chemical analysis of the nematicide could be made.   Other tomato

plants which had been dipped for 24 hr were grown for 16 days at

$25^{\circ}C$ before the tissues were frozen.   The plants were divided into

roots , stems and foliage and these subsamples were analysed as

outlined in Section 4 a).

7.   The Effect on ^{The} <u>In Vitro</u> Egg Hatch of Meloidogyne incognita.

The hatch of eggs of <u>Meloidogyne</u> spp. and other nemat-
ode genera has been studied in many artifical environments , on moist
sterile soil (Bergeson , 1959) , in petri dishes containing water or
nutrient solutions (Loewenburg , Sullivan and Schuster, 1960) and using
agar coated slides to show the effects of temperature , moisture and nema-
ticides (Johnston and Shamiyeh , 1968). The optimal conditions for the
best hatch of <u>M. incognita</u> were found to be high moisture and $25^{\circ}C$
which produced a 70% hatch.

The <u>in vitro</u> effect of aldicarb and its oxygen analogues
on <u>Meloidogyne</u> egg hatch was investigated by means of a modified
Baermann extraction technique.   Gauze baskets 20 x 20 x 15 mm
of mesh size 0.1 mm , were suspended in 50 ml plastic beakers containing
1 , 5 , 10 , 50 and 100 ppm aqueous solutions of aldicarb , its sulphoxide
and sulphone (Figure 3).
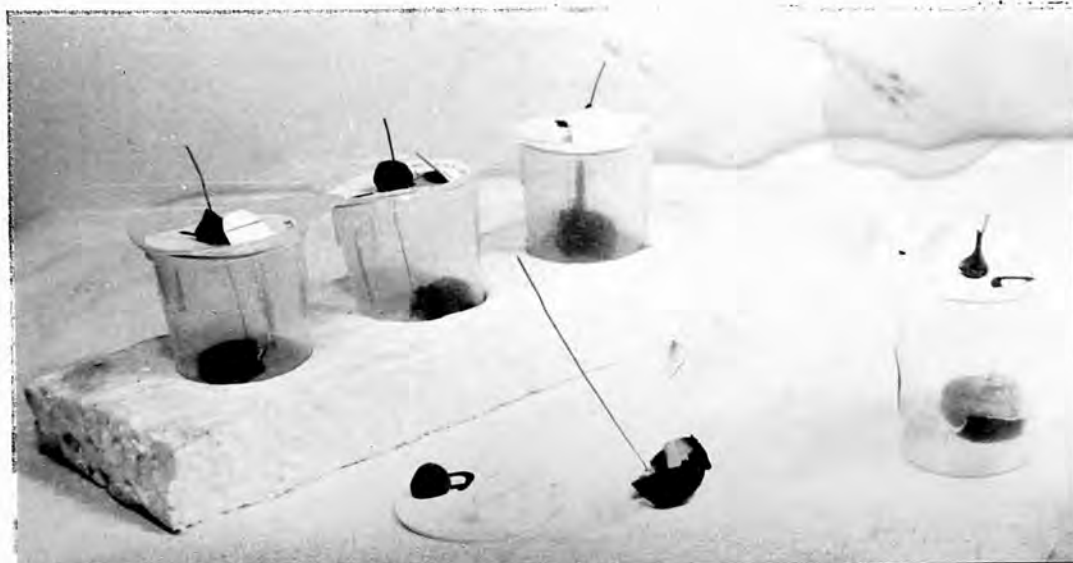
<u>Figure 3</u>   Simple apparatus for hatch evaluation tests.

Four egg masses of approximately equal size were
transferred by hand to each basket.   The confidence limits for
the number of eggs/ 4 egg masses was 1360 ± 80.4 for eggs used
in the aldicarb experiment and 1562 ± 340.7 for the sulphoxide and
sulphone experiment.   The egg masses were immersed in 10 ml of the
treatment solution so   that   it   just   covered the egg masses.
The hatch solutions were maintained in the dark to prevent photo -
decomposition and incubated at 25°C.   The hatched second stage larvae
were collected every fifth day for 20 days by transferring the baskets
containing the egg masses to new containers in which freshly prepared
nematicide was poured.   The total number of larvae hatched was
determined by counting the numbers collected in the plastic beakers
by pouring them into a open larval counting tray and counting with
the low power of a binocular microscope.

The results were calculated as percentage of the total
hatch of the untreated control groups which was regarded as 100%.
The regression lines were calculated from angular transformation of
the data with aldicarb , its sulphoxide and sulphone and the slopes
of the regression lines compared.

8.   The Effect of In Vitro Treatments with Aldicarb and its

Oxygen Analogues on the Body Contents and Infectivity of

Second Stage Meloidogyne incognita Larvae.

Second stage larvae of Meloidogyne   of uniform

age , all being collected in a 24 hr hatching period were treated

with 10 ml aldicarb , its sulphoxide and sulphone in aqueous solutions

at 1 , 10 and 100 ppm .   The experiment maintained at 20°C in the

dark , the nematodes suspended in solution by agitation at low speed

on a variable speed shaker.   After 20 days the nematodes were

washed in distilled water and then stained in buffered 1% osmium

tetroxide for 12 hr .   Larvae were then examined and a visual

comparison of the stained and unstained body contents of the intest-

inal region   of   nematicide treated and control groups with

freshly hatched larvae   was made.

In a separate series of experiments , 300 second stage

larvae were exposed to aqueous solutions of aldicarb at 1 , 10 and

100 ppm for 1 or 7 days at 20°C.   At exposure the larvae were

washed with 250 ml distilled water and collected on a 3 μ Millipore

filter pad.   The larvae were transferred by wash with water to

syracuse dishes and inoculated into soil containing a 7 week old

tomato seedling cv MoneyMaker.   The plants were grown for a further

er period of 2 weeks after the soils had been inoculated , at 25°C

on a 16 hr photoperiod.   The plants were separated from the pot

soils and the roots carefully washed , and the number of larvae within

roots estimated as described in Section 5.

The invasion of tomato roots by larvae treated in water
for 1 or 7 days was regarded as 100% and the reduction of larval in-
vasion caused by nematicide treatments were calculated as percentages
of the control invasion.   The resulting data was transformed to
angles and regression lines for the effect of exposure duration on
the reduction of invasion were calculated.

9.   The Motile Response of Nematodes   Treated with Aldicarb in

    Aqueous Solutions , in Soils or on Agar Media.

    a)   The Extraction of Larvae from Treated Soils.

            Approximately 300 freshly hatched second stage larvae of

Meloidogyne   were inoculated into 300 g soil in small pots.   The soils

were then drenched with aldicarb , its sulphoxide or sulphone at 4 ppm

(1200 µg of each nematicide dissolved in 50 ml water).   Three replicates

of each treatment were used.   The soils were maintained at 18°C

and were watered at daily intervals.   The soils were transferred to

Whitehead Trays after 1 , 7 and 14 days and the larvae extracted from the

soils over a period of 36 hr .   The total number of larvae which had

migrated from the soils were collected and counted.

    b)   Larval Mobility in Aqueous Treatment Solutions.

            Second stage larvae of Heterodera   were hatched from

cysts soaked in tomato root diffusate , the larvae from this study were

collected 5 days after hatching commenced.

            The experimental exposure combined two phases , an init-

ial 24 hr nematicidal treatment and a subsequent 24 hr water treatment.

After the hatched larvae were washed in distilled water approximately

20 nematodes were immersed in freshly prepared 1, 5 , and 10 ppm solutions

of aldicarb its sulphoxide and sulphone made up in aerated distilled

water.   The experimental solutions in flat-bottomed syracuse dishes

were kept at 20°C in the dark.   Hourly observations of larval activ -

ity were made for 6 hr and finally at 24 hr .   The nematodes were then

thoroughly washed three times with distilled water and placed in aerated

distilled water under the same conditions as before and observations

made at 1 , 3 , and 24 hr . The tests were replicated six times.

The observations consisted of counting the body undulations or wave

frequency of 10 nematodes in 30 seconds , one undulation taken to re-

present one complete cycle of movement at the anterior end (Wallace ,

1962 ; Croll , 1966). Nematodes in physical contact with one an-

other were not recorded , to overcome this more than 10 nematodes were

used in each sample replicate.

The percentage of larvae exhibiting abnormal stylet

movement was determined from the original observations in a similar

manner during each observation interval. The stylet movement was

defined as an axial extrusion and retraction of the stylet.

A separate series of nematicidal contact tests were

designed to establish an ED_{50} for the experimental compounds against

second stage larvae of Heterodera and Meloidogyne , adult Xiphinema

and Aphelenchus. According to Trevan (1927) an ED_{50} is the median

effective dose which will produce a response other than death in half

the treated population. With the exception of Xiphinema where

30 hand picked nematodes were used these tests were conducted with

between 500 and 1000 nematodes.

The nematodes were treated with a range of concentrations

between 0.1 and 1000 ppm of aldicarb and its oxygen analogues for a

24 hr period in 10 ml pyrex tubes in the dark at 20 ^{o} C . The nema-

todes were kept in suspension by agitation of the solutions with a

variable speed shaker , at low speed. The exposure period was term-

inated when the nematodes were washed with 250 ml of distilled water and

collected on a 3μ Millipore^R filter pad over a Millipore glass

sinter filter.   The pad was then inverted over a Coldstreem^R

(Boots , Nottingham) milk filter on a 90μ nylon seive immersed in

water as described by Townshend (1964).   Those nematodes which had

migrated through the filter and seive in 24 hr were collected and

subsquently counted.

The number of nematodes recovered from each treatment

were expressed as a percentage of those nematodes which were recovered

in the control groups.   The percentage reduction in nematode recov-

ery was converted into probits and probit response lines plotted , so

that the ED_{50} for the particular nematicide could be determined.

c)   Movement Patterns   of Nematodes on Treated Agar Films.

The movement patterns of nematodes on agar has been

studied by several workers (Rode and Staar , 1961 ; Standstedt ,

Sullivan and Schuster , 1961 ; Rode , 1970 ; Croll , 1971).

The effect of aldicarb , its sulphoxide and sulphone

on the movement patterns of nematodes was investigated.   These

compounds were made up in distilled water and introduced into 1%

ionagar prior to pouring at about $60^{\circ}C$.   The treated agar films

produced in 8.8 cm plastic petri dishes   about 3 to 4 mm thick.

To improve the quality of the track left by nematodes the petri

dishes were stored at $10^{\circ}C$ for 2 or 3 days.

The treatment consisted of two phases , a 1 hr nematicide

period followed by a 2 hr period on untreated agar.   The treatment

period was limited to short exposure because of the difficulty of

tracking nematode accurately over long distances. The period

nematodes were left on untreated agar was also limited for the same

reason. Where nematode recovery was not observed the period the

nematode remained was extended for upto 16 hr.

Second stage larvae of Heterodera and Meloidogyne which

had been freshly extracted from hatching solutions were used for this

investigation. The larvae were inoculated by hand onto the agar

at marked loci by using a mounted hair. The plates were stored in

the dark at 20 °C during treatment. Each treatment was replicated

ten times.

The position of the larvae at the end of the treatment

period was marked and the nematodes transferred by means of the mounted

hair without washing to untreated media. The plates were returned to

the dark and at the end of the exposure period the position of the larvae

were marked before they were finally removed from the media.

The effect of these compounds on the activity of larvae

during the two treatment phases was determined from measurement made

from scale drawing of the tracks or slicks left behind on the surface of

the agar by the larvae. A negative contact print of 1 mm square

graph paper was placed beneath the petri dishes and by alternate adjus-

tment of the microscope the track of the nematode and the scale could

be focused. These tracks were then copied onto 1 cm square graph

paper which represented a 10 fold increase in the size of the actual

larval movement pattern.

The total number of undulations , the distance which

nematode's travelled and the furthest dispersal during the two treat-

ment phases were determined from the tracks.   The reduction in

any of these three activity parameters was calculated as percentages

based upon the activity of the control groups which was regarded as

100% .   The data was transformed to angles and regression lines

calculated so that the effect of these three compounds could be com-

pared.

In a separate series of experiments the dispersal of Panag-

rellus adults was investigated.   Ten nematodes were transferred

using a mounted hair onto different loci on 1% ionagar which had been

treated with aldicarb at a range of concentrations from 1 to 1000 ppm.

The agar was divided into 4 concentric zones of approximately 10 mm

width and the position of the nematodes after 24 hr in relation to

these zones was observed.   Each treatment was replicated ten times.

The mean number of nematodes in each zone was then determined and

expressed as a percentage of the total number of nematode used.   The

affect of the different concentrations on the dispersal of Panagrellus

were compared.

The dispersal of Aphelenchus adults and Heterodera second

stage larvae were compared on prepared concentration gradients of the

three nematicides in diffusable 1% ionagar.   Twenty five micro-

litres of aldicarb its sulphoxide or sulphone containing 100 μg   in

distilled water were added by means of a graduated syringe to 5 x 3 mm

1% ionagar blocks.   When the dose had been absorbed , the blocks were

transferred to the centre of 8.8 cm petri dishes poured with 1% ion-

agar to a depth no greater than 4 mm .   The agar was divided into

4 zones of approximately equal width such that they increased linearly
in weight and volume.   The blocks were removed from the agar media
12, 24 and 48 hr later, the agar divided into predetermined zones and
analysed by TLC to determine the total concentration of nematicide in
each zone.   The gradients formed after 48 hr (Figure 4) were   the
most satisfactory and therefore chosen as suitable gradients for all
subsequent experiments.   These gradients were highest in the inner
zones but were found to decline more slowly in the outer two zones.

agar   divided   into four zones
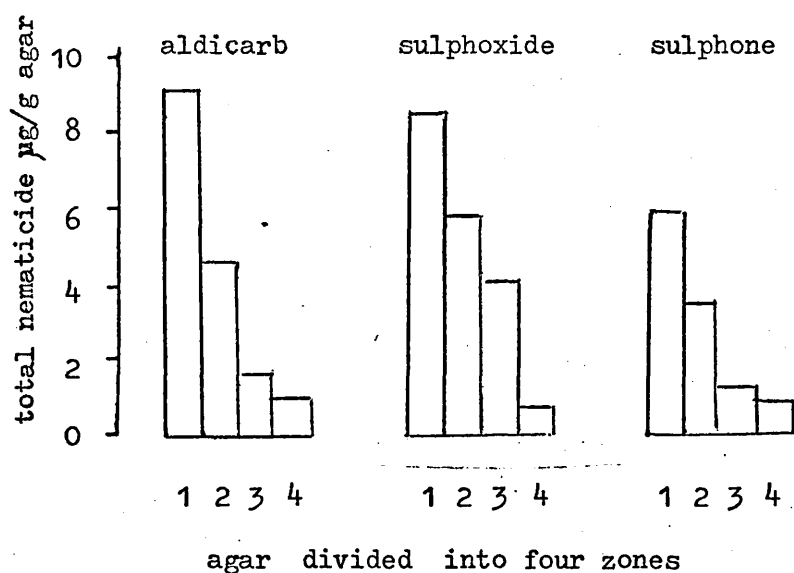
Figure 4   The diffusion of aldicarb and its oxygen analogues

in 1% ionagar media 48 hr after 100 µg of each nematicide

in small agar blocks was added to the centre of the media.

Key: 1 central zone , 4 outer zone , zone width approximately

10 mm.

The concentration of nematicide in the central zone ranged from $6\,\mu g$ to $9\,\mu g/g$ and in the outer zone from $0.7\,\mu g$ to $1.4\,\mu g/g$ agar.

Ten second stage larvae of <u>Heterodera</u> and adult <u>Aphelen-</u><u>chus</u> were inoculated by hand onto the treated agar 15 mm from the centre of the media where the agar block had been placed some 48 hr before, so that they were initially located at points on a concentric circle which demarked the central zone 1 from zone 2. Nematodes remained on the agar in darkness at $20\,^{\circ}C$ for 24 hr, their position in relation to the four zones were noted before they were removed from the media. The mean number of nematodes in each zone were determine from 10 replicates of each treatment. The percentage of nematodes remaining on the media after 24 hr were compared.

10.   The Effect of Culturing Mycophagous Nematodes on Treated

Fungi on Their Population Size.

The three carbamoyloximes were incorporated into

PDA culture media as described by Oliff (1965) for organophosphate

Thionazin.   Known weights of each compound were dissolved in

small volumes of acetone which were diluted with distilled water.

The acetone was evaporated off under a stream of nitrogen gas at 40° C

and the solutions made up by the addition of more water.

Solutions of 3.9% PDA were autoclaved at 15 p. s.i

for 15 minutes and cooled to 60°C.   One millilitre volumes of each

compound was then added to the PDA and the mixture then shaken vigour-

ously.   Concentrations of 0.1 , 1.0 , 5.0 and 10 ppm were made up .

The agar was then poured into 8.8 cm sterile plastic petri dishes

which were allowed to stand until the agar had solidified.

When cool , the plates were inoculated with fungi,

Fusarium oxysporum   and Botrytis cinerea for bioassay with Aphelenchus

avenae and Ditylenchus myceliophagus   respectively.   The cultures

were incubated at 25°C in darkness until the fungi had covered the

media , normally 3 to 5 days.

The cultures were then inoculated with approximately

600 to 700 surface sterilized nematodes , mainly adults but included

some larvae , in 0.5 ml of sterile water.   The cultures were incubated

at 25°C until they were harvested 3 , 7 or 14 days after nematode

inoculation.   The nematodes were extracted from the agar cultures

by dicing and filtration described by Townshend (1964).

Preliminary experiments showed that a 36 hr extraction period was adequate to collect over 90% of the total number which were extractable by this method. When the total number of nematodes extracted exceeded 400 the collection solution was diluted to 10 ml and three 1 ml aliquots were counted. The number of nematodes obtained from these treated cultures as a mean of three replicates were determined and their numbers expressed as the unit increase or decrease in the population size when compared with the control group population at the time of harvesting (Oliff, 1965).

Aphelenchus were treated exposed in vitro to aldicarb and its sulphoxide at different concentrations for 19 days at 25°C. The exposure period was terminated by washing the nematodes, collect-them on filter pads and allowing them to migrate through milk filters in 24 hr as described in section 7 b). Their numbers were counted and 10 adult females from each treatment sample were inoculated by hand onto isolates of Fusarium cultured on PDA at 25°C. After 25 days the nematode populations were determined by the method described above. The subsequent fecundity of Aphelenchus after in vitro treatments was determined from the population growth rate compared with the control population growth rate.

Analysis was made of the fate of aldicarb, its sulph-oxide and sulphone in the Fusarium and Botrytis cultures after they had been incubated for 26, 33 and 40 days at 25°C. Fusarium was also cultured in aldicarb and sulphoxide treated nutrient potato dex-trose broth at 10 ppm, buffered to pH 5.6. Cultures incubated at 25° C were washed three times with acetone and water on a glass sinter after 40 days. The fungal cakes were frozen until TLC analysis was made.

11 .   The Effect of Aldicarb on the Oxygen Consumption of

Nematodes.

The consumption of oxygen by Panagrellus redivivus

and Aphelenchus avenae   were measured by means of a YSI Model 53

Biological Oxygen Monitor at $30^{\circ}C$.   The instrument detector

used a Clark type polarographic electrode situated on a probe within

a test chamber.   The electrodes , a platinum cathode and a silver

anode are immersed in 2 - 3 μl of saturated potassium chloride and

covered by a thin permeable membrane.   The oxygen consumpted   by

the sensor is less than 10^{-7} g/ hr in air , and the rate of consumpt-

ion is in direct proportion to the current produced by the sensor

across the electrodes.   Operational errors may be manifest unless

the system was kept in a well controlled environment.   Oxygen leak

rate error , sensor membrane defects , movement of oxygen from the

electrolytes into the sample solution , fluctuations in operating

temperatures and barometric pressure changes cannot be neglected when

the size of the nematodes in the test sample is small.   Corrections

were made for these errors , but there was still considerable variation

between different batches of the test organism.   The size of the

stirrer was reduced as the one specified by the manufactures . crushed

the nematodes.   It was replaced by a small glass covered stirrer

and this minor modification has also been suggested by Marks and Sor-

ensen (1971).

Panagrellus extracted from cultures in 3 hr and Aphel-

enchus by the modified Seinhorst mistifier in 24 hr were cleaned by

centrifugal flotation.   These nematodes were then added to aqueous

solutions of aldicarb in 100 ml flasks and the solution diluted to
60 ml by the addition of aerated distilled water, to produce treatment
concentrations of 1, 10 and 100 ppm. Two replicates of each treatment
were used. Throughout the duration of the exposure the nematodes
were kept in suspension by gently shaking the flasks in a water bath
at $30^{\circ}C$ for 3 hr. Six millilitre aliquots of the treatment solutions
containing the test organisms were pipetted into the test chamber of
the Biological Oxygen Monitor apparatus. Approximately 3.8×10^5
nematodes were used in each aliquot and this number was used as a basis
for the measurement of oxygen consumption. Before aliquots were
transferred to the test chamber the treatment solutions were resaturated
with oxygen which was bubbled into the solutions suspended in the water
bath at $30^{\circ}C$.

The solutions in the test chamber were stabilized and
the plunger - probe inserted so that the excess air was expelled thro-
ugh the access slot of the plunger. The stirrer brake was then relea-
sed and the nematodes suspended (Stirrer motor, 480 rpm), 30 seconds
were allowed for thermal equilibration of the sample and sample chamber.

The oxygen consumption rate was then recorded as a trace
measured for 5 minutes at a chart speed of 10 mm/min. When the indivi-
dual readings had been taken the test samples were dicarded and new
samples taken for the next reading, which were made half hourly for 3
hr.

The mean dry weight of _Panagrellus_ obtained when 10 ali-
quots were freeze dried was 31 mg and the oxygen consumption rate based
upon this value was calculated in μl/mg/min at $30^{\circ}C$.

12 .   The Histochemical Determination of Acetylcholinesterase

Inhibition in Xiphinema diversicaudatum by Aldicarb and its

Oxygen Analogues.

The effect of aldicarb and its oxygen analogues on

the inhibition of acetylcholinesterase in Xiphinema using Gomori's

Method (Pearse , 1960).

Intact , freshly extracted nematodes were pretreated for

4 hr in 4% neutral formalin and then for 1 hr in a saturated solution

of sodium sulphate containing aldicarb , its sulphoxide or sulphone at

0.1 , 1.0 and 10 ppm , at 37.5°C. Thirty nematodes in batches of six

after the initial treatment were transferred to a 5 ml solution of acet-

ylcholine iodide and buffered copper ions and were then incubated at

37°C for 24 hr.   Enzymatic hydrolysis of the acetylcholine iodide

yields thiocholine in the form of a copper thiolate.   The incubated

nematodes were rinsed in saturated sodium sulphate and then treated

with concentrated ammonium sulphide for 1 to 2 minutes.   A dark

brown cupric sulphide deposit was formed with tissues in those sites

where thiocholine was released , while areas where enzymatic hydrolysis

did not occur remained unstained.

The percentage of treated nematodes in which hydrolysis

was inhibited was determined by the absence of a brown deposit at the site

of the nerve ring of Xiphinema behind the base of the spear , were

compared.

13. Autoradiography of Xiphinema diversicaudatum Treated in

^{14}C Labelled Aldicarb.

Xiphinema treated in a 3.016×10^{-4}M solution of labelled aldicarb for 6 hr at 20°C were washed in distilled water and then treated in two ways. Nematodes were either mounted whole onto slides which had been coated with Meyer's albumen containing a little formalin , or the individual nematodes divided transversely into 3 sections and fixed in a solution of gluteraldehyde (Gordons) for 2 hr. The nematodes were rinsed in a buffer pH 7.2 and placed in veronal acetate and osmic acid for a further hour, when they were washed and dehydrated by means of a graded series of alcohols. The tissues were then transferred to 'araldite' which was polymerised at 60°C for 48 hr . The blocks were shaped and transverse sections of Xiphinema were cut at 900 A^{o} on a Reichart Ultra-microtome. The sect-- ions were collected on small peices of glass coverslip and the specimens flattened by means of vapour from chloroform.

The whole mounted nematodes or transverse sections on microscope slides were then covered with stipping film , Kodak AR 10. The emulsion was floated on distilled water at 22° C and allowed to expand (Doniach and Pelc, 1950). The microscope slide containing the specimens was then submerged beneath the film and then slowly withdrawn bearing the film. Excess water was removed with blotting paper and the film allowed to dry before it was transferred to a light proof box containing silica gel and stored for 25 days at 4°C to expose the film.

The stripping film was then developed in Kodak 19b at $11^{\circ}C$ for

20 minutes   , washed in distilled water and then transferred to Amfix

for 3 minutes.   The slides were then washed in running distilled

water   for 30 minutes and then dried.

The   specimens were covered with a   glass cover-

slip after the addition of Euparal[R]   to the surface of the stripping

film adjacent to the specimen.   The   whole mounts and   transverse

sections were then viewed under oil immersion to determine if there was

any localization of the silver grains on the emulsion of the stripping

film.

14. <u>The Uptake and Egress of ^{14}C Labelled Aldicarb in</u>

<u>Saprophagous and Mycophagous Nematodes.</u>

This investigation was divided into two parts:

a) <u>Uptake Studies</u>

<u>Aphelenchus</u> <u>avenae</u> and <u>Panagrellus</u> <u>redivivus</u> were used as the test organisms because of the ease in which large numbers could be cultured. <u>Panagrellus</u> were harvested from cultures after 25 days , while <u>Aphelenchus</u> after 30 days. Extraction was done on a modified Seinhorst Mistifier , <u>Panagrellus</u> culture media poured onto Coldstreem filters and the nematodes collected in 3 hrs. The <u>Fusarium</u>-PDA culture media containing the <u>Aphelenchus</u> were diced prior to placing in the mistifier and the nematodes extracted over a 24 hr period.

<u>Panagrellus</u> were either used immediately or starved for 24 hr at 20°C before use , so that the permeability of nematodes to labelled aldicarb and its subsequent breakdown by starved and unstarved animals could be compared. Nematodes were not otherwise stored before use.

1   <u>Size of nematodes</u>

The biomass of <u>Aphelenchus</u> and <u>Panagrellus</u> used in uptake experiments was determined by estimation of the weight and volume using formulae suggested by Andrássy (1956). The lengths and maximium widths of 30 adult and larvae <u>Aphelenchus</u> and <u>Pana-</u> <u>grellus</u> in F :A 4 : 10 were measured on a microscope slide as it was

would be in the order of 1 to 4%.   The   total number

of nematodes in each treatment aliquot and the ratio of adult to larval

stages was calculated.   The biomass of each sample was then estimated

from the mean weights of larvae and adults.   This procedure was

adopted since although it was possible to seive nematodes to remove

the smaller second and third stage larvae , some could not be excluded

and together with the fourth stage larvae their biomass if not taken into ,

account may enlarge the errors incurred.

The quantification of labelled material as $\mu g/g$

of nematode was considered to be more accurate than the use of m moles

of labelled material / ml of nematode as used by Marks et al (1968).

The breakdown of aldicarb in nematodes produced compounds of different

molecular weight which would need to be taken into consideration., since

the   labelled material detected in the residue fraction of · nemat-

ode's was not identified.it was not known in which form it was present

or its molecular weight.

2.   Exposure technique.

Approximately 50000 Panagrellus of biomass which varied

from 89 - 160 mg were exposed to a 20 ml solution of a $3.016 \times 10^{-4} M$ c^{14} -

labelled aldicarb solution (50 ppm) , contained in 100 ml stoppered flasks.

The treatment solutions were kept in darkness at $20^{o}C$ and the flasks gently

agitated to keep the nematodes in suspension.   The same procedure was used

with Aphelenchus where approximately 1,000,000 nematodes of biomass from

45 - 70 mg .

The exposure period was terminated by rapid fitration of

the treatment solution using a medium porosity glass sinter - stuck sug-

gested by Marks et al (1968),   The nematodes   were

Stage	Panagrellus redivivus			Aphelenchus avenae		
	Length μ	Width μ	Biomass mg	Length μ	Width μ	Biomass mg
Adult	1476	49.3	0.00224	666	19.2	0.00015
Larva	973	31.3	0.00059	307	10.3	0.00002

Table   2   A comparison of the mean lengths , widths and estimated biomass of Panagrellus and Aphelenchus used in uptake studies.

systemically traversed and their biomass calculated (Table 2).

While the mean length of adult Panagrellus was similar to that reported by Goodey (1943) their widths were smaller , and the overall size of Aphelenchus was smaller than had been anticipated. Evans and Fisher (1970) suggested that the size of Aphelenchus would vary considerably with the type of culture media and would decrease in size with the age of the culture.

Andrássy (1956) suggested that his rapid method for estimating weights and volumes gave errors of between 1 to 4 % when compared to data obtained from precise and exact measurement. In this investigation the quantification of labelled nematicide per gram of nematode using Andrássy's formula would mean that the errors

washed three times with alternate 10ml volumes of acetone and water

in less than 1 minute.   The labelled nematicides were then extracted

from the nematodes and the treatment solution as has been described

in Section 4 b) para 2.

All the results obtained from these investigations were the

averages of no less than two replicates.

b)   Egress of Labelled Nematicides

The egress of aldicarb and its

breakdown products from Panagrellus · was investigated.   While it

was not purposely intended the test organisms were starved for 24 hr

before they were exposed to   20ml of a 3.016×10^{-4}M solution of

^{14}C labelled aldicarb for 1 hr.   These nematodes were washed in

distilled water using a filter stick to remove the washings , and after

30 seconds returned to a solution of non-labelled aldicarb at 50 ppm.

The treatment solutions were kept in   darkness at 20°C and the flasks

gently shaken to keep the nematodes in suspension.

The exposure of nematodes in non-labelled aldicarb was termin-

ated after 10 , 20 , 40 or 60 minutes when the nematodes were washed

three times as described above for the uptake studies.   The washings

were added to the treatment solutions and the labelled and non- labelled

nematicides were partitioned into chloroform as described in Section

4 b) para 4 .

Quantification was made of the amount of labelled material in

the residue fraction of the nematodes after centrifugation   (Section 4

b) and of the labelled compounds detected in the supernatant by TLC.

All the results were the averages of no less than two replicates.

RESULTS

1.   The Effect of Aldicarb Applied as Soil Drenches on the

Control of Meloidogyne incognita Larvae on Tomatoes.

Several aspects of the control of Meloidogyne were

investigated.

a) The Effect on Hatch of Egg Masses of Meloidogyne and Root

Invasion by Second Stage Larvae.

The hatch of second stage larvae from egg masses

described in Section 5 , took place rapidly.   Within 1 week a total

of more than 200 larvae were found in the soil and tomato roots of

control groups, and the total hatch in treated soils was not signif-

icantly reduced at a concentration less than 8 ppm (Figure 5).

