THE EFFECTS OF ALDICARB, A NEMATICIDAL CARBAMOXYLOXIME, AND ITS OXYGEN ANALOGUES ON SOIL AND PLANT PARASITIC NEMATODES.

A thesis submitted for the degree of
Doctor of Philosophy in The University of London

by

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September 1971
To My Wife

Riki

for her patience and understanding
This study was sponsored by the Agricultural Research Council of Great Britain and carried out at Imperial College Field Station, in the Department of Zoology and Applied Entomology.

I would like to express my gratitude to Professor T.R.E. Southwood for his encouragement and support, and to Dr W. Mordue for his helpful advice and criticism throughout this work.

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Union Carbide Corporation, New York, USA donated the experimental compounds used in this investigation.

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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 sulphone 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 sulphone, the nematodes becoming almost motionless.

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

Of the three compounds, aldicarb had the greatest affect on
stylet movement, stimulating abnormal protraction except in *Xiphinema*. Maximum stylet protraction corresponded with maximum body inactivity.

The permeation of two nematode species by $^{14}$C-labelled aldicarb was investigated. These were permeable to aldicarb which accumulated within them. An equilibrium was reached between the nematicide inside the nematode and that in the exposure solution. After penetration 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 susceptible 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 unusually 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 ascertain 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 *Aphelenchus avenae* were only affected by Thionazin 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 nematode 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 carbamates 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®, 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 investigation. 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 effective 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. *Xiphinema 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 dibromochloropropane (DBCP) penetrate nematodes and reach an equilibrium distribution 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 Tarjan, 1955; Santmyer, 1956; Kampfe, 1964; Spurr, 1965) and *Aphelenchus 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 mycoparasitic 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 **Lycopersicum 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 stream® milk filter over a 90μm mesh nylon sieve. The larvae which had migrated through the sieve 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; Moriarity, 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 behavioural 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 sulfoxide [2-methyl-2-(methylsulphinyl) propionaldehyde O(methylcarbamoyl) oxime = Temik Sulphoxide], and aldicarb sulphone [2-methyl-2-(methyl-sulphonyl) propionaldehyde O(methylcarbamoyl) oxime = Temik sulphone].

\[
\begin{align*}
\text{CH}_3 & \\
\text{CH}_2\text{SC} & \quad \text{CH} = \text{NOCO.NHCH}_3 \\
\text{CH}_3 & 
\end{align*}
\]

_**Aldicarb** Molecular weight 190.3

b) **Labelled Compounds**

Studies on the uptake of aldicarb and its break-down in nematodes were made with $^{14}$C labelled nematicide [2-methyl-2-(methyl-$^{14}$thio) propionaldehyde O (methylcarbamoyl) oxime] (sp. activity 2.03 mCi/mmole). This compound was obtained from Messers Mallinckrodt Ltd, St Louis, U.S.A.

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 homogenised 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 mixture 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 added 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 discarded, but the addition of 100 ml acetone to the top of the column was then collected. This eluate was then concentrated 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 20-30μl.
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 plate. 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 6% 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 Rₜ 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 ¹⁴C 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 preparative 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 soluble products would be formed during short exposure intervals.

Labelled aldicarb was used to study uptake and breakdown 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°C. The supernatant liquid was collected by centrifugation 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 could be spotted on TLC plates.

The nematode precipitate was stored at -4°C in glass phials until such time as quantitative estimation of the radioactivity 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 dryness 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 autoradiography utilizing Kodarex Estar\textsuperscript{R} medical X-ray film which was exposed for 20 days before developing in Phen-X\textsuperscript{R} (Ilford Ltd) and fixed in Amfix\textsuperscript{R} (Ilford Ltd) (Figure 1).

The radioactive spots were also detected with the chromogenic agents described in 4 a) and then oversprayed with 7% 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 unknown compounds detected on the silica plates could be identified against the \( R_f \) values of the known standards shown in Table 1.
Fig. 1. 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 - acetonitrile. The labelled compounds were then located by exposure of the plate to medical X-ray film for 20 days.
3:1 Methylene Chloride - Acetonitrile

2:1 Ether - Hexane + 20% Acetone
<table>
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<th>Code</th>
<th>Compound</th>
<th>( R_f ) value in solvent systems</th>
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<tr>
<td></td>
<td></td>
<td>2:1 ether-hexane</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( \text{+} )</td>
</tr>
<tr>
<td>T</td>
<td>2-methyl-2-(methylthio) propionaldehyde</td>
<td>( 0.40 ) (0.61)</td>
</tr>
<tr>
<td></td>
<td>( \text{Q(methylcarbamoyl) oxime (Aldicarb).} )</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-methyl-2-(methylsulphonyl) propionaldehyde</td>
<td>( 0.01 ) (0.05)</td>
</tr>
<tr>
<td>T</td>
<td>( \text{Q(methylcarbamoyl) oxime (Aldicarb sulphoxide).} )</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-methyl-2-(methylsulphonyl) propionaldehyde</td>
<td>( 0.11 ) (0.10)</td>
</tr>
<tr>
<td>T</td>
<td>( \text{Q(methylcarbamoyl) oxime (Aldicarb sulphone).} )</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-methyl-2-(methylsulphonyl) propionaldehyde</td>
<td>( 0.08 ) (0.13)</td>
</tr>
<tr>
<td></td>
<td>( \text{oxime (Oxime sulfoxide).} )</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>2-methyl-2-(methylsulphonyl) propionaldehyde</td>
<td>( 0.27 ) (0.61)</td>
</tr>
<tr>
<td></td>
<td>( \text{oxime (Oxime sulphone).} )</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>2-methyl-2-(methylsulphonyl) propionitrile.</td>
<td>( 0.23 ) (0.38)</td>
</tr>
<tr>
<td>T</td>
<td>2-methyl-2-(methylsulphonyl) propionitrile.</td>
<td>( 0.14 ) (0.57)</td>
</tr>
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</table>

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 correction curve gave a percentage counting efficiency of 89 and 85% respectively.
A quench correction curve for the $^{14}$C counting efficiency against the channel ratio.

The channel ratios were found at between 4 and 5 which gave a counting efficiency of 89% to 85.6% respectively.
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/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 100ml and after agitation three 20ml 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® 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 remaining nematodes finally suspended in 10ml 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 μg/g wet weight of plant tissue.
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°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°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 nematicide 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°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).
The Effect on In Vitro Egg Hatch of Meloidogyne incognita.

The hatch of eggs of Meloidogyne spp. and other nematode genera has been studied in many artificial 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 nematicides (Johnston and Shamiyeh, 1968). The optimal conditions for the best hatch of M. incognita were found to be high moisture and 25°C which produced a 70% hatch.

