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EFFECT AND MODE OF ACTION  
OF SOME SYSTEMIC NEMATOCIDES

BIBLIOTHEEK  
DER  
LANDBOUWHOGESCHOOL  
WAGENINGEN

J. A. BUNT

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EFFECT AND MODE OF ACTION  
OF SOME SYSTEMIC NEMATOCIDES

*(with a summary in Dutch)*

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD  
VAN DOCTOR IN DE LANDBOUWWETENSCHAPPEN  
OP GEZAG VAN DE RECTOR MAGNIFICUS  
DR. IR. J. P. H. VAN DER WANT  
HOGLERAAR IN DE VIROLOGIE  
IN HET OPENBAAR TE VERDEDIGEN OP  
VRIJDAG 3 OKTOBER 1975  
DES NAMIDDAGS TE VIER UUR  
IN DE AULA  
VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN

## STELLINGEN

### I

Er zijn aanwijzingen, dat de effecten van organofosfaten en organo(oxim)-carbamaten op nematoden worden veroorzaakt door remming van acetylcholin-esterase.

### II

De bescherming van de plant tegen nematodenaantasting door systemische nematiciden wordt door sommige auteurs alleen aan de systemische <sup>a)</sup> en door anderen alleen aan de contactwerking <sup>b)</sup> toegeschreven. Beide stellingen zijn onvolledig.

a) OUDEN, H. DEN (1971). Meded. Rijksfak. LandbWet., Gent 36 (3-4): 889-903.

SEINHORST, J. W. (1974). Meded. No 666 Inst.Plziektenk.Onderz., Wageningen.

b) PAIN, B. F. & HAGUE, N. G. M. (1971). Meded. Rijksfak. LandbWet., Gent 36 (1): 161-173.

### III

De wijze waarop voor oxamyl en phenamiphos basipetaal transport in planten werd aangetoond, is niet betrouwbaar.

TAYLOR, C. E. & ALPHEY, T. J. W. (1973). Ann.appl.Biol. 75: 464-467.

RICH, J. R. & BIRD, G. W. (1973). J. Nematology 5 (3): 221-224.

### IV

De voor systemische nematiciden vermelde selectieve werking op nematoden berust op een verkeerde interpretatie van onderzoekresultaten.

WEISCHER, B. (1969). Mitt. biol. Bundesanstalt f.land.- u. Forst-wirtsch. Berlin-Dahlem 136: 104-109.

### V

De stelling van Baines & Small, dat de populatie-dichtheid van *Tylenchulus semipenetrans* in wortels toeneemt onder invloed van metaboliëten van oxamyl, is onjuist.

BAINES, R. C. & SMALL, R. H. (1974). J.Nematology 6: 135.

## VI

De aanwezigheid van virusdeeltjes in het distale eind van het voedselkanaal van bladluizen wijst niet op de overdracht van virus door braken.

TAYLOR, C. E. & ROBERTSON, W. M. (1974). *Phytopath. Z.* 80 (3): 257-266.

## VII

Het effect van chemicaliën op organismen in vloeibare- of gasvormige media moet niet worden uitgedrukt in LD- of ED waarden (LD = letale dosis, ED = effectieve dosis).

## VIII

De gevaren verbonden aan het gebruik van chemische hulpstoffen bij de voortbrenging en conservatie van voedingsmiddelen zijn kleiner dan vaak wordt gesteld, dank zij onder andere het vermogen van organismen om hun biochemische samenstelling te regelen.

## IX

Er is een tijdschrift nodig waarin samenvattingen van de resultaten van doctoraalstudies bij de Landbouwhogeschool verschijnen.

## X

De evolutietheorie wordt niet overtuigend door feiten gesteund. Het hierop gerichte onderzoek en onderwijs verdienen minder aandacht dan ze thans krijgen.

## ABBREVIATIONS

CP	chloropicrin
DBCP	dibromochloropropane
DD	dichloropropene-dichloropropane mixture
EDB	ethylenedibromide
MB	methylbromide
MIT	methylisothiocyanate
Ox	oxamyl = thioxamyl = the active ingredient in Vydate
Phen	phenamiphos = the active ingredient in Nemacur
EC-50,90	effective concentration, which causes an effect on 50, respectively 90% of a nematode population
ED-50,90	effective dose, which causes an effect on 50, respectively 90% of a nematode population
LC-50,90	lethal concentration, which causes a kill of 50, respectively 90% of a nematode population
LD-50,90	lethal dose, which causes a kill of 50, respectively 90% of a nematode population
LSD	least significant difference
a.i.	active ingredient

These, and other used abbreviations – e.g. for statistical analysis – are also at least one time explained in the text (cf. 2.6 and Table 1).

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# 1. INTRODUCTION AND LITERATURE REVIEW

## 1.1. THE SOIL FAUNA

A ml of cultivated or naturally grown soil contains 10 to 50 specimens of small multicellular animals (*Metazoa*). Nematodes (*Nematoda*) are the most abundant and widespread group comprising 80–90% of all multicellular animals. OOSTENBRINK (1970) indicated that 30–50% of the nematodes in soil are known or suspected plant parasites, i.e. feeding on living plant cells. The rest of the nematode community consists of saprozoic or microbivorous and predatory species, which vary widely in their diet. Various stages of animal and human parasitic nematodes may also be found in soil, but irregularly spread and often at low densities.

Certain groups of microarthropods, particularly mites (*Acarina*) and spring-tails (*Collembola*), are also found in numbers in the soil; these groups also comprise plant parasitic, saprozoic and predatory species. Some microarthropods are predators of plant parasitic nematodes and are for this reason included in this study.

### A. *Nematodes*

The main agricultural significance of nematodes is related to the damage which they cause to plants, but the economic losses they cause on a world wide basis have not been thoroughly evaluated. Earlier estimates (OOSTENBRINK, 1957) as well as a recent, detailed inventory by the Committee on Crop Losses of the American Society of Nematologists (ANONYMOUS, 1971) indicate an overall crop loss of about 10%, despite control measures. Local damage due to heavy infestations by certain species is often higher.

The saprozoic and other nematodes may not be of much economic significance, but they are taken into account in this study because they are good indicators of soil treatments with pesticides.

Plant parasitic nematodes are often 'classified' into ectoparasitic and endoparasitic species, depending on their feeding position on or in plant tissue. Most ectoparasitic species are root parasites which stay at the surface of the roots and never leave the soil. They have stylets to pierce plant tissues and to feed on them from the outside. Species of *Tylenchorhynchus* and *Paratylenchus* are the main ectoparasites included in this study.

Endoparasitic species invade plant tissue and spend at least part of their lives inside the plant, either in roots or aerial parts of the plant. Several important species of this group were used in experiments: *Ditylenchus dipsaci* for in vitro and in vivo studies on systemic nematicides, and *Pratylenchus penetrans*, *Pratylenchus crenatus*, *Heterodera rostochiensis* and *Meloidogyne incognita* for studying the response of nematodes in vivo to treatments with such compounds.

It is not always possible to discern sharply between ectoparasitic and endo-



parasitic species. Part of the population of some ectoparasites may penetrate roots, and the endoparasitic *D. dipsaci* may under certain circumstances live ectoparasitically on leaves of host plants.

### B. Other Metazoa

Other soil animals influence nematode density by competition and may be influenced directly or indirectly by soil treatments with nematicides. This makes it desirable to study the response of mites and springtails to nematicide treatments. Other members of the soil fauna such as earthworms, enchytraeid worms, molluscs, myriapods, dipterous larvae and others, although influenced by soil disinfection with nematicides were not studied.

SHARMA and WINDRICH (1966) and VAN DE BUND (1971) studied predation of nematodes by gamasid mites. VAN DE BUND also participated in the present study as far as microarthropods were involved. The main microarthropod species studied were the springtail *Tullbergia krausbaueri* and the mite *Rhodacarellus silesiacus*, which under laboratory conditions, in vitro and in soil, reduced nematode populations.

## 1.2. SOIL TREATMENT WITH NEMATICIDES

Chemical treatment of soils is developing rapidly for nematode control in certain agricultural areas and appears to be an improvement on such methods as farm hygiene, crop rotation, growing resistant varieties and physical control.

The area treated in the Netherlands per year has risen from 10,000 to 50,000 ha in a few years (EISSA, 1971; NOLLEN, personal communication). For soil application of nematicides dosages are required, which are 10–100 times as high as those of most other pesticides. In spite of the fact that the area treated with fungicides and insecticides is much larger, the amount of nematicides used in The Netherlands is now already higher than that of fungicides and insecticides together (OOSTENBRINK, 1973).

The main nematicides used at the moment in The Netherlands and elsewhere, are fumigants. In this study these are only used for the evaluation of some screening methods, and for comparison with the non-volatile systemic nematicides which are the main topic of this study.

### A. Fumigants

One of the earliest reports on application of fumigants against nematodes is by KÜHN (1881) who tried to eradicate *Heterodera schachtii* by means of carbonbisulfide. Between the two world wars there were reports on volatile compounds being nematicidal by BIARS (1919), who controlled *Meloidogyne* spp. by means of hydrocyanic gas and by HURST and FRANKLIN (1938) and SMEDLEY (1936, 1938), who reported the nematicidal properties of cyanamide and isothiocyanate compounds, respectively. The broad-spectrum biocide chloropicrin was found to be an effective nematicide by MATTHEWS (1919). Most reports on the

nematicidal, fungicidal, insecticidal and herbicidal effects of chloropicrin are, however, published after 1940. HOESTRA (1968) found chloropicrin also suitable to control the specific apple replant disease, for which no organism has been found to be responsible up to now. The nematicidal properties of methylbromide, which is widely used as an insecticide, were reported by RICHARDSON and JOHNSON (1935).

A break-through to large-scale application of nematicides followed discovery of the nematicidal effect of the fumigant 1,3-dichloropropene (DD) by CARTER in 1943.

After the discovery of DD only few other fumigants have reached wide application, e.g. ethylene dibromide (EDB) reported as nematicide by CHRISTIE in 1945, 1,2-dibromo-3-chloropropane (DBCP) reported by MCBETH in 1954 as nematicidal in the Nemagon formulation, and compounds releasing methylisothiocyanate (MIT), e.g. Monam.

Although hardly used in the Netherlands, DBCP is given some special attention. It is the only fumigant which may be used as a curative without causing phytotoxic effects on several growing crops, e.g. ornamental plants, fruit trees, banana a.o., owing to its low phytotoxicity and its relative high nematicidal activity, which makes dosages of 10–20 kg per hectare effective. Its relatively low volatility restricts this nematicide to sub-tropical and tropical regions, where it is widely used, often side by side with EDB and DD. Before introduction of the systemics, DBCP was the only nematicide used as a chemotherapeutic agent in practice (GOOD and TAYLOR, 1965).

The knowledge about the mode of action of alkyl halide nematicides such as DD, EDB and DBCP, is still incomplete. There are hypotheses that these chemicals are involved in oxidation processes or in alkylation of the SH-group of cysteine molecules on protein chains (CASTRO, 1964 and MOJE, 1960, respectively), but in what way these reactions are lethal to the nematodes is still unknown. Most alkyl halide compounds cause narcosis and, depending on concentration, finally death. At high concentrations rapid kill has been observed (MOJE, 1960). Under field conditions at normal concentrations DBCP caused much slower kill than the other fumigants and, therefore, samples to determine its effectiveness must be taken several weeks or even months after application.

The high dosages used of the conventional fumigants, the special equipment required for their application and the long waiting periods due to their phytotoxic properties, have stimulated the search for less phytotoxic non-volatile systemic nematicides which can be applied also during the growing season of the plant.