Exposures of 2 or 3 weeks duration were found to have a marked affect

on total hatch. While large numbers of larvae were extracted from the

aldicarb treated soils less were extracted from control soils after 2 weeks

The reduction in root invasion by second stage larvae

after soil drenches was greatest after 1 week , at 1 ppm 90% and at

8 ppm it was 99% of the total number of hatched larvae.   Invasion

of roots after a 3 week treatment was approximately 65% of the total

number of hatched larvae in the control groups , reduced to 46% at

1 ppm and was only 9% in the 8 ppm treatment .

While second stage larvae in the untreated control

soils invaded tomato roots rapidly it was evident that aldicarb treat-

did not inhibit hatch but the second stage larvae accumulated in the

soils and not in the plant roots.   In pot soils aldicarb delayed

Figure 5   The effect of aldicarb applied as a soil drench

on the hatch and root invasion of _Meloidogyne_ larvae

from egg masses at 25°C in 3 weeks.   The total larval

hatch was the summation of the larvae in treated soil

and tomato roots.

TOTAL LARVAL INVASION

TOTAL LARVAL HATCH
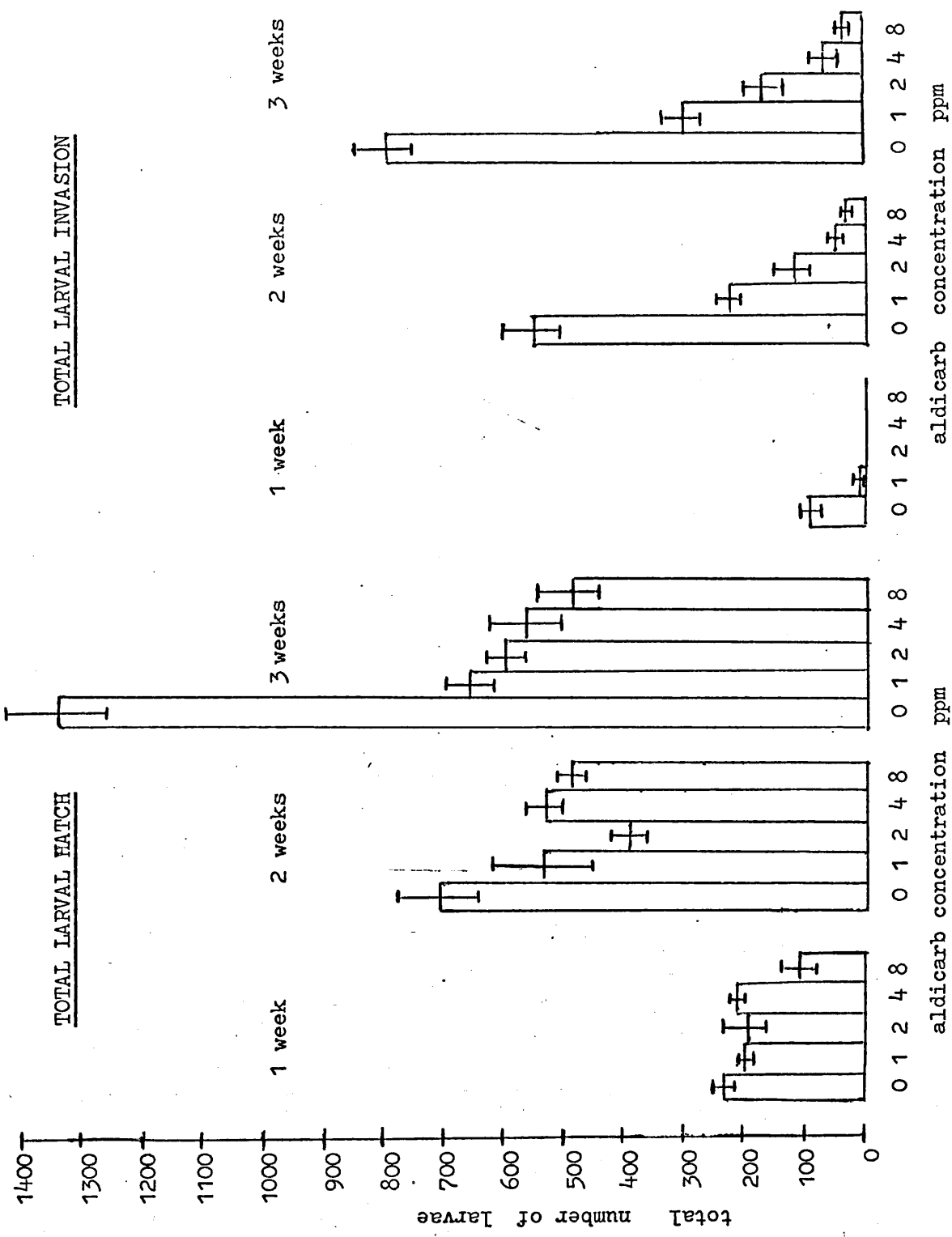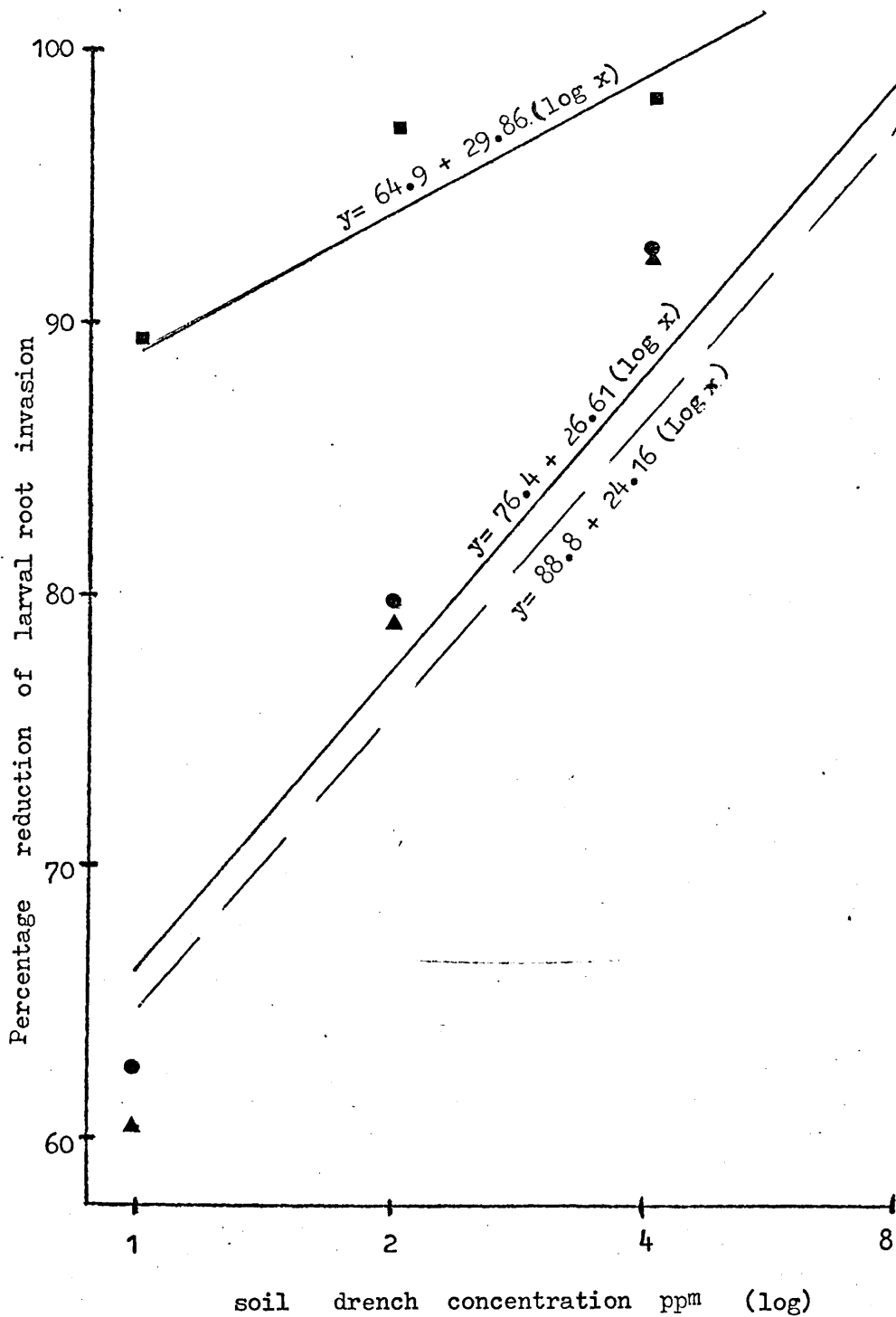
Figure 6   The effect of aldicarb on the invasion of tomato roots

by second stage larvae of Meloidogyne in 3 weeks at 25°C.

Key: ■———■ 1 week;   ▲———▲ 2 weeks;   ●———● 3 weeks.

b) The Effect on Root Invasion of Second Stage Meloidogyne

Larvae.

To eliminate the effect of aldicarb on the hatch of

second stage larvae , drenched soils were inoculated with freshly hatch -

ed larvae.   The tomato plants were carefully separated from pot soils

at weekly intervals for 4 weeks , the roots washed and stained.

After 1 week 21% of the total inoculum in untreated soils had invaded

roots , 31% after 2 weeks and 60% after 3 weeks.   The invasion of

larvae into tomato roots in aldicarb treated soils was significantly

reduced at all concentrations. The number of larvae / g root did not

exceed 7.2 with any treatment compared to 60.5 / g in the untreated

control roots after 1 week (Table 3).   However the weight of treated

and untreated tomato roots infected with larvae were similar , at 1

week the control mean root weight was 1.8 g, 3.6 g after 2 weeks and

6.7 g after 3 weeks.   .

Aldicarb at 1 ppm applied as a soil drench reduced

the invasion of larvae into roots by 80%.

Length of treatment	Number of larvae / g root				
	0	1 ppm	2 ppm	4 ppm	8 ppm
1 week	60.5	5.0	3.3	1.8	1.6
2 " s	43.8	7.2	3.4	1.9	0.5
3 "	45.1	5.0	3.0	1.9	0.5
4 "	49.1	7.0	3.8	1.3	0.7

Table 3   The number of Meloidogyne larvae per gram tomato root

after approximately 500 larvae were inoculated into pot soils

drenched with aldicarb.

. c) <u>The Effect on Extraction of Larvae from Treated Soils.</u>

Hatch and root invasion are affected by the presence

of aldicarb in soils , but a number of other soil parameters may also

be involved.

It was found that <u>Meloidogyne</u> larvae inoculated into

soils as described in Section 8 a), were less extractable from treated

soils (Figure 7). When larvae were treated in soils for 24 hr , ex-

traction using a Whitehead Tray for 36 hr was reduced by 70% by aldi-

carb , 51% by sulphoxide and 19% by sulphone treated soils. Two week

exposure reduced larval extraction by approximately 50% with all treat-

ments.

C T T_1 T_2 C T T_1 T_2 C T T_1 T_2

24 hr   7 days   14 days

Length of treatment after drenching
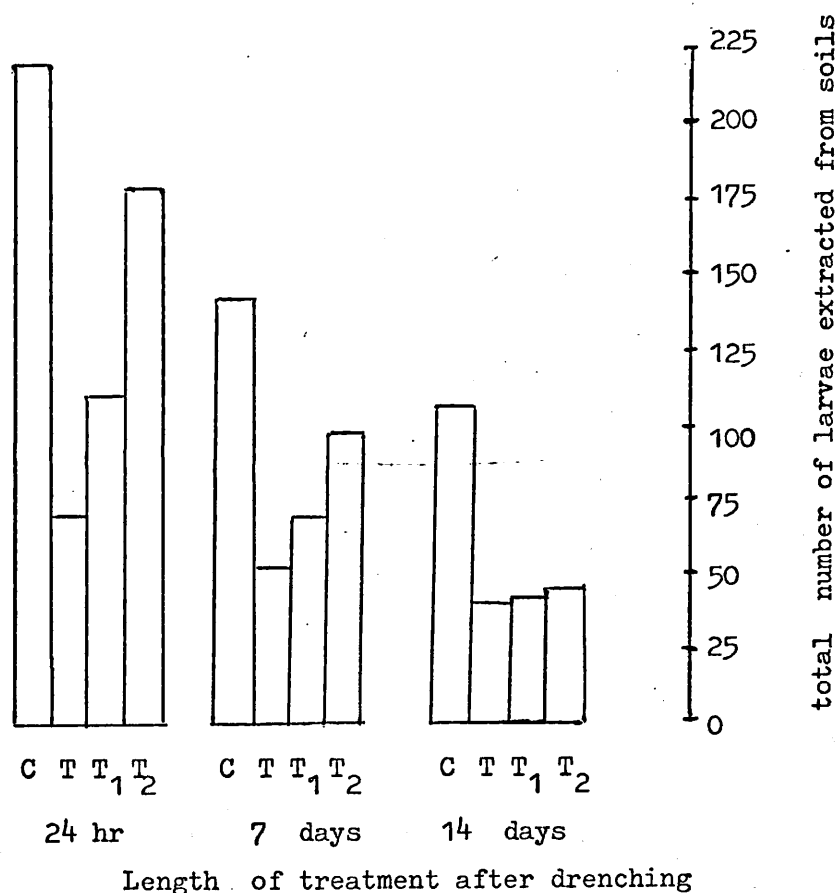
<u>Figure 7</u>   The effect of aldicarb and its oxygen analogues applied

as soil drenches at 4 ppm on the extraction of <u>Meloidogyne</u> larvae

by Whitehead Tray. Key: C control , T aldicarb , T_1 sulphoxide ,

T_2 sulphone.

2.   The In Vitro Effect of Aldicarb and its Oxygen Analogues on
the Hatch and Invasive Capacity of Second Stage Larvae of
Meloidogyne.

Two parameters were investigated.

a)   The Effect on Egg Hatch.

The procedure was   described in Section 7.
In 20 days the total hatch of Meloidogyne larvae in water was approx-
imately 88 to 95% of the total number of eggs.   Hatching commenced
within 5 days and was completed within 20 days , when the exposure per-
iod was extended few larvae were recovered.

Aldicarb at 50 and 100 ppm was found to completely sup-
press egg hatch , a few larvae were recovered within the first 5 days
of treatment   but hatching then ceased (Figure 8).   The hatch
of second stage larvae was reduced significantly at all treatment con-
centrations after a 10 day exposure.
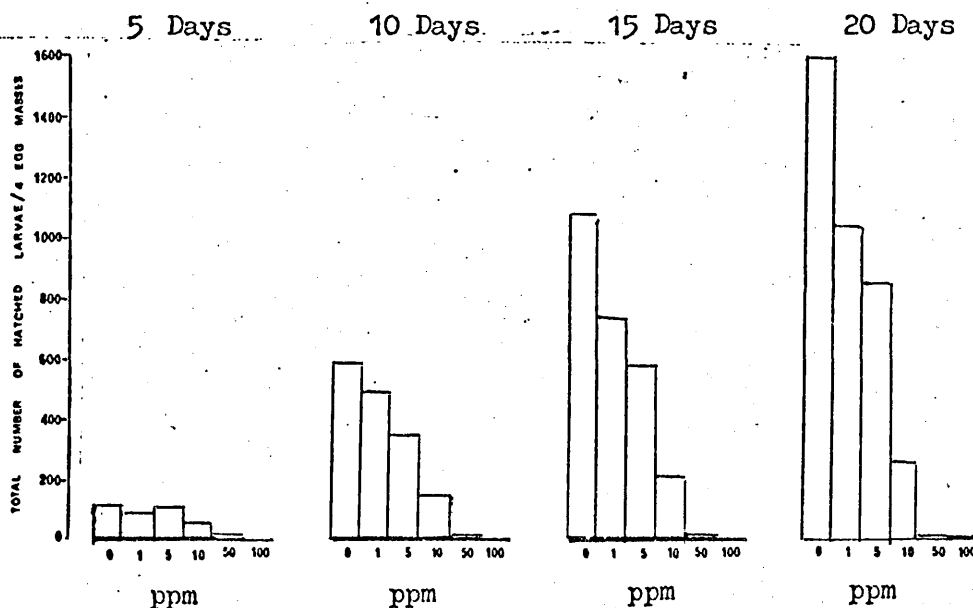
Figure 8   The in vitro effect of aldicarb on the cumulative hatch
in 20 days at 25°C of Meloidogyne larvae

Figure 9   The effect of aldicarb on the <u>in vitro</u>   hatch of

<u>Meloidogyne</u> eggs in 20 days at 25°C.   Regression lines

represent the depression of the culumulative hatch at 5 day

intervals.   Key: ▣————▣ 5 day; ●— — —● 10 day;

▲—— —— ——▲15 day; ▢—— ——▢20 day.
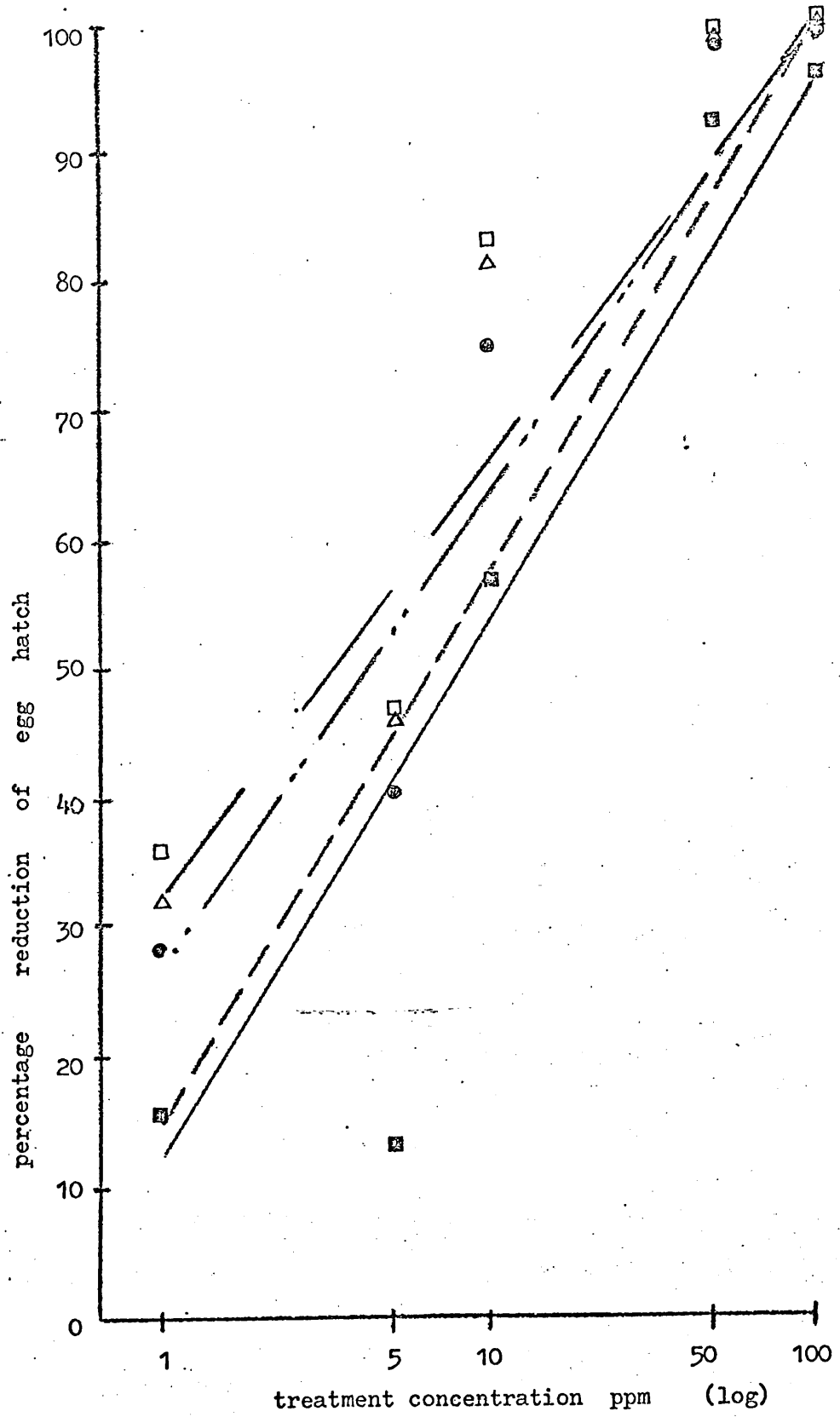
Nematicide and treatment (ppm)		Percentage reduction of egg hatch during treatment			
		5 Days	10 Days	15 Days	20 Days
1	T	28.53	15.96	32.22	35.12
	T_1	10.63	nil	nil	nil
	T_2	nil	nil	nil	nil
5	T	13.17	40.92	46.30	46.65
	T_1	24.56	21.39	21.09	20.96
	T_2	nil	nil	nil	nil
10	T	57.37	75.17	81.15	83.90
	T_1	25.48	13.16	11.49	10.98
	T_2	nil	25.00	26.59	26.90
50	T	92.79	98.50	99.20	99.42
	T_1	66.29	60.63	61.49	60.87
	T_2	34.00	31.00	32.70	33.00
100	T	96.25	99.13	99.51	99.61
	T_1	88.89	87.83	88.79	88.33
	T_2	65.00	66.00	66.49	66.20

Table 4   The in vitro effect of aldicarb and its oxygen analogues on the hatch of Meloidogyne eggs from egg masses at $25^{\circ}C$. Percentage reductions were based upon egg hatch in water which were regarded as 100%. Key: T aldicarb, T_1 sulphoxide, T_2 sulphone.

The hatch ED_{50} with aldicarb calculated from the re-
gression lines (Figure 9) was approximately   8 ppm after 5 days and
3 ppm after a 20 day exposure.

The hatch was also affected if aldicarb sulphoxide or
the sulphone were substituted , but neither compound was as effective in
reducing egg hatch as aldicarb (Table 4).   At 1 ppm for 20 days
the sulphoxide and sulphone did not reduce hatching , the ,hatch ED_{50}
calculated from the regression lines (Figure 10) after 20 day exposures
were 24 ppm for the sulphoxide and 66 ppm for the sulphone.
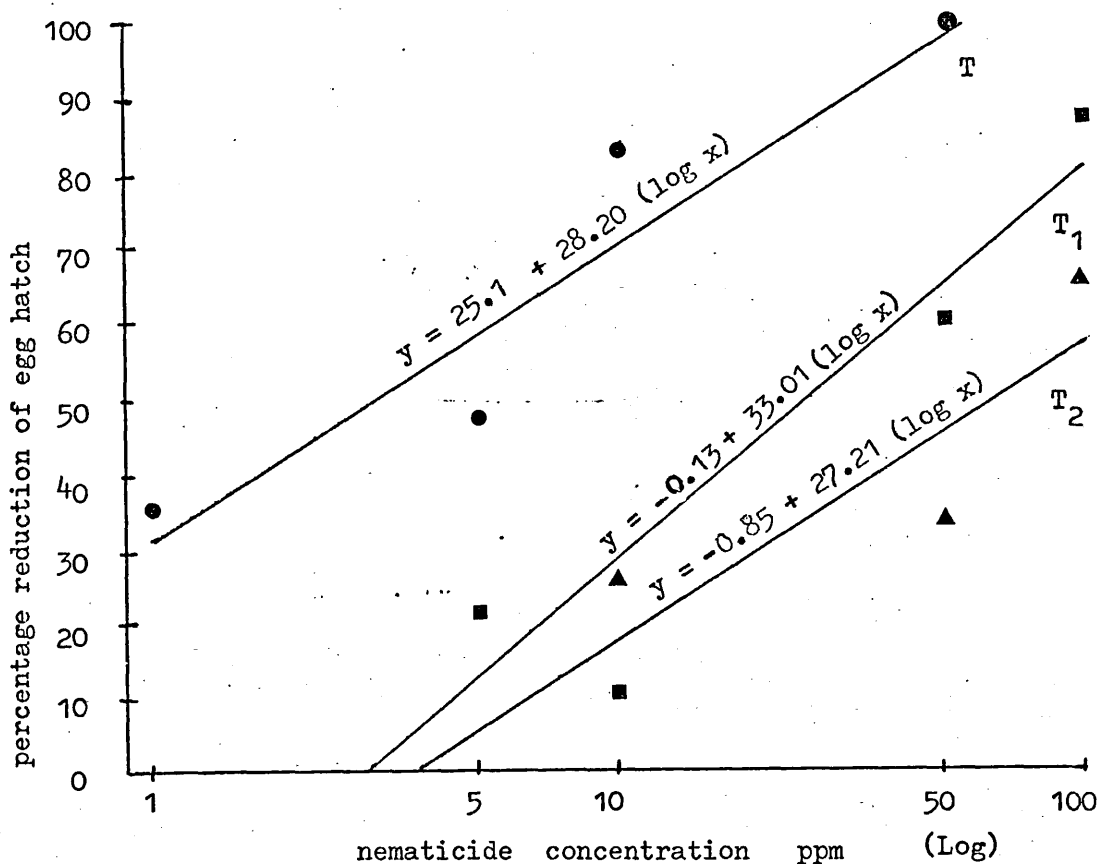
Figure 10   The in vitro effect of aldicarb and its oxygen analogues
on the reduction of egg hatch of Meloidogyne larvae after
20 days at 25°C. Key: ●——● T , ■——■ T_1, ▲——▲ T_2.

b)   The Effect on Root Invasion by Second Stage Larvae.

The method used for this study was described in Section 7.
The reduced larval invasion may have been a direct result of exposure
to aldicarb in soils on the infective capacity of second stage larvae.

This was tested by _in vitro_ exposure of approximately
300 freshly hatched larvae of 1 or 7 days at $20^\circ C$ with aldicarb solu-
tions.   The root invasive capacity of larvae treated in water
for 1 day was 60% but was reduced to 35% when treated in water for 1
week (Table 5).   Exposure to aldicarb at low concentrations did
not reduce their invasive capacity , while at 100 ppm invasion was
reduced by 54% .   Treatment for   1 week was found to have a marked
effect at all concentration , an ED_{50} for invasion was calculated from a
regression line   (Figure   11)   as 23 ppm aldicarb.

Treatment (ppm)	24 hr Treatment		7 Day Treatment	
	mean number of larvae in roots	% of ino-culum in roots	mean number of larvae in roots	% of ino-culum in roots
0	180.0	60.0	104.2	34.7
1	179.2	58.4	93.3	31.1
10	177.5	59.0	56.7	18.9
100	98.3	32.0	17.5	5.8

Table 5   The invasion of tomato roots by approximately 300 freshly

hatched Meloidogyne larvae after in vitro treatment   with

aldicarb for 1 day or 1 week.

62.
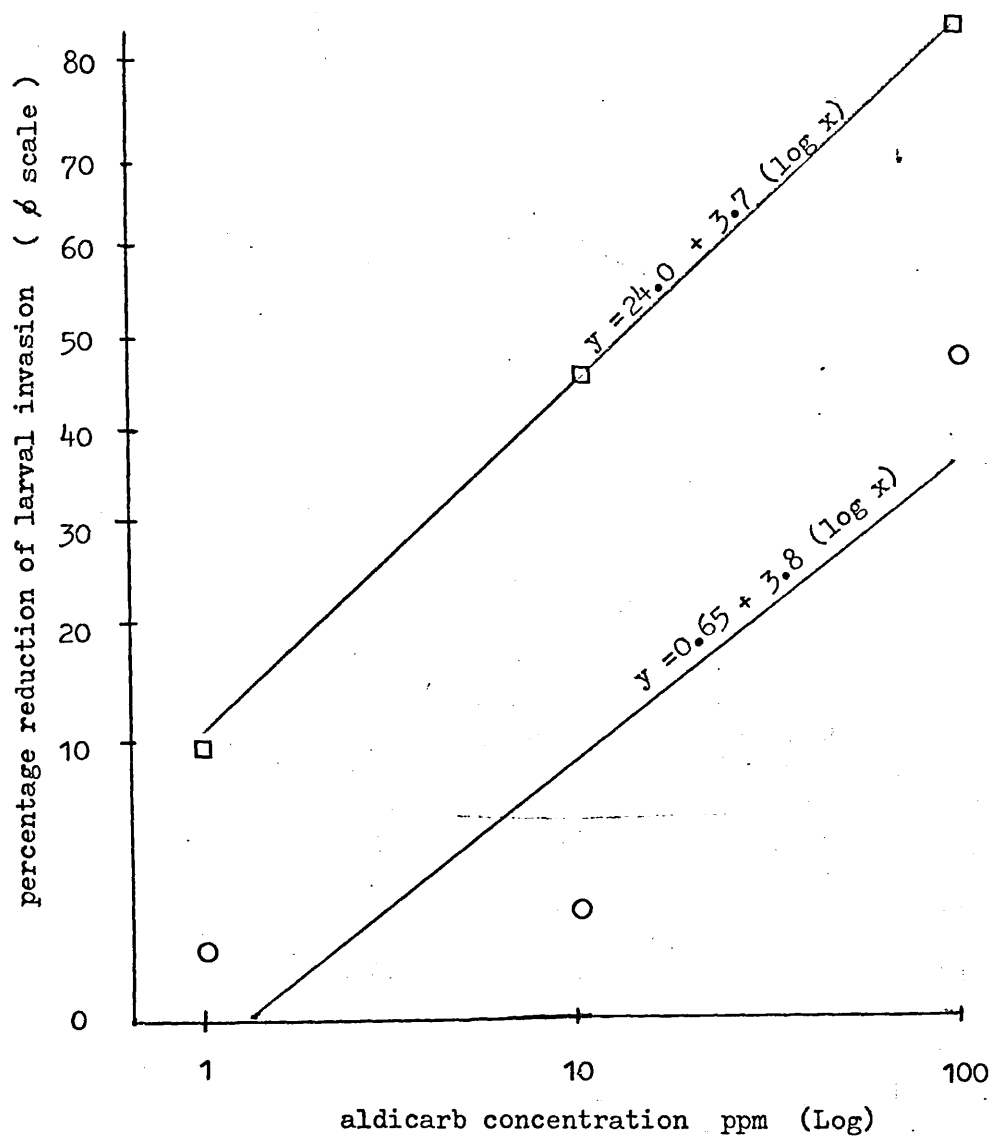
Figure 11. The reduction of tomato root invasion by second stage Meloidogyne larvae treated in vitro with aldicarb for 1 day or 1 week and inoculated into soil containing tomato plants for 2 weeks at 25°C . Key: o——o 1 day, □———□ 1 week.

3.   The Effect of Dipping Tomato Roots in Aldicarb on the

Invasion of Second Stage Meloidogyne Larvae.

The invasion of freshly hatched Meloidogyne larvae
into tomato roots which had been dipped in solutions of aldicarb (out-
lined in Section 6) was investigated. Invasion of roots by larvae which had
been dipped in water was between 57 and 64%, but roots treated in
aldicarb were found to have fewer nematodes (Table 6).

Whenlarvae were inoculated into soils surrounding treated
tomato plants two or nine days after the plants were repotted, their
invasive capacity was reduced by 39 - 50% and 29 - 47% respectively with
all treatments.   Although when the interval between dipping and ino-
culated was lengthened   the effect upon invasion declined, there was
no apparent difference between the treatment concentrations.