The in vitro effect of aldicarb and its oxygen analogues on Meloidogyne 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).

Figure 3 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 $1360 \pm 80.4$ for eggs used in the aldicarb experiment and $1562 \pm 340.7$ 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 photodecomposition and incubated at $25^\circ C$. 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 intestinal 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 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 invasion 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 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 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 activity 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-
represent 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°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® filter pad over a Millipore glass sinter filter. The pad was then inverted over a Coldstream® (Boots, Nottingham) milk filter on a 90μ nylon sieve immersed in water as described by Townshend (1964). Those nematodes which had migrated through the filter and sieve in 24 hr were collected and subsequently 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 recovery 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°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°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 up to 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 adjustment 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 compared.

In a separate series of experiments the dispersal of Panagrellus 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 diffusible 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% ionagar 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 choseh 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.

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 µg to 9 µg/g and in the outer zone from 0.7 µg to 1.4 µg/g agar.

Ten second stage larvae of Heterodera and adult Aphelenchus 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 °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.
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 vigorously. 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, collecting 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 sulphoxide 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 dextrose 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.
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°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 ml of saturated potassium chloride and covered by a thin permeable membrane. The oxygen consumed by the sensor is less than 10^{-7} g/hr in air, and the rate of consumption 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 manufacturer crushed the nematodes. It was replaced by a small glass covered stirrer and this minor modification has also been suggested by Marks and Sorensen (1971).

Panagrellus extracted from cultures in 3 hr and Aphelenchus 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°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 $3.8 \times 10^5$ 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°C.

The solutions in the test chamber were stabilized and the plunger - probe inserted so that the excess air was expelled through the access slot of the plunger. The stirrer brake was then released 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 individual readings had been taken the test samples were discarded 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 aliquots were freeze dried was 31 mg and the oxygen consumption rate based upon this value was calculated in μl/mg/min at 30°C.
The Histochemical Determination of Acetylcholinesterase Inhibition in *Xiphinema diversicaudatum* by Aldicarb and its Oxygen Analogues.


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 acetylcholine 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 $3.016 \times 10^{-4}$ M solution of labelled aldicarb for 6 hr at $20^\circ C$ 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 $60^\circ C$ for 48 hr. The blocks were shaped and transverse sections of *Xiphinema* were cut at $900 \AA$ on a Reichart Ultra-microtome. The sections were collected on small pieces 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 stripping film, Kodak AR 10. The emulsion was floated on distilled water at $22^\circ C$ 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 $4^\circ C$ to expose the film.
The stripping film was then developed in Kodak 19b at 11°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® 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.
The Uptake and Egress of $^{14}$-C Labelled Aldicarb in Saprophagous and Mycophagous Nematodes.

This investigation was divided into two parts:

a) Uptake Studies

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

Panagrellus 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 Size of nematodes

The biomass of Aphelenchus and Panagrellus used in uptake experiments was determined by estimation of the weight and volume using formulae suggested by Andrásy (1956). The lengths and maximum widths of 30 adult and larvae Aphelenchus and Panagrellus 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 sieve 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 µg/g of nematode was considered to be more accurate than the use of 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 nematode'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 $3.016 \times 10^{-4} \text{M C}^{14}$-labelled aldicarb solution (50 ppm), contained in 100 ml stoppered flasks. The treatment solutions were kept in darkness at 20°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 filtration of the treatment solution using a medium porosity glass sinter-stuck suggested by Marks et al (1968). The nematodes were
Table 2  A comparison of the mean lengths, widths and estimated biomass of *Panagrellus* and *Aphelenchus* used in uptake studies.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Panagrellus redivivus</th>
<th>Aphelenchus avenae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Length μ</td>
<td>Width μ</td>
</tr>
<tr>
<td>Adult</td>
<td>1476</td>
<td>49.3</td>
</tr>
<tr>
<td>Larva</td>
<td>973</td>
<td>31.3</td>
</tr>
</tbody>
</table>

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 $3.016 \times 10^{-4}$ 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 $20^\circ$C and the flasks gently shaken to keep the nematodes in suspension.

The exposure of nematodes in non-labelled aldicarb was terminated 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 significantly 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 6% of the total number of hatched larvae in the control groups, reduced to 4% at 1 ppm and was only 3% in the 8 ppm treatment.

While second stage larvae in the untreated control soils invaded tomato roots rapidly it was evident that aldicarb treated 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.
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 hatched 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%.

<table>
<thead>
<tr>
<th>Length of treatment</th>
<th>Number of larvae / g root</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>1 week</td>
<td>60.5</td>
</tr>
<tr>
<td>2 weeks</td>
<td>43.8</td>
</tr>
<tr>
<td>3 weeks</td>
<td>45.1</td>
</tr>
<tr>
<td>4 weeks</td>
<td>49.1</td>
</tr>
</tbody>
</table>

*Table 3* The number of *Meloidogyne* larvae per gram tomato root after approximately 500 larvae were inoculated into pot soils drenched with aldicarb.
c) The Effect on Extraction of Larvae from Treated Soils.

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 *Meloidogyne* larvae inoculated into soils as described in Section 8a), were less extractable from treated soils (Figure 7). When larvae were treated in soils for 24 hr, extraction using a Whitehead Tray for 36 hr was reduced by 70% by aldicarb, 51% by sulfoxide and 19% by sulphone treated soils. Two week exposure reduced larval extraction by approximately 50% with all treatments.

![Graph showing the effect of aldicarb and its oxygen analogues on larval extraction](image)

**Figure 7** The effect of aldicarb and its oxygen analogues applied as soil drenches at 4 ppm on the extraction of *Meloidogyne* larvae by Whitehead Tray. Key: C control, T aldicarb, T1 sulfoxide, T2 sulphone.

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 approximately 88 to 95% of the total number of eggs. Hatching commenced within 5 days and was completed within 20 days, when the exposure period was extended few larvae were recovered.