#### *B. Non-volatile nematicides with systemic properties*

In recent years several systemic nematicides have been developed, but only some are approved for limited use because there are still uncertainties regarding residues in crops and soil. Most systemic nematicides and many other pesticides with nematicidal activity are listed under 4.1. in table 5 and under 4.2. in table 6 and figure 12.

In this study 'systemic nematicides', or 'systemics', means any chemical that can be taken up by the plant, via roots, leaves or both, and is effective inside the plant, in its original or altered chemical state, in protecting the plant against nematode attack. Strictly the word 'nematicide' means 'chemical which will kill nematodes'. Usually this word is used in a broader sense, including those compounds which protect plants against nematode attack by other actions, e.g. antifeeding, antireproduction, antipenetration, inhibition of hatching of larvae from eggs, or repellence. The literature on systemic nematicides mentions all these types of action. To what extent each of them is involved in nematode control, is often not made clear. The relevant literature dealing with nematode control by systemic nematicides is reviewed under 1.4.

### C. Scheme of interactions

Figure 1 illustrates the main relations or interactions between pesticide, soil fauna, soil flora and the soil itself when a systemic nematicide is applied to the soil or to plants. The scheme is a simplification, because every relation or interaction is complex itself. All pathways given in figure 1 may be important for the result of a nematicidal treatment. The main ones are  $A \leftrightarrow B$  and  $A \leftrightarrow C$  and our study deals primarily with them. The interaction  $A \leftrightarrow D$ , however, may reflect decomposition of the chemical by the soil flora and therefore persistence or it may determine whether the nematicide has been converted by the plant or the soil flora into a more effective derivative. In this study we had, for practical reasons, to avoid complex experiments and to concentrate on the main effects of systemics on nematodes.

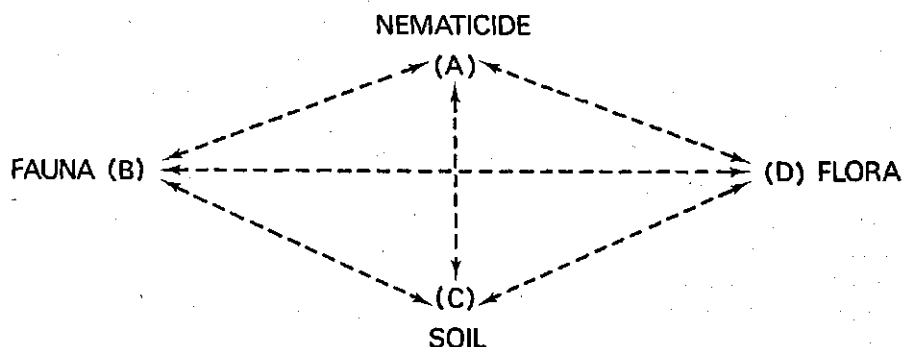


FIG. 1. Scheme of the main relations or interactions if a systemic nematicide is applied to the soil or to the plant.

### 1.3. THE SEARCH FOR NEMATICIDES

Most nematicides which are now used in practice, are detected by chance, or by large-scale screening of chemicals. There are at least 20 industries which pass most or all of the chemicals which they investigate for biocidal activity through

a test for nematocidal activity. The number of new chemicals tested per year may exceed 100,000.

Few nematocides are developed on the basis of synthesis guided by expectation derived from activity of related chemicals, and none, to our knowledge, was synthesised on the basis of conceptual ideas alone.

The development of new chemicals is desirable not only for the producer, but also to ensure and improve food production for the growing world population (SHINDO, 1974), to safeguard and improve the financial revenue of the individual farmer, and to replace currently used nematocides. Although this applies to pesticides in general it is particularly true of nematocides because effective, safe, cheap and easy-to-handle compounds for general application in agriculture have not yet been developed.

Nematocide application is nevertheless developing rapidly in agriculture, despite the high dosages needed for currently used nematocides, the high costs and the risk for soil pollution. Substitutes which are effective at low dosages may solve risks as well as the cost problems. Systemic nematocides seem to offer such possibilities, and therefore require careful scrutiny.

It must be stressed that the discovery of new nematocides depends strongly on the techniques used for screening. Also after discovery of effectiveness, a series of investigations by industries, license-furnishing authorities and farm advisors must usually be done before a nematocide can be introduced into agriculture. For most of these chemicals this developmental stage takes ten or more years. Several authors have commented on the procedure of searching for and testing of nematocides in general (MYERS, 1972; ARLT, KÄMPFE and THIEDE, 1971; MCBETH, 1969; FENWICK and MOHAMMED, 1967; BIJLOO, 1965; JOHNSON and LEAR, 1962; KLEIN and ALLISON, 1957; TAYLOR, FELDMESSER and FEDER, 1957; TARJAN, 1955; OOSTENBRINK, 1954; MCBETH and BERGESON, 1953).

It is remarkable, and at the same time disquieting, that the most-widely used nematocides, dichloropropene and ethylenedibromide, which have both been found by chance, are still prevalent some 30 years after the first reports on their efficacy. The common procedure is that large series of chemicals are systematically passed through routine tests for nematocidal and other biocidal activities. Sometimes a specific series related to an active principle are made by chemists and then inserted into the primary screen.

MCBETH (1969) describes the successive tests which a candidate nematocide may have to undergo before official registration and license for application in agriculture is obtained in the USA. After a primary screen within a practical range of concentrations, secondary tests follow to obtain an impression of its applicability (soil injection, mix or drench, bare-root dip, foliar spray, seed treatment and others). Then field tests against different nematodes in various soils and climatic conditions are needed, and at the same time residual effects in soil and plants, toxic effects on different organisms including effects of long-term feeding to mammalia, and other specific influences of the chemical must be determined. The practical applicability and economics often require additional large-scale experiments.

The choice of the test method determines the success or failure of finding various types of nematicides. Interpretation of results obtained with systemics is only possible if one has knowledge of the techniques used. The conventional methods used up to now may not be useful for studying the effect of systemic nematicides, their mode of action and their fate in soil and plants. Because we must consciously choose from the existing methods, or develop new ones ourselves for our study on systemic nematicides, a review is given below of published techniques, viz. (A) primary screens, (B) secondary tests and (C) studies of residual activity of nematicides in soil and plants.

#### *A. Primary screen methods*

##### **Water screen**

A primary screen should select from candidate chemicals those that require further (secondary) testing (MYERS, 1972). Before systemic nematicides were known, a simple water screen was often used as the primary test; i.e. observing the toxic effect in watery solutions or suspensions of chemicals on test nematodes immersed in them for 1–2 days (PETERS, 1952; MCBETH and BERGESON, 1953; WELLE, 1964; FENWICK and MOHAMMED, 1967; MCBETH, 1969; HANDELÉ, 1971; FELDMESSER, 1972). When systemic nematicides, such as the oximcarbamate aldicarb and the organophosphate thionazin were developed (1960–1970), the need for a new primary screen method was born, because systemic nematicides often have little or no direct killing effect on the nematodes (MOTSINGER, 1961; BIJLOO, 1965; FELDMESSER, 1972; NELMES, TRUDGILL and CORBETT, 1973). Also chemicals with specific effects on nematode behaviour, reproduction and feeding, are not detected if nematodes are immersed in a watery preparation of a chemical for short periods. The method may also be ineffective in tracing other groups of nematicides, as indicated under soil screen.

##### **Soil screen**

Nematicides which are active only in the vapour phase, or after break-down or transmission in the soil, are not detected in the water screen. OOSTENBRINK (1954) developed a soil screen, for which infested soil is mixed together with the candidate nematicide in jars and the rate of killing is measured after a few days by extraction of the active nematodes. In this method chemicals may be evaluated for their nematicidal effect in the solid, liquid or vapour phase. TAYLOR et al. (1957) modified the method by using little glass vials filled with sand, nematicide and nematodes. The vials are closed for 48 hours and then the nematodes are washed free from the sand and counted as dead or alive. The fact that these soil screen methods are based upon killing or inducing inactivity only, makes them unsuitable for tracing systemic nematicides, or nematicides which have no contact effect but another mode of action.

##### **Pisum test**

This test, described by BIJLOO (1965) is the first test with plants which was

specially developed to sort out systemic nematicidal substances. Candidate chemicals are dissolved or suspended in water and 15 dry seeds of pea, *Pisum sativum* cv. Unica, are placed in 25 ml of the chemical solution or suspension in flasks, which are placed in the dark at 20°C for 24 hours. The seeds are then taken from the flask and washed in running tap water for 5 minutes to remove adhering chemical from the seed coats. The seed coats are removed with a scalpel and 50 *Ditylenchus dipsaci* larvae are placed between the cotyledones on the plumule. These prepared seeds are planted in trays with river sand, together with control seeds. Two weeks later the *D. dipsaci* attack is evaluated to measure the protective effect of the nematicide taken up by the seeds.

The method can be used to trace systemic nematicidal effects, but is rather time-consuming for testing great numbers of compounds. Another disadvantage is that it does not detect contact nematicides.

**Biological assay with root-knot nematodes, *Meloidogyne* spp.**

This method is used by several industries to test candidate nematicides for their effect on the degree of galling of indicator plants (tomato, tobacco, cucumber or others). This method is evaluated by KLEIN (1957), JOHNSON and LEAR (1962), and others. The test is as follows. Naturally or artificially infested soil with the test nematode is treated with the nematicide by mixing, drenching or otherwise. After a waiting period, to avoid phytotoxic effects, the treated soil and untreated soil as a control are planted or seeded with the indicator plant. Four to six weeks later, the plants are uprooted, washed free from adhering soil particles and rated for the degree of galling, which gives an indication for the nematicidal efficacy of the chemicals tested. For the detection of chemicals with different modes of action, this method is more acceptable than the others discussed above. However, the method is time-, labour- and material-consuming and gives little or no information about the mode of action by which the chemicals are effective. An other disadvantage is the need of a high-temperature greenhouse for the tropical *Meloidogyne* species used as test organisms.

Effective nematicides which are strongly absorbed to soil are not detected in this biological assay in soil, whereas they may still be of interest. At some laboratories, therefore, this *Meloidogyne* test is used as a secondary screen, whereas the primary screen is done in another way.

### *B. Secondary tests*

#### Greenhouse tests

Tests with infested soil in pots or other containers can investigate the effect of dosage and the method of application, e.g. injection with or without cover, soil mix, drench, plant dip or others. The interaction of soil type, the effects on various nematode communities and phytotoxicity can be investigated. Temperature, humidity of the soil and other experimental conditions may also be varied.

Biological assay with *Meloidogyne* species may be used as a primary or a secondary screen: the test is usually done in greenhouses, but in warm regions may be done out of doors.

Volatile materials may be tested in closed containers: less volatile compounds may be injected into or mixed with the soil, with or without a 'water seal' or another cover. Waiting times may vary, suitable host plants may be chosen and the nematode densities may be estimated by relevant methods in the roots and/or in the soil depending on the species of nematode.

It may be necessary to determine the efficacy of the nematicides at different times after treating the soil, e.g. 1, 2, 4 and 8 weeks after treatment, partly because of the danger of phytotoxicity but also because some chemicals may have a slow but long-term effect (e.g. DBCP) and others may be active only for short time after treatment (most fumigants).

#### Indication tests in the field

The tests described above can also be done in small field trials as treated strips with test plants cross-over, with all kind of variations except for soil and climatic conditions.