Treatment (ppm)	inoculated after 2 days		inoculated after 9 days	
	Mean number of larvae in roots	% reduction of larval invasion	Mean number of larvae in roots	% reduction of larval invasion
0	191.7	nil	170.0	nil
1	114.3	40.4	116.7	31.4
4	100.7	47.5	120.0	29.4
8	116.3	39.3	116.7	31.4
12	95.0	50.4	90.0	47.1

Table 6   The invasion of second stage Meloidogyne larvae into
tomato roots which were dipped in aldicarb for 24 hr, washed
and subsequently grown for 2 or 9 days at 25°C before the
soils were inoculated with approximately 300 larvae.   The
roots were examined after growing for a further 14 days.

4. ## The Uptake and Fate of Aldicarb in Tomato Plants after

Root Dipping.

The presence of   some   of the toxic nemat-
icide in plant roots may influence the invasive capacity of Meloido-
gyne.   The uptake and fate of aldicarb applied as root dips to
tomato plants was investigated so that the relationship between the
amount of nematicide within plant tissues and the degree of larval
invasion   could be clarified.

The method used was described in sections 6 and
4 a).   Aldicarb was found to be rapidly taken up into tomato
roots and translocated to the aerial portions of the plant (Table 7).
Aldicarb was not distributed evenly throughout the plants and was
broken-down rapidly to the sulphoxide and sulphone analogues.
Aldicarb was found in the root and stems of plants which had been
sampled immediately after a 6 and 12 hr dip , but it was not detected
in the leaves of any treatments.   Plants which had been dipped for
24 hr were found to contain less aldicarb, detected only in the stems
and greater amounts of the sulphoxide but little sulphone.   The
sulphone produced was only detected in the roots of plants dipped
for 12 or 24 hr (Table 7 and Figure 12).   The amount of nematicide
detected in plant root and greens increased with increasing dip
concentration , and the amount in the greens exceeded the amount in
the roots after a 24 hr treatment.   At 12 ppm for 24 hr the aerial
portion of tomato plants contained approximately 9 μg/g of nematicide ,
the roots only 5 μg/g .

Plants sampled immediately after 24 hr root dip.
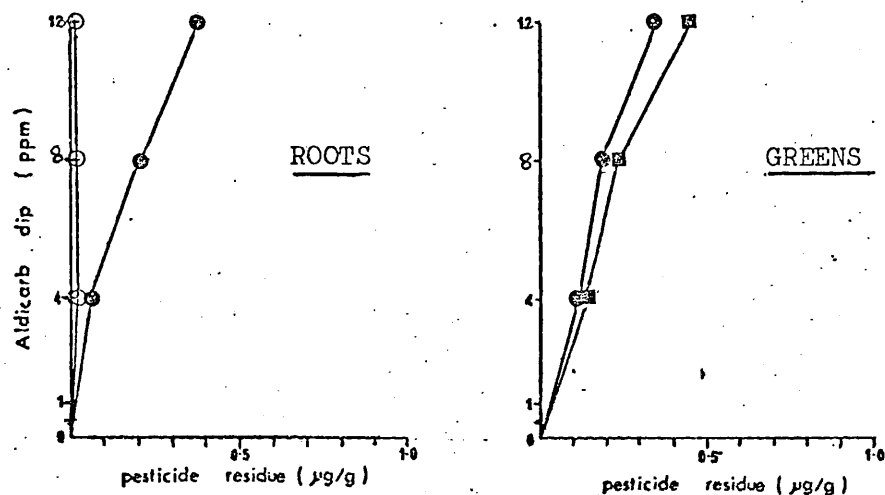
Plants dipped and grown for 16 days at 25°C
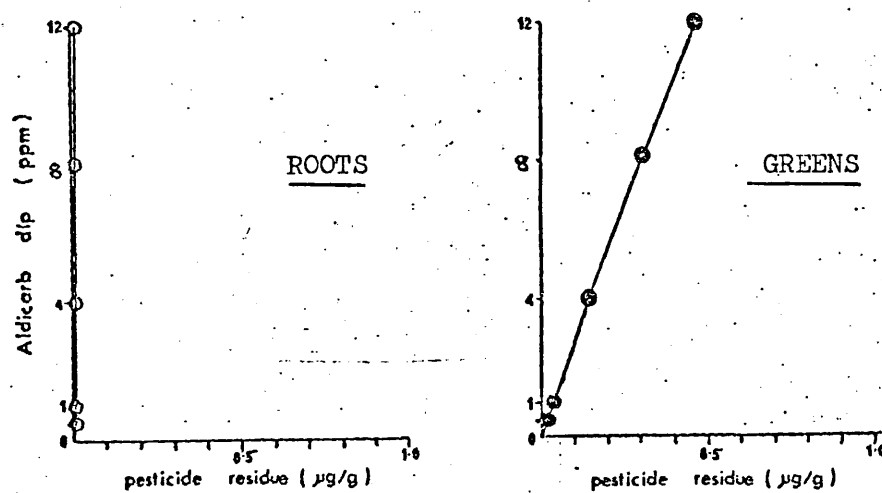
Figure 12   The distribution and fate of aldicarb and its

oxygen analogues in tomato plants cv MoneyMaker ,

immediately after root dipping in aldicarb for 24

hr or repotted and grown for 16 days.

Key : ■——■ T , ●——● T$_1$, ○——○ T$_2$.

Plant Region	ROOT			STEM			LEAF		
Residue Detected	T	T_1	T_2	T	T_1	T_2	T	T_1	T_2
Length of dip									
6 hr	+	+	ND	+	+	ND	ND	+	ND
12 hr	+	+	+	+	+	ND	ND	+	ND
24 hr	ND	+	+	+	+	ND	ND	+	ND

Table 7   The fate of aldicarb and its oxygen analogues in tomato plants which were dipped for 6 , 12 and 24 hr in a 4 ppm aldicarb solution at 20°C.

Key: + compound detected ,   ND compound not detected.

The amount and distribution of aldicarb and its oxygen analogues in tomato plants was investigated after the plants dipped in 1, 4 , 8 and 12 ppm of aldicarb for 24 hr , were repotted and grown at 25°C for 16 days.   These parameters were both markedly affected by the interval between treatment and sampling. Aldicarb was no longer detected in the plant system (Figure 12) , the sulphoxide was found to constitute the major detectable nematicide residue in the plant.   The sulphoxide was not evenly distributed throughout the plant for it was not found in the roots , while sulphone present in the roots was not detected in the aerial portions.

Residue Detected	Soil Drench Conc (ppm)		
	1	4	8
T	ND[a]	ND	ND
T_1	ND	TRACE[b]	0.05[c]
T_2	ND	ND	ND

Table 8   The fate of aldicarb in the lower, tomato

fruit-sets of plants grown for 15 weeks in soil

which had been drenched with aldicarb   at 8 ppm.

Key : a ND compound not detected , b   TRACE less

than 0.01 ppm , c amount in $\mu g/g$ plant tissue.

The amount of sulphone detected in roots

was not found to increase with increasing treatment concentration ,

and was not in excess of 0.01 ppm .   The amount of sulphoxide in

the aerial portions did increase with treatment concentration , at

4 ppm after 16 days it was 0.15 $\mu g/g$ and at 12 ppm increased to 0.45

$\mu g / g$ plant tissue.

Nematicide residues of aldicarb accumulated

rapidly in the aerial portions of tomato plants and not in the roots.

Only the presence of trace amounts of aldicarb sulphone in tomato

roots at 16 days after treatment is likely to influence the invasion

of Meloidogyne larvae.

When the tomato fruit-sets of dipped plants

were analysed by TLC after the plants had been grown for 15 weeks ,

no detectable nematicide residues were found.   Residues of the sulph-

oxide were only detected in fruits of plants which had been grown in

aldicarb drenched soils at 8 ppm (Table 8) none at lower concentrations.

5.   The Effect of Aldicarb and its Oxygen Analogues on

        Nematode Mobility.

        The mobility of larvae in treated soils could

be adversely affected for several weeks by the presence of the

nematicidal compounds.   The mobility of nematodes in soils is crucial

for the further continuance of their life cycle within plant tissues ,

or for the supply of food.   A reduction of nematode mobility,either

temporary or permanent would be likely to delay or entirely suppress

nematode development.   Several parameters were investigated.

        a)   The Dispersal of Nematodes on Treated Agar.

        The experimental methods were described in

Section 8 c).   Preliminary investigations were made of the disper-

sal of Panagrellus redivivus on aldicarb treated 1% ionagar.   Only

9 % of the control groups were found on untreated agar after 24 hr ,

but with increasing aldicarb concentration the number of nematodes

remaining on the agar increased (Table 9).   At 1 ppm 33% remained ,

at 100 ppm it was 68% and at 1000 ppm 87% of the nematodes inocula-

ted were observed on the treated agar.

        Dispersal of Panagrellus decreased with the

increase in treatment concentration.   Treated at 1 ppm for 24 hr

17% of the nematodes had not migrated   distances   greater than a rad-

ius of 10 mm .   At 1000 ppm , 38% were within a radius of 10 mm and

another 41 % a further 10 mm distance.

Treatment	Number of Nematodes in Zones after 24 hr [a]			% of total
(ppm)	0 - 10 mm	10 - 20 mm	20 - 26 mm	on media
0	2	4	3	9
1	4	14	15	33
10	17	19	12	48
100	27	27	14	68
500	37	31	10	78
1000	38	41	8	87

Table 9   The effect of aldicarb in 1% ionagar on the dis-

persal of Panagrellus in 24 hr in darkness.

Key : a   number was the mean of 10 replicates of 10

individual nematodes.

Although aldicarb did reduce the dispersal of

Panagrellus at 1000 ppm,13 % of the total number treated were able

to migrate a distance much in excess of 26 mm which was the radius

of the agar medium.   After 24 hr the activity of nematodes remaining

on the treated media was reduced and at concentrations of 100 ppm and

more the nematodes were motionless.

b)   The Movement Patterns of Nematodes on Agar.

The movement patterns of second stage Heterodera
and Meloidogyne larvae were affected by the presence of aldicarb , its
sulphoxide and sulphone in 1% ionagar when treated   for only 1 hr.
The effect of aldicarb on Meloidogyne larvae in treated agar was
marked at all concentrations (Figure 13), the tracks were reduced
in length but became more tortuous.

Meloidogyne larvae treated at 50 or 100 ppm
aldicarb became completely inactive within an 1 hr , but at lower
concentrations the larvae were still moving across the media.
Larvae treated with the sulphoxide and sulphone were also affected
(Table 10), not so marked however at the lower concentrations.
With the sulphone at 1 ppm the activity and movement pattern of Melio-
dogyne larvae was not affected.

Three activity parameters were investigated
(Table 10) and the least affected in 1 hr was the furthest disper-
sal of Meloidogyne larvae .   Aldicarb and its sulphoxide at 1 ppm
reduced the total number of undulations by between 20 and 30%, while
at 100 ppm they were reduced by 55 to 65%. The sulphone had the least
effect of the three compounds.

Dividing the total number of undulations against
the total distance in 1 hr , the rate   for untreated larvae was
37.   Only aldicarb was found to affect . this relationship at
10 ppm and above , indicating the the number of undulation per distance
travelled increased (Figure 14) during exposure.
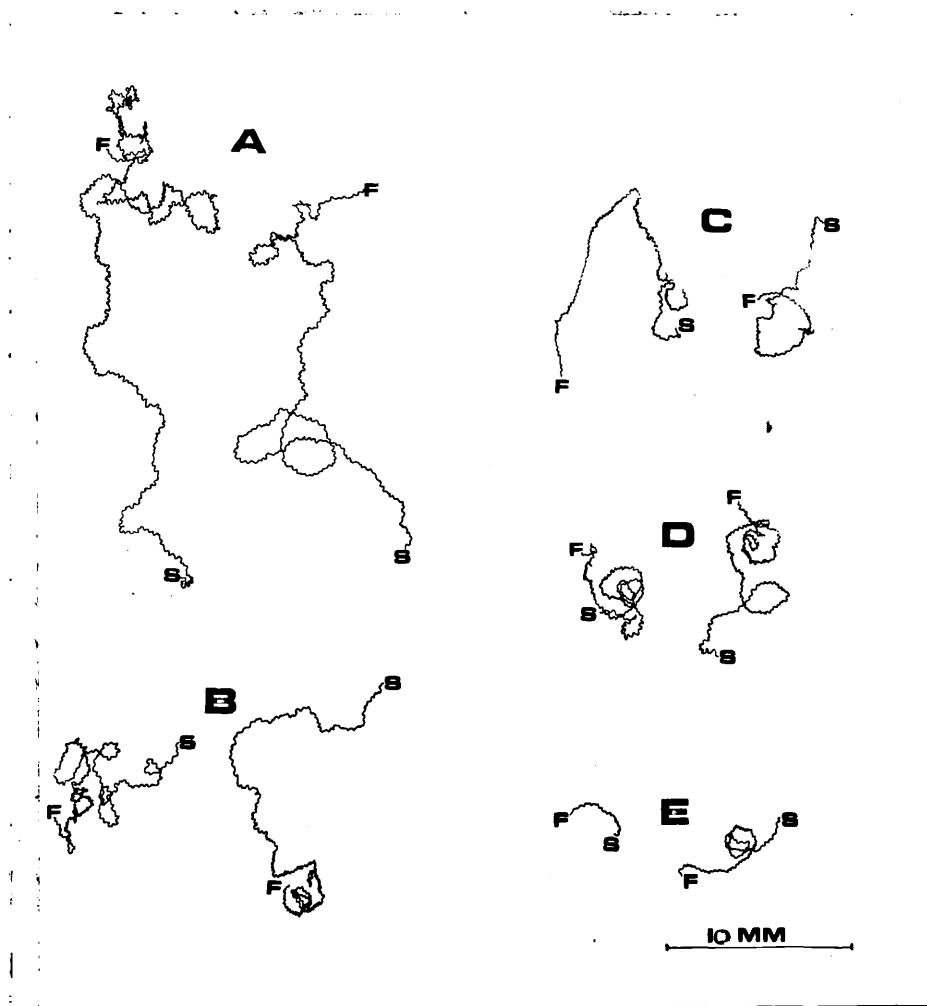
Figure   13   The movement patterns of second stage larvae

of <u>Meloidogyne</u> on aldicarb treated 1% ionagar   in

1 hr at 20°C in darkness.

Key: A   control ,   B 1 ppm ,   C   10 ppm ,

D 50 ppm ,   E   100 ppm ,   S   start ,

F   finish.

Table 10   The effect of aldicarb and its oxygen analogues   on the mobility of second stage <u>Meloidogyne</u> larvae on treated agar   after 1 hr and subsequent 2 hr on untreated media

TREATMENT PERIOD - 1 HR

Treatment (ppm)	total distance cm	S.E	total number undulations	S.E	furthest dispersal cm	S.E
0	6.08	± 0.34	222.6	±13.2	1.50	± 0.12
T 1	4.51	0.26	149.1	4.5	0.99	0.15
T 10	2.50	0.49	126.3	10.0	0.90	0.09
50	2.45	0.13	111.0	5.1	0.77	0.09
100	1.55	0.20	72.8	9.7	0.57	0.05
1	4.71	0.27	174.8	9.9	1.53	0.20
T_1 10	3.06	0.05	107.8	3.4	0.96	0.09
50	2.57	0.20	95.8	12.2	0.85	0.73
100	1.80	0.18	70.5	7.3	0.61	0.07
1	7.09	0.43	260.8	16.6	1.34	0.12
T_2 10	5.03	0.25	177.4	7.6	0.89	0.11
50	3.69	0.26	146.3	10.5	0.86	0.07
100	2.10	0.15	82.4	5.9	0.93	0.12

RECOVERY PERIOD - 2 HR

Treatment (ppm)	total distance cm	S.E	total number undulations	S.E	furthest dispersal cm	S.E
0	6.42	± 0.42	248.5	±16.3	1.56	±0.19
1	5.79	0.38	206.5	14.7	1.03	0.09
T 10	4.50	0.39	198.5	11.9	1.27	0.11
50	0.05	0.95	2.0	0.8	0.01	0.03
100	0.00	-	0.0	-	0.00	-
1	3.63	0.33	168.2	12.6	0.87	0.07
10	3.83	0.19	191.5	11.2	0.87	0.06
T_1 50	2.98	0.35	121.7	13.4	0.65	0.08
100	0.89	0.26	36.1	10.6	0.14	0.05
1	7.55	0.45	268.9	14.2	1.55	0.17
10	4.76	0.36	208.5	13.7	0.99	0.14
T_2 50	2.10	0.15	93.7	10.3	0.70	0.08
100	0.83	0.20	31.8	8.3	0.18	0.03

There was no significant difference in the
slope of the regression lines for treatment of Meloidogyne larvae
with aldicarb or its sulphoxide. The ED_{50} of total movement in
1 hr was approximately 10 ppm with aldicarb , 15 ppm with the sulpho -
xide and 60 ppm with the sulphone.

Meloidogyne larvae treated with aldicarb and its
sulphone at low concentrations recovered rapidly in the subsequent 2-
hr on untreated media. With aldicarb at 1 ppm the total distance
travelled by larvae in 2 hr was reduced by 17% , while at 100 ppm the
reduction was 100%. Aldicarb treated larvae were not observed to
recover with less than a 6 hr interval on untreated media (Table 11).
The recovery of treated larvae after being transferred to untreated
media was not instantaneous , since the mobility parameters would
not otherwise have differed from the control groups. Larvae treated
at high concentration with the sulphoxide and sulphone regained their
vigour quicker than those treated with aldicarb.

The regression lines , especially that of the
effect of the sulphoxide on the recovery of treated larvae did not
fit the points on the graph well, but it was apparent that larvae
treated with this compound at low concentrations did not recover
as rapidly as those larvae treated with the other two compounds.
The sulphoxide and sulphone treatments had a considerable affect on
the relationship between the total number of undulations and the total
distance travelled in 2 hr (Figure 14). The number of undulations
per mm travelled increased to nearly 50 while the control larvae was
an average of less than 40.

Treatment Period - 1 hr

Recovery Period - 2 hr
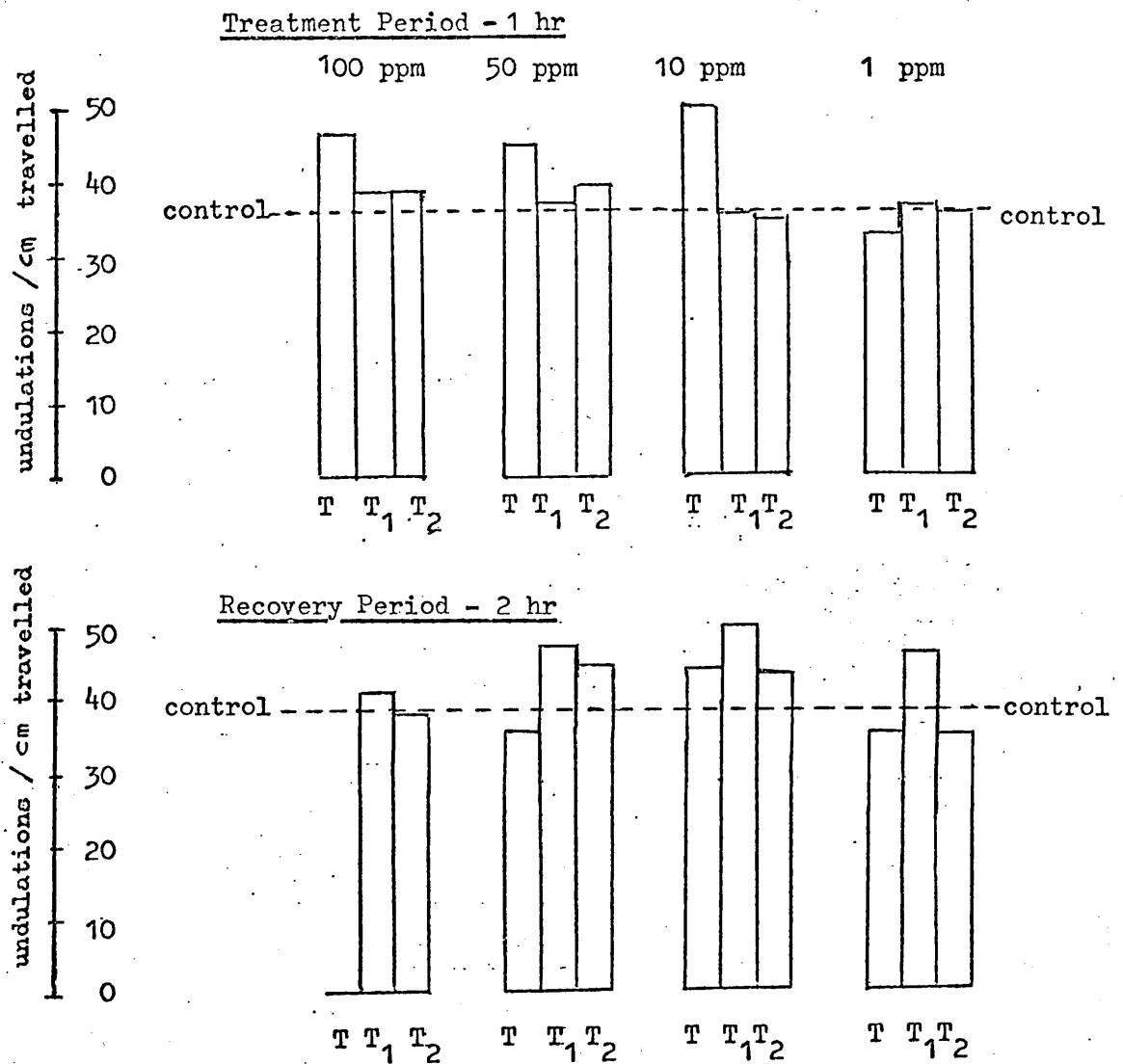
Figure 14   The effect of aldicarb , its sulphoxide and sulphone

in 1% ionagar on the relationship between the total

number of undulations made by Meloidogyne larvae in 1

hr and the total distance the larvae travelled.

Figure 15   The effect of aldicarb , its sulphoxide and

sulphone in treated 1% ionagar on the reduction

in mobility of second stage <u>Meloidogyne</u> larvae.

The percentage reduction of total movement , the

total number of undulations and the furthest

dispersal were   plotted as regression lines.

The larvae were treated for 1 hr and transferred

to untreated media for 2hr.

Key : A   1 hr treatment , B 2 hr recovery ,

O————O T , ▲————▲ T_1,   ■————■ T_2.

EFFECT ON THE TOTAL NUMBER OF UNDULATIONS

percentage reduction

T $y= 31.0 + 8.8$ (log x)
T_1 $y= 24.4 + 12.9$ (log x)
T_2 $y= 0.05 + 24.2$ (log x)

A

T $y= 6.2 + 35.6$ (log x) T
T_1 $y= 21.1 + 14.2$ (log x)
T_2 $y= -0.3 + 33.9$ (log x) T_2

T_1

B
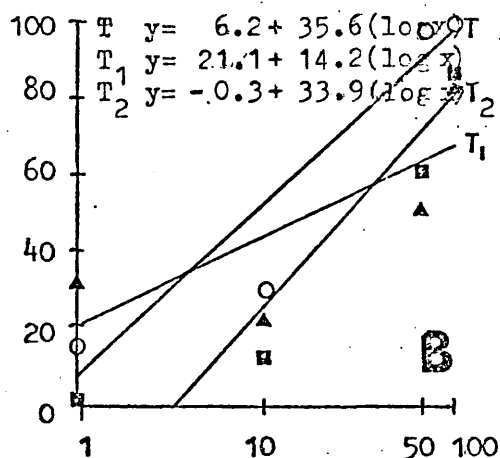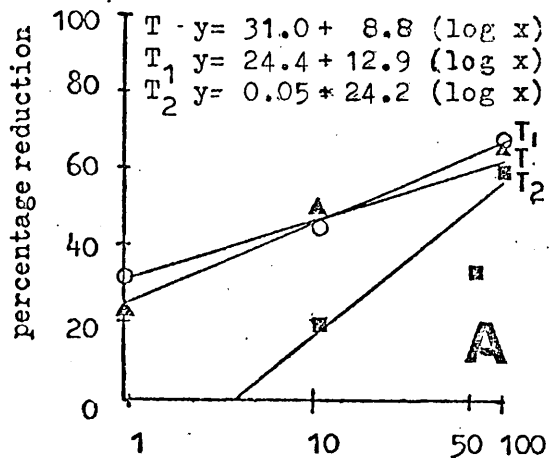
EFFECT ON THE TOTAL DISTANCE TRAVELLED

percentage reduction

T $y= 27.6 + 13.1$ (log x)
T_1 $y= 23.3 + 13.5$ (log x)
T_2 $y= -0.5 + 25.6$ (log x)

A

T $y= 4.1 + 37.5$ (log x) T
T_1 $y= 34.1 + 12.2$ (log x)
T_2 $y= -0.2 + 35.4$ (log x) T_2

T_1

B
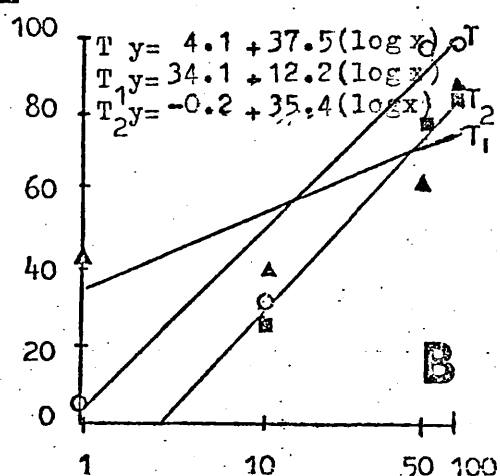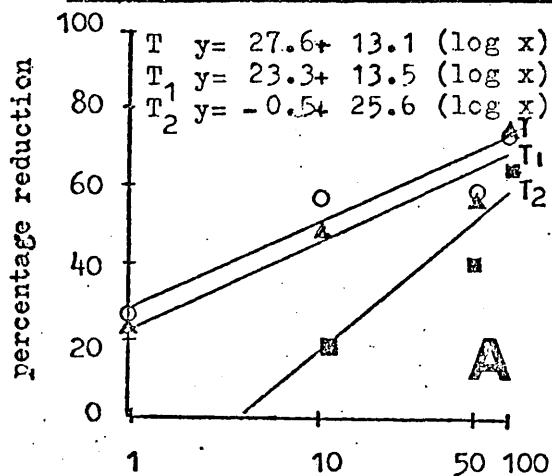
EFFECT ON THE FURTHEST DISPERSAL

percentage reduction

T $y= 32.1 + 7.7$ (log x)
T_1 $y= 16.3 + 9.2$ (log x)
T_2 $y= 0.5 + 34.0$ (log x)

T_1

T_2

A

T $y= 16.1 + 30.2$ (log x) T
T_1 $y= 27.6 + 16.8$ (log x) T_1
T_2 $y= 0.4 + 30.7$ (log x) T_2

B
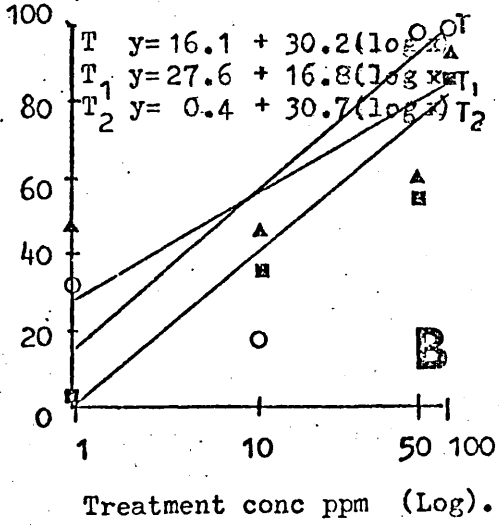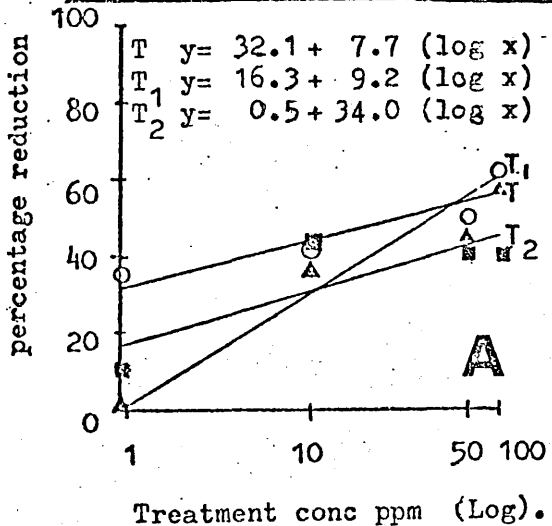
Treatment conc ppm (Log).

Treatment conc ppm (Log).

5 mm
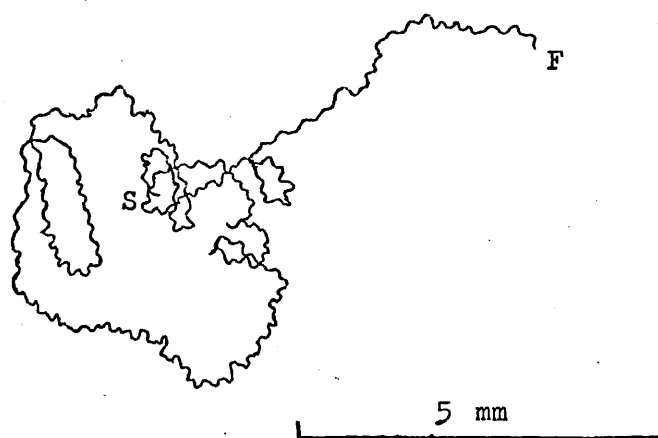
Figure 16   The movement pattern of a second stage

larvae of Meloidogyne on aldicarb treated agar at 50

ppm , when transferred after 1 hr to untreated med-

ium for 2 hr.

Key : S. position of larvae immediately after tre-

atment , F. position after 2 hr.

The movement pattern of larvae observed during the recovery

period was the reverse of the pattern during treatment. Larvae treat-

ed at high concentrations which had become motionless , on regaining

mobility their tracks were very tortuous (Figure 16). Progressive

recovery was observed , the pattern changed from closed to open spirals

and the number of undulations / cm travelled declined.

The velocity of Meloidogyne larvae of the control

group declined during the recovery period , the average undulation

rate / minute was reduced from 3.7 during the first hour to 2.1 in the

Recovery Period after treatment		distance travelled cm	total number of undulations	furthest dispersal cm
	T	0.00	0.0	0.00
4 hr	T_1	3.77	162.8	0.59
	T_2	3.40	118.0	0.87
	T	0.00	0.0	0.00
6 hr	T_1	2.37	92.0	0.38
	T_2	7.12	261.4	1.38
16 hr	T	10.23	338.2	1.38

Table 11   The recovery of second stage larvae of Meloidogyne

on untreated agar after an initial exposure to aldicarb ,

its sulphoxide and sulphone at 100 ppm .

subsequent 2 hr (Table 12).   Although the velocity of the untreated

larvae decreased by 44% , the average number of body undulations /

cm travelled in 2-hr was not affected.   There was little apparent

difference between the velocity of Heterodera and Meloidogyne   larv-

ae in 1 hr on untreated agar , but in the following 2 hr declined by

less than 20 %.