Aldicarb at 50 and 100 ppm was found to completely suppress 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 concentrations 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 \textit{in vitro} hatch of \textit{Meloidogyne} eggs in 20 days at 25°C. Regression lines represent the depression of the cumulative hatch at 5 day intervals. Key: $\square$ 5 day; $\bigcirc$ 10 day; $\Delta$ 15 day; $\square$ 20 day.
<table>
<thead>
<tr>
<th>Nematicide Percentage reduction of egg hatch during treatment and treatment (ppm)</th>
<th>5 Days</th>
<th>10 Days</th>
<th>15 Days</th>
<th>20 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>28.53</td>
<td>15.96</td>
<td>32.22</td>
<td>35.12</td>
</tr>
<tr>
<td>1</td>
<td>10.63</td>
<td>nil</td>
<td>nil</td>
<td>nil</td>
</tr>
<tr>
<td>5</td>
<td>13.17</td>
<td>40.92</td>
<td>46.30</td>
<td>46.65</td>
</tr>
<tr>
<td>50</td>
<td>nil</td>
<td>nil</td>
<td>nil</td>
<td>nil</td>
</tr>
<tr>
<td>100</td>
<td>nil</td>
<td>nil</td>
<td>nil</td>
<td>nil</td>
</tr>
<tr>
<td>T</td>
<td>57.37</td>
<td>75.17</td>
<td>81.15</td>
<td>83.90</td>
</tr>
<tr>
<td>T</td>
<td>25.48</td>
<td>13.16</td>
<td>11.49</td>
<td>10.98</td>
</tr>
<tr>
<td>50</td>
<td>nil</td>
<td>25.00</td>
<td>26.59</td>
<td>26.90</td>
</tr>
<tr>
<td>100</td>
<td>92.79</td>
<td>98.90</td>
<td>99.20</td>
<td>99.42</td>
</tr>
<tr>
<td>T</td>
<td>66.29</td>
<td>60.63</td>
<td>61.49</td>
<td>60.87</td>
</tr>
<tr>
<td>50</td>
<td>34.00</td>
<td>31.00</td>
<td>32.70</td>
<td>33.00</td>
</tr>
<tr>
<td>100</td>
<td>96.25</td>
<td>99.13</td>
<td>99.51</td>
<td>99.61</td>
</tr>
<tr>
<td>T</td>
<td>88.89</td>
<td>87.83</td>
<td>88.79</td>
<td>88.33</td>
</tr>
<tr>
<td>100</td>
<td>65.00</td>
<td>66.00</td>
<td>66.49</td>
<td>66.20</td>
</tr>
</tbody>
</table>

Table 4  The in vitro effect of aldicarb and its oxygen analogues on the hatch of Meloidogyne eggs from egg masses at 25°C. Percentage reductions were based upon egg hatch in water which were regarded as 100%. Key: T aldicarb, T₁ sulphoxide, T₂ sulphone.
The hatch ED$_{50}$ with aldicarb calculated from the regression 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°C with aldicarb solutions. 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.

<table>
<thead>
<tr>
<th>Treatment (ppm)</th>
<th>24 hr Treatment</th>
<th>7 Day Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean number</td>
<td>% of inoculum</td>
</tr>
<tr>
<td></td>
<td>of larvae in</td>
<td>in roots</td>
</tr>
<tr>
<td></td>
<td>roots</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>180.0</td>
<td>60.0</td>
</tr>
<tr>
<td>1</td>
<td>179.2</td>
<td>58.4</td>
</tr>
<tr>
<td>10</td>
<td>177.5</td>
<td>59.0</td>
</tr>
<tr>
<td>100</td>
<td>98.3</td>
<td>32.0</td>
</tr>
</tbody>
</table>

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.
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: ○—○ 1 day, □—□ 1 week.
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 (outlined 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).

When larvae 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 inoculated was lengthened the effect upon invasion declined, there was no apparent difference between the treatment concentrations.

<table>
<thead>
<tr>
<th>Treatment (ppm)</th>
<th>inoculated after 2 days</th>
<th>inoculated after 9 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean number of larvae in roots</td>
<td>% reduction of larval invasion</td>
</tr>
<tr>
<td>0</td>
<td>191.7</td>
<td>nil</td>
</tr>
<tr>
<td>1</td>
<td>114.3</td>
<td>40.4</td>
</tr>
<tr>
<td>4</td>
<td>100.7</td>
<td>47.5</td>
</tr>
<tr>
<td>8</td>
<td>116.3</td>
<td>39.3</td>
</tr>
<tr>
<td>12</td>
<td>95.0</td>
<td>50.4</td>
</tr>
</tbody>
</table>

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 nematicide in plant roots may influence the invasive capacity of Meloidogyne. 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 4a). 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, • • T₁, ○ ○ T₂.
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.
Table 8

<table>
<thead>
<tr>
<th>Residue Detected</th>
<th>Soil Drench Conc (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>T</td>
<td>ND&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>ND</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>ND</td>
</tr>
</tbody>
</table>

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 µg/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 µg/g and at 12 ppm increased to 0.45 µg/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 sulphoxide 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 sulphoxide 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 dispersal 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 inoculated 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 radius of 10 mm. At 1000 ppm, 38% were within a radius of 10 mm and another 41% a further 10 mm distance.
<table>
<thead>
<tr>
<th>Treatment (ppm)</th>
<th>Number of Nematodes in Zones after 24 hr&lt;sup&gt;a&lt;/sup&gt;</th>
<th>% of total on media</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 - 10 mm</td>
<td>10 - 20 mm</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>10</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>100</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>500</td>
<td>37</td>
<td>31</td>
</tr>
<tr>
<td>1000</td>
<td>38</td>
<td>41</td>
</tr>
</tbody>
</table>

Table 9 The effect of aldicarb in 1% ionagar on the dispersal 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 *Meloidogyne* larvae was not affected.

Three activity parameters were investigated (Table 10) and the least affected in 1 hr was the furthest dispersal 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 *Meloidogyne* 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 *Meloidogyne* larvae on treated agar after 1 hr and subsequent 2 hr on untreated media.
### TREATMENT PERIOD - 1 HR