Because most nematicides aim at control of nematodes under field conditions, field tests are necessary for a final evaluation. In the field a chemical is less effective than in pot tests due to irregularities in the soil, loss of the chemical into the air or into the soil, and other environmental factors. Since field tests are rare in this study, the techniques are not discussed in detail.

#### Application around or on living plants or plant parts

Active materials of low phytotoxicity may be applied around or near to growing plants or harvested plant products. Also foliar sprays against leaf, stem or some root nematodes may be useful. If a chemical is effective as a spray against these nematodes, soil contamination is prevented, but then systemic activity must be involved and special tests are desirable. Substances found to be active in a primary screen may also be tested as bare-root dips on living plants. The root systems of plants infested with endoparasites, e.g. *Pratylenchus* spp., are then soaked for different periods in a series of concentrations of the chemical and replanted in nematode-free soil. After different periods, phytotoxic effects on the plants and root infestation are evaluated.

Non-phytotoxic substances could also be used for treatments of seeds, seed potatoes or other infested planting materials. Because most nematodes attack plants after the seeds germinate and roots and foliage are formed, nematode control by seed treatment alone is usually not effective. A systemic, however, may penetrate the seed and prevent nematode infestation of the seedling. Accurate treatment of seeds with chemicals is generally difficult (LORD and JEFFS, 1971).

#### C. Residual activity in soil and in plant tissue

Chemical control of nematodes in soil usually involves some residual activity. Only some fumigants combine quick kill of the nematodes with short persistence in the soil. Promising new nematicides with great persistence in the soil or in the plant, are usually discarded because of phytotoxicity or due to residues in food crops or in the environment.

For systemic nematicides some persistence inside the plant is a prerequisite, because without persistence therapeutic effect may hardly be expected. If persistence is too great, the compound may, however, be unsafe on food crops. Persistence of systemics, therefore, has to be determined quantitatively, and acceptable tolerances, if any, for associates of the target nematodes or consumers of the plant yields have to be known.

Persistence of chemicals in soil or plants is usually assessed by extraction and determination quantitatively by means of physical methods, such as gas-chromatographic analyses, or by chemical analysis, such as specific reactions based on titration techniques. For some pesticides biological assays are also used. To estimate the longevity of systemic nematicides in soil and other media, a biological assay based upon nematode behaviour is described under 7.2. Biological assays are generally attractive because they indicate residual effects, which may be noxious or sometimes favourable, of the parent compound and of its breakdown products or metabolites.

#### 1.4. LITERATURE REVIEW ON SYSTEMIC NEMATICIDES

A review of the literature is given on nematicidal effects of systemics in vitro (A), and on the results of nematode control by applying systemics (B).

##### A. *In vitro* effects of systemics on nematodes

The investigations with nematodes in vitro, without using host plants under normal growing conditions, furnish data on nematode kill and on feeding, behaviour, and other non-lethal effects.

##### Lethal effects

The literature reveals great discrepancies in opinion about the lethal effect of systemics on nematodes. KAAI (1972) concludes that systemic nematicides are only active if taken up by the plant and that they have little or no contact effect on nematodes in soil or water in vitro. MYERS (1972), using axenic cultures of *Aphelenchoides rutzgeri* in salt solutions, found a killing effect, expressed as LC-50<sup>1</sup>, for aldicarb of 57 ppm, for phenamiphos of 29 ppm, for thionazin of 46 ppm and for fensulphothion of 29 ppm, whereas for oxamyl and carbofuran no LC-50 value could be given because it was probably above 1000 ppm of the active ingredient. If, however, MYERS (1972) calculated the effect of these systemics on reproduction of the nematodes, expressed as EC-50<sup>2</sup>, then he found a much greater effect. The EC-50 was 5 ppm for aldicarb, about 0.5 ppm for phenamiphos, 2.3 ppm for thionazin, about 1 ppm for fensulphothion, 5 ppm for oxamyl and 2.5 ppm for carbofuran. On the basis of his extensive investigations with

<sup>1</sup> LC-50 = median lethal concentration; i.e. the concentration which causes 50% kill in a given time.

<sup>2</sup> EC-50 = median effective concentration; f.i. the concentration which results in a nematode reduction to 50% of the control in a given time. Both values are expressed in ppm, which means mg chemical per kg test medium (for soil sometimes per litre instead of per kg).



*A. rutgersi*, MYERS also recorded that at some concentrations the influence on egg hatch resulted in accumulation of unhatched eggs and in lower numbers of nematodes, and that the adult to larvae ratio is sometimes affected. MYERS' experiments with *A. rutgersi* have given much information on screening chemicals for non-lethal effects on nematodes, but the method used is too complicated for general use in screening programs. Besides this, the method cannot be used for fumigant nematicides.

KONDROLLOCHIS (1971) concluded that less than 50% of *Ditylenchus dipsaci* were killed if placed for 6 days in 1000 ppm a.i. thionazin, whereas the other 50% became active again after transfer to water. OSBORNE (1973) observed strong inhibition of larval hatch in *Heterodera rostochiensis* as long as cysts were dipped in 1 ppm a.i. aldicarb. When treated cysts were transferred into potato root diffusate without nematicide, the ability of the larvae to hatch was restored. HARRISON (1971) found evidence that also oxamyl inhibited hatch of *Heterodera rostochiensis* larvae from cysts. Inhibition of larval hatch was also found for *Meloidogyne arenaria* in oxamyl, phenamiphos and aldicarb by BERGÉ and CUANY (1972b). The results obtained by FELDMESSER (1972) by dipping *Panagrellus redivivus* in oxamyl solutions, indicated a LC-50 of about 150 ppm.

Although lethal effects of systemic nematicides on nematodes at concentrations used in the field are recorded in literature, behaviour effects are considered more important (KONDROLLOCHIS, 1971, 1972; MYERS, 1971, 1972; NELMES et al., 1973). Reduced nematode reproduction, as found by MYERS (1971, 1972), may also be due to interruption of feeding, because nematodes then also stop laying eggs.

#### Disruption of feeding and behaviour

KONDROLLOCHIS (1972) stated that the main effect of thionazin was the interruption of feeding and penetration when *Aphelenchus avenae* on *Rhizoctonia solani* was treated with the nematicide. Few minutes after adding the nematicide to the culture, feeding stopped and abnormal stylet protractions occurred. When *D. dipsaci* was treated no invasion of onion seedlings and no egg production occurred. *Meloidogyne incognita* larvae, exposed to agar containing 1 ppm aldicarb, decreased the number of body undulations (NELMES et al., 1973). Systemic nematicides also affected the movement of nematodes in soil (MOTSINGER, 1961; PAIN and HAGUE, 1971a).

In vitro investigations about the effect of oxamyl and phenamiphos on nematodes are still scarce. Other carbamates and phosphates, characterised by inducing acetylcholinesterase inhibition effects, are more nematostatic than nematicidal. The same effects may be expected for oxamyl and phenamiphos.

#### B. Nematode control with systemic nematicides

Results of many greenhouse and field experiments with systemics are published, but they are difficult to evaluate and to integrate. It is, for instance, doubtful whether conclusions can be drawn about basipetal transport of systemic nematicides following foliar sprays, because much spray liquid runs off from the leaves onto and into the soil; this is certainly so if spraying is followed

by rain. Foliage treatments had in many cases rather be considered as soil drenches. Nevertheless, an attempt is made to scrutinize and evaluate literature on plant-nematode-nematicide relations and the mode of action of systemic nematicides. The data are grouped according to application under: soil treatment, root treatment, foliage treatment, and seed treatment.

#### Soil treatment

##### a. Against *Heterodera* species

Application of systemics for the control of *Heterodera* species is not yet an accepted practice but results have been promising.

WHITEHEAD, TITE and FRASER (1973) and WHITEHEAD et al. (1973a, b) reported an effective control of *Heterodera rostochiensis*, when granules of oxamyl, phenamiphos and other similar compounds were mixed into the soil at rates of 11 kg a.i. or less per ha. In organic soils control of *H. rostochiensis* with aldicarb 5–10 kg a.i./ha was better than for 1000 kg DD/ha; in sandy clay soils small amounts of aldicarb gave better control than large amounts of dazomet or DD. PAIN and HAGUE (1971a, b) also found aldicarb effective in reducing multiplication rate of *H. rostochiensis* and increasing potato yields. They concluded that aldicarb acts as a contact poison and affects the behaviour of *H. rostochiensis* larvae by disturbing their orientation towards the roots. At 5 ppm a.i., aldicarb did not affect the emergence of encysted larvae, and caused accumulation of second stage larvae in the soil till 6 weeks after treatment, apparently because these larvae were unable to find or to penetrate roots. At higher concentrations hatch of *H. rostochiensis* larvae from cysts decreased. If larvae treated with 5 ppm a.i. aldicarb were afterwards washed free from the chemical, 50% were able to invade potato roots. Aldicarb treatments caused a marked increase in the male: female ratio. This effect on sex ratio was also found by DEN OUDEN (1971) who, however, concluded that aldicarb acts only 'via the host' and not as a contact poison.

The influence of systemics on growth of potato in soil heavily infested with *H. rostochiensis* was examined by HOMEYER (1971b). Development of the potatoes was much improved in soil treated with 10 kg a.i. fensulphothion per ha; this was also true for nematode-resistant varieties, which is understandable because they may suffer from penetrating larvae. BUNT (1972) obtained 98–100% control of *H. rostochiensis* when oxamyl was mixed into the soil at the low rate of 2.5 mg a.i. per litre soil, potatoes were planted, and larvae inoculated onto the roots 4 weeks later. Aldicarb gave a similar result. Weight of the potato plants increased with amount of the nematicides.

Other records of good control of *H. rostochiensis* by soil treatments with small amounts of systemic nematicides are by DESHMUKH and WEISCHER (1971) and by HIDE and CORBETT (1973). Aldicarb gave varying results against different populations of *H. rostochiensis* (DESHMUKH and WEISCHER, 1971). KÄMPFE (1973) found no influence on *H. rostochiensis* of relatively large amounts of aldicarb applied before winter; normal development of new cysts occurred after planting potatoes in the next spring.

Control of *Heterodera schachtii* on beet, Brussels sprouts, cabbage and other

cruciferous crops by soil treatments with systemics has been reported (GRIFFIN, 1973; GRIFFIN and GESSEL, 1973; JORGENSEN, 1969; KOTLINSKY, 1973; LEAR, 1972; POTTER and MARKS, 1971; STEUDEL and THIELEMANN, 1968; STEUDEL, 1972a, b). The living contents of *H. schachtii* cysts declined when they were exposed to 10 ppm a.i. aldicarb for 45 days (STEUDEL, 1972b).

Other reports dealing on successful treatments against *Heterodera* spp. with systemics are by MILLER (1969, 1970, 1972), who found that oxamyl and phenamiphos did not kill the cyst contents, but were nevertheless highly effective in protecting the host plant tomato against *H. tabacum*. MILLER (1969) and HIDE and CORBETT (1973) achieved reduced root invasion by cyst nematodes after application of benomyl and thiabendazole. THOMASON and HOUGH (1973) concluded that aldicarb interfered with the movement and orientation of nematodes, rather than causing lethal effects. Aldicarb increased beet yields on a clay soil by more than 12 tons/ha if given as a 4.5 kg/ha side-dressing at planting time. THIELEMANN and STEUDEL (1973) also recorded yield increases of sugar beet by aldicarb application to *H. schachtii* infested fields, even when beet was grown without rotation for several years.