The mobility of Heterodera   was affected on

aldicarb treated agar , although they were not completely inactive

at the end of the recovery period , only 3 % produced a   track at all.

Treatment (ppm)	undulations / cm	undulations / min	progression cm / min
1 hr 0	26.7 (36.6)	2.2 (3.7)	0.08 (0.10)
100	28.3 (46.9)	1.1 (1.2)	0.03 (0.03)
Recovery			
2 hr 0	25.6 (38.6)	1.8 (2.1)	0.07 (0.05)
100	23.3 (-)	1.2 (-)	0.00 (-)

Table   12   The effect of aldicarb at 100 ppm in agar on the activity parameters of second stage <u>Heterodera</u> larvae treated for 1 hr and transferred to untreated media for 2 hr. Key: ( ) effect on <u>Meloidogyne</u> larvae.

<u>Aphelenchus</u> in contrast to the other nematodes tested did not become completely motionless . Although not progressing in a closed or concentric spirular pattern, the nematodes were observed to coil and recoil slowly on the treated agar medium.

c) The Dispersal of Nematodes In Nematicide Gradients

in Agar.

The formation of nematicide gradients in agar
was described in Section 8 c).   When Aphelenchus   were inoculated
onto untreated media and their position observed after 24 hr , only
41% of the original number were located (Table 13) on the media.
With Heterodera larvae treated in a similar manner ,83% were remain-
ing on the media.   When either species was placed on the nematicide
gradients the percentage of nematodes remaining on the media rose
markedly, except on sulphone gradients and Aphelenchus where 34% had
still migrated from the media.

With other than the sulphone gradients neither
Aphelenchus nor Heterodera were found to move across the agar to reg-
ions containing less of the toxic nematicide.   On the aldicarb grad-
ient with Aphelenchus 62% in the more toxic central two zones , 77%
on sulphoxide agar and 17% on sulphone treated agar.   The number of
Heterodera larvae remaining on the inner two, more . concentrated zones
was between 72 and 86% with all three compounds.

After 24 hr neither species was moving activity
across the treated media so that their migration was impeded.   Altho-
ugh the total amount of each compound in the zones was quantified , the
actual concentration in the surface of the agar in contact with the nem-
atode was unknown.   However exposure to 6.1 to 9.3 μg / g on these zo-
nes for short periods was not thought to seriously affect mobility , so
that nematodes could have responded by migrating to less toxic regions
before the nematicides brought about inactiviation.

Treatment	Mean number of nematodes on nematicide gradients[a]						% total nematodes on media
	individual zones				grouped zones		
	1[b]	2	3	4[c]	1 - 2	3 - 4	
Aphelenchus avenae							
O	0.50	1.16	0.91	1.50	1.66	2.40	40.7
T	2.83	3.41	1.75	1.85	6.24	3.40	98.6
T_1	4.25	3.41	1.41	0.91	7.66	2.31	99.8
T_2	0.91	0.83	1.85	3.00	1.74	4.85	65.9
Heterodera rostochiensis							
O	1.58	1.91	2.75	2.08	3.49	4.83	83.2
T	4.08	4.50	1.25	0.16	8.58	1.41	99.9
T_1	3.91	3.75	1.50	0.83	7.66	2.33	99.9
T_2	3.25	4.00	1.58	1.08	7.25	2.66	99.1

Table   13   The effect of aldicarb, its sulphoxide and sulphone in chemically defined gradients on the dispersal   of Aphelenchus and Heterodera   after 24 hr in darkness at 20°C.   Nematodes were inoculated   15 mm from the centre of the media demarking zones   1 and 2.

Key :   [a] 10 replicates of 10 individual nematodes,

[b] zone with the highest nematicide concentration,

[c] zone with the lowest nematicide concentration.

d)   The Effect on Nematode Feeding on Treated Fungal

   Cultures.

Preparation of Fusarium cultures treated with the three carbamoyloximes was described in Section 9.   Observation of Aphelenchus avenae showed that abnormal behaviour (feeding and locomotion) increased with increased nematicide concentration in the culture medium (Table 14).

The effect of aldicarb and its sulphoxide were most apparent at low concentrations , locomotion was reduced and feeding was not observed. At 1 ppm the stylets were extruded from between the lips , while at higher concentrations the nematodes had a flat-spiral posture , the nematodes only   coiled and uncoiled occasionally.

Aphelenchus normally distributed themselves throughout the entire medium following the channels formed by the growing hyphae.   While the sulphone did not affect their distribution , the other compounds restricted their dispersal and they were found only on the surface to which they were inoculated.

Response observed	0.1 PPM			1.0 PPM			10 PPM		
	T	T_1	T_2	T	T_1	T_2	T	T_1	T_2
Feeding	+	+	+	-	-	-	-	-	-
Locomotion	+	+	+	+	+	+	-	-	+
Abnormal stylet movement	-	-	-	+	+	-	-	-	-
Coiled - posture	-	-	-	-	-	-	+	+	-

Table 14   The effect of aldicarb , its sulphoxide and sulphone

on the general behaviour of Aphelenchus on treated Fusarium

cultures. Key: + response observed , - response absent.

e)   The Effect on Nematode Mobility in Aqueous
      Treatment Solutions.

The response of nematodes to these carbamoyloximes in aqueous treatment solutions was investigated by considering four parameters : body movement , stylet thrusting , nematode migration through filters and the loss of body contents during exposure. These are considered separately although they formed part of the same experiment   described in Section 8 b).

1.   Body Movement

A wide variation in the amplitude of the undulation rate was found between batches of Heterodera second stage larvae and between treatments.   The control undulation rate was 9 / minute after 1 hr , dropped to 7.5 / minute after 24 hr and yet again after 48 h r to 4 / minute.   The decline in the rate of undulation was not necessarily so great as in this example.   Thus to obtain a direct comparison , the percentage of larvae actively undulating during each observation interval was calculated from the original observations. This reduced much of the variation as before nematode were termed inactive no undulation should occur during each observation interval.

Of the three carbamoyloximes tested. aldicarb was the most effective.   Inhibition of body activity was rapid at the high dosage levels of 5 and 10 ppm (Figure 16 .1).   After the initial 24-hr nematicide treatment all of the Heterodera larvae treated with 10 ppm aldicarb were inactive , compared to 85% inactivity with the sulphoxide and 65% with the sulphone.   The majority

Figure 16.1   Percentage of   Figure 16.2   Percentage of

second stage <u>Heterodera</u> larvae   second stage <u>Heterodera</u> larvae

that exhibited undulatory   that exhibited abnormal stylet

body movements.   movement.

All larvae were treated with different carbamoyloximes at

1 , 5 and 10 ppm for 24 hr , followed by a 24-hr aerated water

treatment.

      Key :   ●——●   Control , □——□ 1 ppm , O——O 5 ppm ,

      ■——■ 10 ppm.

The effect of aldicarb
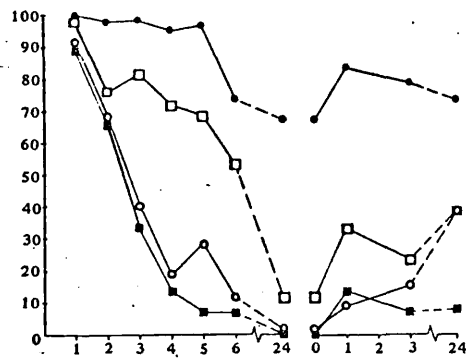
The effect of aldicarb
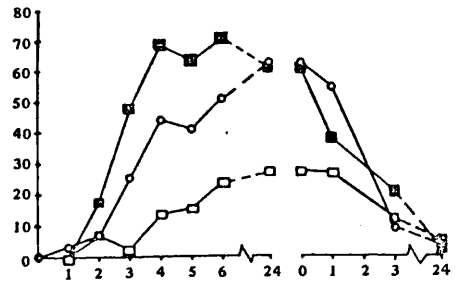
The effect of the sulphoxide
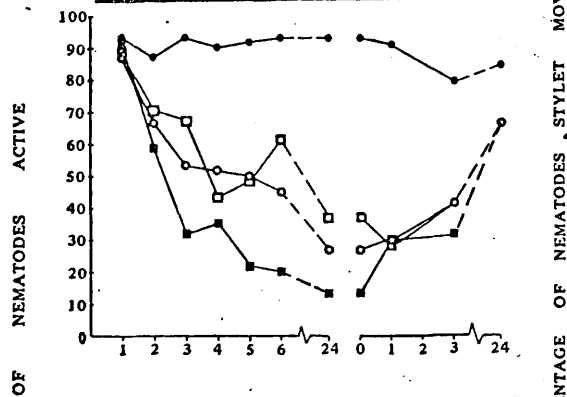
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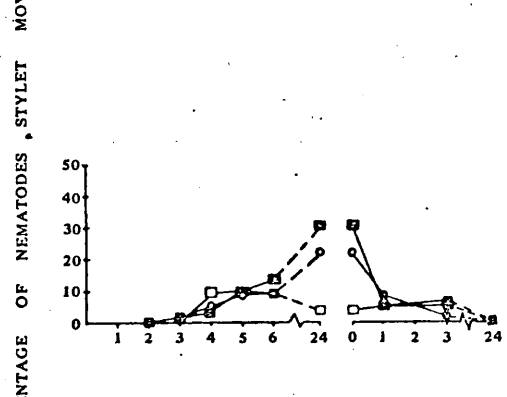
The effect of the sulphone

The effect of the sulphone

PERCENTAGE   OF   NEMATODES   ACTIVE

PERCENTAGE   OF   NEMATODES , STYLET   MOVING

NEMATOCIDE TREATMENT
(Hr)

WATER TREATMENT
(Hr)

NEMATOCIDE TREATMENT
(Hr)
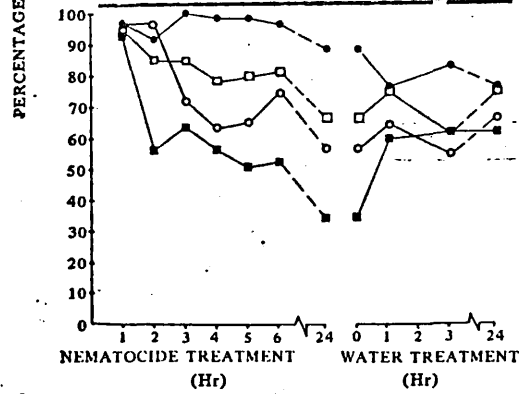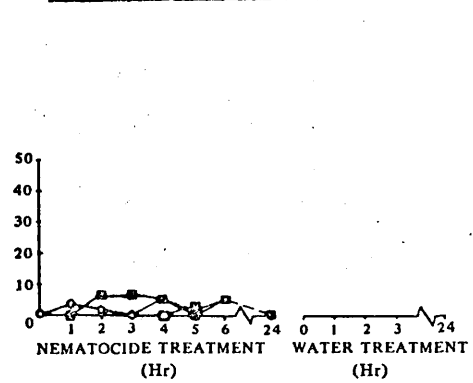
WATER TREATMENT
(Hr)

of larvae treated at the 10 ppm dosage recovered some of their former

vigour during the subsequent water treatment, 67% of those with

aldicarb sulphoxide and 62% with aldicarb sulphone.   Effective

recovery of larvae after treatment with 10 ppm of the primary toxicant

did not occur , only 8% were able to undulate after 24 hr , and their

vigour was much reduced.

2   Stylet Thrusting

The percentage of Heterodera larvae exhibiting

abnormal stylet movement was greatest during exposure to the nematic-

ides , and very little during the water treatment (Figure 16.2).

Of the three compounds , the response was most marked with aqueous

solutions of aldicarb.   It was not immediately apparent , for 2 hr

after the commencement of the treatment only 20% of the larvae at

10 ppm responded.   The number continued to increase until it reach-

ed a maximium of more than 70% of the total number treated in 6 hr .

The sulphoxide even applied at 10 ppm did not produce a response of the

same magnitude as that of aldicarb.   A maximium of 30% was reach-

ed after 24 hr exposure.   The sulphone did not cause a greater

response than 10% when applied for upto 24 hr.

Continuous treatment with aldicarb for 24 hr was

found to cause the stylets of treated nematodes to become extruded

between the lips (Plate 1). The stylets were withdrawn at inter-

vals of between 20 - 40 seconds and withheld for approximately 10

seconds before they were extruded once more.

Stylet thrusting of ᴴeterodera larvae with
aldicarb at 10 ppm attained a maximium of 4.5 thrusts / minute
after a 4 hr exposure , and declined to 1.2 / minute after 24 hr.
Transfer of aldicarb treated larvae to water had a marked affect on
stylet activity.   After an hour stylet activity was reduced and
within 24 hr no abnormal action was detected.   The stylet had
been withdrawn   as the body vigour of the larvae increased.
ᵀhe sulphone was not found to affect stylet activity at any time
during water treatment period.

10 ᴜ
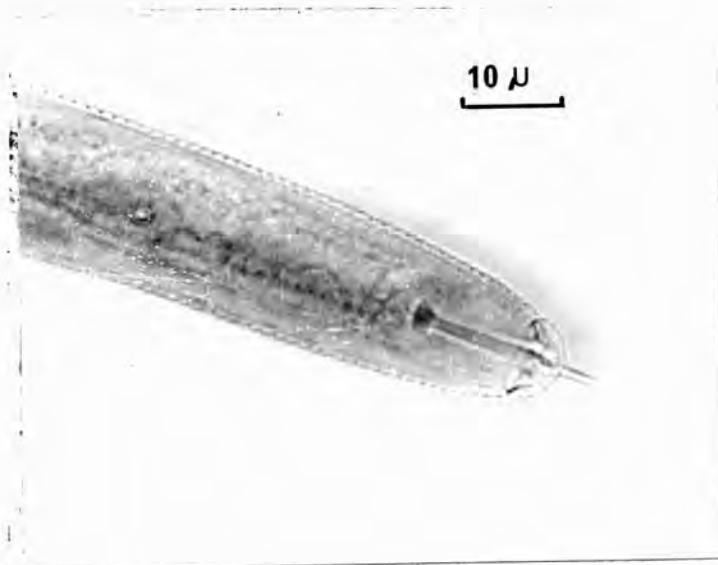
Plate 1   Anterior portion of second stage   larva of
Heterodera rostochiensis   showing the stylet
partially extruded from between the lips.   The
larva was treated in a 100 ppm solution of aldicarb
for 4hr.

3. Migration Through Filters

The ability of treated nematodes to migrate through milk filters was used to support the behavioural observations in aqueous solutions.

When Heterodera larvae were treated with aldicarb and its sulphoxide for 24 hr , when allowed a futher 24 hr to migrate through filters their migration was reduced (Table 15), although the sulphone at 500 ppm had no effect.

Treatment (ppm)	Mean number collected	% reduction of migration
Aldicarb [a]		
0	423.7	0
0.1	367.7	13.2
0.5	334.0	21.2
1.0	282.0	33.4
5.0	216.7	48.5
10.0	163.0	61.5
Aldicarb sulphoxide [b]		
0	296.3	0
50	281.3	5.0
75	261.7	11.7
100	237.0	20.0
125	237.3	19.7
150	216.3	27.0

Table 15   The effect of aldicarb and its sulphoxide on the recovery of second stage larvae of Heterodera   treated for 24 hr and then allowed to migrate through filters in 24 hr. Key: [a] approximately 300 ; [b] approximately 500 larvae treated.

The ED$_{50}$ of migration was 4.3 ppm   for aldicarb and 280 ppm for

the sulphoxide determined from Figure 17 . ^{H}eterodera   larvae

recovered more rapidly after the sulphoxide rather than the aldicarb

treatment.

Meloidogyne larvae treated in a similar manner

with aldicarb were found to recover more effectively than Heterodera

(Table 16).   Unfortunately the effect of the sulphoxide and the

sulphone was not investigated.
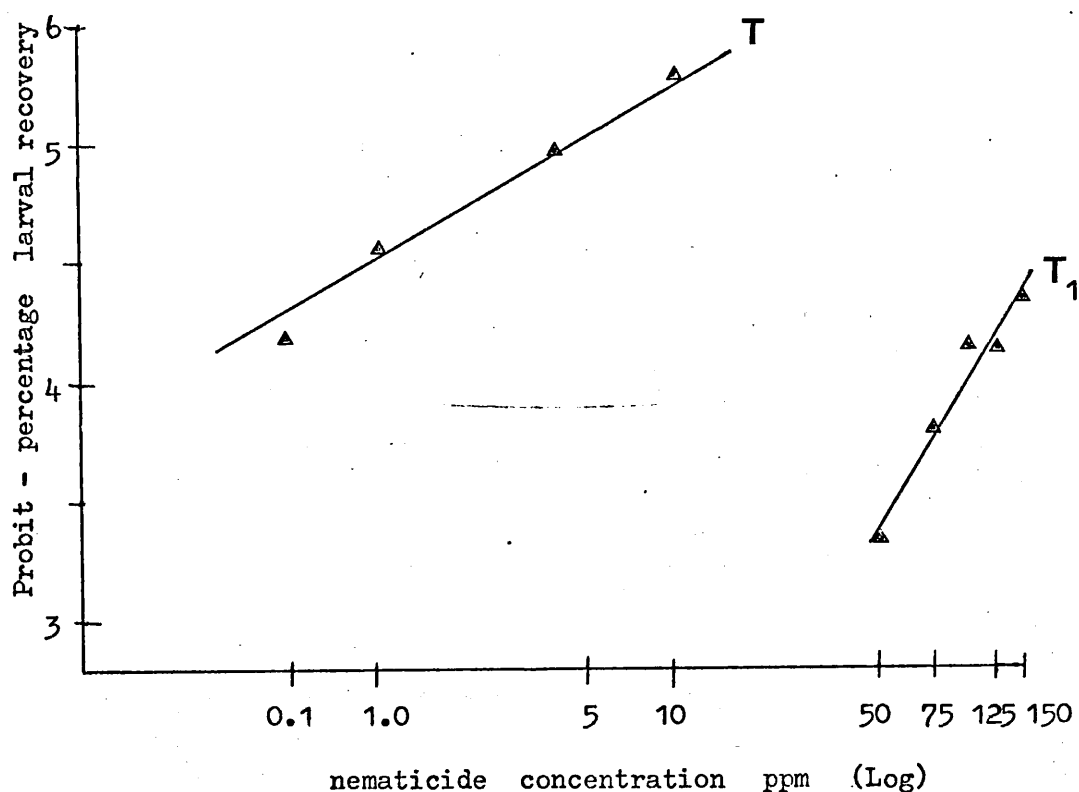
Figure   17   The effect of aldicarb and its .sulphoxide   on the

percentage recovery of second stage larvae of Heterodera

through filters in 24 hr after a 24 hr nematicide treatment,

plotted as probits.

Treatment (ppm)	Mean number collected	% reduction of migration
Aldicarb [a]		
0	272.3	0.0
1	274.0	0.0
5	267.7	3.5
10	265.0	4.4
50	263.3	5.1
100	251.0	9.5

Table 16   The effect of aldicarb on the recovery of second

stage larvae of Meloidogyne treated for 24 hr and

then allowed to migrate through filters in 24 hr.

Key : [a] approximately 300 larvae treated.

Treatment (ppm)		24 hr nematicide % of nematodes [a] active	24 hr water % reduction of migration
	0	96.1	100.
1	T	91.5	89.4
	T_1	91.5	100.
	T_2	82.2	100.
10	T	76.5	69.5
	T_1	48.3	100.
	T_2	33.3	100.
100	T	0.0	80.6
	T_1	31.5	100.
	T_2	24.4	100.

Table 17   The effect of aldicarb , its sulphoxide and sulphone

on the percentage of Xiphinema diversicaudatum active

after a 24 hr treatment and their subsequent migration

through filters in 24 hr. Key : [a] Slight flexing movements

were discounted.

While the activity of _Xiphinema_ was found to decline when treated for 24 hr with aldicarb and its oxygen analogues at 100 ppm the majority of the treated nematodes were able to migrate through filters in 24 hr (Table 17). Aldicarb was most effective in reducing body activity during treatment but had little effect in reducing their migration through filters.

Aphelenchus avenae were more tolerant to aldicarb than _Heterodera_ or _Meloidogyne_ larvae when treated for 24 hr and allowed to migrate through filters (Table 18). Migration was only reduced by 9% after treatment at 1000 ppm. When the length of exposure was increased to 19 days , aldicarb and its sulphoxide we were both effective in reducing mobility (Table 19). In aldicarb at 50 ppm migration through filters was reduced by 31 % and by 25% after sulphoxide treatment.

Ten nematodes extracted in 24 hr were inoculated onto freshly prepared _Fusarium_ cultures and incubated at 25°C for 21 days. Aldicarb and sulphoxide treatment was found to affect the fecundity of _Aphelenchus_ (Table 20), reducing the production of larvae. Pretreatment with aldicarb was found to have the greatest affect. Unfortunately some of the cultures were contaminated with mites which destroyed the nematodes and invalidated some results. Sufficient number of replicates remained uncontaminated to allow the effect of these compounds at 0.5 ppm and 10 ppm to be determined. The effect of the sulphone was not investigated.

Treatment (ppm)	Mean number collected	% reduction of recovery
0	1422.9	0
100	1466.0	0
1000	1290.0	9.3

Table 18   The effect of aldicarb on the migration of Aphelenchus through filters in 24 hr after exposure to the nematicide for 24 hr.   Key : [a] approximately 1700 nematodes treated in each sample.

Treatment (ppm)		Mean number[a] collected	% reduction of migration
0		264	0.
0.5	T	225	15.9
	T_1	262	2.0
1.0	T	203	23.3
	T_1	261	1.3
10.	T	222	16.1
	T_1	212	20.3
50.	T	183	30.8
	T_1	198	25.2

Table 19   The effect of aldicarb and its sulphoxide on the migration of Aphelenchus through filters in 24 hr after a 19 day exposure.   Key :[a] 300 nematodes treated for 19 days.

Treatment (ppm)		Population Growth[a]
	0	58.0
0.5	T	26.8
	T_1	46.2
10.0	T	14.9
	T_1	40.0

Table 20   The population growth of Aphelenchus on

Fusarium - PDA   media after 21 day at 25°C. Nematodes

were pretreated with aldicarb and its sulphoxide for

19 days , recovered after migration through filters

over 24 hr and 10 young adults from each sample were

inoculated on the fungal medium.

Key : [a] Final population / initial population.

4. <u>Loss of Nematode Body Contents</u>

The procedure used was described in Section 7.
The osmium tetroxide stained the lipid contents of nematodes black
while the non lipid remained a yellowish brown. Although lipids
do not constitute the entire body content of nematodes , such food
reserves are important in the maintenance of essential energy (p 128).

The lipid content of <u>Meloidogyne</u> larvae treated
in solutions of the three carbamoyloximes were found to be proportion-
ally more than the content of those larvae in water (Plates 2 & 3) .
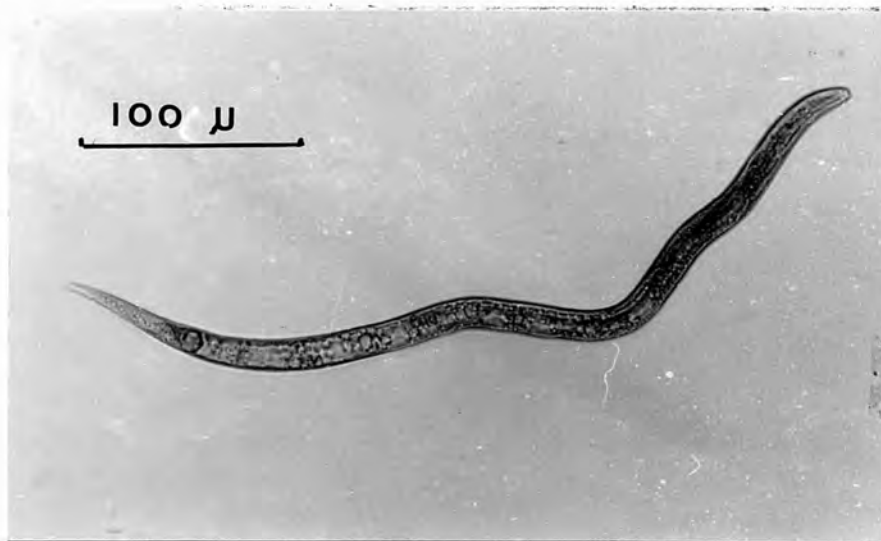
Plate 2   Second stage larva of <u>Meloidogyne</u> stained with
osmium tetroxide after treating in water at $25^{\circ}C$
for 3 weeks.

The water treated larvae were characterized by the complete absense
of lipid in their intestinal region after 3 weeks , in contrast
at 100 ppm in aldicarb the intestinal region of larvae was found to
contain large amounts of dark brown globules and granular material
stained by the osmium tetroxide.   The three carbamoyloximes
used were found to have similar affects on the total lipid content ,
at low concentrations (1 ppm)   the amount was reduced , more of the
posterior and anterior portion was unstained (Table 21).

Larvae treated in aldicarb at 100 ppm for   3
weeks resemble freshly hatched Meloidogyne larvae in the relative amount
of stainable lipid material present in the   intestinal region.
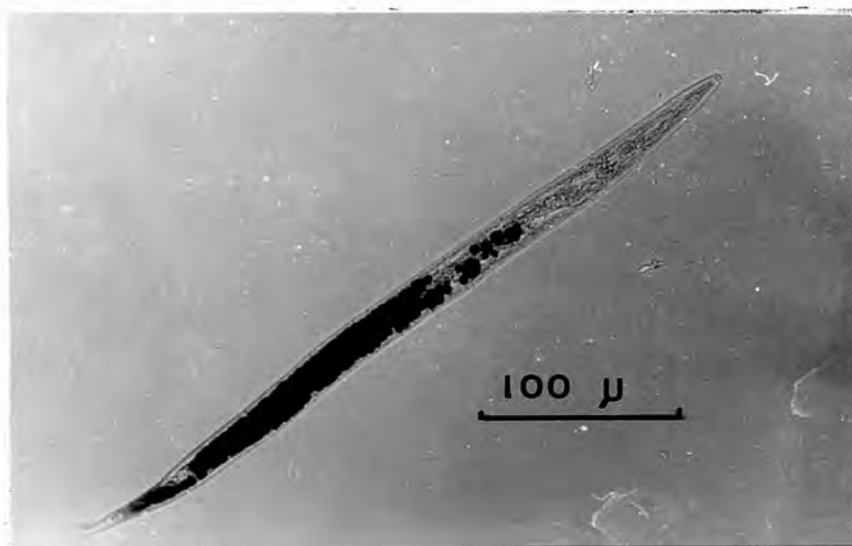
Plate 3   Second stage larva of Meloidogyne stained with
osmium tetroxide after treated in aldicarb at 100
ppm at 25°C for 3 weeks.

Treatment (ppm)		Presence of stain-able lipids
0		−
1	T	+
	T_1	+
	T_2	+
50	T	+ +
	T_1	+ +
	T_2	+ +
100	T	+ + +
	T_1	+ + +
	T_2	+ + +

Table 21   The amount of lipid in ^{M}eloidogyne larvae detected

by staining with 1% osmium tetroxide   , after treatment

with aldicarb , its sulphoxide and sulphone for 3 weeks.

Key: −   absence of lipid in the intestinal region.

+   small amounts ,   + + medium amounts ,

+ + + large amounts of lipid occupying almost

the   entire   intestinal region.

Although lipid body contents in the intestinal region   were conserved ,

as quantitative methods were not used to determine and identify these

lipids , is is not possible to known which lipid fractions are util-

ized by nematodes or whether nematicide treatment alters consumption

of some and   not other lipid fractions.

6.   The Effect of Aldicarb and its Oxygen Analogues on the

Population Growth of Mycophagous Nematodes.

The method used for this investigation as descri-
bed in Section 9 , demonstrated that the fecundity of Aphelenchus and
Ditylenchus was affected when these species were cultured on nematicide
treated fungal media.

The recovery of Aphelenchus   measured by their ex-.
traction from aldicarb treated Fusarium cultures after treatment for
24 hr was not affected (Figure 18).   An extraction period of 48 hr re-
covered between 64% and 70% of the total number of nematodes initially
inoculated , extension of the extraction period by 24 hr did not imp-
rove the percentage which were recovered.   When the period nematodes
were treated on   aldicarb - Fusarium - PDA was increased , the extraction
of Aphelenchus   decreased.   This was apparent after 3 , 5 or 7 day
exposures (Table 22).