<table>
<thead>
<tr>
<th>Treatment (ppm)</th>
<th>total distance cm</th>
<th>S.E</th>
<th>total number undulations</th>
<th>S.E</th>
<th>furthest dispersal cm</th>
<th>S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.08 ± 0.34</td>
<td></td>
<td>222.6 ± 13.2</td>
<td></td>
<td>1.50 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4.51 ± 0.26</td>
<td>0.49</td>
<td>149.1 ± 4.5</td>
<td>0.99</td>
<td>0.90 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>2.45 ± 0.13</td>
<td>9.7</td>
<td>111.0 ± 5.1</td>
<td>0.77</td>
<td>0.09 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>1.55 ± 0.20</td>
<td></td>
<td>72.8 ± 9.7</td>
<td>0.57</td>
<td>0.05 ± 0.05</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment (ppm)</th>
<th>total distance cm</th>
<th>S.E</th>
<th>total number undulations</th>
<th>S.E</th>
<th>furthest dispersal cm</th>
<th>S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>1</td>
<td>4.71</td>
<td>9.9 ± 1.56</td>
<td>1.53</td>
<td>0.20 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>3.06 ± 0.05</td>
<td>9.6</td>
<td>107.8 ± 3.4</td>
<td>0.85</td>
<td>0.73 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>2.57 ± 0.20</td>
<td>8.5</td>
<td>95.8 ± 12.2</td>
<td>0.61</td>
<td>0.07 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>1.80 ± 0.18</td>
<td>7.3</td>
<td>70.5 ± 7.3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment (ppm)</th>
<th>total distance cm</th>
<th>S.E</th>
<th>total number undulations</th>
<th>S.E</th>
<th>furthest dispersal cm</th>
<th>S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>1</td>
<td>7.09</td>
<td>16.6 ± 1.34</td>
<td>1.34</td>
<td>0.12 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>5.03 ± 0.25</td>
<td>7.6</td>
<td>177.4 ± 7.6</td>
<td>0.89</td>
<td>0.11 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>3.69 ± 0.26</td>
<td>10.5</td>
<td>146.3 ± 10.5</td>
<td>0.86</td>
<td>0.07 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>2.10 ± 0.15</td>
<td>5.9</td>
<td>82.4 ± 5.9</td>
<td>0.93</td>
<td>0.12 ± 0.07</td>
<td></td>
</tr>
</tbody>
</table>

### RECOVERY PERIOD - 2 HR

| 0               | 6.42 ± 0.42       |     | 248.5 ± 16.3             |     | 1.56 ± 0.19           |     |
| 1               | 5.79 ± 0.38       | 14.7| 206.5 ± 14.7             | 1.03| 0.09 ± 0.07           |     |
| T               | 4.50 ± 0.39       | 11.9| 198.5 ± 11.9             | 1.27| 0.11 ± 0.07           |     |
| 50              | 0.05 ± 0.95       | 8.0 | 2.0 ± 0.8                | 0.01| 0.03 ± 0.03           |     |
| 100             | 0.00 ± 0.05       | 0.0 | 0.0 ± 0.0                | 0.00| -                     |     |

| 1               | 3.63 ± 0.33       | 12.6| 168.2 ± 12.6             | 0.87| 0.07 ± 0.07           |     |
| T1              | 3.83 ± 0.19       | 11.2| 191.5 ± 11.2             | 0.87| 0.06 ± 0.06           |     |
| 50              | 2.98 ± 0.35       | 13.4| 121.7 ± 13.4             | 0.65| 0.08 ± 0.08           |     |
| 100             | 0.89 ± 0.26       | 10.6| 36.1 ± 10.6              | 0.14| 0.05 ± 0.05           |     |

| 1               | 7.55 ± 0.45       | 14.2| 268.9 ± 14.2             | 1.55| 0.17 ± 0.07           |     |
| T2              | 4.76 ± 0.36       | 13.7| 208.5 ± 13.7             | 0.99| 0.14 ± 0.09           |     |
| 50              | 2.10 ± 0.15       | 10.3| 93.7 ± 10.3              | 0.70| 0.08 ± 0.08           |     |
| 100             | 0.83 ± 0.20       | 8.3 | 31.8 ± 8.3               | 0.18| 0.03 ± 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 sulphoxide 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.
Figure 14 The effect of aldicarb, its sulfoxide 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.
The effect of aldicarb, its sulphoxide and sulphone in treated 1% agar on the reduction in mobility of second stage *Meloidogyne* 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 2 hr.

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

- O---O T
- ▲▲▲ T₁
- ■■■ T₂
EFFECT ON THE TOTAL NUMBER OF UNDULATIONS

\[ T \cdot y = 31.0 + 8.8 \ (\log x) \]
\[ T_1 \cdot y = 24.4 + 12.9 \ (\log x) \]
\[ T_2 \cdot y = 0.05 + 24.2 \ (\log x) \]

\[ T \cdot y = 6.2 + 35.6 \ (\log x) \]
\[ T_1 \cdot y = 21.1 + 14.2 \ (\log x) \]
\[ T_2 \cdot y = -0.3 + 33.9 \ (\log x) \]

EFFECT ON THE TOTAL DISTANCE TRAVELED

\[ T \cdot y = 27.6 + 13.1 \ (\log x) \]
\[ T_1 \cdot y = 23.3 + 13.5 \ (\log x) \]
\[ T_2 \cdot y = -0.5 + 25.6 \ (\log x) \]

\[ T \cdot y = 4.1 + 37.5 \ (\log x) \]
\[ T_1 \cdot y = 34.1 + 12.2 \ (\log x) \]
\[ T_2 \cdot y = -0.2 + 35.4 \ (\log x) \]

EFFECT ON THE FURTHEST DISPERAL

\[ T \cdot y = 32.1 + 7.7 \ (\log x) \]
\[ T_1 \cdot y = 16.3 + 9.2 \ (\log x) \]
\[ T_2 \cdot y = 0.5 + 34.0 \ (\log x) \]

\[ T \cdot y = 16.1 + 30.2 \ (\log x) \]
\[ T_1 \cdot y = 27.6 + 16.8 \ (\log x) \]
\[ T_2 \cdot y = 0.4 + 30.7 \ (\log x) \]

Treatment conc ppm (Log).
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 medium for 2 hr.

Key: S. position of larvae immediately after treatment, F. position after 2 hr.

The movement pattern of larvae observed during the recovery period was the reverse of the pattern during treatment. Larvae treated 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
<table>
<thead>
<tr>
<th>Recovery Period after treatment</th>
<th>distance travelled cm</th>
<th>total number of undulations</th>
<th>furthest dispersal cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>0.00</td>
<td>0.0</td>
<td>0.00</td>
</tr>
<tr>
<td>4 hr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T₁</td>
<td>3.77</td>
<td>162.8</td>
<td>0.59</td>
</tr>
<tr>
<td>T₂</td>
<td>3.40</td>
<td>118.0</td>
<td>0.87</td>
</tr>
<tr>
<td>6 hr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>0.00</td>
<td>0.0</td>
<td>0.00</td>
</tr>
<tr>
<td>T₁</td>
<td>2.37</td>
<td>92.0</td>
<td>0.38</td>
</tr>
<tr>
<td>T₂</td>
<td>7.12</td>
<td>261.4</td>
<td>1.38</td>
</tr>
<tr>
<td>16 hr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>10.23</td>
<td>338.2</td>
<td>1.38</td>
</tr>
</tbody>
</table>

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.