#### b. Against *Meloidogyne* species

There are several reports on use of systemic nematicides against *Meloidogyne* species. Root-knot nematodes are also difficult to control due to their occurrence inside plant roots, and because eggs are laid in and on roots in protective egg sacs. The best time to control *Meloidogyne* spp. is probably when that larvae emerge from the eggs and move through the soil to reach their host. If the larvae penetrate the damage to the plant is done and the nematodes are then difficult to kill.

*M. incognita* and *M. hapla* were controlled well by mixing infested soil with 1–5 ppm a.i. phenamiphos (HOMEYER, 1971a). A high degree of control of four different *Meloidogyne* spp. (*javanica*, *arenaria*, *hapla* and *incognita*) was also obtained under field conditions using less than 10 kg phenamiphos per ha (BURNETT and INGLIS, 1971). MILLER and NOEGEL (1970) investigated the influence of application method on eradication of *M. incognita* in infested *Gardenia* plants by means of phenamiphos. They obtained control if 40 ppm a.i. was applied as soil drench or as soil mix. Bare-root dips and broadcast application of granules without soil cover were less effective.

Soil treatment with 3 kg oxamyl per ha gave good control of *M. naasi* on wheat (ANONYMOUS, 1971). *Calathea makoyana* plants heavily infested with *M. arenaria* were practically freed from infestation by drenching the soil with oxamyl (ANONYMOUS, 1971). From trials with fumigant and systemic nematicides, BRODIE and GOOD (1971) concluded that systemics were equal or better than some of the fumigants in controlling *M. incognita* and in improving tobacco yields. They found also that the method of application influenced the effect of systemics. Phenamiphos incorporated into the top 5–10 cm of the soil at 4.2 kg/ha gave better results than DD at 84 liters/ha.

BRODIE and DUKES (1972) stated that the yield of tobacco on soil infested with

*M. javanica* depends only on the degree of infestation during the first months after transplanting and was not related to the root-knot indices at harvest time, 4 months after transplanting. Six nematicides, systemics and fumigants, all reduced the infestation and subsequently increased yield, but at harvest time root-knot indices were practically the same for all treatments, including untreated plants.

COOLEN, HENDRICKX and D'HERDE (1971, 1972) reached near-complete control of *Radopholus similis* and *M. arenaria* on *Calathea makoyana* and *Monstera deliciosa* with 6–12 gram a.i. aldicarb/m<sup>2</sup>. They found no influence of the formulation, granules or liquor, and therefore preferred granules which are likely to be less dangerous to the grower.

For control of *M. javanica* in ginger rhizomes, phenamiphos was satisfactory, but Mocap, aldicarb and oxamyl did not give the desired degree of nematode control (COLBRAN, 1972). COLBRAN and BROUWER (1972) concluded from field trials with EDB and several systemic nematicides, that only EDB reached an acceptable level of control of *M. javanica* in tobacco.

Positive results of *Meloidogyne* control on tobacco, tomato and other tropical crops by systemics were recorded by FERRER, AYALA and CUEBAS (1972); FELDMESSER (1969, 1972); JOHNSON (1969 a, b); LAMBERTI (1972), and others.

Nearly all publications on soil treatments with systemic nematicides against *Meloidogyne* species lead to the conclusions that the best time of application is just before or at the moment of planting and that the best method is incorporating the nematicide intimately into the top soil. The general conclusion may be that the systemics are as effective as the fumigants in protecting the plant against *Meloidogyne* species.

The mode of action by which systemic nematicides control *Meloidogyne* spp. was investigated by REDDY and SESHADRI (1971). Thionazin and aldicarb inhibited the rate of root invasion by *M. incognita* larvae. Thionazin reduced the fecundity of *M. incognita* females from more than 200 to less than 50 eggs per egg mass if the dosage of the chemical was increased from 4–16 kg/ha. Aldicarb had no effect on egg production. Larvae from eggs produced on with thionazin and aldicarb treated plants were not adversely affected by the treatments. Hatchability of *Meloidogyne* eggs was not affected by organophosphorus and organocarbamate nematicides if they were subsequently transferred to water (REDDY and SESHADRI, 1971; MOTSINGER, 1961). For thionazin only the highest dose of 1000 ppm was lethal to eggs of *M. incognita*, but at the dosages recommended for nematode control in the field, a 5-days' exposure had no effect on the number of larvae extracted from egg masses. It seems that the gelatinous layer of the matrix has some protecting properties, possibly by acting as a barrier against penetration of the chemicals.

KONDROLLOCHIS et al. (1970) concluded that systemics act mainly as narcotics like the organo-halide contact nematicides. NELMES (1971) found that the systemic aldicarb was taken up quickly by the nematode *Panagrellus redivivus* and was metabolized by the nematode to sulphoxide and sulphone and some

other metabolites. The degree of transformation was related to the physiological condition of the nematodes. The concentration of aldicarb within the nematodes was about twenty times higher than in the treatment solution, which confirmed an earlier finding of MARKS, THOMASON and CASTRO (1968) for EDB and the nematode *Anguina tritici*.

c. Against other plant parasitic nematodes

Soil treatments against ectoparasitic nematodes, f.e. *Paratylenchus*, *Tylenchorhynchus*, *Rotylenchus* and *Trichodorus* species, seem to be more effective than for endoparasites, f.e. *Pratylenchus*, *Radopholus* and *Rotylenchulus* species. The sedentary endo- or semi-endoparasitic *Heterodera* and *Meloidogyne* species can probably best be controlled by treating the freeliving larval stages before they penetrate the root, as indicated earlier. This may not hold for true systemics, because they influence the nematodes via the plant. As for the *Heterodera* and *Meloidogyne* spp., the best treatment with systemics against freeliving nematodes may be mixing the chemicals into the top 10 cm of the soil just before or shortly after sowing or planting.

HOMEYER (1971a) reported that soil treatment by 10 ppm phenamiphos gave good control of freeliving plant-parasitic species for periods of 2-3 months after application. Field studies indicated that *Belonolaimus longicaudatus* and *Trichodorus christiei* were controlled more effectively by systemic than by fumigant nematicides (JOHNSON and CHALFANT, 1973). Aldicarb, however, did not reduce the population of *Criconemoides ornatus* if measured one and three months after treatment. Studying nematicides for control of *Pratylenchus* species in fern plants, OVERMAN (1971) found that carbofuran and Mocap were effective for at least 21 weeks after treatment, whereas roots from oxamyl-treated plots had higher populations than the control plots 12 weeks after treatment. A similar result, higher populations in treated roots, was found by BUNT (1973) for *Pratylenchus penetrans* in *Pyrus malus* seedlings at 25°C, but at 15° and 20°C the numbers were the same and at 10°C they were lower than for roots of control plants. In chapter 6.2. this phenomenon is discussed in more detail.

*Pratylenchus penetrans* and other freeliving endoparasites have been studied extensively for their reaction on systemic nematicides. JOHNSON and BURTON (1971) compared soil treatments with 11.4 kg a.i./ha aldicarb and phenamiphos against a mixed nematode community of *Criconemoides*, *Pratylenchus*, *Trichodorus*, *Belonolaimus* and *Xiphinema* species, by investigating soil samples one, three and four months after treatment. Most species were significantly controlled up to three months after treatments, compared to the increased densities of control populations. *Criconemoides* species, however, were significantly higher in the aldicarb-treated plots than in the control plots at four months after treatment. They concluded that *Criconemoides* could increase because aldicarb had disappeared early and the nematode could reproduce free from competitors. *Trichodorus* spp. were found at the same densities as in the untreated plots for both nematicides. *Belonolaimus* populations were still suppressed after four months. MARKS, ELLIOT and TU (1971) concluded from their experiments with

*P. penetrans*, that it was not the numbers of nematodes at harvest, but the numbers during the first few weeks in the roots that determined tobacco yield. Mocap at the rate of 11.2 kg/ha was more effective than DD at 224.6 litre a.i. per ha. They concluded that tobacco could tolerate high numbers of *P. penetrans* if the plant is well established before the nematodes penetrate the roots.

PERRY, SMART and HORN (1971) found that the fumigant DBCP was not phytotoxic when injected into golf courses, and turf-grass growth was excellent for at least one year after treatment. For nematode control on golf courses, however, systemic nematicides are used more frequently than DBCP.

Systemic nematicides at the low rate rate of 1–2 kg a.i./ha, applied at or before planting resulted in better yield of field corn attacked by *Belonolaimus longicaudatus* (RHOADES, 1971).

*Ditylenchus dipsaci*, the stem and bulb nematode, is known to be noxious to several crops in temperate climates.

Systemics are promising against this nematode because the nematode moves towards the stem of the plants and may thus cause damage even at low population densities in the soil. Besides this, *D. dipsaci* may survive desiccation and planting materials, such as seeds, bulbs and tubers often harbour living nematodes. TÉLEZ, HEREDIA and CASTRO (1971) illustrate the damage by *D. dipsaci* to garlic and the efficacy of phenamiphos to control the nematode. They found all fields infested in the main garlic production area in Mexico, resulting in 10–100% loss in 1967. If 18 kg/ha of phenamiphos was sprinkled onto an infested field, the yield increased from 100 kg to more than 4000 kg/ha. Nearly 100% of the bulbs in the untreated field were decaying, against only 6% in the treated field.

In The Netherlands *D. dipsaci* is a serious pest of onion, flower bulbs and several other crops. Good control of *D. dipsaci* in onion on clay soils was obtained with systemics by KAAI (1967, 1969, 1972), KAAI and DEN OUDEN (1966, 1969) and KAAI, KOERT and HOEFMAN (1967). The texture of clay soils makes fumigant nematicides more difficult to apply and less effective. Control of *D. dipsaci* by systemic nematicides, including oxamyl and phenamiphos, in flower bulbs, was studied in The Netherlands by WINDRICH (1969, 1971, 1973) and in the U.K. by WINFIELD (1970, 1971, 1973). Thionazin, the most intensively studied systemic, did not always give satisfactory results. Because flower bulbs are used for planting new crops and for export, stem nematodes must be absent or fully exterminated, and it appears difficult to reach this by treating infested plants with systemics.

An interesting aspect of the use of systemic nematicides concerns control of virus-transmitting nematodes. *Longidorus elongatus*, vector of raspberry ring-spot and tomato black ring viruses, and *Xiphinema diversicaudatum*, vector of arabic mosaic and strawberry latent ringspot viruses, were controlled and virus transmission was prevented by 12.5 ppm a.i. oxamyl as a soil mix (TAYLOR and ALPHEY, 1973). BROWN and SYKES (1973) found oxamyl, Mocap, phenamiphos, aldicarb, thionazin and phorate effective in controlling 'spraying' disease of potato caused by tobacco rattle virus, transmitted by *Trichodorus* species. The

amount of active ingredient of the systemics needed for an adequate control of 'spraing' was only 2–5 kg a.i. per ha. Remarkable is the result reported by COOPER and THOMAS (1971), who found that DD and methomyl both reduced the incidence of 'spraing', although the effect was not clearly correlated with kill of the vectors, *Trichodorus* species. Methomyl controlled virus infection more effectively than killing *Trichodorus* spp, indicating that methomyl, like most systemics, is nematostatic rather than nematicidal.

### Root treatment

Plant materials infested with endoparasitic nematodes can be disinfested by bare-root dips in systemics. This holds for infested woody stock, bulbs, tubers and other transplants. Reports in literature deal mostly with bare-root dips against *Meloidogyne* and *Pratylenchus* species, but the treatment may also be effective against *Heterodera* cysts adhering to plant materials.