Treatment (ppm)	Mean number nematodes recovered after exposure[a] (days)			
	1	3	5	7
0	1104.0	1188.6	1589.7	1660.5
0.1	1195.8	833.9	1230.7	1542.6
1	1123.9	721.6	543.7	503.9
10	1098.3	593.3	542.7	358.7

Table 22   The effect of aldicarb in Fusarium-PDA media   on the

extraction of Aphelenchus treated for 1 , 3 , 5 or 7 days.

through filters in 48 hr. Key : [a] initial nematode inocu-

lum approximately 1700.
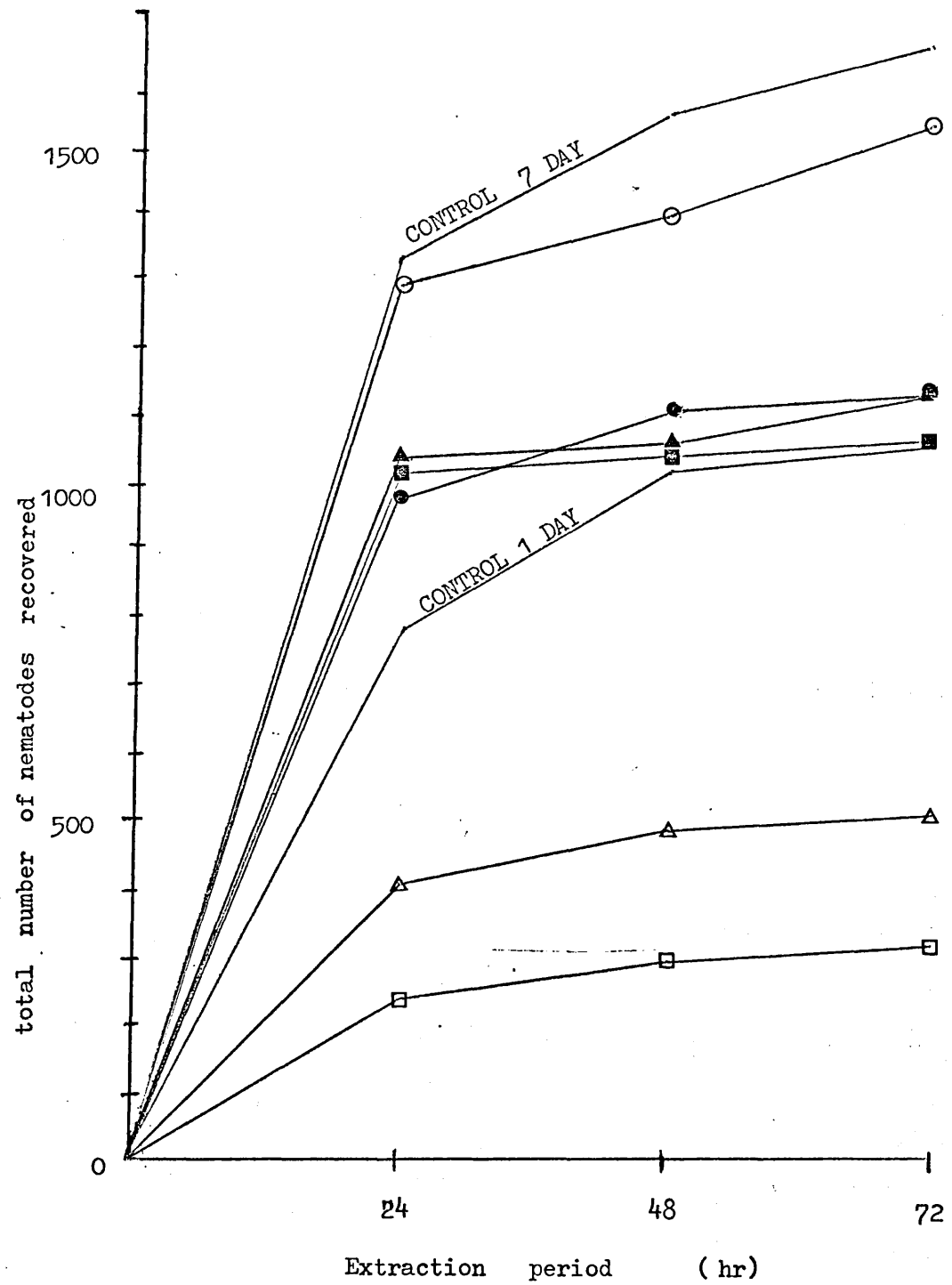
Figure 18   The effect of aldicarb - Fusarium media on the

extraction of Aphelenchus by migration through filters.

Key: 24 hr exposure - ●——● 0.1 ppm , ▲——▲ 1 ppm,

▣——▣ 10 ppm ; 7 day exposure - ⊙——⊙ 0.1 ppm,

△——△ 1 ppm , ☐——☐ 10 ppm .

The number of a _Aphelenchus_ recovered after aldicarb treatment for 1 week at 0.1 ppm did not influence the numbers extracted , while at 1 ppm the numbers extracted were markedly reduced. When nematodes were treated at 0.1 ppm for 2 weeks larval production was slightly reduced (Figure 19). The greatest decline in fecundity was found with the sulphoxide treatments at 0.1 ppm and 1 ppm after 7 and 14 days , while the sulphone was generally least effective (Figure 19).

The population growth of the control batches were very variable , ranging from 13 to 22 fold increases in the number of nematodes at the time of harvesting. Thus to make a comparison of the effect of the three carbamoyloximes on _Aphelenchus_ and _Dityl-enchus_ the population growth determined at 3 , 7 and 14 days were based on the total numbers in the control groups at the harvest times , having a population growth of one (Table 23 and Figure 19).

Treatment (ppm)		Nematode population growth		
		3 DAYS	7 DAYS	14 DAYS
0		1	1	1
1	T	0.60	0.35	0.25
	T_1	0.80	0.77	0.15
	T_2	0.72	0.53	0.17
5	T	0.42	0.34	0.04
	T_1	0.53	0.17	0.05
	T_2	0.69	0.35	0.06
10	T	0.37	0.22	0.03
	T_1	0.48	0.09	0.04
	T_2	0.72	0.35	0.04

Table 23 The effect of aldicarb and its oxygen analogues in _Botrytis_ cultures on the population growth of _Ditylen-chus_ after 3 , 7 and 14 days at $25^{\circ}C$.

Figure 19

The effect of aldicarb, its sulphoxide and sulphone in

Fusarium - PDA cultures on the population growth of Aphelenchus

incubated at $25^{\circ}C$ for 3, 7 and 14 days. Treated nematodes

were harvested from the cultures by the dicing of the cult-

ure media and extracting the nematodes over filters. The

population growths were determined from the numbers harvested

from the control groups which was regarded at 1 at each interval.

Key: ●——● T , ▶——▶ T_1 , □——□ T_2 .
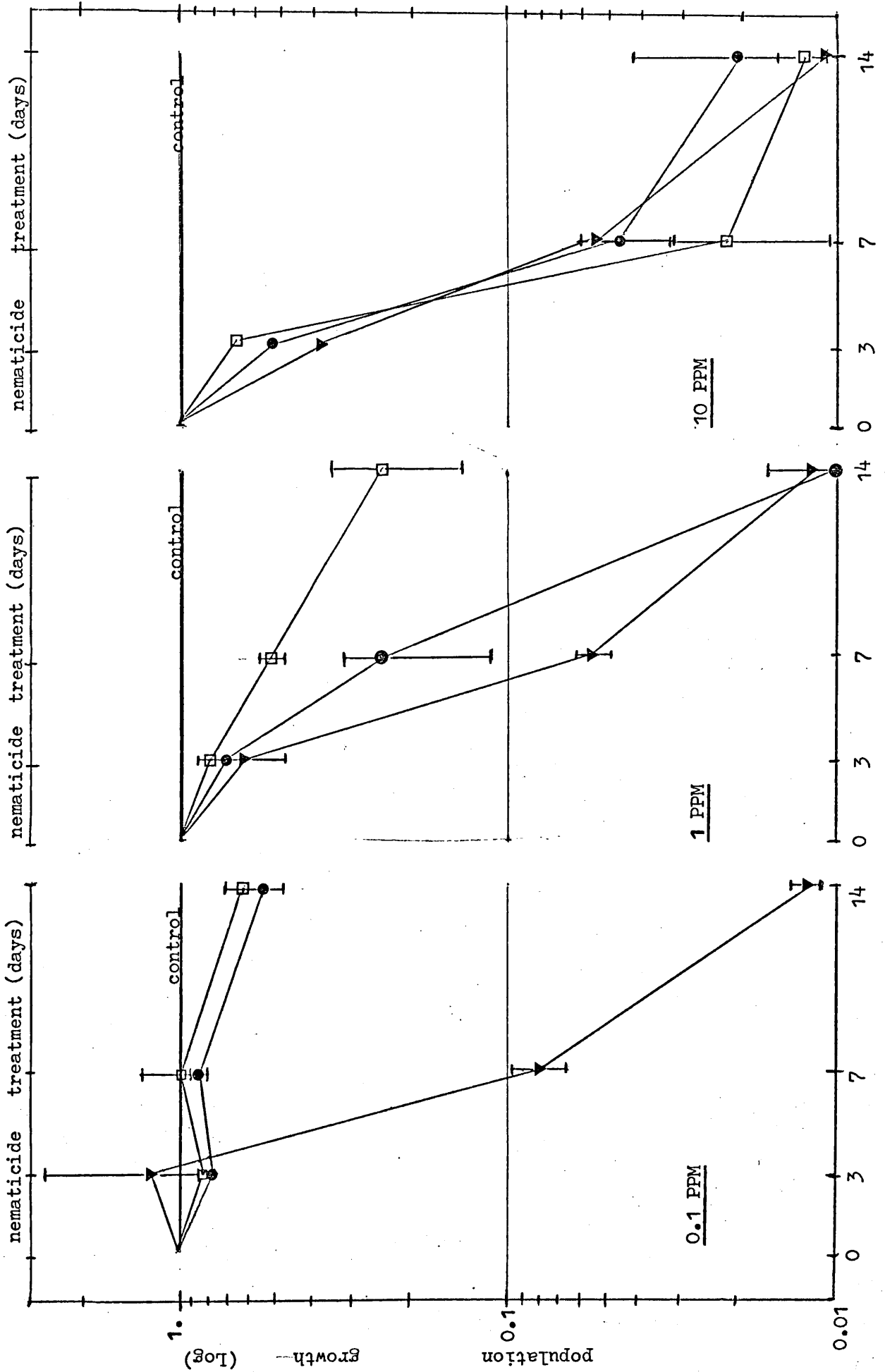
<u>Ditylenchus</u> on treated <u>Botrytis</u>   were found to be affected like

<u>Aphelenchus</u>   , the population growth was reduced   within 3 days

exposure.   The sulphone was again least effective , the sulphoxide

causing the most rapid decline in the population growth after a 1 week

treatment at 5 and 10 ppm.   At 10 ppm with the three nematicides the

growths approached zero for while the numbers extracted from the

treated media declined the numbers on the untreated cultures increa-

sed considerably.   After 14 days exposure the population growth

results with all three compounds at 5 and 10 ppm were similar (Table 23)

and so were the results at 10 ppm with <u>Aphelenchus</u> (Figure 19).

7.   The Fate and Persistence of Aldicarb and its Oxygen Analogues
     in Fungal Cultures.

When mycophagus nematodes are treated on nematicide-
fungal media for periods longer than 24 hr , it was not strictly accur-
ate to regard the results as being solely due to the presence of the
test compound.   The breakdown of aldicarb and its oxygen analogues
to products of increased or diminished toxicity may affect the fecund-
ity or extraction of the nematode from the cultures.   Thus   the
breakdown of these compounds was investigated in Fusarium and Botrytis ,
as described in Sections 9 and 4 a).

Aldicarb was found to be broken-down in Fusarium-
PDA and Fusarium - PD broth cultures at 25°C very slowly (Table 24).
After 26 days   80% of the parent compound remained unchanged , and at
40 days 50% was still detected,   the only breakdown product formed
was the sulphoxide analogue.   Sulphone in trace amounts was found
in Fusarium-PDA cultures which had been treated with aldicarb sulph-
oxide , while 40% after 40 days had been presumably broken-down along
other pathways to compound not detectable by the TLC method employed.
The breakdown of sulphone in Fusarium cultures was extremely slow ,
only 15% after 40 days.   The breakdown of aldicarb was found to be
similar in Botrytis-PDA cultures.

Neither aldicarb nor its oxygen analogues at 10
ppm was found to affect the growth rate of Botrytis or Fusarium.
The only affect was to reduce the mycelial pigmentation of Fusarium
over 3 weeks.   Normally in aging cultures the mycelia turned deep

pink but at 10 ppm with aldicarb the mycelia appeared completely

white and at 0.1 ppm only slightly pale pink in colouration.

Treatment & Nematicide Residue	% of original and breakdown product remaining		
	26 Days	33 Days	40 Days
Aldicarb - Fusarium -PDA			
T	80	60	50
T_1	20	30	40
T_2	ND^a	ND	ND
Sulphoxide- Fusarium - PDA			
T_1	90	60	50
T_2	10	10	10
Sulphone - Fusarium - PDA			
T_2	90	90	85

Table 24   The fate of aldicarb and its oxygen analogues

in Fusarium - PDA   cultures at 25°C , incubated

in darkness.

Key [a] ND compound not detected.

8. Acetylcholinesterase Inhibition in Xiphinema diversicaudatum
Treated with Aldicarb , and its Oxygen Analogues.

The method used was outlined in Section 11.
The presence of a dark brown stain in the area of the nerve ring of
Xiphinema which had been treated in water indicated the site at which
acetylcholine had been released from the acetylthiocholihe. The
nerve ring was deeply stained (Plate 4).
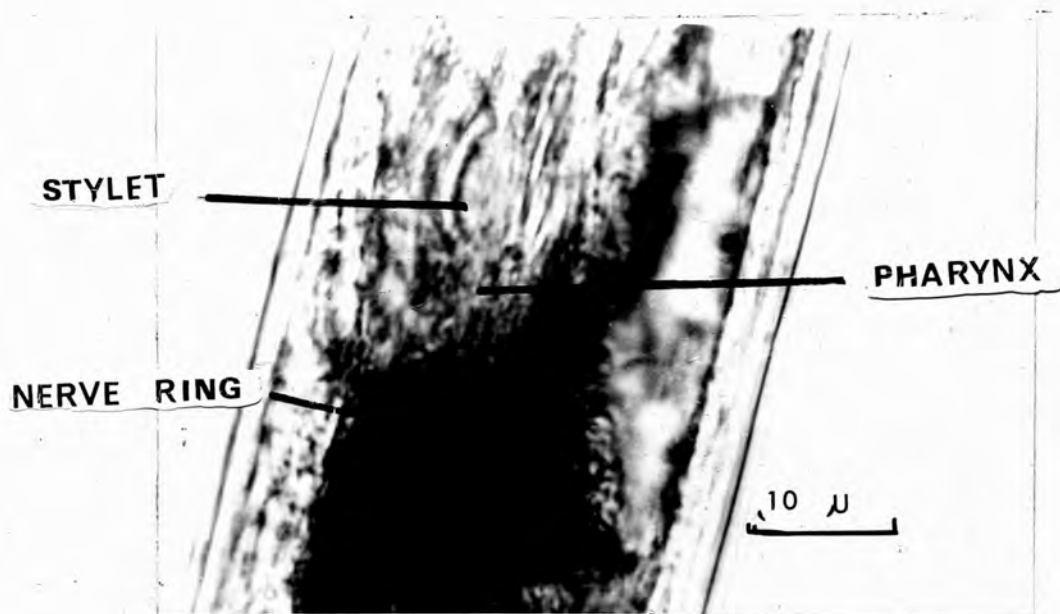
Plate 4 Location of acetylcholinesterase in the nerve
ring of Xiphinema behind the base of the spear.
Acetylcholine release caused the tissues to become
darkly stained by the deposition of copper thiolate.

Aldicarb sulphoxide was demonstrated by the
complete absence of stain in the nerve ring of 5.4% of the total
number of _Xiphinema_ treated to have the greatest anti-acetylcholin-
esterase activity. Neither aldicarb nor its sulphone had any in-
effect at this concentration or at 1 ppm. _Xiphinema_ treated at
10 ppm with aldicarb and its sulphoxide was found to inhibit the
action of acetylcholinesterase in all nematodes , while the sulphone
was the least effective (Figure 20).
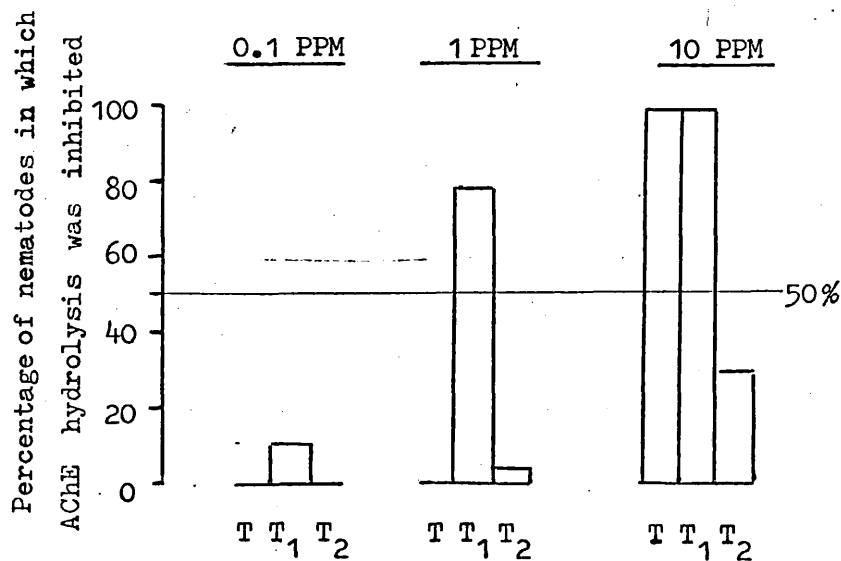
Figure 20   The effect of aldicarb , its sulphoxide and
sulphone on the inhibition of acetylcholine-
sterase in _Xiphinema_ detected in the nerve ring
histochemically.

9.   The Effect of Aldicarb on the Oxygen Consumption of Treated

Nematodes.

The procedure used for determining the effect of aldicarb

on the oxygen consumption of Panagrellus and Aphelenchus was descri-

bed in Section 10.

Aldicarb was found to affect the consumption rate of

Panagrellus and not Aphelenchus . Using Panagrellus (Figure 21)

there was an immediate increase in the oxygen consumption rate (measured

within 8 minutes of the start of the experiment) at all treatment

concentrations. The consumption rate at 1 ppm was maintained at

a rate higher than the control groups in water throughout the

3 hr exposure. Oxygen consumption of nematodes treated at 10 and

100 ppm although initially increased , eventually declined after 2 hr

to a rate below the control group. The oxygen consumption by the

controls was reasonably constant throughout the experiment , although

it declined at the end of the experiment.

Aldicarb was not found to alter the oxygen consumption rate of

Aphelenchus when treated for upto 3 hr at $30^{\circ}C$ at 100 ppm.
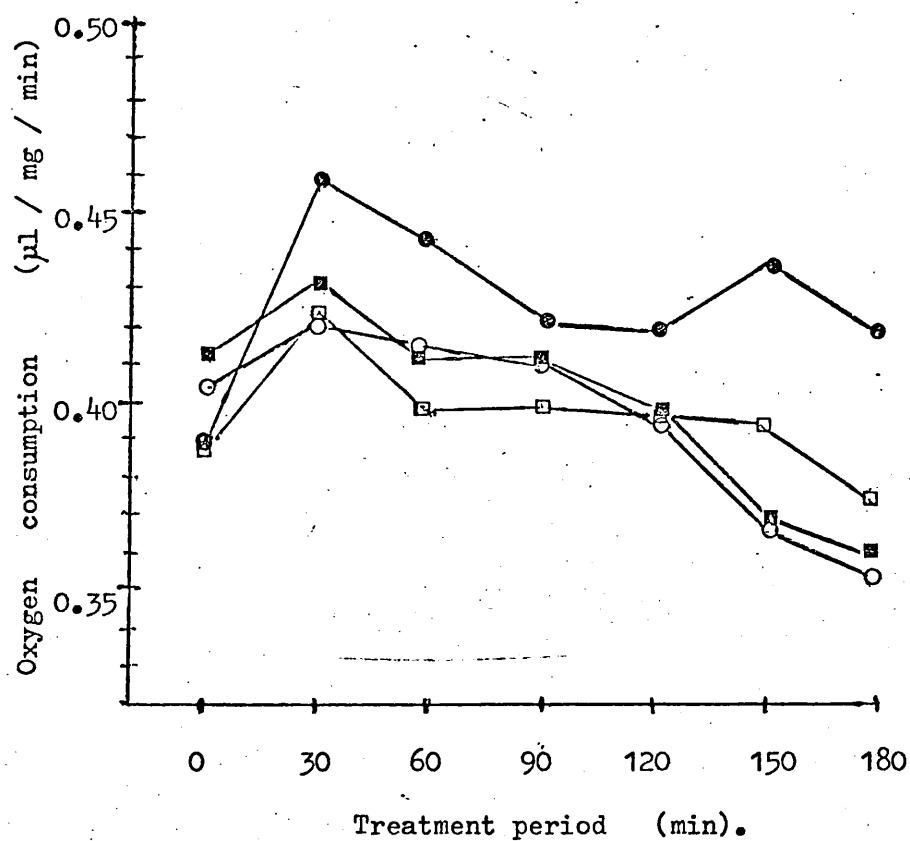
Figure 21   The effect of aldicarb on the oxygen consumpt-
ion of Panagrellus in 3hr at 30°C.
Key: □——□ Control ,   ●——● 1 PPM ,
    ■——■ 10 PPM ,   O——O 100 PPM.

10.   <u>Autoradiography of Xiphinema diversicaudatum after Treat-
ment with [14]C Labelled Aldicarb.</u>

The procedure used was outlined in Section 12.
When <u>Xiphinema</u> which had be pretreated in 5ml of a 3.016 x 10^{-4}M
solution of ^{14}C labelled aldicarb for 6hr , and then exposed to
AR 10 stripping film for 20 days , there was no localisation of
silver on the films after exposure.

The silver grains which formed the background
were randomised and no localised with any particular part of the
nematode (Plate 5) or the nematode's cuticle (Plate 6).
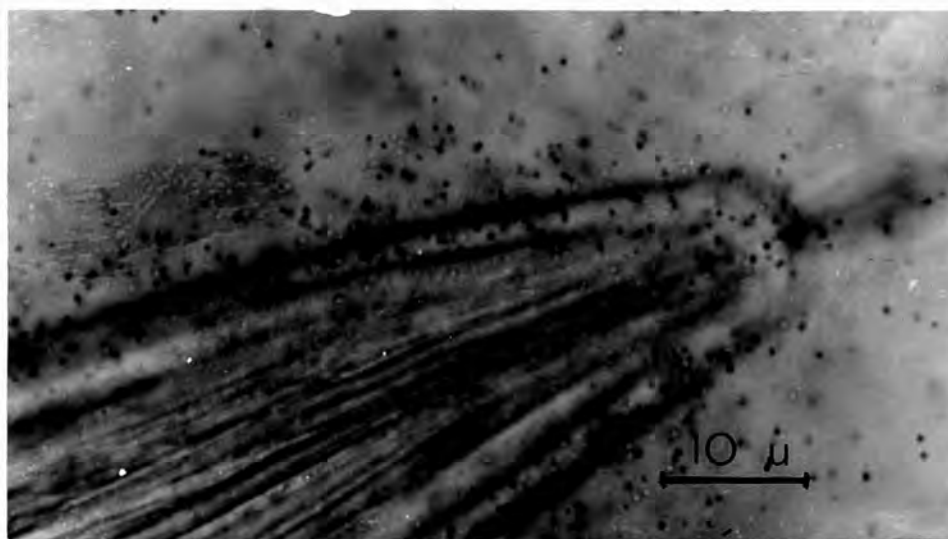
Plate 5   The anterior end of <u>Xiphinema</u> after pretreat-
ment with ^{14}C labelled aldicarb and then exposed
to AR 10 stripping film for 20 days at -4°C.
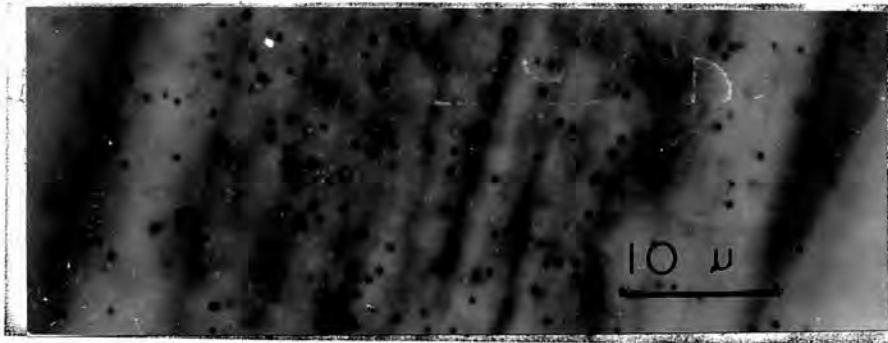
Plate 6   Cuticle of _Xiphinema_   after pretreatment
with ^{14}-C labelled aldicarb for 6 hr , washed
and then exposed to AR 10 stripping film for
20 days at -4°C.

When _Xiphinema_   which had been pretreated
in labelled aldicarb for 6hr and the sectioned ,   there was a
localisation of silver grains in transverse sections (Plate 7).
Although the background of silver grains was rather high , silver
was located mainly in the region of body wall.   The grain
size varied from 0.4 to 0.9 μ in width , were rather large .

The labelled nematicide or a derivative was
found in the region of the body wall between the inner cuticular
layer and the somatic musculature of the nematode , in the hypodermis
(Plate 8).   This could be observed when the sections were subse-
quently stained with pyronin and methylene green.
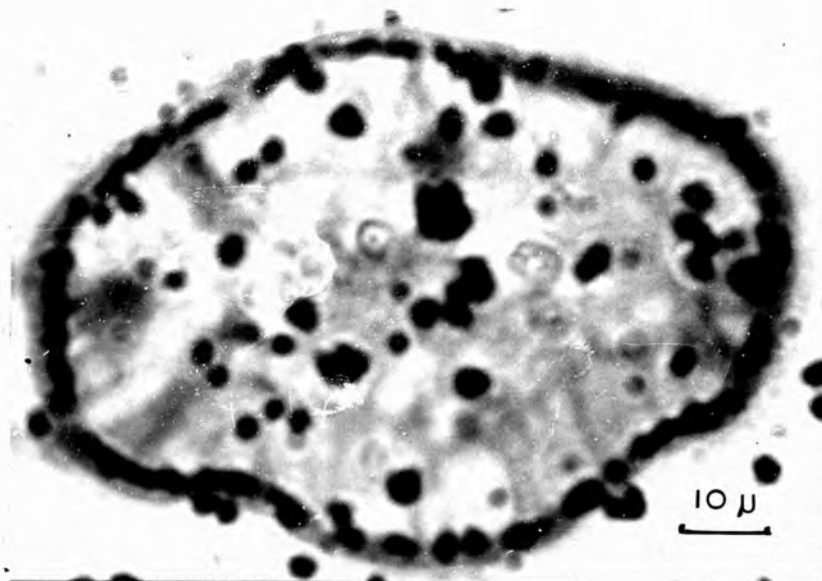
Plate 7   Transverse section of <u>Xiphinema</u> cut from
the posterior portion of a while nematode which
had been exposed to ^{14}C labelled aldicarb for 6 hr,
then exposed to AR 10 stripping film for 25 days at
-4°C.
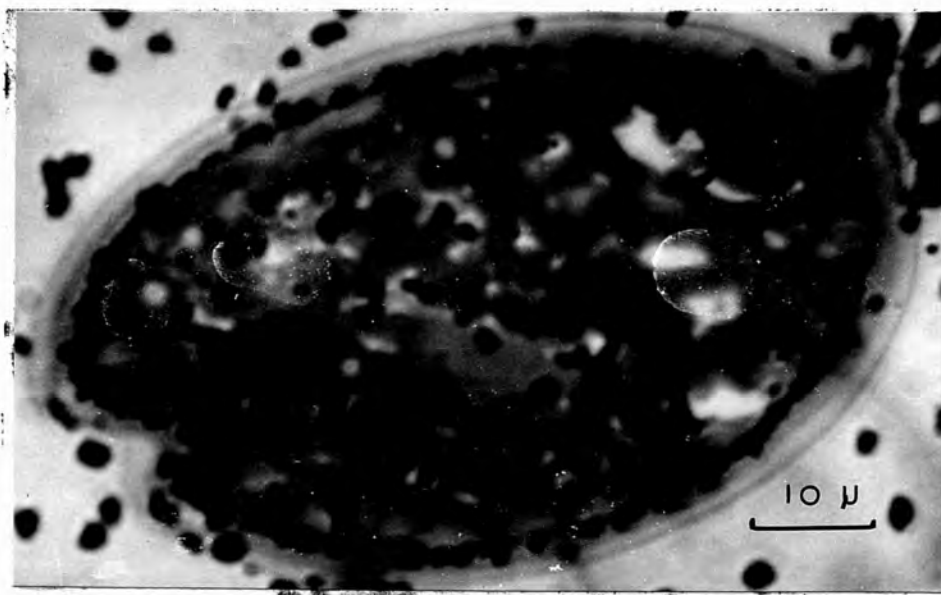
Plate 8   Transverse section of <u>Xiphinema</u> similar to Plate
7 , but stained with pyronin and methylene green
after exposure to AR 10 stripping film for 25 days.

11.   The Permeability of Nematodes to ^{14}C Labelled Aldicarb.

The   permeability of nematodes to aldicarb
was investigated as described in Sections   13 and 4 b).   This   study
was   divided   into   two parts -   the uptake of labelled aldicarb into
Panagrellus and Aphelenchus and secondly the egress of the labelled
compounds from treated Panagrellus.

a)   Uptake of Labelled Aldicarb

When Panagrellus   was   treated in labelled aldicarb
for 10 , 20 and 30 minute exposures , the labelled compound was found
to be taken up.   The treated nematodes divided into two fractions,
the supernatant   and pellet were both found to contain radioactivity
(Table 24).   The amount in the supernatant fraction was found to
increase with the length of treatment   while the amount remaining in the
homogenate fraction   was only between 6 and 13% of the total radioact-
ivity of the nematode.   The results obtained with Aphelenchus   were
similar to those with Panagrellus , the labelled nematicide accumulat-
ing in the supernatant fractions with increased exposure (Figure 23).

In   a 30 minute exposure the labelled nematicide
was found to have not only accumulated in the supernatant   fraction of
nematodes but was no longer present as a single compound.   In
Panagrellus   the   concentration of aldicarb was 3.6 µg/g   and the sulph-
oxide derivative was 1.1 µg/g nematde after a 30 min   exposure period
(Figure 22).   Increasing the length of nematicide treatment in-
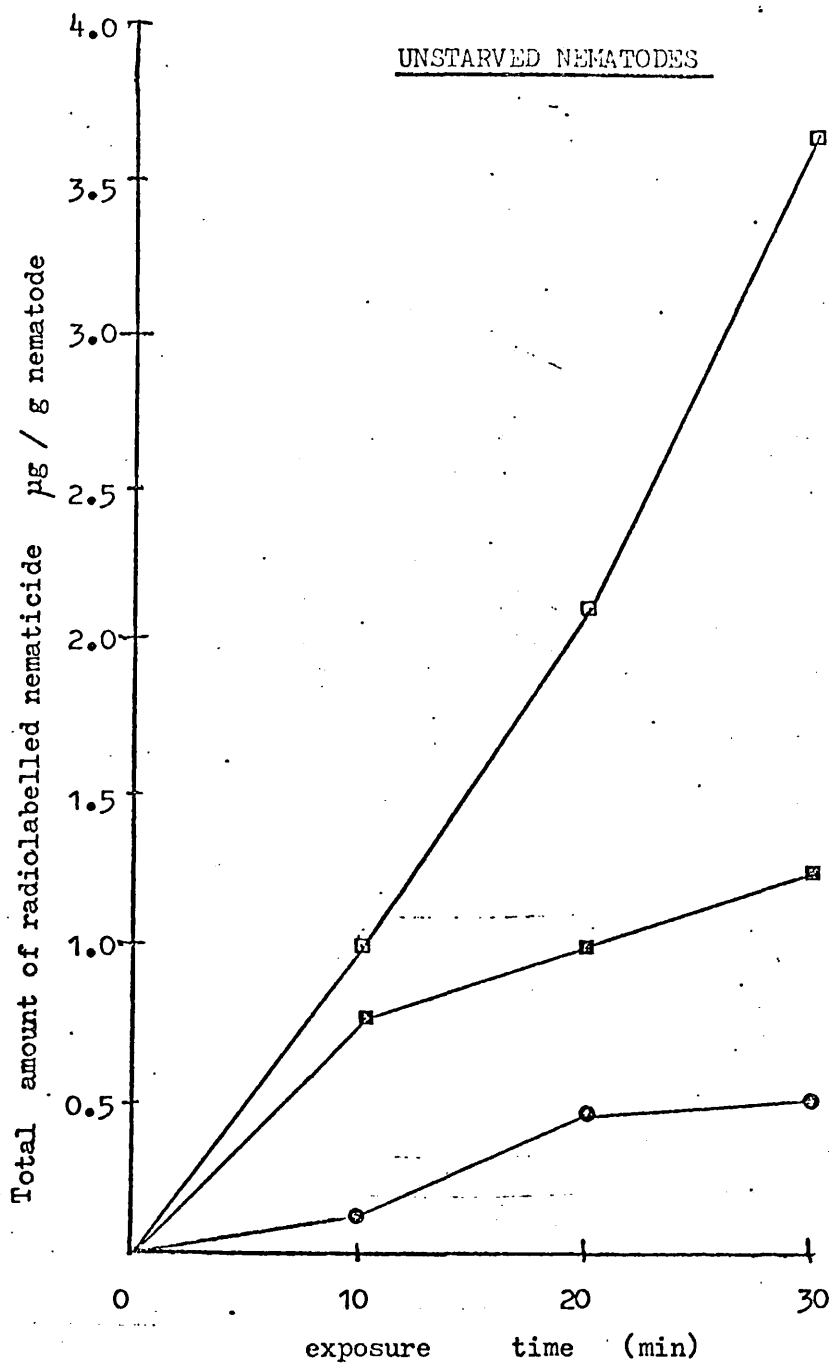
Figure 22   Uptake and fate of ^{14}C labelled aldicarb

in Panagrellus during a 30 minute exposure at

3.01 x 10^{-4}M solution.   The labelled material

in the nematode supernatant and residue was

quantified.   Key: Supernatant □——□ T ,

■——■ T$_1$;   Residue   ◉——◉   unknown

Treatment time (min)	Nematode supernatant		Nematode residue	
	Total dpm[a]	% of Total	Total dpm[a]	% of total
10	1845	94.1	116	5.9
20	3361	87.0	562	13.0
30	5143	91.7	564	8.3

Table 24   A comparison of the radioactivity in the super-
natant and residue fractions of Panagrellus   which
had been treated in a 3.01 x 10^{-4}M solution of ^{14}C
labelled aldicarb for up to 30 minutes.