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* larvae 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.
### Table 12

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

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

*Aphelenchus* 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 remaining 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 regions containing less of the toxic nematicide. On the aldicarb gradient 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. Although the total amount of each compound in the zones was quantified, the actual concentration in the surface of the agar in contact with the nematode was unknown. However exposure to 6.1 to 9.3 µg/g on these zones 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 inactivation.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean number of nematodes on nematicide gradients</th>
<th>% total nematodes on media</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>individual zones</td>
<td>grouped zones</td>
</tr>
<tr>
<td></td>
<td>1&lt;sup&gt;b&lt;/sup&gt; 2 3 4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1 - 2 3 - 4</td>
</tr>
<tr>
<td>A. avenae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.50 1.16 0.91 1.50</td>
<td>1.66 2.40 40.7</td>
</tr>
<tr>
<td>T</td>
<td>2.83 3.41 1.75 1.85</td>
<td>6.24 3.40 98.6</td>
</tr>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>4.25 3.41 1.41 0.91</td>
<td>7.66 2.31 99.8</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.91 0.83 1.85 3.00</td>
<td>1.74 4.85 65.9</td>
</tr>
<tr>
<td>H. rostochiensis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.58 1.91 2.75 2.08</td>
<td>3.49 4.83 83.2</td>
</tr>
<tr>
<td>T</td>
<td>4.08 4.50 1.25 0.16</td>
<td>8.58 1.41 99.9</td>
</tr>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>3.91 3.75 1.50 0.83</td>
<td>7.66 2.33 99.9</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>3.25 4.00 1.58 1.08</td>
<td>7.25 2.66 99.1</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Response observed</th>
<th>0.1 PPM</th>
<th>1.0 PPM</th>
<th>10 PPM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T T₁ T₂</td>
<td>T T₁ T₂</td>
<td>T T₁ T₂</td>
</tr>
<tr>
<td>Feeding</td>
<td>+ + +</td>
<td>- - -</td>
<td>- - -</td>
</tr>
<tr>
<td>Locomotion</td>
<td>+ + +</td>
<td>+ + +</td>
<td>- - +</td>
</tr>
<tr>
<td>Abnormal stylet movement</td>
<td>- - -</td>
<td>+ + -</td>
<td>- - -</td>
</tr>
<tr>
<td>Coiled - posture</td>
<td>- - -</td>
<td>- - -</td>
<td>+ + -</td>
</tr>
</tbody>
</table>

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 hr 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 second stage *Heterodera* larvae that exhibited undulatory body movements.

Figure 16.2 Percentage of second stage *Heterodera* larvae that exhibited abnormal stylet 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, ○—○ 5 ppm, ■—■ 10 ppm.
The effect of aldicarb

The effect of the sulphoxide

The effect of the sulphone
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 nematicides, 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 reached a maximum 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 maximum of 30% was reached after 24 hr exposure. The sulphone did not cause a greater response than 10% when applied for up to 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 intervals 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 maximum 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.

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 4 hr.
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 sulphone *b* for 24 hr, when allowed a further 24 hr to migrate through filters their migration was reduced (Table 15), although the sulphone at 500 ppm had no effect.

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

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. *Heterodera* 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](image_url)

**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.
<table>
<thead>
<tr>
<th>Treatment (ppm)</th>
<th>Mean number collected</th>
<th>% reduction of migration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldicarb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>272.3</td>
<td>0.0</td>
</tr>
<tr>
<td>1</td>
<td>274.0</td>
<td>0.0</td>
</tr>
<tr>
<td>5</td>
<td>267.7</td>
<td>3.5</td>
</tr>
<tr>
<td>10</td>
<td>265.0</td>
<td>4.4</td>
</tr>
<tr>
<td>50</td>
<td>263.3</td>
<td>5.1</td>
</tr>
<tr>
<td>100</td>
<td>251.0</td>
<td>9.5</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Treatment (ppm)</th>
<th>24 hr nematicide % reduction of migration</th>
<th>24 hr water % of nematodes active</th>
<th>24 hr water % reduction of migration</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>96.1</td>
<td>100.</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>91.5</td>
<td>89.4</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>T₁ 91.5</td>
<td>100.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T₂ 82.2</td>
<td>100.</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>T₁ 76.5</td>
<td>69.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T₂ 48.3</td>
<td>100.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T₂ 33.3</td>
<td>100.</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>T₁ 0.0</td>
<td>80.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T₂ 31.5</td>
<td>100.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T₂ 24.4</td>
<td>100.</td>
<td></td>
</tr>
</tbody>
</table>

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 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.
<table>
<thead>
<tr>
<th>Treatment (ppm)</th>
<th>Mean number collected</th>
<th>% reduction of recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1422.9</td>
<td>0</td>
</tr>
<tr>
<td>100</td>
<td>1466.0</td>
<td>0</td>
</tr>
<tr>
<td>1000</td>
<td>1290.0</td>
<td>9.3</td>
</tr>
</tbody>
</table>

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: approximately 1700 nematodes treated in each sample.

<table>
<thead>
<tr>
<th>Treatment (ppm)</th>
<th>Mean number collected</th>
<th>% reduction of migration</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>264</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>T 225</td>
<td>15.9</td>
</tr>
<tr>
<td></td>
<td>T1 262</td>
<td>2.0</td>
</tr>
<tr>
<td>1.0</td>
<td>T 203</td>
<td>23.3</td>
</tr>
<tr>
<td></td>
<td>T1 261</td>
<td>1.3</td>
</tr>
<tr>
<td>10.</td>
<td>T 222</td>
<td>16.1</td>
</tr>
<tr>
<td></td>
<td>T1 212</td>
<td>20.3</td>
</tr>
<tr>
<td>50.</td>
<td>T 183</td>
<td>30.8</td>
</tr>
<tr>
<td></td>
<td>T1 198</td>
<td>25.2</td>
</tr>
</tbody>
</table>

Table 19 The effect of aldicarb and its sulphone on the migration of *Aphelenchus* through filters in 24 hr after a 19 day exposure. Key: a 300 nematodes treated for 19 days.
<table>
<thead>
<tr>
<th>Treatment (ppm)</th>
<th>Population Growth&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>58.0</td>
</tr>
<tr>
<td>0.5 T</td>
<td>26.8</td>
</tr>
<tr>
<td>0.5 T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>46.2</td>
</tr>
<tr>
<td>10.0 T</td>
<td>14.9</td>
</tr>
<tr>
<td>10.0 T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>40.0</td>
</tr>
</tbody>
</table>

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: <sup>a</sup> Final population / initial population.
4. **Loss of Nematode Body Contents**

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 *Meloidogyne* larvae treated in solutions of the three carbamoyloximes were found to be proportionally more than the content of those larvae in water (Plates 2 & 3).