DE GRISSE and MOUSSA (1970) found that aldicarb does not directly attract or repel larvae of *H. rostochiensis*, but more larvae were attracted by treated than by untreated roots. They concluded that aldicarb may stimulate formation and secretion of root diffusates.

BINDRA and KAUSHAL (1971) reported the effectiveness of *dimethoate* as bare-root dip of tomato plants infested with *M. incognita*. A 6 hours' root dip in 0.025% dimethoate caused a near complete eradication of the nematode. Root dip of Chinese gooseberry infested with *M. hapla* in thionazin was effective, whereas dips for 1 hour in 0.1% fensulphothion and parathion gave inadequate control (DALE and MESPEL, 1972).

Dipping root pieces of Mimosa, *Albizia julibrissin*, in solutions of phenamiphos, fensulphothion and other systemics were effective against *M. incognita* (JOHNSON and GILL, 1972). JOHNSON, RATCLIFFE and FREEMAN (1970) reached near-complete control of root-knot nematodes with bare-root dips of dogwood seedlings in concentrations of 1000 ppm of systemics for 15–30 minutes. Phenamiphos as a dip treatment was effective against *M. incognita* in *Gardenia* plants, but less so than drenches and soil mix treatments (MILLER and NOEGEL, 1970). PONCHILLIA (1973) found fensulphothion and thionazin effective as bare-root dips against *M. incognita* in peach trees, but oxamyl, methomyl and Mocap were ineffective even at the highest dose of 5000 ppm, which also caused phytotoxicity.

STOKES and LAUGHLIN (1970) reported that Mocap, fensulphothion and phenamiphos eradicated *P. penetrans* from roots of infested leatherleaf ferns up to 12 weeks after treatments after dipping roots in 800 ppm a.i. for 30 minutes.

### Foliage treatment

Because oxamyl had shown little phytotoxicity in early experiments, several investigators applied it as foliar spray. STOKES and LAUGHLIN (1970) were first to report that oxamyl had systemic nematicidal properties if used as a foliar spray. The roots of ferns were kept practically free from *P. penetrans* up to 12 weeks after spraying oxamyl on the foliage of the plants. Other reports on *P.*

*penetrans* control in the roots by foliage sprays with oxamyl are by WILLIS and THOMPSON, 1973; ABAWI and MAI, 1971, 1972; BUNT, 1972; DICKSON and SMART, 1971 and others. The amount of oxamyl needed varied from 1–10 kg a.i./ha, depending also on the amount of water used.

It is difficult to conclude with certainty from the literature that oxamyl has basipetal systemic properties, because an amount of 1 kg per hectare dripping on the soil may already cause nematode control in the soil, roots or foliage, directly or after acropetal transport. In greenhouse experiments investigators try to prevent contamination of the soil with the chemical, but contamination may nevertheless occur along plant stems through the secretion of water drips by leaf hydathodes or due to watering of the plants. For nematode control proper it may not matter which mode of action affects the nematodes and spray treatments may at any rate be attractive for practical reasons.

Other nematodes controlled for several weeks following oxamyl sprays are *Rotylenchulus reniformis* (BIRCHFIELD, 1971), *H. schachtii* (POTTER and MARKS, 1971), *M. arenaria* (DICKSON and SMART, 1971), *H. rostochiensis* (HARRISON, 1971), *M. incognita* (MILLER, 1971; RADEWALD et al., 1970; AYALA et al., 1971) and others.

Little information is available about the effect of phenamiphos. ZECK (1971) reports 90–100% control of *Rotylenchulus reniformis* on pineapple after foliage treatment. Stem application with a paste of phenamiphos caused good control of *P. penetrans* (TARJAN, 1972). HOMEYER (1971a) found that foliar dips of tobacco plants reduced subsequent galling of the roots by *M. incognita*. It seems that application of phenamiphos as a foliar spray is restricted by its phytotoxic properties.

Several investigators also reported insecticidal effects of oxamyl, phenamiphos and other systemics, if applied to the soil or onto the foliage. Attack by and control of insects in plant leaves and stems, however, is outside the scope of this study.

#### 'Seed' treatment

Thionazin is used already for many years against *D. dipsaci* in flower bulbs and onions (HAGUE, 1972). Recently phenamiphos, oxamyl and other systemics are also examined for their nematocidal effects in 'seed' treatments.

Phenamiphos showed good control of *D. dipsaci* in onions (INFANTE and SOSA-MOSS, 1971). Immersion of coffee seed in oxamyl was effective against root rot caused by *Pratylenchus coffeae* (ESCOBAR and ABREGO, 1972). Also the highest dosage used (10,000 ppm a.i.) was not phytotoxic to the coffee seeds or to the plants growing from the treated seeds. Immersion of seeds of the string bean *Phaseolus vulgaris* L. in oxamyl caused phytotoxicity effects and decreased germination, whereas attack by *M. incognita* on plants from treated seeds was not reduced (PARISI, TORRES and SOSA-MOSS, 1972). Seed treatments with oxamyl and phenamiphos and other systemics of cotton were also not effective against *M. incognita* and sometimes reduced growth of the crop. Oxamyl, how-



ever, reduced *H. rostochiensis* infestation of potato if seed tubers were dipped in solutions of the chemical before planting (PROUDFOOT and MORRIS, 1972).

### 1.5. SCOPE OF THE STUDY

The literature review indicates that nematode control by systemics shows promise. In several cases systemics controlled nematodes in soil and plants as well or better than the conventional fumigant nematicides. They are mostly less toxic to plants and may reach nematodes which have already penetrated into the plant, and therefore may be used curatively. This is a great advantage over other nematicides, which are all phytotoxic, except DBCP for some plants. Most systemics may further be effective against nematodes and improve plant growth at very low dosages, which may help to make treatments safe and economic.

It is often easier to apply systemics than fumigants, because they may be applied as soil mix, soil drench, foliar spray, or plant dip, often without the use of special equipment.

If tolerance levels with respect to non-target organisms, particularly the danger for grower and consumer, can meet the requirements, then systemics may well replace the present-day fumigants. It is already beyond doubt that systemics will find a place in agricultural practice, either for direct control of nematode infestation or as part of an integrated control scheme (OOSTENBRINK, 1964).

If systemic nematicides can be shown to be safe, effective at low dosages and cheap, then they could cause a major break-through in nematode control, which would be important for agriculture; the priority of various aspects of current nematicide research would also be altered.

There are however several important questions about systemic nematicides and their application at the moment; the present study therefore was aimed to:

1. Improve existing or to develop new techniques for proper and efficient testing of systemic and other nematicides;
2. Trace possible systemic and contact nematicidal effects of a wide range of agricultural chemicals;
3. Study the mode of action of the selected systemic nematicides oxamyl and phenamiphos which belong to different groups of chemicals; and
4. Investigate the influence of the two selected nematicides on nematodes, microarthropods and plant growth and to study residues and side-effects, influence of temperature, soil moisture, soil type and other major environmental factors.

## 2. MATERIALS AND METHODS

Materials and methods generally used in this study are discussed here, whereas special techniques are indicated under the respective experiments. The development of new techniques for testing chemicals on systemic and other nematicidal activity is treated separately in chapter 3.

### 2.1. TEST PLANTS

Young plants for greenhouse or laboratory experiments were sown in normal potting soil and transplanted to obtain well developed, uniform plants. To keep away saprozoic nematodes, seeds were placed in sterilized sand for emergence and then transplanted into the experimental medium. The two plants species *Vicia faba* cv. Dubbel Wit and *Lycopersicum esculentum* cv. Moneymaker were used for the development of new test methods.

#### A. Field bean, *Vicia Faba*

For one of the newly developed screening methods and for the biological assay on nematicidal residues in soil, stem parts of *V. faba* were required. For this purpose three seeds were planted in 10 cm clay pots with normal, sterilized potting soil. In a greenhouse at 18–22°C plants grew about 20 cm high in three weeks and were then used to give 4 cm long stem sections between internodes. Every pot gave about 15–20 stem sections. Extra light was furnished in mid-winter. To obtain straight stems it was necessary to tie up the plants. About 1000 stem sections could be produced in three weeks per square metre of greenhouse space. The use of stem sections is described under 3.2.

#### B. Tomato, *Lycopersicum esculentum*

Tomato plants were used in one new and one modified screening method. Both techniques made use of unrooted cuttings of *L. esculentum*. Roots were cut off just below soil level.

The seeds were sown and emerged in small wood trays and were once transplanted. The soil used was normal, sterilized potting soil mixed 1:1 with sterilized river sand. About three weeks after sowing, when the first true leaves were well developed, the plants were ready for use. In winter the plants were raised in a greenhouse at 25°C with some extra light. When young plants are used a few hundred test plants may be grown per square metre greenhouse space.

#### C. Evaluating plant growth

When plant growth had to be evaluated, the criteria chosen were usually plant height, fresh and dry weights of roots or of total plants, visual plant vigour and specific symptoms of nematode attack, e.g. for *Meloidogyne in-*

*cognita* the rate of root galling and for *Ditylenchus dipsaci* the disruption of tissue, swellings of stems and discoloration of the infection site.

## 2.2. TEST ANIMALS

### A. Nematodes

The main nematode species used for experiments were the bulb and stem nematode, *Ditylenchus dipsaci* (KÜHN, 1858) FILIPJEV, 1936 and one of the root-lesion nematodes, *Pratylenchus penetrans* (COBB, 1917) FILIPJEV & SCHUURMANS STEKHOVEN, 1941.

Other plant parasitic nematodes used in certain investigations are *Heterodera rostochiensis* WOLLENWEBER, 1923, *Meloidogyne incognita* (KOFROID & WHITE, 1919) CHITWOOD, 1949, *Tylenchorhynchus dubius* (BÜTSCHLI, 1873) FILIPJEV, 1936, *Pratylenchus crenatus* LOOF, 1960, *Paratylenchus* spp. and *Helicotylenchus* spp., while saprozoic nematodes were studied in those cases that nematode communities in soil were examined. In the last case all other tylenchid nematodes, comprising *Tylenchus*, *Psilenchus* and other stylet-bearing species, were counted together as a group.

*D. dipsaci* and *P. penetrans*, and also the methods of extraction and counting of nematodes, are discussed in more detail here.

#### *D. dipsaci*, onion race

The need for large numbers of *D. dipsaci* throughout this study made it necessary to maintain a stock culture by multiplying the nematode on a suitable plant. A slightly modified version of a method described by SEINHORST (1959) was used in which potato tubers were used as host. Fresh potato tubers are washed with water and disinfected in a solution of a mixture of streptomycin 0.25% and Aretan 0.05% for 1 hour. The nematodes for inoculation are disinfected in the same way. Then the tubers are given a cut about 1 cm deep with a sterilized knife; a few hundreds specimens of *D. dipsaci* are placed in each cut with a sterilized hypodermic syringe or with a fine needle, and the inoculated tubers are placed, e.g. in a paper bag, at 15 °C.

With the suitable race of this nematode a 100 to 1000 fold increase may be reached in 4–12 weeks; from one tuber 1–2 millions L-4 stage larvae were usually obtained. As soon as symptoms of attack (swellings, discoloration and dry rot of the tissue) are seen, potatoes not immediately needed for extraction may be stored at 2–4 °C, and will then yield large numbers of extractable nematodes even after several months of storage. After extraction the nematodes may be kept viable in water without aeration in a refrigerator for about two weeks. If dried and stored, they can be reactivated and used even after several years. Extraction of the nematodes from the tubers was done by placing small potato slices on a double cottonwool filter in an extraction dish, or by the funnel-spray method described by OOSTENBRINK (1960) for the extraction of endoparasitic nematode species from plant tissues. In this study only *D. dipsaci* of the onion race reproduced in potato tubers were used, directly after their extraction.