Key: [a] values are the averages of two replicates,
total obtained by the summation of the radioactivity
of all compounds located by TLC.

creased   the number of breakdown products formed and their
quantity.   The sulphoxide oxime .and its corresponding nitrile were
detected after a 60 minute   exposure in small amounts , but when
unstarved Panagrellus were treated for 24 hr   the amounts formed in-
creased to 41% and 18% of the total nematicide in the supernatant
respectively (Table 25).

After a 60 minute exposure the total internal
concentration of labelled nematicide in unstarved and starved Pana-
grellus was 8.74 µg/g and 9.15 µg/g nematode   respectively
(Table 26).   After 24 hr exposure although there was little
difference in the amounts which were extracted from the nematode

Residue detected	% of recovered radioactivity in the nematode supernatant fraction after exposure to aldicarb				
	Exposure interval (min)				
	10	20	30	60	24 (hr)
Unstarved Nematodes					
T	55.8	68.1	75.0	80.0	18.5
T_1	44.2	31.9	25.0	15.0	19.5
T_2	...	...	...	...	...
T_1O	...	...	...	2.0	41.5
T_2O	...	...	...	...	...
T_1N	...	...	...	1.8	18.1
T_2N	...	...	...	...	...
unknown	...	...	...	1.2	2.4
24 hr Starved Nematodes					
T				29.7	60.3
T_1				60.2	26.3
T_2				...	...
T_1O				0.9	4.8
T_2O	NOT INVESTIGATED			...	...
T_1N				4.8	5.9
T_2N				...	...
unknown				4.4	2.7

Table 25   The metabolism of ^{14}C labelled aldicarb in unstarved and starved Panagrellus   after exposure for periods upto 24 hr.

supernatant , the percentage which remained in the nematode residue

fractions increased to 15 % in unstarved and 19% in starved nematodes.

The total internal concentrations therefore continued to rise after

exposure for 1 hr although the rate of increase was presumeably reduced.

Although the point at which equilibrium was reached was not determined ,

it occurred in Panagrellus with exposures longer than 1hr.

Starvation of Panagrellus had no effect upon the

amount of labelled nematicide accumulated with nematodes after 1 hr

exposure. While quantitatively there was not difference in the total

internal concentration , qualitative differences were marked.

After 1 hr aldicarb was found to be only 30% of the total nematicides

in 24 hr starved Pangrellus , the sulphoxide forming the majority

of the breakdown products formed (Table 25). On the other hand , in

unstarved Panagrellus aldicarb constituted 80% of the total amount

in the supernatant fraction, and the sulphoxide : again formed the

Treatment (time)	Amt., in supernatant (μg / g)	% of total radioactivity in residue	Total internal concentration (μg / g)
Unstarved Nematodes			
1 hr	8.26	5.5	8.74
24 hr	9.05	15.5	10.63
24 hr Starved Nematodes			
1 hr	8.81	3.7	9.15
24 hr	8.01	19.5	9.94

Table 26 A comparison of the amount of labelled nematicide

detected in starved and unstarved Panagrellus after

a 1 hr or 24 hr exposure to labelled aldicarb.

Residue detected	% of recovered radioactivity in the treatment solution at the end of the exposure period	
	1 hour	24 hours
Unstarved Nematodes		
T	91.97	87.54
T_1	6.91	9.69
T_2	...	...
T_1	0.48	0.54
T_1	0.20	0.50
unknown	0.12	0.29
unknown	0.29	0.05
unknown	...	0.56
24 hr Starved Nematodes		
T	92.47	95.70
T_1	5.60	3.41
T_2	...	...
T_1	1.04	0.10
T_1	0.24	0.53
unknown	0.26	0.23
unknown	0.31	...
unknown	...	...

Table   27   A comparison of the percentage amounts of aldicarb and its breakdown products detected by TLC in the treatment solution,after starved and unstarved *Panagrellus* treated for either 1 or 24 hr had been removed and the nematode washings added.

major breakdown product.

In unstarved nematodes after a .treatment period of 24 hr the breakdown of the parent compound had increased , only 18% remained unchanged while 19 % occurred as the sulphoxide , 41% as the sulphoxide oxime , and 18% as its corresponding nitrile. However the results from the 24 hr starved nematodes after a 24 hr treatment period were not expected . Breakdown of aldicarb had not continued , the majority of the labelled material was present in the form of the parent compound , the sulphoxide forming 26% of the remainder. When the accumulation of nematicide with ^{P}anagrellus had reached a maximium of 10.63 µg / g in unstarved nematodes after 24 hr , the total amount detected in the nematode aliquot did not exceed 0.4 % of the total concentration of the treatment solution , 4 µg / total number of nematodes in the aliquot.

The concentration of the treatment solution was fairly con.-stant throughout the exposure periods , aldicarb was found to remain in the greatest proportions , in the treatment solution of unstarved nematodes it constituted 91% and 87% of the total amount after a 1 hr and 24 hr treatment respectively (Table 27). The amount unchanged of the 24 hr starved nematode treatments was similar , and in both cases cases the sulphoxide product was found to constitute between 3% and 9% of the total concentration at the end of a 24 hr treatment period.

It was established that extraction and'clean up' of labelled aldicarb from aqueous solution reduced the total recovery efficiency by between 7% to 10% (Table 28).

Treatment time (min)	% total loss of radioactivity
10	7.5
20	9.2
30	10.0

Table 28   A comparison of the percentage loss of labelled material from the treatment solutions after exposure had been terminated. Panagrellus were treated for intervals up to 30 minutes , removed from the labelled solutions , the labelled compounds were then extracted ,'cleaned up' and spotted for detection by TLC.

Residue detected	% of recovered radioactivity in the nematode supernatant fraction after exposure to aldicarb				
	Exposure interval (min)				
	10	20	30	60	24 (hr)
T	51.68	53.55	53.83	44.76	20.57
T_1	48.32	46.44	41.76	41.90	55.72
T_2	...	...	...	...	2.70
T_1O	...	...	...	6.67	9.92
T_1N	...	...	4.41	3.49	9.82
unknown	...	...	...	...	1.82

Table 29   The metabolism of ^{14}C labelled aldicarb in Aphelenchus after exposure for periods upto 24 hr.

The permeation of <u>Aphelenchus</u> by labelled aldicarb was faster than that found with <u>Panagrellus</u> , reaching a maximimum concentration in the supernatant of 12.9 µg / g/nematode after a 30 minute exposure in the same concentration (Figure 23). This is compared to only 8.26 µg / g in unstarved <u>Panagrellus</u> after a 1 hr exposure. When <u>Aphelenchus</u> was treated for 1 hr in labelled aldicarb the total concentration of labelled material in the supernatant fraction was 10.25µg/ g , a decline of 2.6 µg/g. This may be accounted for by the increase in the amount of radioactive material in the residue which was found to have increased to 14% of the total concentration in the nematode. This figure is higher than would normally have been expected , and no explanation can be offered for the poor extraction from the nematode residue.

An equilibrium between the amount within the nematode and the concentration of nematicide in the treatment solution was reached within 30 to 60 minutes exposure (Figure 23). After a 10 minute exposure the parent compound was not detected alone but had moreover been breakdown to the sulphoxide which constituted 48% of the total amount in the supernatant (Table 29). After a 30 minute exposure small quantities of the sulphoxide nitrile were detected , and within an hour the sulphoxide oxime was also present in minor amounts. Although the amount of the sulphoxide and aldicarb were similar after a 60 minute exposure , after 24 hr the sulphoxide was found to be the major nematicide residue in the supernatant. Only 20% of the parent compound within <u>Aphelenchus</u> remained unchanged.
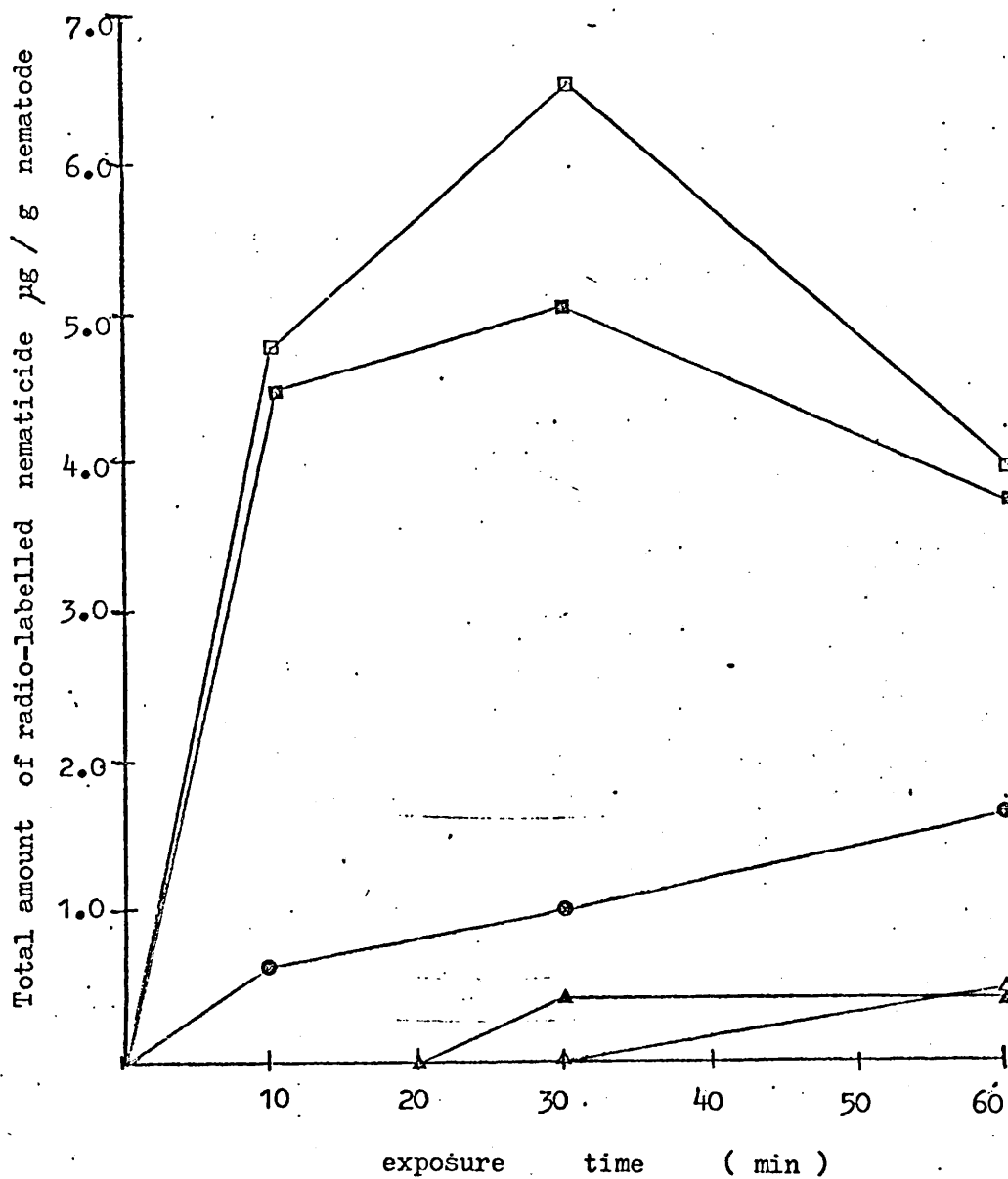
Figure 23   Uptake and fate of ^{14}C labelled aldicarb in
Aphelenchus   during a 60 minute exposure at 3.01×10^{-4}
M solution.   The labelled material in the nematode
supernatant and residue was quantified.

Key: Supernatant   □——□ T ,   ■——■ T_1,   ▲——▲ T_1O ,

△——△ T_1N ;   Residue   ⊘——⊘ unknown.

b) <u>Egress of Labelled Nematicides</u>

    The release of labelled aldicarb and its break-
down products was investigated in <u>Panagrellus</u>. Erroneously 24 hr
starved nematodes were used , for at the time it was not appreciated
the differences in breakdown which could result from a period
of starvation. Nematodes treated for 1 hr were washed and re-
suspended in non-labelled aldicarb at the same concentration (50 ppm)
for upto 1 hr.

    The amount of labelled material in the nematodes

Residue detected	% of recovered radioactivity in the nematode supernatant fraction after re-exposure				
	Exposure	interval	(min)		
	0	10	20	40	60
T	10.28	...	...	...	...
T_1	47.83	100.	93.85	97.62	100.
T_2	5.04	...	...	...	...
T_1O	6.19	...	6.15	2.38	...
T_2O	...	...	...	...	...
T_1N	30.60	...	...	...	...
T_2N	...	...	...	...	...

<u>Table 30</u> The composition of labelled aldicarb and its
breakdown products in <u>Panagrellus</u> treated in ^{14}C
labelled aldicarb for one hr , washed and transferred
to non-labelled aldicarb for a further hour.

after re-exposure declined (Figure 24). Although the amount in the supernatant fractions declined the amount of radioactivity in the residue fractions remained unaltered. The egress of labelled material from the treated nematodes was most rapidly in the first 10 minutes of re-exposure in non-labelled aldicarb. After 1 hr the final concentration of the supernatant was reduced by 88 %.

The concentration of labelled material after pretreatment in labelled aldicarb for 1 hr was low , approximately 1.2 μg / g/nematode. A satisfactory explanation cannot be given for this low concentration other than by the fact that they were rinsed quickly with water before the labelled nematicides were then extracted with acetone and the nematodes disintergrated by the reasonance of sound waves. Washing with water for 30 seconds may well have reduced the internal concentration , and it was not established what effects were likely. Just prior to re-exposure the supernatant was found to consist of 48% sulphoxide , 31% the sulphoxide nitrile , 10% aldicarb and 6% the sulphoxide oxime (Table 30).

After re-exposure for 10 minutes , only the sulphoxide breakdown product was found in the nematode supernatant, and except for small traces of the sulphoxide oxime it was the only compound detected after 20 , 40 and 60 minutes. Thus the greatest decline in concentration over the first 10 minutes is related more to the loss of aldicarb , the sulphoxide oxime and its nitrile rather than the sulphoxide itself.

The release of aldicarb and its breakdown products continues from treated nematodes even when they are bathed in solution of non-labelled aldicarb at the same concentration.
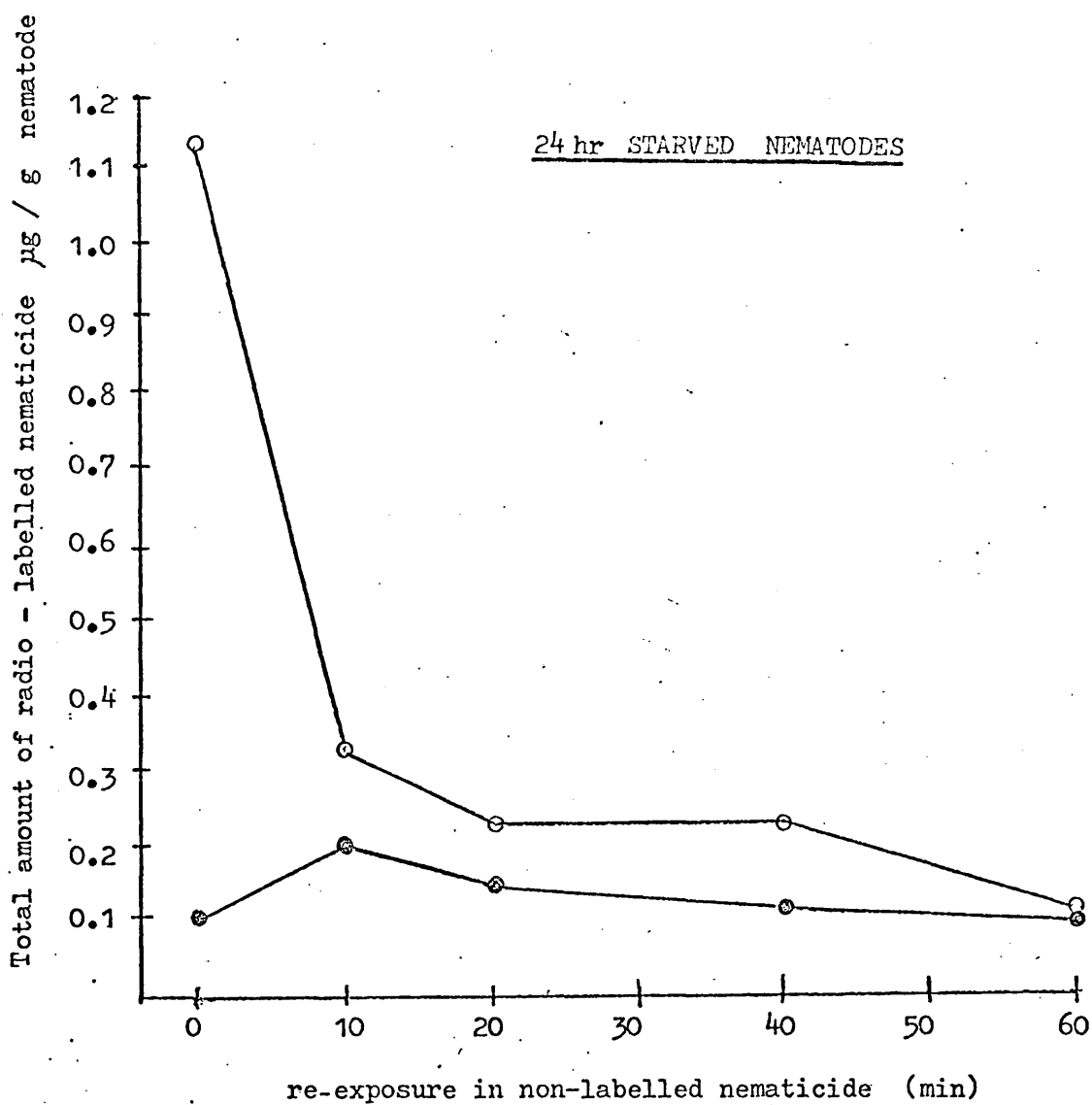
Figure 24   The egress of labelled nematicide from Panagrellus treated in ^{14}C labelled aldicarb for 1hr , washed and returned to non-labelled aldicarb at the same concentration for a further hour. The labelled material in the nematode supernatant and residue fractions were quantified. Key: O————O   total in super - nant , ⊛————⊛   total in residue.

DISCUSSION

1.   The Contact and Systemic Action of Aldicarb.

        The success of a nematicide will depend upon its

ability to reach the target organism in sufficient concentration to

cause the death or paralysis of the nematode.   Aldicarb has been

demonstrated to reduce soil nematode populations for 2 years after

application to the field   (Whitehead et al , 1970)   and also to

affect the invasion of larvae into treated plant roots (den Ouden ,

1971).   Together with its toxic breakdown products , aldicarb

may have a direct influence on the behaviour of nematodes by contact

with them in the soils or treated plant tissues.   On the other

hand plant tissue may become unsuitable for the invasion , feeding or

development of the nematode without necessarily becoming toxic.

The uptake and accumulation of aldicarb and its breakdown products

may alter the biochemistry or physiology of the plant in some way so

the tissues become adverse for nematode development.   Little

evidence is available to support this hypothesis , although the

organochloride pesticide have been shown to dramatically affect the

physiology of green algae (Wurster , 1968).

        The persistence of aldicarb and its breakdown

products will vary in soils and plants due to the differences   in their

physical and chemical properties .   The importance of aldicarb in

these two systems on the activity of nematodes has been investigated.

a)   The Contact Effect of Aldicarb on Nematodes

Aldicarb , its sulphoxide and sulphone have been found to reduced the mobility of nematodes and eventually cause them to become paralysed.   The effects which were dependent upon the nematicide and its   concentration were found to be reversible , the treated animals regained their mobility when the nematicides were removed.   The   action of aldicarb contrasts with the action of the organo-halide nematocides which were found to affect nematodes irreversibly after treatment for several hours (Moje , 1960).

The hatch of ^{M}eloidogyne eggs was reduced when egg masses were treated in vitro with aqueous solutions of these carbamoyloximes , and   aldicarb was found to be the most effective.   The persistence of aldicarb in soils will depend upon the soil type and the climatic conditions (Coppedge et al , 1967; Hendrickson and Meagher, 1968) and is not thought to be much in excess of 3 weeks.   In vitro the action of the sulphoxide and sulphone breakdown products is considerably less effective , but since these will be likely to form the major toxic residues in soils , the efficacy of the nematicide will depend upon their action.   In soils it has been suggested that the sulphoxide forms the most stable component , but its biological half-life was about 1 week (Bull , 1968).

Aldicarb and several other non volatile nematicides have been demonstrated not to effect the hatch of H.rostchiensis

(Hague and Pain , 1970) or H. tobaccum (Miller, 1970). The tolerance

of these cyst nematode egg stages to aldicarb may be greater than that

of M. incognita , but there is little in the literature on the com-

parative biochemical and physiological differences that would help

to explain these results. Desiccated cysts of H. rostochiensis

have been found to be readily permeable to water (Ellenby and

Smith , 1969) , the larvae becoming active within the egg membranes.

These cysts if permeable to water should also be permeable to nematicides

for I would anticipate that they had little selective control over

the uptake of such compounds. Ensheathed nematodes were thought to

less permeable to nematicides , however it was disproved by Marks et

al (1968) who demonstrated that in Pellodera sp. the amount of

EDB taken in was the same in exsheathed and ensheathed larvae.

Of course the fact that nematodes should be permeable to nematicides

does not necessarily infer that they should be more susceptable.

The stage in the nematode's life cycle when the nematicides are applied

seem to be particularly critical , Aphelenchus adults have been found

to be less susceptable to EDB than larvae (Evans and Thomason, 1970).

Furthermore these authors found that moulting larvae were more suscep-

table than their non-moulting counterparts. In vitro treatment

of H. rostochiensis in aldicarb at 1 ppm for 10 weeks has however been

found to reduce egg hatch (Osborne - personal communication).

The failure of other authors to observe an in vivo

supression of hatch with cyst nematodes may have been due to the

rapid breakdown of aldicarb in treated soils. Unpublished data

suggests that large errors can exists between the expected and the

actual concentrations of aldicarb in soils after treatment. In

pot soils at 25°C in a 24 hr treatment only 90% of the total amount of nematicide added to the soils could be detected by TLC , and 5% had been converted to the sulphoxide analogue. Occasionally losses of upto 40% were found after 24 hr.

Assessment of the in vivo hatch and invasion of tomato roots by Meloidogyne in aldicarb treated soils were both found to be affected. The reduction of root invasion was greater than the reduction of egg hatch.   The numbers of second stage larvae increased with the length of exposure in treated soils , for while 63% for of the control larvae had invaded tomato roots , at 1 ppm only 41% and at 8 ppm 9% of the total number hatched were in roots.   H. rosto- chiensis larvae have also been reported to accumulate in aldicarb treated soils (Pain and Hague , 1971).

Extraction of second stage Meloidogyne larvae from soils treated with the three carbamoyloximes with the Whitehead Tray was reduced.   The greatest effect was found after a 24hr treatment with aldicarb , while the sulphone had the least effect. Soil treatment for 2 weeks were still effective , but all three compounds were found to reduce extraction to the same extent.   Thus the absence of nematodes from treated soil samples is not indicative that nematodes are killed , but do not possess the ability to migrate from the soil on the Whitehead Trays. Pain and Hague (1971) have also reported that second stage larvae of H. rostochiensis were less extractable from aldicarb treated soils , the number decreasing with increasing concentration.   Furthermore these authors found that the numbers of larvae extracted from these soils had little or no food reserves stored

in their intestinal region.

In contrast , when Meloidogyne second stage larvae were treated with aldicarb and its oxygen analogues in vitro at 25°C they did not lose their endogenous supply of lipid material from their intestines. On hatching the supply of lipid was large , Van Gundy , Bird and Wallace (1967) estimated that it represented 41 % of the total body weight of M. javanica and was the principal storage material . While the larvae treated in water lost their lipid reserves , the amounts retained after treatment with the nematicides increased with the concentration. It was reported (Van Gundy et al , 1967) that 70% of the total body lipid of M. javanica was consumed within 9 days at 25°C. The inability of treated larvae to utilize their endogenous lipid material appeared to be correlated to the quiescent state of the animal , for immobilised nematodes might be expected to require less energy .

The absence of food reserves in H. rostochiensis in treated soils reported by Pain and Hague (1971) many have resulted from the recovery of the larvae as the nematicidal effects in the pot soils diminished. These larvae being unable to penetrate the roots of host plants which contained nematicidal residues consumed their endogenous food supply as they wandered through the soil.

If paralysed nematodes could remain for many months in treated soils without utilizing all their food supply , thus the longevity of the nematode may actually be increased. Thomason (1962) considered that infective larvae could still invade plant

tissues when their body contents had almost been exhausted. Thus larvae which remained paralysed in soils , consuming little of their food supply would not be prevented from entering a host plants be - cause of the small amount of potential energy remaining .

The movement patterns of nematodes on agar treated with the three compounds was found to be adversely affected. With Heterodera and Meloidogyne larvae the patterns changed from open to concentric spiralling , and the velocity of the treated larvae decreased with increased concentration. The undulation frequency /cm moved during a 1hr exposure on aldicarb treated agar at 10 and 100 ppm increased markedly for Meloidogyne. The larvae became paralysed and remained motionless within 1 hr at high concentrations with all compounds, while at low concentrations of aldicarb and its sulphoxide their activity was reduced. Aldicarb was the most effective compound in reducing activity , after 1 hr the ED_{50} for the total distance travelled was only 9 ppm , the sulp- hoxide was 15 ppm and the sulphone 60 ppm. The least affected par- ameter after 1hr exposure was the furthest dispersal of the larvae, the ED_{50} were 30 ppm with aldicarb , 40 ppm with the sulphoxide and 100 ppm with the sulphone.

The reduction of mobility of Heterodera larvae in aqueous treatment solutions was not found to be so rapid as that with Meloidogyne on treated agar. After 1 hr the undulation rate had declined by only 14% and 10% with aldicarb and its sulphoxide at 5 ppm. The decline in activity was greater after a 24 hr exposure , 98% and 85% with aldicarb and its sulphoxide respectively at 5 ppm.

The sulphone was found to have the least effect on the activity of

Heterodera and Meloidogyne larvae.

The undulation rate of Heterodera larvae in

water was 68% greater than the same activity on ionagar . While

larvae were able to move effectively across the agar medium , its

viscosity and the increase in friction gave adequate purchase but

reduced the frequency of sinusoidal waves.

The tolerance of nematodes to nematicides is

somewhat difficult to assess accurately , for it depends upon the

assay procedure employed which varies for a particular species and

also with an individual stage in its life history. Spurr (personal

communication) found that Pangrellus were more tolerant than Heter-

odera or Meloidogyne larvae to aldicarb when assessed by water contact

tests. Panagrellus has also been demonstrated to be more toler-

ant to the soil fumigant DD than the other species tested (Simard ,

1964). As several assay procedures were used in this investigation

and the effect of these carbamoyloximes on several species of nematode

studied , little comparative data is available. However in the

water contact tests , Aphelenchus , D. dipsaci (unpublished data),

Xiphinema and Meloidogyne larvae were found to recover more rapidly

than Heterodera larvae after a 24 hr exposure. All nematodes with

the exception of Meloidogyne recovered more rapidly from the effects

of the sulphoxide and sulphone rather than aldicarb , the parent compound

was found to be the most effective and the sulphone derivative the least.

Aldicarb and its oxygen analogues were not found to

kill treated nematodes after short treatments at high concentrations ;

while treatment of longer periods was found to reduce the invasive

capacity of Meloidogyne second stage larvae and the fecundity of

Aphelenchus. When the affects of exposure time with phenols and

cresols was investigated (Chitwood , 1952 ; Staniland and Stone ,

1952) and also that of organo-halides (Moje , 1959 ; Evans and Thom-

ason , 1969) they were found to be lethal to nematodes. While

EDB applied for 24 hr was found to kill Aphelenchus the nematodes were

found to recover if the exposure was reduced to 8 hr (Evans and Thom-

ason , 1969).

Permanent paralysis of nematodes by the direct

action of nematicides is . fundamental for their control in soils.

The retention of paralysis long after the treatments had been dis-

continued or the biologically active residues degraded is advantageous.

The longer ectoparasitic phytophagous stages or cyst - nematode infective

larvae are immobilized the better chance the plant has to becoming

established . A rapid recovery would afford the plant little

protection and use of such compounds would be of no economic advantage.

While the activity of nematodes was reduced after a 24 hr exposure to

these carbamoyloximes , with larvae other than Meloidogyne and Heter-

odera they had not lost their capacity to migrate through filters

when transferred to water from 24 hr. Heterodera larvae were found

to recover their body activity more rapidly after sulphoxide than

aldicarb treatment, and an ED_{50} for migration through filters with

these compounds after a 24 hr exposure was 4.5 ppm and 280 ppm respect-

ively.