![Image of *Meloidogyne* larva stained with osmium tetroxide](Plate 2)

**Plate 2**  Second stage larva of *Meloidogyne* stained with osmium tetroxide after treating in water at 25°C for 3 weeks.
The water treated larvae were characterized by the complete absence 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 carbamoylooximes 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 24).

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.
<table>
<thead>
<tr>
<th>Treatment (ppm)</th>
<th>Presence of stainable lipids</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>T</td>
<td>+</td>
</tr>
<tr>
<td>1 T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>+</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>+</td>
</tr>
<tr>
<td>50 T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>++</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>++</td>
</tr>
<tr>
<td>T</td>
<td>+++</td>
</tr>
<tr>
<td>100 T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>+++</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>+++</td>
</tr>
</tbody>
</table>

Table 21: The amount of lipid in *Helidorogyne* 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 utilized by nematodes or whether nematicide treatment alters consumption of some and not other lipid fractions.

The method used for this investigation as described 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 extraction from aldicarb treated *Fusarium* cultures after treatment for 24 hr was not affected (Figure 18). An extraction period of 48 hr recovered between 64% and 70% of the total number of nematodes initially inoculated, extension of the extraction period by 24 hr did not improve 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).

<table>
<thead>
<tr>
<th>Treatment (ppm)</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1104.0</td>
<td>1188.6</td>
<td>1589.7</td>
<td>1660.5</td>
</tr>
<tr>
<td>0.1</td>
<td>1195.8</td>
<td>833.9</td>
<td>1230.7</td>
<td>1542.6</td>
</tr>
<tr>
<td>1</td>
<td>1123.9</td>
<td>721.6</td>
<td>543.7</td>
<td>503.9</td>
</tr>
<tr>
<td>10</td>
<td>1098.3</td>
<td>593.3</td>
<td>542.7</td>
<td>358.7</td>
</tr>
</tbody>
</table>

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 inoculum 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 *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 *Ditylenchus* 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).

<table>
<thead>
<tr>
<th>Treatment (ppm)</th>
<th>Nematode population growth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 DAYS</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>T</td>
</tr>
<tr>
<td></td>
<td>T</td>
</tr>
<tr>
<td></td>
<td>T</td>
</tr>
<tr>
<td>5</td>
<td>T</td>
</tr>
<tr>
<td></td>
<td>T</td>
</tr>
<tr>
<td></td>
<td>T</td>
</tr>
<tr>
<td>10</td>
<td>T</td>
</tr>
<tr>
<td></td>
<td>T</td>
</tr>
<tr>
<td></td>
<td>T</td>
</tr>
</tbody>
</table>

Table 23 The effect of aldicarb and its oxygen analogues in *Botrytis* cultures on the population growth of *Ditylenchus* after 3, 7 and 14 days at 25°C.
Figure 19 The effect of aldicarb, its sulfoxide and sulphone in Fusarium - PDA cultures on the population growth of Aphelenchus incubated at 25°C for 3, 7 and 14 days. Treated nematodes were harvested from the cultures by the dicing of the culture 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, ▼▼ T₁, □□ T₂.
Ditylenchus on treated Botrytis were found to be affected like Aphelenchus, 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 increased 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 Aphelenchus (Figure 19).

When mycophagus nematodes are treated on nematicide-fungal media for periods longer than 24 hr, it was not strictly accurate 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 fecundity 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 sulphoxide, 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.

<table>
<thead>
<tr>
<th>Treatment &amp; Nematicide</th>
<th>% of original and breakdown product remaining</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>26 Days</td>
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<tr>
<td>Aldicarb - Fusarium -PDA</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>80</td>
</tr>
<tr>
<td>T₁</td>
<td>20</td>
</tr>
<tr>
<td>T₂</td>
<td>ND&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sulphoxide- Fusarium - PDA</td>
<td></td>
</tr>
<tr>
<td>T₁</td>
<td>90</td>
</tr>
<tr>
<td>T₂</td>
<td>10</td>
</tr>
<tr>
<td>Sulphone - Fusarium -PDA</td>
<td></td>
</tr>
<tr>
<td>T₂</td>
<td>90</td>
</tr>
</tbody>
</table>

Table 24 The fate of aldicarb and its oxygen analogues in Fusarium - PDA cultures at 25°C, incubated in darkness.

Key: <sup>a</sup> ND compound not detected.
8. **Acetylcholinesterase Inhibition in *Xiphinema diversicaudatum* Treated with Aldicarb, and its Oxygen Analogue.**

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 acetylthiocholine. 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 sulfoxide 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-acetylcholinesterase activity. Neither aldicarb nor its sulphone had any effect at this concentration or at 1 ppm. Xiphinema treated at 10 ppm with aldicarb and its sulfoxide was found to inhibit the action of acetylcholinesterase in all nematodes, while the sulphone was the least effective (Figure 20).

![Graph showing percentage of nematodes inhibited](image)

**Figure 20** The effect of aldicarb, its sulfoxide and sulphone on the inhibition of acetylcholinesterase in Xiphinema detected in the nerve ring histochemically.

The procedure used for determining the effect of aldicarb on the oxygen consumption of Panagrellus and Aphelenchus was described 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 up to 3 hr at 30°C at 100 ppm.
Figure 21 The effect of aldicarb on the oxygen consumption of *Panagrellus* in 3hr at 30°C.

Key: □□ Control, ●●● 1 PPM,

■■ 10 PPM, ○○○ 100 PPM.
10. **Autoradiography of Xiphinema diversicaudatum after Treatment with $^{14}$C Labelled Aldicarb.**

The procedure used was outlined in Section 12. When *Xiphinema* which had been pretreated in 5ml of a $3.016 \times 10^{-4}$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 *Xiphinema* after pretreatment with $^{14}$C labelled aldicarb and then exposed to AR 10 stripping film for 20 days at $-4\,^\circ\text{C}$. 
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 $-4^\circ$C.

When *Xiphinema* which had been pretreated in labelled aldicarb for 6 hr 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 $\mu$ 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 subsequently stained with pyronin and methylene green.
Plate 7  Transverse section of Xiphinema cut from the posterior portion of a while nematode which had been exposed to \(^{14}\text{C}\) labelled aldicarb for 6 hr, then exposed to AR 10 stripping film for 25 days at \(-5^\circ\text{C}\).

Plate 8  Transverse section of Xiphinema 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 4b). 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 radioactivity of the nematode. The results obtained with Aphelenchus were similar to those with Panagrellus, the labelled nematicide accumulating 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 $\mu$g/g and the sulphoxide derivative was 1.1 $\mu$g/g nematode 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 $3.01 \times 10^{-4}$M solution. The labelled material in the nematode supernatant and residue was quantified. Key: **Supernatant** □□□□ $T_1$, ■■■■ $T_1$; **Residue** ●●●● unknown
Table 24  A comparison of the radioactivity in the supernatant and residue fractions of Panagrellus which had been treated in a \(3.01 \times 10^{-4}\) solution of \(^{14}\text{C}\)-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.