### *P. penetrans*

*Pratylenchus* species are suitable for studying systemic nematicides as they are endoparasitic cortical feeders. If high numbers of *P. penetrans* were required the nematode was cultured on a suitable host plant in heavily infested soil in the greenhouse.

*Pyrus malus* and *Ligustrum ovalifolium* seedlings were planted in soil with about 500 specimens of *P. penetrans* per 100 ml soil, and after about 10 weeks high numbers of the nematode were present in the roots. For extraction the plants were uprooted, washed free from soil, crushed in a blender with 100 ml water per 10 gram roots and separated from root debris on cottonwool filters for 48 hours by the method described by STEMERDING (1963).

If infested plants were needed for experiments the desired species were sown in sterilized potting soil and transplanted to *P. penetrans* infested soil. If sieved and well-mixed soil was used, uniform infection of the plants occurred, with more than 50% of the nematodes penetrating the roots within a week.

#### Extraction and enumeration of nematodes

Estimation of nematode densities in soil and roots was done by the methods described by OOSTENBRINK (1960), or slight modifications.

For soil the modified decantation method was often used, as follows. Wet soil, mostly 100 ml, is placed into a 2 litre pan and 1 litre water is added. The suspension is stirred for about 10 seconds and is decanted after a further 10 seconds into a container of 4 litre. This procedure is repeated twice more and the suspension poured on 4 sieves (44  $\mu$  mesh). Further extraction was done using OOSTENBRINK'S (1960) elutriation method.

For roots the method described by STEMERDING (1963) was also used (see above).

Depending on the nematode density in the water after extraction either the whole suspension was counted under the dissecting microscope, or 5, 10 or 25 ml samples of the 100 ml suspension was examined. Usually suspensions with less than 200 specimens were counted as a whole; if higher numbers were present the suspension was stirred with an airpump and subsamples were pipetted into counting dishes.

The numbers of nematodes were expressed per 100 ml of soil and per root system, unless otherwise stated.

### B. Other Metazoa

The other *Metazoa* investigated in this study were *Collembola* and *Acarina*. Except for some small laboratory investigations, only natural soil populations were studied with respect to their response to systemic nematicides mixed into the soil.

The species of *Collembola* studied was *Tullbergia krausbaueri* BÖRNER, 1901 and the *Acarina* species was *Rhodacarellus sileciacus* WILLMANN, 1936.

For examination of soil samples the modified Tullgren funnel method described by SHARMA (1971) was used. Metal sieves containing 62.5 ml of soil are placed above a funnel which is placed on a tube containing water to collect the

animals. Above the soil, lamps are placed to heat and dry the soil and to force the animals downwards. The tubes are cooled with running tap water and the animals fall through the bottom sieve gauze into the tubes when fleeing from the gradually heated and dried soil. After 1 week most small animals of the sample have been collected in the tubes and can be determined after fixing them in 60% alcohol. By this method about 80% of the active microarthropods may be caught.

### 2.3. SOILS AND OTHER MEDIA

Three soils were used for experiments, namely Ellecom soil, Winschoten soil and sterilized potting soil. These soils are discussed in more detail.

#### A. *Ellecom soil*

A large quantity of this sandy-loam soil was collected from a farm field in autumn. The soil was dried slightly in the greenhouse till sieving was possible. Then stones, roots and other large particles were removed and the soil was mixed to obtain a homogeneous distribution of nematodes and microarthropods. Representative samples were then collected to assess the nematode and microarthropod densities and to determine pH, organic matter and clay content of the soil.

The pH-KCl was 5.7, organic matter content was 2.8% and clay content (fraction below 2  $\mu$ ) was 6%. Nematode and microarthropod densities are recorded under the experiments concerned.

This soil was used to set up experiments about the dynamics of nematode and microarthropod populations following treatments of the soil with systemics, with and without the growth of a host plant (chapter 6) and laboratory experiments about the persistence of systemics in soil (chapter 7).

#### B. *Winschoten soil*

The Winschoten soil from a farm field was also slightly dried for sieving and mixing and then used for experiments, or stored in concrete rings in the open air and planted with apple and rose seedlings to maintain the *P. penetrans* population at a high level.

This sandy soil contained 7.7% organic matter and had a pH-KCl of 5.2.

All experiments in chapter 6 (*P. penetrans*) were done with this soil. If other soils were used the details are given in the respective experiments. The Winschoten soil contained about 500 *P. penetrans* per 100 ml, whereas other plant parasitic nematodes were rare.

#### C. *Sterilized potting soil*

This soil contained an organic matter content of about 50%. It was a fertile soil used for growing plants in other investigations (cf. 2.1.), and for special ex-

periments on the influence of organic matter content on the efficacy of systemic nematicides by mixing this soil with different amounts of sterilized river sand. For experiments this soil was also sieved to remove coarse materials. The pH-KCl was 6.0.

#### *D. Other media*

Other media used for laboratory experiments included agar plates, fertilizer solutions, sterilized river sand and finally pure silver sand washed with hydrochloric acid and heated to remove organic matter and other unwanted substances. Silver sand was used in laboratory tests to estimate the toxicity of chemicals to nematodes, because of its low adsorptive capacity and its constant, homogeneous composition. In the newly developed test methods for nematicidal activity described in chapter 3 silver sand was used.

## 2.4. PESTICIDES AND THEIR APPLICATION

Data on chemical composition and biological activity of the pesticides used in this study are listed in Table 5 (cf. 4.1.).

Application of granular systemic nematicides was done by mixing the chemicals thoroughly with the soil. Most systemics are non-phytotoxic and therefore planting followed immediately after treatment unless otherwise stated.

Liquid formulations (emulsified concentrates) diluted with water were applied either as drenches to the top soil or sprayed on top of a soil layer which was then mixed thoroughly on a plastic sheet. In many experiments a 1 ml solution of pure chemicals was pipetted into small vials containing 5 ml of silver sand and 1 ml of a nematode suspension, after which the sand was just saturated. Details for application of these techniques are given in chapter 3.

Spraying leaves with nematicides was done with a paint-sprayer with a fine nozzle connected with an air pressure line. Leaves were sometimes moistened with chemical solutions by dipping.

In vitro tests (Chapter 5) were set up with the chemical solutions in water in glass vials. Nematodes were dipped in these solutions and then examined under the dissecting microscope and either photographed, or tested in biological assays to determine their viability. The new test methods, described in chapter 3, although conducted in glass vials, are biological assays, because after exposure of the nematodes to the chemicals in sand their ability to reach and invade the host was estimated by means of a test plant or part of a plant.

Experiments were set up with sufficient replicates and concentration ranges and at controlled or at least registered environmental conditions to make statistical analysis of the data possible.

## 2.5. EXPERIMENTAL CONDITIONS

Experiments were usually conducted in the laboratory at room temperature (18–22 °C), or under more rigidly controlled conditions in greenhouse compartments, constant temperature chambers or series of thermostats.

### *A. Greenhouse compartments*

In the greenhouse, compartments with controlled temperature were available. To investigate the influence of temperature on nematode control by means of systemics, sections were used with temperatures of 10, 15, 20 and 25 °C ( $\pm 2$  °C). In mid-summer at high temperatures outdoors, sometimes higher temperatures occurred at mid-day, notably in the 25 °C compartment first because this was not furnished with a cooling system. These peaks were rare and appeared for a few hours only, so that the soil temperature was hardly influenced. In every compartment temperature during experimentation was systematically recorded.

### *B. Constant temperature chambers*

Some experiments were conducted in large constant temperature chambers. Temperature, light and air moisture were automatically controlled. Temperatures used were 10, 15 and 20 °C ( $\pm 1$  °C). Artificial light was given to reach assimilation periods of 16 hours per day. Air moisture was about 90% relative humidity.

### *C. Series of thermostats*

A series of thermostats was used to obtain controlled temperature conditions without light. The temperatures showed variations of 1 °C. Temperature ranged between 0 and 45 °C, from which several different compartments were used for special experiments mentioned later.

### *D. Special incubators*

Other incubators with heating or cooling systems were used incidentally and are mentioned under the experiments.

## 2.6. STATISTICAL ANALYSIS

Experiments were set up with replicates (usually 4–10) to calculate statistical significance of the results. Because most trials were set up with a range of concentrations of the chemicals, regression and correlation tests were often used. Numbers of nematodes were usually transformed to log. scale or log. ( $x+1$ ) before use in calculations. Some results had to be expressed as percentages and were then transformed to probits, which are often recorded against log. concentrations.

Other statistical methods used were analysis of variance, Tuckey's multiple range test and calculations on the parallelism of regression lines. Significance

of differences is given in LSD values or with indications of probability as follows: ns = not significant; \* =  $P \leq 0.10$ ; \*\* =  $P \leq 0.05$ ; \*\*\* =  $P \leq 0.01$ , unless stated otherwise.

Some trials and series of data required other statistical methods. The main part of the results is expressed in graphs. For reasons of comparison the results are often expressed as median effective concentration or median lethal concentration (EC-50 and LC-50, respectively).



### 3. NEW TECHNIQUES FOR TESTING NEMATOCIDES

#### 3.1. INTRODUCTION

In this chapter three new test methods for a primary screen of candidate substances on nematocidal activity are described. From the literature review (1.3.) it was clear that a simple, cheap and non-laborious screen to examine nematocidal effects within a few days was not available. Moreover the primary screens in use do not cover the range of possible modes of action of systemic nematocides. Often two or more primary screens must be used side by side to avoid that potentially effective nematocides are overseen.

#### 3.2. THE PENETRATION INHIBITION TEST ('PI TEST')

The PI test rates the property of all known nematocides to inhibit invasion by nematodes of plant tissue, independent of the mode of action: kill, paralysis, repellance, narcosis or antifeeding.

##### *Description*

The PI test is described with reference to figure 2. Into a 10 ml glass vial containing 5 ml of dry silver sand (A) a *D. dipsaci* suspension of 1 ml comprising about 400 specimens (B) is pipetted. Another 1 ml with the substance to be tested in the desired concentration (C) is also pipetted into the vial. The sand is then just saturated. After 24 hours incubation at room temperature, a 4 cm stem section of field bean, *Vicia faba* L., is placed a few mm into the moist sand (D). After another 24 hours, the L-4 stage larvae of *D. dipsaci* have invaded partic-

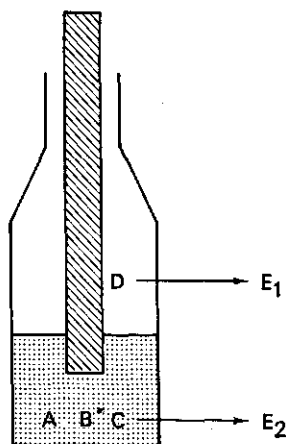


FIG. 2. Scheme of the penetration inhibition test ('PI test'). A = 5 ml silver sand in a 10 ml glass vial; B = 1 ml suspension of *Ditylenchus dipsaci*; C = 1 ml solution of candidate nematocide; D = stem section of *Vicia faba*; E1 = extract of nematodes from the stem section; E2 = extract of nematodes from the silver sand.