Heterodera and Meloidogyne both remain inactive

for some time after treatment , ^{H}eterodera larvae were still inactive

after 24 hr in water after treatment at 10 ppm for 24 hr and Meloidogyne

larvae treated with aldicarb for 1 hr at 100 ppm remained in active for

more than 6 hr. Observation of the movement patterns of Meloidogyne

larvae during their recovery showed that their tracks were initially

very tortuous , as the period was increased a pattern of open spiral-

ing resulted. Larvae treated with the sulphoxide or sulphone at

10 ppm or 50 ppm for 1 hr , the subsequent average undulation rate /

cm travelled was in excess of the control average. None of the

compounds at 1 ppm altered this rate and although they were not

as active as the controls , their movement pattern was normal. In

aqueous solutions Heterodera larvae after treatment with the sulph-

oxide and sulphone were found to have increased their undulation

rate / minute to a level similar to the control which declined by

2 undulations / minute during the recovery period.

Exposure of Aphelenchus on nematicide treated

Fusarium cultures for less than 3 days did not reduce the number of

nematodes which could be extracted from the media. Longer exposures

of Aphelenchus and Ditylenchus on treated cultures were found to re-

duce their fecundity , all compounds having similar effects except

at low concentrations when the sulphoxide was found to be the most

effective and the sulphone the least.

In vitro treatment of second stage Meloidogyne

in aqueous solutions of aldicarb reduced their subsequent capacity

to invade tomato roots , the longer the initial treatment the greater

the reduction. A 24 hr exposure at 100 ppm reduced invasion by 40%

while after 1 week an ED_{50} of invasion was estimated at 23 ppm.
A similar affect has been reported by Pain and Hague (1971) when
Heterodera larvae extracted from treated soils and then inoculated
into pot soils containing a host plant , did not invade its roots.

From the evidence it is not satisfactory to ass-
ume that second stage larvae of cyst nematodes which can be extract-
ed from treated soils or those which have become active once more ,
can continue their life cycle as if nothing had happened. Be -
cause Meloidogyne larvae are unable to utilize the endogenous food
supply and the larvae not killed it seem likely that would remain
viable as root parasites for longer than untreated larvae. How-
ever the length of exposure seem to be critical in determining
their capacity to invade roots.

Miller and Kring (1970) suggested that Pratylenchus
penetrans and Tylenchorhynchus claytoni were able to migrate in
aldicarb treated soils to regions containing less chemical.
The response of nematode to the presence of aldicarb or its oxygen
analogues in soil is dependent upon their being detected by the sen-
sory receptor organs of the nematode. Nematodes will be unable to
detect the presence of such compounds if they do not possess adequate
sensory receptor sites or have suitable receptor sites but need very high
threshold levels for these compounds. The latter would result in the
nematodes becoming paralysed before they had the opportunity to detect
the presence of the nematicide and respond.

The migration of Heterodera and Aphelenchus on

concentration gradients of aldicarb , its sulphoxide and sulphone

formed in ionagar was not observed.   These nematodes were placed near

the highest part of the gradient , but at a concentration which were

not thought to cause deleterious symptoms (approximately 6 µg/g to

9 µg/g of nematicide)   did not migrate down the gradient to areas of

lower concentration.   The nematodes within 24 hr were found to

have travelled much shorter distances than the control groups , and

were either less active or motionless on the treated media.   Only

on the sulphone gradient did Aphelenchus   migrate towards the less

toxic peripheral region of the agar.

For a chemical such as aldicarb to act as a repel-

ent the nematode must perceive either an increase or decrease in the

concentration , which will depend upon the gradient itself , the speed

of the nematode and its movement in relation to the gradient as

suggested by Green (1971).   A range of volatile and non-volatile

compounds including root exudates (Widdowson , Doncaster and Fenwick ,

1958) , sex attractants (Santos , 1969) and carbon dixoide (Johnston

and Viglierchio , 1961 ; Klinger , 1963)   have been found to affect

nematode behaviour.   Some bacteria from rhizosphere of beet plants

are known to repel H. schactii   (Bergman and Van Duuren , 1959) , and

some fungi , actinomycetes and bacteria can attract and repel Aphel-

enchoides parietinus (Katznelson and Henderson, 1963).   Whether

nematodes can detect aldicarb or its breakdown products is a matter of

conjecture.   Where a greater number of nematodes have been extract-

ed from soil between areas to which band application of aldicarb was

more likely to be related to the immobilization of nematodes in treated soil and their poor extraction rather than an active migration as suggested by Miller and Kring (1970).

b) The Systemic and Contact Effect of Aldicarb in Plant Roots on Nematode Invasion.

Although fumigants act solely by contact with nematodes in soils (Good and Feldmesser, 1967) other nematicides may be taken into plant roots where they may form a possible second line of defense against the attack of plants by phytophagous nematodes. Hague and Kondrollochis (1969b) suggested that the presence of as little as 1 µg/g of Thionazin in dipped bulbs was sufficient to suppress the invasion of D. dipsaci for several months.

Tomato roots which had been dipped in aqueous solutions of aldicarb for 24 hr were found to contain less Meloidogyne larvae after they had been grown for nearly 4 weeks. Root invasion by larvae in pot soils 2 days after treatment was less than when larvae were inoculated into soils 9 days after the roots had been dipped. At between 1 and 8 ppm there was found to be no difference in the level of larval reduction which ranged from 40 % to 50% and from 30 % to 47% when larval were inoculated into soils 2 and 9 days after treatment respectively.

Tomato plant roots which had been dipped in aldicarb for 24 hr were found to contain large amounts of aldicarb sulphoxide which increased in concentration with the tissues with the dip concentration. Also present were trace amounts of the

the sulphone.   Thus the presence of the sulphoxide derivative

in roots   immediately after treatment is likely   affect the invasion

of <u>Meloidogyne</u> larvae.   Roots examined for the residues of aldicarb after

16 days   contained   only the sulphone derivative , while the sulph-

oxide was found in the aerial portion of the tomato plants.   The

amount of sulphone in the roots was not found to increase with the in-

crease in dip concentration, and was not greater than $0.1\,\mu g/g$ of plant

material (wet weight).   Thus after a period of 3 weeks any protect-

afforded by the nematicide will be due to the presence of small amounts

of the sulphone in the roots originally treated with aldicarb.

Several other authors have suggested that the sulp-

hone may have important toxicological significance on pests which feed

on treated plant material (Metcalf <u>et al</u> , 1966 ;   Coppedge <u>et al</u> , 1967;

Skrentny and Ellis , 1970).   The uptake of aldicarb and its sulph-

oxide into plants is considered to be an active process , while that of

the sulphone is passive and therefore much slower (Skrentny , 1970).

While the sulphone was not found to be generally very effective at

low concentrations in the reduction of nematode activity , in plants

the presence of $0.1\,\mu g/g$ of this compound and the level of root in-

vasion suggests that in the environment of the root   tissues it is

more potent.

Aldicarb and its breakdown products are known to

be translocated to the foliage and accumulate in the leaves and in

meristematic tissue (Bull , 1968).   The sulphoxide in the roots

of tomato plants immediately after dipping has been translocated to the

foliage where it remained,   and the parent compound still present

in the aerial portion of the plant was broken down.   No sulphone was

found in the aerial portion of the tomato plants, only the roots. Thus the small quantities of sulphone which had formed in the roots after 16 days are not translocated to the foliage.

A more comprehensive investigation of the formation and distribution of the other degradative products was not made, as the TLC method used to detect non-labelled aldicarb and its breakdown products was restricted to the two oxygen analogues. Bartley et al (1970) has demonstrated that the other breakdown products in plant tissues had little toxic significance.

Dipping infected plants in aldicarb has been reported to repel Heterodera larvae (den Ouden, 1971) but whether this is because the plant has become an unfavourable or a toxic host is a matter of conjecture. Larvae were found to flee from treated potato roots when the roots had been infected with second stage larvae just prior to treatment. When the period between infection and treatment was extend at subsequent evasion of larvae or the development of the cyst were not affected (den Ouden, 1971). If the nematicide was present in the roots in amounts which were normally toxic to the nematode, it would be anticipated that the animal would become paralysed rather than migrate out from the tissues. On the other hand the nematodes within treated tissues may be able to respond to the presence of the toxic nematicides by escaping before the deleterious symptoms take effect. The alternative is that the plant is rapidly altered physiologically due to the uptake of nematicide, the careful balance within the tissues is upset and the root environment becomes unfavourable rather than toxic. Further investigation is

is necessary to ellucidate the role of nematicides in plant tissues
on the invasion and development of nematodes. Several workers
have shown that aldicarb and other nematicides increases the male
population of Heterodera which form in treated plants (den Ouden,
1971 ; Pain and Hague , 1971 ; Trudgill - personal communication)
thus altering the sex ratio of the developing nematodes.

c)   The Effect on Mycophagous Nematodes in Aldicarb   Treated
      Fungal Cultures.

The breakdown of aldicarb in Botrytis and Fusarium -
PDA cultures was found to contrast with the rate of breakdown in
higher plants. In these cultures the half-life of aldicarb was approximately 3 weeks , whereas in tomato plants it was
reached before the end of a 24 hr dip. Skrentny (1970) was unable
to detect aldicarb in the tissues of bean plants when they were dipped ,
no matter how short the duration. The breakdown of the sulphoxide
and sulphone in fungal culture was also very slow.

A variety of enzyme systems referred to as " mixed
functional oxidases" are considered to be responsible for the met-
abolism of certain pesticides in higher plants (Metcalf et al , 1966 ;
Shrivastava , Tsukamoto and Casida , 1967). These enzyme systems
are probably absent in fungi and compounds like mushroom tyrosine
oxidase was not found to enhance the breakdown of aldicarb (Kuhr
and Casida , 1967).

This soil fungi could form a natural reservoir

for active pesticide residues , which could affect the mycophagous

nematode population. However this hypothesis would be difficult to

establish. The organophosphate Parathion[R] has been detected

in soils to which it was applied 16 years earlier (Steward , Chisholm ,

and Ragab , 1971). About 0.1% of the initial amount applied to

the soil was detected and it was suggested that this reservoir of

Parathion could have been associated with the lipid phases in the soil

organic matter. In this way it was protected against degradat-

ion and hydrolysis. The importance of soil fungi which can act as

a reservoir of pesticides needs further investigation.

An unsuccessful attempt was made to distinguish

between the effect of ingestion of the nematicide in fungal contents

thought to be important by Oliff (1965) and Hague and Kondrollochis

(1969a) , and the contact effect of the nematicide through the nematode's

cuticle (unpublished data).

Both Fusarium and Botrytis were both found to

translocate aldicarb and its oxygen analogues (unpublished data).

These compounds were detected in untreated media which was separated

from the nematicide treated media when both fungi were allowed to

grow across glass bridges which separated the media (Schutte, 1956).

Had celluloid films been used to separate the hyphae from the untreat-

ed media it may have been possible to demonstrate that the nematici-

des translocated were able to diffuse out from the hyphae into the

untreated media.

When Aphelenchus were inoculated by hand onto

untreated Fusarium-PDA cultures into which aldicarb , its sulphoxide

and sulphone were known to have translocated , the nematodes stopped

feeding and adopted a coiled-posture. It was only on the sulphone

treated media that the nematodes were still found to feed. The del-

eterious symptoms caused by the presence of aldicarb and its sulphoxide

may not result from the ingestion of nematicidal fluid from fungi

but due to the presence of the nematicide in the medium on which the

nematodes lay. Kondrollochis (personal communication) has demon-

strated that the pulsation of the pharyngeal wall of Aphelenchus is

stopped very rapidly after the nematode was transferred onto Thionazin

-fungal-PDA media , and that feeding and the ingestion of fluid also

stopped.

2. The Permeability of Nematodes to Aldicarb.

Penetration of nematodes by nematicides is normally a prerequiste to the physiological reactions associated with death and paralysis. There is an initial delay between the application of the nematicides and the appearance of the symptoms of narcosis. Heterodera and Meloidogyne larvae for example did not show any apparent symptoms until 20 - 30 minutes after treatment in aldicarb at 50 ppm. While Panagrellus was not immediately affected they were able to migrate before eventually becoming paralysed , but it was demonstrated that their oxygen consumption rate was immediately increased. The median effective dose require to produce a 50% response does not give any indication of the quantity of nematicide which needs to be taken into the nematode to produce the response.

Panagrellus and Aphelenchus were demonstrated to be permeable to aldicarb which was then accumulated within them , eventually reached an equilibrium distribution between the nematode and the treatment solution. This supports the evidence of Marks et al (1968) who demonstrated that EDB and DBCP were taken up into nematodes and also reached an equilibrium.

While attempts were not made to determine the time at which this equilibrium was reached , it was obviously different for the two species investigated. It was reached quickly in Aphelenchus after approximately 30 minutes but in Panagrellus more slowly some time after treatment for 1 hr.

The initial uptake slopes for aldicarb into these nematodes was not determined because of insufficient quantities of labelled material. The amount taken up into Aphelenchus was greater than that into Panagrellus when equilibrium was thought to have been reached. After 30 minutes the internal concentration was 12.8 $\mu g/g$ in Aphelenchus and after 1 hr in Panagrellus it was 8.7 to 9.1 $\mu g/g$ for unstarved and starved nematodes respectively. Thus the uptake rate of labelled aldicarb into Aphelenchus was much faster than that into Panagrellus . It is not known whether the uptake of aldicarb into these nematodes was an active or a passive process and this remains to be investigated. The uptake of EDB and DBCP into 3 species of plant parasitic nematode was established as passive (Marks et al , 1968) and the uptake of aldicarb may be also considered to be passive.

Little aldicarb will gain entry through the body openings , the amount detected within treated nematodes could not be explained by the small amounts which would be likely to enter by this method. The uptake of the bulk of labelled aldicarb must occur through the nematode's cuticle. The entry of aldicarb per oreum may occur initially but would soon cease , when mycophagus nematodes were treated in aldicarb the rhythmic pulsations of the pharyngeal wall and the uptake of fluid through the mouth stopped. Second stage larvae of cyst nematodes do not feed until a later stage in their life cycle , thus the effect of the nematicide can only be a result of uptake through the nematode's cuticle. Autoradiography of Xiphinema which had been exposed to labelled aldicarb did not show

any localization of labelled   nematicide around the vulva (unpublish -
ed data).   Glucose , a polar compound has been demonstrated to
penetrate nematodes very slowly (Marks et al , 1968) but it has been
shown to be selectively absorbed by the intestine of marine nematodes
in perference to the nematode's cuticle (Warwick and Chia, 1969).
Marine nematode treated with glucose passed the labelled compound
into its intestine from the mouth by an effective pumping mechanism
while nematodes treated with nematicides were no longer able to
operate their pharyngeal pumps.

Once aldicarb had been   taken up into Panagrellus
and Aphelenchus   it did not remain unchanged within these nematodes
but was transformed into several compounds which increased in number
and concentration with increase in the exposure interval.   On the
other hand the breakdown of EDB was not reported to occur when nema-
todes were treated (Marks et al , 1968).

Aldicarb sulphoxide was formed rapidly within treated
nematodes and after a 10 minute exposure represent between 44% and 48%
of the total labelled nematicide in the supernatant fractions of
Panagrellus and Aphelenchus respectively.   No other breakdown products
were detected in treated nematodes until exposures of more than 1 hr were
used , except for small traces of the sulphoxide nitrile formed within
a 30 minute exposure   with Aphelenchus.

In Aphelenchus   the amount of aldicarb and its
sulphoxide after an hour exposure were approximately 1:1 but in
unstarved Panagrellus   it was 5:1, the amount of aldicarb conversion

to the sulphoxide was less.   In starved <u>Panagrellus</u>   after

1 hr however the conversion of aldicarb to the sulphoxide was greater

the ratio was 1.5 : 3 , but was almost the reverse after a 24 hr expo-

sure the ratio being 2.3 : 1 .   When unstarved <u>Panagrellus</u> and

and <u>Aphelenchus</u> were also treated in labelled aldicarb for 24 hr ,

the amount of the parent compound remaining unchanged was low , 18%.

and 20% respectively.   The amounts of sulphoxide detected in the

two species was different, more was found to have been further degraded

in <u>Panagrellus</u> than in <u>Aphelenchus</u> in which it formed over 50% of the

total residue in the supernatant.

The formation of the sulphoxide derivative in treated nema-

todes corresponds to the breakdown pathway   which have been described

for aldicarb in different organisms (Metcalf <u>et al</u> , 1966 ;   Dorough and

Ivie , 1968 ; Bartley <u>et al</u> , 1970). The further conversion of the sul-

phoxide to the sulphone was found to be a minor oxidative pathway , no

sulphone was detected except in <u>Aphelenchus</u> which had been treated for

24 hr .   The major degradative pathway of the sulphoxide resem-

bled that found by other workers (Metcalf <u>et al</u> , 1966 ; Bartley <u>et al</u>,

1970) the sulphoxide nitrile either being formed directly from the

sulphoxide or indirectly after the sulphoxide had been converted to

its oxime.   In <u>Aphelenchus</u>   the sulphoxide nitrile was detected

before any sulphoxide oxime was formed , and after a 1 hr exposure the

amount of the oxime exceeded its nitrile.

The sulphoxide oxime has been suggested as an inter-

mediate in the transformation of the nematicide from organic to 'water' -

soluble products (Bartley <u>et al</u> , 1970).   This oxime was found to

be less than 10% of the total nematicide in nematodes after treatment ,
except in unstarved Panagrellus, where it represented 41 % of the total
labelled material in the supernatant. The accumulation of the
sulphoxide oxime in these nematodes cannot be explained. As chemical
analysis was not made of the 'water' - soluble products it is not known
to what extent they are likely to be formed during these exposure
periods.

The sulphone oxime and its corresponding nitrile
were not detected in the supernatant of either species investigated.
These products were not detected in plants either (Bartley et al ,
1970) and thus represent products of a minor degradative pathway
not found in nematodes.

The sulphoxide being fairly stable in nematodes
must be responsible for some of the deleterious effects of the nemat-
icide , for its degradation to the oxime and nitrile would produce
compounds of diminished biological activity.

The contrast between the amount of aldicarb break-
down in 24 hr starved and unstarved Panagrellus after a 1 hr exposure
was interesting. The starved nematodes were unable to maintain
the conversion of aldicarb to the sulphoxide when the exposure was
extended to 24 hr, but the unstarved nematodes (which were them-
selves starved for 24 hr at the termination of the experimental
exposures) had little of the parent compound remaining unchanged.

The resistance of Panagrellus to alkyl halides
was found to have increased when the nematodes were starved for 24 hr

before treatment (Santmyer , 1956) . The alteration of nematode

resistance was only associated with an alteration in the respiratory

quotient of starved nematodes , and a satisfactory explanation could

not be reached. The greater rate of aldicarb breakdown in starved

Panagrellus is not indicative that they are more tolerant to aldicarb

than their corresponding unstarved partners, but it is not an imp-

robable hypothesis. However it is difficult to correlate the

tolerance of Panagrellus to two dissimilar nematodes, the alkyl halides

with an affinity for the cytochrome systems of nematodes (Castro ,

1964) and the oxime carbamates with nematode esterases (Hastings et

al , 1970). Thus the enzyme systems which are likely to be

responsible for the biotransformation of these two groups of nematicides

is probably different.

In nematodes the rapid rate of aldicarb breakdown

is probably caused by some of the oxidoreductases , although few

oxidases act on sulphur molecules. The oxygen carriers likely to

be involved are flavin nucleotides and cytochromes which have been

demonstrated in nematode homogenates (Krusberg , 1960). Peroxidases

are probably not involved for they have not been detected in nematode

extracts (Bird and Rogers , 1965). The demethylation of the

sulphoxide or sulphone analogues would require still further enzyme

systems. Metcalf et al , (1966) suggested that small quantities of

aldicarb were converted to formaldehyde and ultimately to carbon dioxide.

What biochemical or physiological changes occur in

Panagrellus in a 24 hr starvation period are not known. Sivapalan

and Jenkins (1966) reported that during starvation the fatty acids from

the neutral lipid fractions and not those from the phospholipids were

utilized.   An alteration in the respiratory quotient of starved
nematodes indicating that they are utilizing stored lipids as a
source of food (Santmyer, 1956) and the role of enzymes used for
lipid metabolism in the breakdown of nematicides are not known.

It has been reported that the medium on which
nematodes are cultured will affect their permeability to organohalides
(Castro and Thomason , 1971).   Aphelenchus cultured on Rhizoctonia-
wheat had a greater uptake rate of EDB than Aphelenchus cultured on
Rhizoctonia - PDA.   If the environment in which the nematodes are
cultured can have such an important affect upon their permeability
to exogenously applied compounds , then it would not be improbable to
consider that starvation of nematodes would produce different rates
of aldicarb breakdown.   The internal concentration of starved
and unstarved nematodes after a 24 hr exposure could not be considered
different ,   for   the errors in estimating the weight of the
treated nematodes are in excess   of 4% .

The amount of unextractable labelled nematicide
which remained in the nematode residue fractions after centrifugation
varied in Panagrellus and Aphelenchus.   These amounts varied from
between 3 and 5%   of the total labelled material in nematodes which
were treated for 1 hr , and in Panagrellus were found to increase to
between 15 and 19% when the exposure period was extended   to 24 hr.
The increase of labelled material in the residue may represent an
accumulation of the nematicide in lipoidal fractions of the nematode
or an increase in the amount of non organo - soluble products which
were not extractable from the nematode with less polar solvents.

Marks et al (1968) reported that the percentage of EDB in the residues of

3 plant parasitic nematodes was between 1 and 2%. Lazarus and

Rogers (1951) using phenothiazine on Ascardia galli found the

residue contained approximately 10% of the total material. Marks

(1967) discussing these results concluded that most of the unextract-

able material was probably in the intestinal or reproductive tissues,

with no more than probably 2% in the cuticle. Further investigation

with aldicarb is needed to clarify the extent to which it can accumulate and

for which tissues it has a greater preference.

Labelled nematicide was not detected by autoradio-

graphy in the outer cuticular layers of Xiphinema which had been expos-

ed to labelled aldicarb for several hours. This is contrary to

the evidence reported by Brown and Dunn (1965) using EDB which they had

demonstrated was evenly distributed throughout the cuticle of treated

Meloidogyne larvae. Labelled nematicide was located in the

hypodermis of Xiphinema which were similarly treated and sectioned

before mounting and exposing the stripping film. Labelled nemati-

cide was not located in other of the nematode's tissues, and this was not

expected.

The hypodermis is a dense synctium, very osmophilic

and containing many protoplasmic inclusions (Roggen, Raski and Jones,

1967). This region is in imtimate contact with the internal tissues

and particularly the pseudocoelomic cavity. The hypodermis is of

fundamental importance in the transference of essential compounds

between the external and the internal nematode environement through

the cuticle, and vice versa. The role of the hypodermis in the

the transport and retention is obviously important.   Several

authors (Chitwood , 1952 ;   Roggen et al , 1967 ;   Marks et al, 1968)

have discussed the presence of a penetration barrier between the cuticle

and the basal lamella of the body wall , and thought that the select-

ive mechanism was in the region of the hypodermis.   The outer osmo-

philic layer of the cuticle is thought not to act as a barrier to the

entry of fat soluble substances or water (Rogers , 1962).

The total concentration of aldicarb and its break-

down products in Panagrellus or Aphelenchus   was never more than

0.4% of the total amount in the external medium.   Similarly ,

Marks et al (1968)   found that following EDB treatment the total

concentration did not exceed 1% of the concentration in the treatment

solution.

Aldicarb taken up into nematodes and converted to

oxidative and degradative products were also found to be released

from the treated nematodes.   Panagrellus which had been exposed to

a solution of labelled aldicarb for 1 hr , when transferred to non -

labelled aldicarb at the same concentration were found to release the

nematicide into the non-labelled treatment solution.   The amount

of radioactivity in the nematode's supernatant declined most rapidly

during the first 10 minutes of re-exposure by 71% , and subsequently

declined.   The amount of radioactivity remaining in the residue

and supernatant fractions after 1 hr in nonlabelled aldicarb were the

approximately the same at 0.1 µg/g nematode.   After exposure for

10 minutes the sulphoxide constituted the major breakdown product

remaining in the nematode and its egress appeared to be slowest.

Trim (1944) found that the concentration of hexyl-
resorcinal in _Ascaris lumbricoides_ did not decrease when the
nematodes were replaced in saline. While these compounds diffused
inwardly through the cuticle , they could neither move outwardly by
passive diffusion through the nematode's cuticle nor be expelled
actively. The release of EDB and DBCP from plant parasitic
nematodes has also be demonstrated (Marks et al , 1968). The
release rate constants for these compounds were less than their uptake
constants into the same nematodes , and presumably this would also happen
with aldicarb , otherwise an equilibrium would not have been reached.
The results would suggested that the retention of the sulphoxide
by treated nematodes and its slow rate of egress from them may be
an important factor in the period between the discontinuation of the
treatment and the recovery of the nematode. It would be valuable
if the amount in nematodes which would allow recovery to commence
could be established , but the importance of the nematicide in the
residue fractions of treated nematodes is unknown. If the nematici-
de were bound into the lipid phases of the body wall or food storage
material then the nematode may not show any signs of narcosis.

The rapid rate of aldicarb uptake and its concurrent
conversion to the sulphoxide suggests that oxidation could occur while
the parent compound is passing through the cuticle . Bird (1957)
provided evidence to suggest that the cuticle was in a constant state
of metabolic activity. Subsequently it was demonstrated that the
inner layer of the cuticle and hypodermis contain enzymes which are
responsible for the formation of structural proteins in the cuticle
(Anya , 1966). Energy production which allows protein synthesis

to occur is oxidative and may therefore affect aldicarb breakdown.

Enzymic prepartions of nematode extracts have not been used to investigate the. affect they may have on the breakdown of nematicidal compounds. The basic reaction requires the direct attachment of an oxygen molecule to the sulphur molecule of aldicarb to form the sulphoxide , and a second oxygen molecule to form the sulphone. In vitro the oxidation of aldicarb made up in buffered solutions was slow , at pH 5 only 30 % after 1 month and none at pH 9 (Skrentny - personal communication).

Experiments with Meloidogyne larvae on aldicarb treated agar containing the synergist piperonyl butoxide (unpublished data) did not increase the effectiveness of the nematicide or prolong the period when the nematode was inactive. Similar results have been reported in the housefly (Weiden , 1968). Piperonyl butoxide is thought to have little effect upon the sulphoxidation reaction since a highly efficient inhibitor of sulphoxidation would not only prevent this reaction but would also antagonise the activity of aldicarb (Weiden, 1968).

It has been suggested that dorylamids may have a greater susceptability to nematicides than tylenchids (Taylor , Thomas , Robertson and Roberts , 1970) since the uptake of dyes (Hollis and Jordan , 1962) and labelled glucose into dorylamids was greater (Mayo and Thomas , 1971). While there do appear to be some distinctive differences between the two groups of nematodes, it is not possible to catagorise susceptibility with their permeability to

these compounds   or nematicides.   The permeability of nematodes
to nematicides may not be as important as the breakdown of these   com-
pounds within the nematode to less toxic compounds and their egress.

Nematicides have been shown to penetrate into
nematodes more rapidly than water and acetone (Castro , Thomason and
Beld , 1970) , than polar compounds like Glucose (Marks et al , 1968)
and ionised molecules (Banage and Visser   , 1965 ; Fiakpui , 1967 ;
Marks et al , 1968).

When applied topically to insects (Weiden , 1968)
aldicarb penetrates the housefly cuticle more rapidly than the sulph-
oxide or the sulphone.   A correlation may exist between the lip-
oidal constituents of nematodes and the molecular penetration of
nematicides.   Collander (1959)   considered that lipid
solubility and molecular size were major factors which determined
the rate of penetration of membranes.   Quantification of the
fatty acids and neutral lipids of some saprophagous nematodes have
been made (Krusberg , 1967 ;   Lower , Willett and Hansen , 1970) , and
eventually it may be possible to correlate certain lipid fractions with
the amount of nematicide which penetrated or accumulated in nematodes.

Traüble (1970) has suggested that small mole-
cules may be aided across membranes.   Kinks in phospholipid
chains are mobile and can push small molecules from one side to
the other side of a membrane.   The cuticle of dorylamids have been
found to contain pore canals which cross the cortical layer (Roggen
et al , 1967).   These canals ramify through the cuticle and are
considered to be involved with the transport of compounds   (Wright ,

1963).   Aldicarb with a molecular weight of 190 , is a small

sized molecule which may well lend itself to transport across

membranes.

3.   Some Physiological Effects of Aldicarb on Nematodes.

Treatment of nematodes with aldicarb, its
sulphoxide and sulphone caused them to become contracted , although
the degree of contraction was by no means uniform.

Second stage larvae of Heterodera or Meloidogyne
and adult Panagrellus became straight , although larval stages of
Panagrellus become helically coiled.   All stages of Aphelenchus
and D. myceliophagus adopted a flat-coiling posture , but the
adult stage of Xiphinema was not apparently affected.

Cryptobiosis of Aphelenchus induced by desiccation
resulted in the nematode becoming tightly coiled (Evans , 1968 ;
Mankau and Mankau , 1963) , but if it was induced by the lack of oxygen
then the nematode became straight (Cooper , 1969).   The helical-
posture found in Panagrellus also occurs normally in some nematodes
(Crofton , 1971).   This posture can be produced temporarily by the
distorting of a flat-spiral posture or it can occur naturally if there
is a slight twist of the nematode's body about its own central body
axis.

The flat-coiled posture implies that aldicarb and
its oxygen analogues has caused the ventral muscle cells of the nematodes
to become contracted to there minimal length while the dorsal muscle
cells have become extended maximally.   In this coiled form the
nematode's have no locomotory power , although there is slight mus-
cular antagonism which allows them to alter the size by the nematode
being able to coil and recoil slowly.

The straight posture of _Meloidogyne_ and _Heterodera_ larvae must be caused by the equal contraction of both the dorsal and ventral muscle cells , the overall length of _Meloidogyne_ treated with aldicarb were found to decrease markedly . When _Aphelenchus_ which had been treated in aldicarb for 3 weeks were fixed in F:A 4:10 and measured they were not found to differ significantly in their overall lengths from the controls nematodes treated in water for 3 weeks. While contraction of nematode was not a violent reaction it has been observed that nematodes treated with nematicides at high concentration were found to egest their intestinal contents (Taylor - personal communication).

Rhode (1960) found that Thimet^R caused muscular spasms especially in the tails of nematodes which were exposed to sublethal doses. Aldicarb was found to cause little muscular twitching except just prior to the complete inactivation of the nematode , although the extrusion of the stylet of _Heterodera_ larvae was presumably caused by the contraction of the stylet protractor muscle.