<table>
<thead>
<tr>
<th>Treatment time (min)</th>
<th>Nematode supernatant</th>
<th>Nematode residue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total dpm(^{a})</td>
<td>% of Total</td>
</tr>
<tr>
<td>10</td>
<td>1845</td>
<td>94.1</td>
</tr>
<tr>
<td>20</td>
<td>3361</td>
<td>87.0</td>
</tr>
<tr>
<td>30</td>
<td>5143</td>
<td>91.7</td>
</tr>
</tbody>
</table>

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 increased 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 Panagrellus was 8.74 \(\mu\)g/g and 9.15 \(\mu\)g/g nematode respectively (Table 26). After 24 hr exposure although there was little difference in the amounts which were extracted from the nematode
<table>
<thead>
<tr>
<th>Residue</th>
<th>% of recovered radioactivity in the nematode supernatant fraction after exposure to aldicarb</th>
<th>Exposure interval (min)</th>
<th>Unstarved Nematodes</th>
<th>Starved Nematodes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>T</td>
<td></td>
<td>55.8</td>
<td>68.1</td>
<td>75.0</td>
</tr>
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<td>44.2</td>
<td>31.9</td>
<td>25.0</td>
</tr>
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<td>...</td>
<td>...</td>
<td>...</td>
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<td></td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;&lt;sup&gt;O&lt;/sup&gt;</td>
<td></td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt;&lt;sup&gt;N&lt;/sup&gt;</td>
<td></td>
<td>...</td>
<td>...</td>
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<td>...</td>
</tr>
<tr>
<td>unknown</td>
<td></td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>24 hr Starved Nematodes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
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<tr>
<td>unknown</td>
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</tr>
</tbody>
</table>

Table 25: The metabolism of <sup>14</sup>C labelled aldicarb in unstarved and starved Panagrellus after exposure for periods up to 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 presumably reduced. Although the point at which equilibrium was reached was not determined, it occurred in *Panagrellus* with exposures longer than 1 hr.

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 *Panagrellus*, 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

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

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.
<table>
<thead>
<tr>
<th>Residue</th>
<th>% of recovered radioactivity in the treatment solution at the end of the exposure period</th>
<th>1 hour</th>
<th>24 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td>Unstarved Nematodes</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>91.97</td>
<td>87.54</td>
<td>92.47</td>
</tr>
<tr>
<td>T_1</td>
<td>6.91</td>
<td>9.69</td>
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<td>92.47</td>
<td>95.70</td>
<td>95.70</td>
</tr>
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<td>T_1</td>
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<td>0.53</td>
<td>0.24</td>
</tr>
<tr>
<td>unknown</td>
<td>0.26</td>
<td>0.23</td>
<td>0.31</td>
</tr>
<tr>
<td>unknown</td>
<td>0.31</td>
<td><strong>...</strong></td>
<td><strong>...</strong></td>
</tr>
<tr>
<td>unknown</td>
<td><strong>...</strong></td>
<td><strong>...</strong></td>
<td><strong>...</strong></td>
</tr>
</tbody>
</table>

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 sulphone, 41% as the sulphone 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 sulphone forming 26% of the remainder. When the accumulation of nematicide with Panagrellus had reached a maximum 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 constant 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 sulphone 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).
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.

<table>
<thead>
<tr>
<th>Treatment time (min)</th>
<th>% total loss of radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>7.5</td>
</tr>
<tr>
<td>20</td>
<td>9.2</td>
</tr>
<tr>
<td>30</td>
<td>10.0</td>
</tr>
</tbody>
</table>

Table 29  The metabolism of $^{14}$C labelled aldicarb in *Aphelenchus* after exposure for periods up to 24 hr.
The permeation of Aphelenchus by labelled aldicarb was faster than that found with Panagrellus, reaching a maximum 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 Panagrellus after a 1 hr exposure. When Aphelenchus was treated for 1 hr in labelled aldicarb the total concentration of labelled material in the supernatant fraction was 10.25 µg/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 Aphelenchus remained unchanged.
Figure 23. Uptake and fate of $^{14}$C labelled aldicarb in *Aphelenchus* during a 60 minute exposure at $3.01 \times 10^{-4}$ M solution. The labelled material in the nematode supernatant and residue was quantified.

**Key:**
- **Supernatant** ○○ ○ $T$;
- **$T_1$** ■ ■ $T_1$;
- **$T_1^0$** ▲ ▲ $T_1^0$;
- **$T_1 N$** △ △ $T_1 N$;
- **Residue** ● ● unknown.
b) **Egress of Labelled Nematicides**

The release of labelled aldicarb and its breakdown products was investigated in *Panagrellus*. 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

<table>
<thead>
<tr>
<th>Residue detected</th>
<th>Exposure interval (min)</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>40</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td></td>
<td>10.28</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td></td>
<td>47.83</td>
<td>100</td>
<td>93.85</td>
<td>97.62</td>
<td>100.</td>
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<tr>
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<tr>
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<td></td>
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<td>...</td>
<td>6.15</td>
<td>2.38</td>
<td>...</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;&lt;sup&gt;N&lt;/sup&gt;</td>
<td></td>
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<td>...</td>
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<td>...</td>
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<td>...</td>
</tr>
</tbody>
</table>

**Table 30** The composition of labelled aldicarb and its breakdown products in *Panagrellus* treated in \(^{14}\text{-C}\) 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 disintegrated by the resonance 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.
24 hr STARVED NEMATODES

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:** ○○ total in supernatant, ●●● 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.
The Contact Effect of Aldicarb on Nematodes

Aldicarb, its sulfoxide 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 *Helodogyne* 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 sulfoxide 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 sulfoxide 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. tobacum (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 comparative 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 be less permeable to nematicides, however it was disproved by Marks et al (1968) who demonstrated that in Peltodera 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 susceptible. 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 susceptible to EDB than larvae (Evans and Thomason, 1970). Furthermore these authors found that moulting larvae were more susceptible 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 suppression 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 exist 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 up to 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% 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. rostochiensis* 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 24 hr 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) may 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 because 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 sulphoxide was 15 ppm and the sulphone 60 ppm. The least affected parameter 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 *Panagrellus* were more tolerant than *Heterodera* or *Meloidogyne* larvae to aldicarb when assessed by water contact tests. *Panagrellus* has also been demonstrated to be more tolerant 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 effects 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 Thomson, 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 Thomson, 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 discontinued 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 *Heterodera* 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 respectively.