When B and C are added to A, 24 hours is allowed before the stem section D is inserted. Again 24 hours later extraction takes place, which takes another 24 hours. The whole test therefore takes  $3 \times 24$  hours, apart from short periods for preparing the vials and counting the nematodes.

ularly the lowest 1 cm of the stem, which is washed free from adhering sand and cut off. After cutting the stem section once longitudinally the pieces are placed in a counting tray in 5 ml water for 24 hours to extract the number of penetrated but still viable nematodes (E1), which are examined and counted after extraction. Untreated stem pieces in water instead of chemical solution usually have about 100 nematodes. The silver sand can also be analysed for surviving *D. dipsaci* by placing the sand sample on a nematode extraction filter in a counting tray (E2).

Figure 3 shows a set of 208 test vials placed in holes of wooden blocks. For a first screen 4 replicates suffice to detect compounds which inhibit nematode invasion into the plant. One person can set up, handle and count a test of several hundred compounds per week. To fill the vials with 5 ml sand, an apparatus was developed by which one person can fill 500 vials per hour. As indicated in figure 2, the whole procedure takes 3 days.

Before field bean stem sections were found suitable as bait for *D. dipsaci*, pieces of potato tubers and carrots and also stem sections of potato and other plants were tested. Results could be obtained with some of them, but they became more often infected with bacteria and fungi and were less easy to handle than stem sections of field bean.

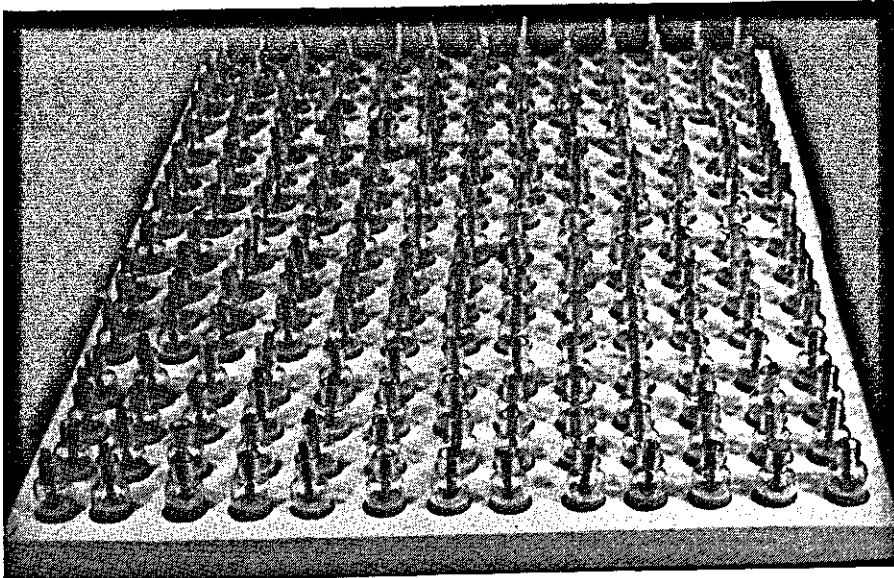


FIG. 3. A set of test vials with *Vicia faba* stem parts in the PI test (cf. Fig. 2).

#### Evaluation

Results of 5 different experiments are recorded here to illustrate the value and flexibility of the PI test. Firstly the effects of four known nematicides are compared (a). Secondly the influence of the immersion period of *D. dipsaci* in

oxamyl on subsequent nematode penetration was studied (b), and finally the systemic properties of oxamyl are shown (c). Only in the first test (a) the PI test was used according to the description of figure 2.

a. Comparison of four nematicides (Exp. 1)

In a preliminary PI test, with one replicate and four concentrations of the nematicides (0.1, 1, 10 and 100 ppm a.i.), an estimate was made of the effective concentration ranges. The nematicides were two systemics, oxamyl (Vydate) and phenamiphos (Nemacur) and two fumigants, DBCP (Nemagon) and methamsodium (Monam). For detailed information on nematicides see table 5 under 4.1.

A four-replicated experiment was then set up with the concentration range for each of the nematicides as specified in table 1. The test was conducted according to the scheme set out in figure 2.

The results are summarised in table 1. Because only 3 of the 4 concentrations gave inhibition of the penetration and were, therefore, usable for calculation of the regression, the derived EC-50's for DBCP and methamsodium should be treated with caution.

TABLE 1. Regression analysis of results obtained in the PI test with two systemic (oxamyl and phenamiphos) and two fumigant (DBCP and methamsodium) nematicides. Concentrations are recorded in ppm a.i. ( $\mu\text{g}$  of active ingredient per ml sand =  $\mu\text{g}$  a.i./ml sand);  $y$  = probit of the percentages penetrated nematodes against the control;  $x$  = concentration of the nematicides;  $r$  = correlation coefficient; EC-50 = concentration of the nematicides, in ppm a.i., which causes 50% inhibition of penetration (median effective concentration). For indications of statistical probability cf. 2.6.

Nematicides	Concentrations tested, in ppm a.i.	Regression formulae	$r$	EC-50 in ppm a. i.
oxamyl	1/16, 1/4, 1, 4,	$y = -0.64x + 6.80$	-0.92*	0.77
phenamiphos	1/64, 1/16, 1/4, 1	$y = -1.03x + 6.74$	-0.99***	0.04
DBCP	1, 4, 16, 64	$(y = -1.01x + 8.45)$	-0.95 <sup>ns</sup>	(28.22)
methamsodium	1/4, 1, 4, 16,	$(y = -1.01x + 7.47)$	-0.98 <sup>ns</sup>	(1.84)

From this experiment the following conclusions may be drawn: 1) a test giving 24 hours exposure of the nematodes to the nematicides appears to reveal significant nematicidal effects which can be expressed quantitatively; 2) at low concentrations phenamiphos, and to a lesser extent oxamyl, greatly reduce the penetration of *D. dipsaci* into bean tissue compared to the fumigants.

b. Influence of exposure time on subsequent rate of penetration of *D. dipsaci* (Exp. 2 and 3)

In the PI test *D. dipsaci* was exposed to the test chemical for 24 hours, before bean stem sections are placed to allow the nematodes to penetrate. The influence of shorter and longer exposure on penetration was studied in two experiments, test conditions were the same as before.

In the first of these experiments (Exp. 2) bean stem sections were placed in

sand moistened by 0, 4, 16 and 64 ppm a.i. oxamyl immediately after adding the nematodes and the chemical solutions.

Replication was 3-fold and after a period of 24 hours the nematodes were extracted from the stem tissue and from the sand.

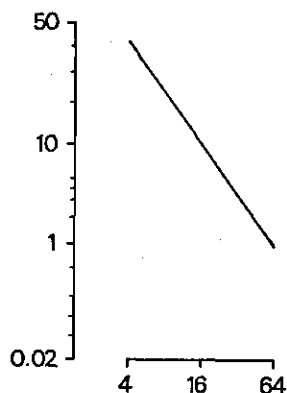
The set-up of Exp. 3 was similar to that of Exp. 2, except that the dipping period was 3 days, after which period stem sections were placed in the sand for 24 hours: replication was 4-fold.

The results of Exp. 2 are recorded in figure 4. The concentrations of oxamyl (ppm a.i. =  $\mu\text{g a.i./ml sand}$ ) on a log. scale are plotted against the probit percentages of penetration (control = 100%). The formula is:  $y = -0.58x + 5.39$ , with  $r = -0.99^*$  and an estimated EC-50 of 3 ppm a.i. oxamyl. Numbers extracted from the stem sections plus the numbers extracted from the sand, i.e. total numbers extracted, did not significantly differ with treatment: they were 776, 853, 1095 and 905 per 3 stem sections, for 0, 4, 16 and 64 ppm oxamyl, respectively.

FIG. 4. Effect of oxamyl upon *D. dipsaci* invasion rate in *V. faba* stem sections in the PI test, if stem sections were inserted at the same moment that nematodes and nematicide were added to the sand, therefore if the initial dip period was 0 hours.

Abscissa: Concentrations of oxamyl in ppm (cf. Table 1); log. scale.

Ordinate: Percentages of invaded *D. dipsaci* larvae; probit scale.



The results of Exp. 3 were not suitable for presentation as a regression line, because inhibition of the penetration was so great for all concentrations used i.e. 98.7, 99.0 and 99.5%, for 4, 16 and 64 ppm oxamyl, respectively. The EC-50 is less than 1 ppm a.i. according to extrapolation of the results. Even this 3 day's dip of *D. dipsaci* in the various oxamyl concentrations did not significantly influence the total numbers of *D. dipsaci* extracted from the stems + the sand, which were 1049, 1293, 1089 and 760, for 0, 4, 16 and 64 ppm a.i. oxamyl, respectively.

These two tests indicate that: 1) oxamyl has not killed the nematodes, even at a concentration of 64 ppm for 4 days; 2) the time for which *D. dipsaci* is treated in oxamyl or into oxamyl-saturated sand is apparently not critical in the PI test. Without an initial treatment of the nematodes higher dosages in the sand are required to reach the same effect as in a 24 hours' dip, but the method is reliable and gives comparable results.

Despite the conclusion in 2) it is clear, that the effect on *D. dipsaci* is greater the longer the nematodes stay in the nematicide. This indicates that oxamyl has a direct effect on the nematodes which makes them less able to penetrate plant tissue but does not kill them.

If desirable the PI test could be done in less time, f.e. in 12 hours, in which case the different steps of the test could be shortened to 3 periods of 4 hours instead of 3 periods of 24 hours, provided that the concentrations of the chemicals are increased. In a 'quick PI test' a higher number of *D. dipsaci*, e.g. 800, must be added to the silver sand in order that the invasion into the untreated bean stems is sufficiently high, i.e. 100–200 specimens. The concentration of the chemicals to be tested should be about 10 times that in the normal PI test, because the EC-50 values are increased by a factor 10. From preliminary experiments with phenamiphos conducted with the quick, the normal and the slow PI test, corresponding with initial dips of 4, 24 and 144 hours, respectively, the following percentages for penetration compared to controls (= 100% penetration) were found for 1/64, 1/16, 1/4, 1, 4, 16 and 64 ppm a.i.:

quick PI test:	100, 95, 91, 78, 33, 6,
normal PI test:	72, 48, 8, 1, 0, 0,
slow PI test:	83, 44, 4, 0, 0, 0.

From these percentages it follows, that a dipping period of more than 24 hours, as in the slow PI test, has no advantages above the normal PI test. The quick PI test may have some advantages if nematocidal or nematostatic activity of a chemical has to be evaluated very quickly. For an efficient screening program, however, the periods of the normal PI test as given in the scheme of figure 2 are preferred.

### c. Systemic properties of oxamyl (Expts 4 and 5)

In two experiments the systemic properties of oxamyl were studied using modifications of the PI test.

In Exp. 4, nematicide application and nematode inoculation of the bean stem sections were given separately in different vials. Firstly bean stems were placed into 5 ml sand saturated with 0, 4, 16 and 64 ppm a.i. oxamyl ( $\mu\text{g}/\text{ml}$  sand) for 3 days. Then they were washed thoroughly with water, placed into clean sand and moistened with 2 ml *D. dipsaci* suspension per 5 ml sand comprising about 400 specimens. The nematodes were allowed to penetrate into the stems for 24 hours, after which they were extracted from the stems by the funnel-spray method. Nematodes were collected from the funnels after 4, 24 and 48 hours.

In figure 5A the nematode numbers are recorded. The first conclusion is, that oxamyl reduced penetration markedly. If these numbers are calculated as percentages of the totals per treatment, there was a significant lower percentage extracted from the oxamyl treated stems, compared to the untreated stems, after 4 hours extraction. The second conclusion is, that oxamyl apparently had a temporary narcotic effect on part of the penetrated larvae in all concentrations, although this was most obvious in the highest concentration.