Aldicarb was found to have the greatest effect on the abnormal stylet activity of nematodes , the sulphoxide and sulphone took a long period of exposure to produce a comparable response. When treated larvae were returned to water the abnormal activity soon ceased, and the stylets which had been almost continuously extruded were withdrawn within the nematode. The rate of the abnormal stylet probing reached a maximium of 3 / minute after 6hr at 10 ppm with aldicarb and then declined. _Aphelenchus_ was found to behave in the presence of these nematicides in a similar manner to that of

Heterodera .   It has been found that the a normal stylet activity

of Aphelenchus on Thionazin treated fungal cultures commenced very

rapidly   on exposure of the nematode (Kondrollochis - personal

communication).

Crofton (1971)   suggested that the coordination of

the stylet action   and the pharyngeal pumping mechanism is probably

achieved by the anatomical relationship of the muscle fibres in rel-

ation to the deformation characteristics of the cuticular lining of

the pharynx.   The contraction of the dorsal and ventral muscle

cells during the paralysis of the nematode caused a distruption of

this balance as the hydrostatic pressure of the animal is increased.

Crofton(1971) considered that nematodes did not necessarily behave

different   even when changes in length of upto 15% occurred during

feeding or defeacation.   The maximium stylet thrusting corresponded

with the time whencomplete immobilization of treated nematodes occurred.

The retraction of the stylet is thought to be caused by the hydrostatic

pressure of the nematode (Doncaster , 1971)   for unlike the dorylamids

(Wright , 1965) , the tylenchids do not possess stylet retractor muscles.

It was indeed interesting that no abnormal stylet activity was observed

in Xiphinema , although protraction of the stylet has been found to occur

in Xiphinema immediately after exposure to Lannate[R] at high concentrations

(Taylor and Gordon , 1970).   The protractor muscles of Heterodera

were found to contract vigourously during to initial phase of the treatment

when the stylet was also withdrawn , but after longer exposure 'the pro-

tractor muscles were almost continuously contracted and the stylet only

briefly withdrawn.

Carbamates are thought to act primarily by the inhibition of acetylcholinesterase in the nervous system of animals (Casida , 1964) and also have a direct effect at the cholinergic receptor sites. He pointed out that dozens of esterases sensitive to inhibition have been demonstrated in insects , but acetylcholinesterase inhibition appears to be the primary biochemical lesion in poisoning. Aldicarb and its oxygen analogues penetrate into nematodes rapidly and presumably combines selectively with the esterases of the nematode to form a chemical complex. Aldicarb has been found to inhibit the action of acetylcholinesterase and other esterase fractions extracted from Panagrellus (Spurr , 1967).

Carbamates are generally accepted to act as reversible inhibitors of acetylchloinesterase (Wilson , Hatch and Grinsburg , 1960); O'Brien , Hilton and Gilmour , 1966), but it is known that they reach a steady state of inhibition very slowly (Hastings , Main and Iverson , 1970). The inhibitory power of aldicarb and its sulphone according to Hastings et al (1970) has been characterised by their relatively poor binding with bovine acetylcholinesterase and also their high carbamylation rate. The stable sulphoxide was found to become firmly attached the these sites. Aldicarb possess charged groups which will allow it to occupy anionic sites present on the acetylcholinesterase molecule , but this alone does not necessarily mean satisfactory binding (Hastings et al , 1970).

Although the sulphoxide was the more potential inhibition of cholinesterase (Payne , Stansbury and Weiden , 1966) , it was not found in nematodes to be the most effective of the three nematicidal compounds investigated . Thus the greater effect of

aldicarb on nematodes   is presumably a consequence of its greater

rate of cuticular penetration rather than its affinity for cholinest-

erase.   The recovery of nematodes from early symptoms of pois-

oning is generally more frequent after treatment with the sulphone

and sulphoxide rather than aldicarb.   However the recovery of

$M_{eloidogyne}$ larvae   treated with the sulphoxide at low concentrations

was slower than with aldicarb even though the latter was found to

reduce activity more rapidly.   Similar effects were found when these

compounds were applied to the ^{M}exican Bean ^{B}eetle (Weiden , 1968) ,

where aldicarb had caused the greatest initial knockdown but after

treatment was found to cause the greatest recovery than with sulphoxide

or sulphone applications.

        It was anticipated that treatment of nematodes

with either aldicarb or its sulphoxide would have been likely to

cause similar effects on the nematode recovery , for aldicarb has been

shown to be converted so rapidly within nematodes to the sulphoxide

and other breakdown products.   Within the nematode aldicarb and the

breakdown products which have been formed will compete with one

another for the occupation of the sites on the cholinesterase molecules.

The slow rate of recovery of second stage Heterodera   larvae after

aldicarb treatment   rather than after sulphoxide treatment   may be

related to a slower rate of breakdown of aldicarb in these larvae.

Aldicarb breakdown may not occur at the same rate or follow the same

pathways in all tissues.   Aldicarb is lipophilic and is conseqent-

ly thought to be taken up by actively metabolising tissues (Weiden ,

1968).   The sulphoxide and sulphone once formed become relatively

more toxic, but these compounds being hydrophilic are thus less likely

to sequestration by the lipid phases of the treated animals

(Weiden , 1968). While aldicarb may be associated with

microsomal tissues the hydrophilic compounds remain free in the tissues

where they are less likely to become rapidly brokendown. Micro-

somal extracts from vertebrate liver cells have been shown to in -

crease the rate of aldicarb breakdown (Oonnithan and Casida , 1968)

and housefly preparations have been reported to have similar effects

(Shrivastava , Tsukamoto and Casida , 1969). In order to be

effective as a nematicide it is essential that the compound exhibits a

balance between it hydrophilic and lipophilic characteristics.

Compounds with the correct balance will presumably be have some

measure of protection against microsomal degradation but without sever-

ely limiting its penetration and distribution within nematodes.

The hypothesis for the action of these carbamoyl-

oximes is based upon their affinity for acetylcholinesterase and the

importance of this compound for the maintenance of the rhythmic action

potentials in the membrane of the muscle syncytia (del Castillo ,

de Mello and Morales , 1963) . Acetylcholine has been found to

be concentrated in the nerve ring of <u>Ascaris</u> , the weight of which

was fifteen times that in the rest of the body (Mellanby , 1955).

Del Castillo <u>et</u> <u>al</u> (1963) maintained that acetylcholine was important

transmittor substance in excitatory synapses and not in inhibitory

synapses which are thought to have different chemical transmittor sub-

stances which are inhibited by chemicals such as y-amino butyric acid

(del Castillo , de Mello and Morales , 1967).

These compound at all concentrations were only ever

found to decrease nematode activity, while Methomyl (Lannate[R]) at

low concentration was found to cause a continuous excitation of

muscular contraction in dorylamids , while at high concentrations the

compounds were immediately toxic (Taylor and Gordon , 1970). Aldi-

carb only increased the activity of stylet movement and the undulation

rate / cm travelled at low concentrations with some tylenchids.

While treatment of Xiphinema with aqueous solutions of

sulphoxide at 1ppm for 24 hr was only found to reduce nematode activity

by 8%. These nematodes were used to determine the extent to which

acetylcholinesterase hydrolysis was inhibited, and at 1 ppm

hydrolysis was inhibited in 68% of the specimens tested. Fixation

and incubation of the nematodes at $37^{\circ}C$ was presumably the major factors

which could alter the cuticular permeability so that the sulphoxide

diffuses in readily. In contrast, aldicarb treatment for 24 hr at 1 ppm

had a greater effect than the sulphoxide on the body activity of the nem-

atode and fewer were able to migrate through filters after exposure. However

nematodes treated in this manner were not found in histochemical tests

to show any signs of acetylcholinesterase inhibition. Spurr

(1966) found that while low concentrations of aldicarb were needed

to produce a 50% inhibition of enzymatic hydrolysis of acetylcholine-

sterase fractions for Panagrellus to produce an equivalent effect in

water contact tests on the activity of Panagrellus extremely high

concentrations were necessary. Rhode (1960) using Thimet also found

that low concentrations in the order of $10^{-6}M$, were all that was needed

to prevent the hydrolysis of acetylcholineasterase in several species

of nematode.

Aldicarb was found to affect the oxygen consumption of treated nematodes. At low concentrations it increased the oxygen consumption rate of Panagrellus , and while initially increasing the rate when nematodes were treated at high concentrations , the rate eventually declined to below that of the controls. Aldicarb was not found to alter the consumption rate of Aphelenchus which were treated for 2 hr at 100 pp m. Marks (1971) reported similar effects when he treated Caenorhabditis and Aphelenchus in EDB , for using the latter it was found that the oxygen consumption was not affected.

Caenorhabditis sp. was found to response in a way very similar to that of Panagrellus with aldicarb. There was an immediate increase in the oxygen consumption rate with all treatment concentrations , and after a period of 2 hr at the high concentrations the rate declined. While other activity parameters were not immed iately affected by the presence of a nematicide , oxygen consumption of Caenorhabditis and Panagrellus are affected. This tool may be useful for the evaluation of the effectiveness of potential nematicides, since a physiological response must come before a behavioural response.

Aldicarb has been found to alter the oxygen consumption rates of Panagrellus and Rhabditis oxycera (Ritzlow and Kampfe , 1971) , however these workers did not observe the initial increases of oxygen consumption when Panagrellus were treated at high concentrations as reported in this thesis. Instead they showed that from the out- set of the treatment only low concentration increased consumption , high- er concentrations causing an immediate decline in the oxygen uptake rate.

The respiratory quotient of Panagrellus (Ritzlow and Kampfe , 1971) and that of Caenorhabditis (Marks , 1971) indicate that these nematodes were utilizing stored lipids. The intergral role of the aerobic and anaerobic respiratory pathways of nematodes and the way in which they are affected by nematicide treatment needs investigating. A satisfactory explanation cannot be reached to explain why Aphelenchus did not respond to aldicarb or indeed EDB. The biochemical and physiological differences between these nematodes which is responsible for increase / or lack of increase in the oxygen consumption needs investigation.

The point at which the oxygen consumption rate begins to decline is in excess of the time take for the nematicide to reach an equilibrium with the nematode. Furthermore , as Panagrellus were not found to increase in body activity with the nematicide concentration used, an increase in oxygen consumption cannot be correlated to an alteration in the motor activity of the nematode.

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Behavioral Responses of *Heterodera rostochiensis* Larvae to Aldicarb and Its Sulfoxide and Sulfone

BY A. J. NELMES

Behavioral Responses of *Heterodera rostochiensis* Larvae to Aldicarb and Its Sulfoxide and Sulfone[1]

A. J. NELMES[2]

Abstract: Temik® aldicarb pesticide [2-Methyl-2-(methylthio) propionaldehyde-O-(methylcarbamoyl) oxime] is an effective contact and systemic compound against a wide variety of agricultural pests. Its metabolism in soils may lead to aldicarb sulfoxide and aldicarb sulfone which are both toxicologically important. The comparative effects of these compounds on body activity and stylet movement of second-stage larvae of the potato cyst nematode, *Heterodera rostochiensis,* were investigated. Temik aldicarb was the most effective contact toxicant, rapidly inhibiting body activity and stimulating abnormal stylet movement. A 24-hr post-nematicide water treatment allowed effective recovery of body vigor and cessation of abnormal stylet movement of the larvae treated with Temik aldicarb at low concentrations, and with aldicarb sulfoxide and aldicarb sulfone at all the dosage levels used. Larvae treated with 10 ppm Temik aldicarb remained paralyzed, the toxic effect being apparently irreversible. Control of *Heterodera rostochiensis* by direct contact toxicity may not be effective in soil since Temik degrades to compounds having reversible toxic effect. *Key Words: Heterodera rostochiensis,* Golden nematode, Potato cyst nematode, Aldicarb, Nematicide, 2-Methyl-2-(methylthio) propionaldehyde-O-(methylcarbamoyl) oxime, Toxicity.

Temik® (Union Carbide Corp., New York, N.Y.) aldicarb pesticide [2-Methyl-2-(methylthio) propionaldehyde O-(methylcarbamoyl)oxime] is taken up by plant roots and moves systemically. Its biological properties are interesting and it is potentially useful against a variety of agricultural pests. As an aphicide it is effective against the pea aphid (*Acyrthosiphon pisum*) on alfalfa seedlings (14) and the black bean aphid (*Aphis fabae*) on broad beans (12), and as an insecticide and acaricide against pests on ornamental plants (11). Nematicidal properties have been reported for the control of the potato cyst nematode, *Heterodera rostochiensis*, on potatoes (9) and for root-knot nematodes on kenaf (*Hibiscus cannabinus* L.) (1).

In soil Temik aldicarb degrades by oxidation or hydrolysis to a number of metabolites (3) several of which have important toxicological properties which merit investigation. Temik sulfoxide (= aldicarb sulfoxide) [2-Methyl-2-(methylsulfinyl) propionaldehyde O-(methylcarbamoyl)oxime] formed from the initial oxidation of Temik (2), is a potent cholinesterase inhibitor in insects (4), fairly persistent in soils and is rapidly translocated in the plant (8). Temik sulfone (= aldicarb sulfone) [2-Methyl-2-(methylsulfonyl) propionaldehyde O-(methylcarbamoyl)oxime] formed along the same oxidative pathway from aldicarb sulfoxide (2) is a weak cholinesterase inhibitor in insects (8).

Since investigators have shown the systemic and contact toxicity of Temik, an investigation of the contact action of the compound and its metabolites on plant parasitic nematodes was desired. This work is a study of the *in vitro* effect of Temik, aldicarb sulfoxide and aldicarb sulfone on body activity and stylet movement of second-stage potato cyst nematode larvae *Heterodera rostochiensis* (= *H. rostochiensis* L2) chosen in view of its economic importance as an agricultural pest in Western Europe.

MATERIALS AND METHODS

H. rostochiensis L2 were hatched from cysts soaked in tomato root diffusate, the larvae for this study were collected 5 days after hatching commenced.

The experimental exposure combined two phases, an initial 24-hr nematicidal treatment and a subsequent 24-hr water treatment. After the hatched larvae were washed in distilled water approximately 20 nematodes were immersed in freshly prepared 1, 5 and 10 ppm solutions of Temik, aldicarb sulfoxide and aldicarb sulfone made up in aerated distilled water. The experimental solutions in flat-bottomed syracuse dishes were kept at 20 C in the dark to prevent photolytic breakdown. Hourly observations of larval activity were made for 6 hr and finally after 24 hr. The nematodes were then thoroughly washed three times with distilled water and placed in aerated distilled water under the same conditions as before and observations made at 1, 3 and 24 hr. The tests were replicated six times.

The experimental observations consisted of counting the body undulations or wave frequency of 10 nematodes in 30 seconds, one undulation taken to represent one complete cycle of movement at the anterior end (10). The results indicated a wide variation in the amplitude of the undulations between both batches and treatments. Thus, to obtain a direct comparison, the percentage of nematodes actively undulating during each observation interval was calculated (16, 5) from the original observations. Nematodes in physical contact with one another were not recorded, thus more than 10 nematodes were used in each sample replicate to compensate for larvae touching and still allow 10 individuals to be observed.

The percentage of larvae exhibiting abnormal stylet movement was determined from the original observations in a similar manner during each observation interval. The stylet movement was defined as axial extrusion and retraction of the stylet.

A separate series of nematicidal contact tests were designed to establish an ED_{50} for the experimental compounds against *H. rostochiensis* L2. According to Trevan (15) an ED_{50} is the median effective dose which will produce a response other than death in half the population. The larvae were treated at various dosage levels for 24 hr, washed and allowed to migrate through a Coldstreem® milk filter (Boots Ltd., Nottingham, U.K.) on a 90-μ nylon sieve immersed in water. Larvae able to migrate through the filter and sieve were collected after 24 hr and counted.

RESULTS

Temik was the most effective toxicant, rapidly inhibiting body activity at the higher dosage level (Fig. 1). After the initial 24-hr nematicide treatment all the larvae treated with 10 ppm Temik were inactive, compared to 86% with the sulfoxide and 65% with the sulfone. The majority of the larvae treated at the 10-ppm dosage recovered some of their former vigor during the subsequent water treatment, 67% of those with aldicarb sulfoxide and 62% with aldicarb sulfone. Effective recovery of larvae treated with 10 ppm of the primary toxicant did not occur, only 8% were able to undulate after 24 hr, and their vigor was much reduced.

The percentage of larvae exhibiting abnormal stylet movement was greatest during exposure to the nematicides, and very little during the water treatment (Fig. 2). The response of the larvae to the high dose was marked after 6 hr, 69% with Temik compared to 15% with the sulfoxide and 5% with the sulfone. After 24 hr Temik 60%, the sulfoxide 30%, while no activity with the sul-

EFFECT OF TEMIK

EFFECT OF TEMIK SULPHOXIDE

EFFECT OF TEMIK SULPHONE

NEMATOCIDE TREATMENT (Hr)　　WATER TREATMENT (Hr)
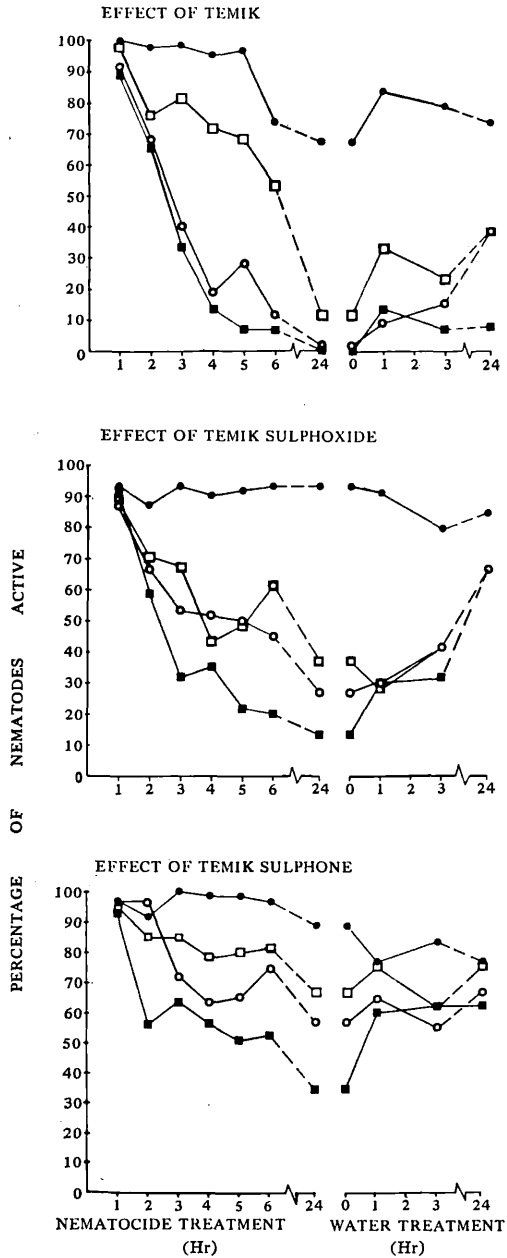
PERCENTAGE OF NEMATODES ACTIVE

FIG. 1. Percentage of *H. rostochiensis* L2 that exhibited undulatory body movements when treated with different carbamoyloximes at 1, 5 and 10 ppm for 24 hr, followed by a 24-hr aerated water treatment. Key: ●—●—● Control, □—□—□ 1 ppm treatment, ○—○—○ 5 ppm treatment, ■—■—■ 10 ppm treatment.

EFFECT OF TEMIK

EFFECT OF TEMIK SULPHOXIDE

EFFECT OF TEMIK SULPHONE

NEMATOCIDE TREATMENT (Hr)　　WATER TREATMENT (Hr)
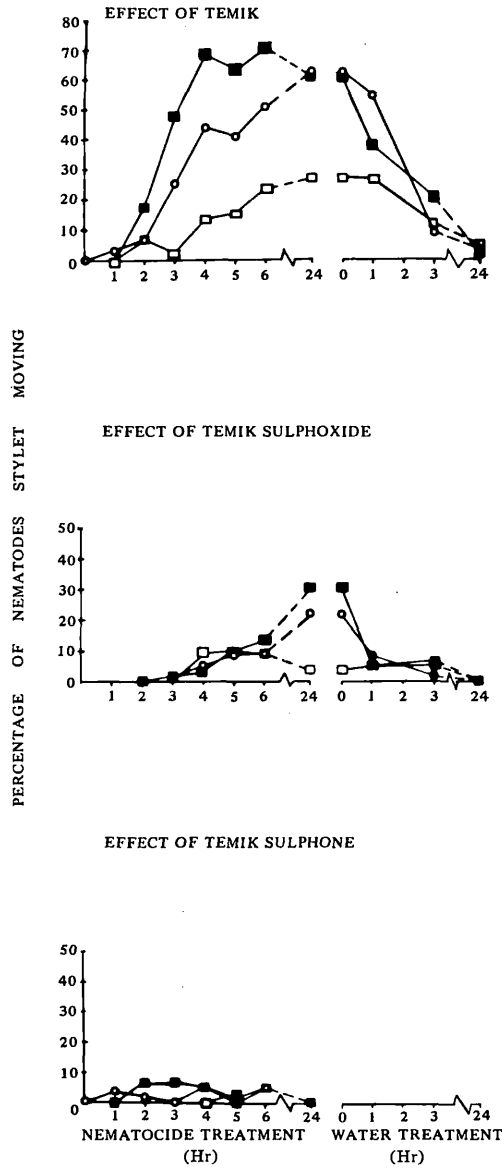
PERCENTAGE OF NEMATODES STYLET MOVING

FIG. 2. Percentage of *H. rostochiensis* L2 that exhibited abnormal stylet movement when treated with different carbamoyloximes at 1, 5, and 10 ppm for 24 hr, followed by a 24-hr aerated water treatment. Key: □—□—□ 1 ppm treatment, ○—○—○ 5 ppm treatment, ■—■—■ 10 ppm treatment.

fone was observed. During the second phase (water treatment) the stylet thrusting activity of the treated larvae rapidly ceased.

An ED_{50} of 4.62 ppm with Temik was calculated from the results of the water contact tests, but with concentrations up to 175 ppm with aldicarb sulfoxide and aldicarb sulfone did not produce this level of response.

DISCUSSION

The inhibitory effect of Temik, aldicarb sulfoxide and aldicarb sulfone on the body activity of *H. rostochiensis* L2 is generally reversible, but at higher concentrations with Temik the effect is permanent at least for the experimental duration. The larvae that were unable to recover were not killed but paralyzed, and this condition may last until all the food reserves were utilized and death followed. The carbamoyloximes stimulate stylet activity in larvae which normally exhibit a high rate of activity during hatching (6), but not when simply immersed in water. As the rate of body activity diminishes so the rate of stylet activity increases when the larvae are treated with the primary toxicant and the sulfoxide. The rate of stylet movement slows towards the end of the nematicide treatment with the Temik-treated larvae, the stylet remains extruded between the lips for periods of time longer than the observation intervals.

The method of calculating an ED_{50} is dependent on the experimental design. Whether the response to the nematicide is measured immediately after exposure to the compounds or after the larvae have had sufficient time to recover in water, will affect the number of larvae that are able to move through a filter to the collection vessel. Data on *Panagrellus redivivus* exposed to Temik (13) would suggest that *H. rostochiensis* L2 are more susceptible to lower concentrations of the pesticide.

The inhibition of body activity may be a reflection of anticholinesterase activity, the irreversible nature with Temik at higher concentrations may be due to its continued binding or interaction with the cholinesterase enzymes of the larvae. Penetration of the cuticle may be dependent on the lipophilic or hydrophilic characteristics of the carbamoyloximes (17). When applied topically, Temik which is lipophilic rapidly penetrates the housefly cuticle, while the hydrophilic aldicarb sulfoxide and aldicarb sulfone penetrate more slowly. Detoxication of carbamates has been demonstrated with insects (7), and it may be an association of this factor with the other physical and chemical properties that effects the rate of toxicity and recovery.

Temik is effective as a contact nematicide but in soil where degradation is rapid, aldicarb sulfoxide would remain the agent of control (3). In early spring when the host plants stimulate hatching of the larvae from cysts, the soils would be well saturated and a high water table would be expected. The effect of the water table may neutralize the contact toxic effect of the aldicarb sulfoxide in the soil and thus allow recovery of the hatched larvae. It would also be pertinent to discover whether larvae which have recovered from the effects of the nematicide would still be able to invade plant roots or respond to sex attractants.

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* LE MODE D'ACTION DE ALDICARB POUR DÉTRUIRE LES NÉMATODES *Meloidogyne incognita* SUR LES TOMATES.

THE MECANISM OF ALDICARB IN CONTROLLING ROOT-KNOT NEMATODES *Meloidogyne incognita* ON TOMATOES.

DER MECHANISMUS VON ALDICARB UM DEN WURZEL-KNOTEN NEMATODEN *Meloidogyne incognita* AUF DEN TOMATEN ZU BEKAEMPFEN.

Temik®, Union Carbide Co., U.S.A. (2-Methyl-2-(methylthio) propionaldehyde 0-(methylcarbamoyl) oxime) an aldicarb pesticide has been used experimentally against a variety of agricultural pests. It has proved successful for the control of plant-parasitic nematodes, and sap-feeding and plant-eating insects. When used for the control of nematodes it has two possible modes of action, its influence in the soil on the larvae or through the media of the plant. In soils it may affect the larval hatching rate or reduce larval activity, while in the plant the roots may be protected from larval invasion due to the up-take of the pesticide or its active metabolites, which make the roots no longer inviting or the cell contents toxic to penetrating larvae. The combination of the two modes of action protect the plant during the initial, most sensitive growth period, for after a short while the aldicarb will have degraded in soils and loose its effectiveness.

To investigate the modes of action, its influence on the free-living stages of root-knot nematodes *Meloidogyne incognita* were studied. It was shown that aldicarb applied as soil drenches at 1 to 8 ppm per gm of soil in small pots reduced root invasion of tomato plants c.v. Moneymaker by 62 and 95 % respectively. Its *in vitro* effect on the rate of larval hatch from egg masses in gauze baskets suspended in solutions of increasing toxicity indicated a suppression of hatch at all treatment levels, significant (P = 0.05) at 10 ppm. Aldicarb was incorporated into 1 % ionagar media at various concentrations. The media was poured as thin films onto which freshly hatched larvae were placed. Nematodes moving across the media left clear tracks from which scale drawings could be made. From these drawings the distances travelled within the exposure period, the displacement and the total number of undulations were calculated. The larvae exposed for 1 h in the dark to 1 ppm, showed a significant reduction (P = 0.05) in the total number of undulations and the distance travelled, while the larvae treated with higher concentrations for the same exposure period became moribund. The larvae were then transferred to pesticide-free media for 2 h, and those treated with 1 or 10 ppm were able

to recover and regain their former activity. Larvae treated with higher concentrations did not revive unless the recovery period was extended beyond 6 h. Nematodes are paralysed by the compound and not killed. It is apparent that unless the nematodes are kept continually dosed with the chemical they will regain their vigour. In soils, its importance in reducing larval activity is possibly the main factor of nematode control.

Root dips of tomato plants c.v. Moneymaker in aldicarb before replanting reduced the level of larval invasion for more than two weeks. Using Thin Layer Chromatography, the presence in all treated roots after 16 days of 0.01 µg/gm aldicarb sulphone was detected, regardless of the initial dip concentrations used. The roots of dipped plants are protection against invasion, but this is governed by the rate of translocation to the foliage and by the rate of degradation of the metabolites within the tissues. Protection of roots is afforded by aldicarb sulphoxide or aldicarb sulphone, and not by aldicarb which is not detected in roots immediately after dipping for 24 h.

Trends in plant chemotherapy for the control of nematodes

by

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Increased resistance of crops by selective plant breeding, and the application of pesticides to soils and plants, are cited as the most effective methods for controlling plant parasitic nematodes. The search for other, novel control measures has been encouraged by the ever-increasing problems relating to residues of pesticides in crops, their persistence in soils and their contamination of different ecosystems.

The control of nematodes is at present best achieved in soils where organophosphate and carbamate compounds exert their chief effect as paralysing agents. Systemic uptake of these compounds into plants through roots causes a temporary halt to nematode feeding and penetration. This is regarded, however, as of subsidiary importance in the general control of nematodes because although the plant may begin its development in a nematode-free environment, the pesticides tend to accumulate in the foliage rather than in roots so that nematode attack soon follows. It is true that the application of the pesticide does result in increased crop yields, but it causes little change in the soil nematode populations, although there may be an initial alteration in the sex ratio of some endoparasitic larvae. Gall-forming nematodes within plant tissue before application of pesticides to roots or leaves appear to continue their development.

Whereas control is at present mainly achieved by treating soils, more effective and persistent control measures may be obtained by the manipulation of the internal environment of the plant making it an unfavourable host. Ideally the application to plants of compounds not intrinsically toxic would be preferable. Such chemotherapeutants could become incorporated into the metabolism of the plant, but be innocuous to it. The translocation of such substances would be essential if foliar application is to be used for the control of root invasion by nematodes. Many exogenous and endogenous compounds are translocated from the roots to the foliage in the transpiration stream, but few compounds are translocated downward because phloem transport is selective.

Unattractiveness of plants to nematodes could be induced in several ways. Roots could be made noxious to nematodes by the production of chemical repellents, substances which deter feeding or penetration responses, substances which inhibit the enzymes normally secreted by penetrating larvae which digest cell membranes during invasion and substances which prevent formation of giant cells from which endoparasitic, gall-forming nematodes obtain their nourishment.

Amino acids are known to be mobile in plants. The antimetabolites DL-methionine, DL-α-alanine, DL-valine and DL-amino-butyric acid all reduce nematode development when applied to plants. Unfortunately, they proved to be of no practical significance because high doses were needed to achieve the necessary control.

Foliar application of sodium fluroacetate, maleic hydrazide, 1,3,5 tricyano 3 - phenyl pentane and derivatives of trichlorobenzoic acid were all found to inhibit nematode invasion for several weeks. These compounds are plant growth regulators which limited their practical usefulness for they were often phytotoxic at levels necessary to achieve nematode control.

Recently the effects of aliphatic and polyhydroxy steroids which act as analogue synergists for hormonal production in insects, have been studied. 2 - 3cis-trans, 6-7 trans farnesyl diethylamine and its methyl ester extracted from plants, when applied to undifferentiated beet nematode larvae stimulated the development of abnormal males. Few steroids have been shown to cause any response and then only at high concentrations. Whether treated plants could influence the further development of nematodes feeding on their tissue is a matter for conjecture.

A recently developed carbamoyloxime, Du Pont 1410 (S-methyl 1-(dimethylcarbamoyl)-N-[(methylcarbamoyl) oxy] thioformimidate) although toxic has proved interesting. It has been reported to control invasion of roots of a variety of plants after foliar application without causing phytotoxic symptoms.

It will be necessary to develop better understanding of the natural mechanisms of resistance in plants if more satisfactory methods of plant protection against nematodes are to be devised. There is clearly a need for non-toxic chemotherapeutants but the effective mechanisms remain to be discovered.