*Heterodera* and *Meloidogyne* both remain inactive.
for some time after treatment, *Heterodera* 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 spiraling resulted. Larvae treated with the sulfoxide 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 sulfoxide 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 reduce their fecundity, all compounds having similar effects except at low concentrations when the sulfoxide 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\textsubscript{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 assume 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. Because 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. However the length of exposure seem to be critical in determining their capacity to invade roots.

Miller and Kring (1970) suggested that Pratylenchus penetrans and Tylelichorhynchus 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 sensory 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 repellant 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 dioxide (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 Aphelenchoides 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 extracted 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 to affect the invasion of *Meloidogyne* larvae. Roots examined for the residues of aldicarb after 16 days contained only the sulphone derivative, while the sulphoxide was found in the aerial portion of the tomato plants. The amount of sulphone in the roots was not found to increase with the increase in dip concentration, and was not greater than 0.1 µg/g of plant material (wet weight). Thus after a period of 3 weeks any protection 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 sulphone may have important toxicological significance on pests which feed on treated plant material (Metcalf *et al.*, 1966; Coppedge *et al.*, 1967; Skrentny and Ellis, 1970). The uptake of aldicarb and its sulphoxide 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 µg/g of this compound and the level of root invasion 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 pres-
ence of the toxic nematicides by escaping before the deleterious sym-
ptoms take effect. The alternative is that the plant is rapidly
altered physiologically due to the uptake of nematicide, the care-
ful balance within the tissues is upset and the root environment
becomes unfavourable rather than toxic. Further investigation is
is necessary to elucidate 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 metabolism 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 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 degradation 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 untreated media it may have been possible to demonstrate that the nematicides 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
erious 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 prerequisite 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 paralyzed, but it was demonstrated that their oxygen consumption rate was immediately increased. The median effective dose required 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 reaching an equilibrium distribution between the nematode and the treatment solution. This supports the evidence of Marks et al. (1968) who demonstrated that EDB and DECP 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 µg/g in *Aphelenchus* and after 1 hr in *Panagrellus* it was 8.7 to 9.1 µg/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 (unpublished 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 preference 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 nematodes 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 Panagrellus 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 Panagrellus and
Aphelechus 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 Panagrellus than in Aphelechus 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 et al, 1966; Dorough and
Ivie, 1968; Bartley et al, 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 Aphelechus which had been treated for
24 hr.

The major degradative pathway of the sulphoxide resem-
bled that found by other workers (Metcalf et al, 1966; Bartley et al,
1970) the sulphoxide nitrile either being formed directly from the
sulphoxide or indirectly after the sulphoxide had been converted to
its oxime. In Aphelechus 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 et al, 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 sulfoxide 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 sulfoxide being fairly stable in nematodes must be responsible for some of the deleterious effects of the nematicide, for its degradation to the oxime and nitrile would produce compounds of diminished biological activity.

The contrast between the amount of aldicarb breakdown 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 sulfoxide when the exposure was extended to 24 hr, but the unstarved nematodes (which were themselves 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.
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 improbable 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 unextractable 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 autoradiography in the outer cuticular layers of Xiphinema which had been exposed 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 nematicide was not located in other of the nematode's tissues, and this was not expected.

The hypodermis is a dense syncytium, very osmophilic and containing many protoplasmic inclusions (Roggen, Raski and Jones, 1967). This region is in intimate 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 environment 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 selective mechanism was in the region of the hypodermis. The outer osmophilic 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 breakdown 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 hexylresorcinol 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 been 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 nematicide 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 preparations 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 susceptibility 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 categorise 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 compounds 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; Fiaqpu, 1967; Marks et al., 1968).

When applied topically to insects (Weiden, 1968) aldicarb penetrates the housefly cuticle more rapidly than the sulphoxide or the sulphone. A correlation may exist between the lipoidal 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.

Trauble (1970) has suggested that small molecules 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,
Aldicarb with a molecular weight of 190, is a small sized molecule which may well lend itself to transport across membranes.
Some Physiological Effects of Aldicarb on Nematodes.

Treatment of nematodes with aldicarb, its sulphone 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 their 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 muscular 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 (1950) found that Thimet caused muscular spasms especially in the tails of nematodes which were exposed to sub-lethal 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 maximum 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 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 relation 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 disruption 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 up to 15% occurred during feeding or defeacation. The maximum stylet thrusting corresponded with the time when complete 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 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 protractor 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 acetylcholinesterase (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 cholinesterase. The recovery of nematodes from early symptoms of poisoning is generally more frequent after treatment with the sulphone and sulphoxide rather than aldicarb. However the recovery of *Meloidogyne* larvae treated with the sulphone 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 Mexican Bean Beetle (Weiden, 1968), where aldicarb had caused the greatest initial knockdown but after treatment was found to cause the greatest recovery than with sulphone 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 consequently thought to be taken up by actively metabolising tissues (Weiden, 1968). The sulphone 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 broken down. Microsomal extracts from vertebrate liver cells have been shown to increase 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 its hydrophilic and lipophilic characteristics. Compounds with the correct balance will presumably be have some measure of protection against microsomal degradation but without severely 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 Ascaris, the weight of which was fifteen times that in the rest of the body (Mellanby, 1955). Del Castillo et al (1963) maintained that acetylcholine was important transmitter substance in excitatory synapses and not in inhibitory synapses which are thought to have different chemical transmitter substances 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\textsuperscript{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). Aldicarb only increased the activity of stylet movement and the undulation rate/cm travelled at low concentrations with some tylenchids.

While treatment of \textit{Xiphinema} with aqueous solutions of sulphoxide at 1 ppm 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°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 nematode 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 acetylcholinesterase fractions for \textit{Panagrellus} to produce an equivalent effect in water contact tests on the activity of \textit{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 ppm. 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 respond 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 immediately 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 outset of the treatment only low concentration increased consumption, higher 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 integral 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

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**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.

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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 ED50 for the experimental compounds against H. rostochiensis L2. According to Trevan (15) an ED50 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 Coldstream® 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-
**Heterodera rostochiensis Response to Aldicarb**

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.

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 affects 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.

**Literature Cited**


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* LE MODE D'ACTION DE ALDICARB POUR DÉTRUIRE LES NÉMATODES Meloidogyne incognita SUR LES TOMATES.
THE MECHANISM 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 O-(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 sulphone or aldicarb sulphone, and not by aldicarb which is not detected in roots immediately after dipping for 24 h.
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-α-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 fluoroacetate, 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. 2:3 cis-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.