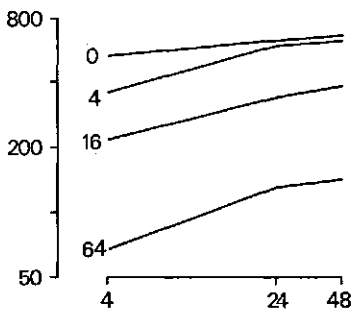
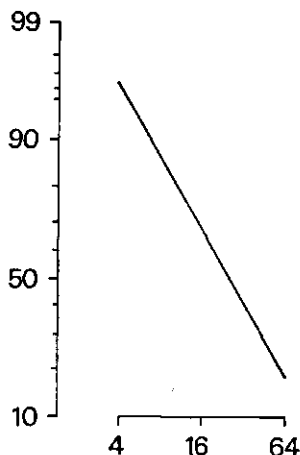


FIG. 5A. Numbers of *D. dipsaci* extracted from stem sections of *V. faba* after extraction periods of 4, 24 and 48 hours. The stem sections had been placed for three days in 0, 4, 16 and 64 ppm oxamyl as indicated in the graphs, and were then transferred to clean sand and exposed to nematode penetration for one day. Abscissa: extraction time in hours; log. scale. Ordinate: number of extracted *D. dipsaci*, log. scale.

FIG. 5B. Final result of the experiment illustrated under figure 5A, expressed as log-probit line. Abscissa: Concentration of oxamyl in ppm, log. scale. Ordinate: Percentages of invaded *D. dipsaci* larvae, probit scale.



In figure 5B the situation at the end of the extraction period is given as a log-probit line. From this figure an EC-50 (concentration which causes 50% inhibition of penetration) of 28 ppm can be read, thus characterising the systemic properties of oxamyl with the PI test in a quantitative way. The formula of the line is:  $y = -1.37x + 8.19$  ( $r = -0.99^*$ ). Compare to the results in figure 4.

In Exp. 5 two major modifications were made, viz. 1) introduction of the nematodes in the middle of the stem sections and not in the sand, and 2) exposure of the stems to the nematicide and to the nematodes extended to 3 weeks. The stem section was placed in sand moistened by 10 ppm oxamyl. Inoculation of *D. dipsaci* in the stem section took place as follows. A bent glass capillary of 2 cm length and 2 mm diameter was fixed against the bean stem, with the opening in the middle of the stem section. The capillary was filled for 1/3 part with water, to which 20 larvae (L-4) of *D. dipsaci* were transferred with a fine needle. The capillary was then filled with dry silver sand which became just saturated with water and thus was suitable for the nematodes to move and therefore to penetrate the stem tissue. Three replicates of each treatment were placed at 10, 15 and 20°C and examined after three weeks to determine the number of adult nematodes (= the originally inoculated L-4 stage larvae) and the number of

young larvae produced. Enough water was added during the experiment to keep the sand moist.

Neither oxamyl nor temperature had any effect on the penetration rate. There was an average of 5 adult *D. dipsaci* in every stem part irrespective of the treatments, indicating that oxamyl has not inhibited the penetration and development of *D. dipsaci* under the conditions of the experiment. The numbers of larvae produced at different treatments and temperatures are shown in figure 6. Treatment, temperature and the interaction of treatment and temperature are all significant ( $P = 0.01$ ). The LSD-values ( $\log x + 1$ ) are 0.52 ( $P = 0.05$ ) and 0.73 ( $P = 0.01$ ), respectively.

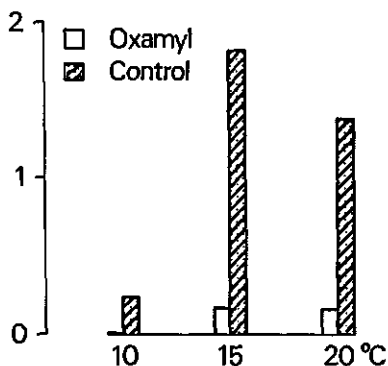


FIG. 6. Reproduction of *D. dipsaci* in three weeks at 10, 15 and 20°C, in *V. faba* stem sections placed in silver sand saturated with 10 ppm oxamyl compared to silver sand saturated with water; average of 3 replicates. Abscissa: temperatures average of 3 replicates as indicated. Ordinate: number of newly-formed larvae of *D. dipsaci*, log. scale.

The conclusion can be drawn that oxamyl has nearly completely inhibited the development of larvae at 15 and 20°C; for 10°C reproduction was very low in the untreated stems and thus no conclusion can be drawn. As seen in the reproduction thermograms, *D. dipsaci* in the untreated stem sections reproduced best at 15°C, which is in accordance with investigations of DAO (1970).

### 3.3. THE THERAPEUTIC TEST ('T TEST')

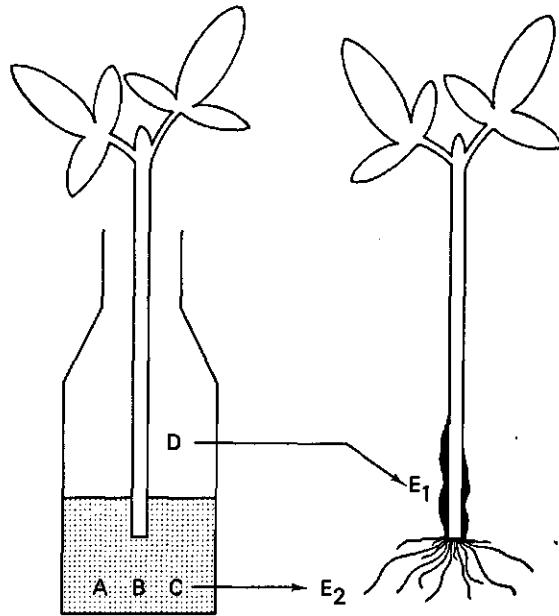
This test was developed to investigate chemicals which might affect nematodes already in infested plants and also to study the effect of delayed treatment when chemicals have to be converted by the plant into nematicidal active derivatives, such chemicals may be missed in the PI test.

#### Description

The T test follows the procedure of the PI test; cf. figure 7. A 1 ml suspension with 400 *D. dipsaci* (B), and 1 ml solution of the test chemical (C), are pipetted into a vial containing 5 ml of dry silver sand (A), just saturating the sand. After incubation at room temperature for 24 hours a tomato cutting is placed few mm in the moist sand (D). At 15–20°C it takes 6–8 days for *D. dipsaci* to cause swellings and discolor of the tomato stem just below to few cm above the soil

FIG. 7. Scheme of the therapeutic test ('T test'). A = 5 ml silver sand in a 10 ml glass vial; B = 1 ml suspension of *Ditylenchus dipsaci*; C = 1 ml solution of candidate nematicide; D = cutting of tomato plant; E1 = extract of nematodes from the stem of the tomato plant and/or visual rating of the symptoms of nematode attack; E2 = extract of nematodes from the silver sand.

When B and C are added to A, 24 hours is allowed before the tomato cutting D is inserted. Depending on the information wanted rating of the symptoms and/or extraction of the nematodes from the stem and/or sand, takes place after 4-14 days. Cf. text for detailed information.



(E1). The silver sand is analysed for surviving nematodes by placing the sand sample on a nematode extraction filter in a counting tray (E2).

An effective nematicide prevents nematode attack and no symptoms are seen. One week after placing the tomato cutting, the attack may be evaluated with the naked eye. Two to four replicates suffice for reliable results over a range of concentrations of a candidate nematicide. Thus some hundreds of compounds can be tested by one person per week. The influence of the chemicals upon egg laying and numbers of larvae produced, may also be determined. If further observation is needed, fertilizer is added and the plants are allowed to grow for 2-3 weeks. The first new larvae have then appeared if an inoculum of L-4 stage larvae is used. If such a longer growth period is desired, vials of a greater content, e.g. 100 ml instead of 10 ml can be used to keep the sand moist. The cuttings can be inoculated in a small vial and the rooted cuttings infested with *D. dipsaci* transplanted to a larger container later on.

For a complete therapeutic test the cuttings were allowed to root in the sand and *D. dipsaci* allowed to invade the stems for 3-4 days before the chemical was introduced. A therapeutic systemic will prevent multiplication or kill the nematodes in the plant tissue. If more than 200 *D. dipsaci* specimens were used as inoculum, the first symptoms are visible after 3-4 days, but they do not become severe if effective systemics are added.

The T test may also be used to study adsorption rate of a chemical on organic matter and on other soil components. Inoculated, rooted cuttings transplanted to various soils are treated with a range of concentrations of the test chemical; the influence of adsorption can be estimated from the test plants by evaluation



of symptoms, egg laying, number of larvae and adults, and sex ratio of the adult nematodes. The best time for evaluation depends on the information wanted; the techniques for nematode extraction are the same as for the PI test.

### Evaluation

The systemics oxamyl and phenamiphos and the fumigants DBCP and methamsodium were tested at a range of concentrations in four-fold replication. For every chemical an untreated series without nematicide was included. Nematodes were extracted from the tomato stems fourteen days after planting. Symptoms were recorded 1 and 2 weeks after planting and inoculation. Of the 500 *D. dipsaci* introduced into the sand, about 250 specimens were found inside the stem tissue after 2 weeks. Few eggs and larvae were present when the experiment finished after 2 weeks. Phytotoxic effect of the chemicals on the tomato seedlings was also determined. Phenamiphos showed symptoms of phytotoxicity in the leaves and phytotoxicity was detected in both stems and roots after treatment with methamsodium; at the highest dosages roots were sometimes formed above the soil. If too many *D. dipsaci* are inoculated, the nematode itself may destroy stem tissue at or above the soil. Oxamyl and DBCP were not phytotoxic at all. In table 2 the experiment and the results are summarised.

The systemics, oxamyl and phenamiphos, were effective at lower dosages than were the fumigants, without being phytotoxic. DBCP was the only compound which had not reached near-complete kill at the highest dose (64 ppm) during the test period of 2 weeks. The appearance of eggs was suppressed first, followed by reduction of *D. dipsaci* symptoms of the plants, indicating that the nematodes

TABLE 2. Results of a screen program with the T test, 2 weeks after treatment with 4 nematicides and 8 dosages. The following criteria were used for evaluation:

- A. Total numbers of *D. dipsaci* per 4 replicates,
- B. Presence of eggs and L-2 stage larvae (+ = present; - = absent),
- C. Symptoms of *D. dipsaci* attack present in more than 1 out of the 4 plants (+ = present; - = absent),
- D. Phytotoxicity symptoms (+ = present; - = absent; l = leave symptoms; s = stem and root symptoms).

Concentration in ppm a.i. (µg/ml sand)	Nematicides and criteria for their effectiveness															
	oxamyl				phenamiphos				methamsodium				DBCP			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	1084	+	+	-	980	+	+	-	1074	+	+	-	990	+	+	-
1/64	1096	+	+	-	1090	+	+	-	1064	+	+	-	1087	+	+	-
1/16	1175	+	+	-	463	-	+	-	1000	+	+	-	1118	+	+	-
1/4	771	-	+	-	13	-	-	-	1035	+	+	-	1024	+	+	-
1	102	-	-	-	4	-	-	-	479	-	+	-	1010	+	+	-
4	31	-	-	-	3	-	-	-	42	-	-	-	840	-	+	-
16	20	-	-	-	3	-	-	+l	3	-	-	+s	491	-	+	-
64	6	-	-	-	0	-	-	+ls	2	-	-	+s	115	-	-	-

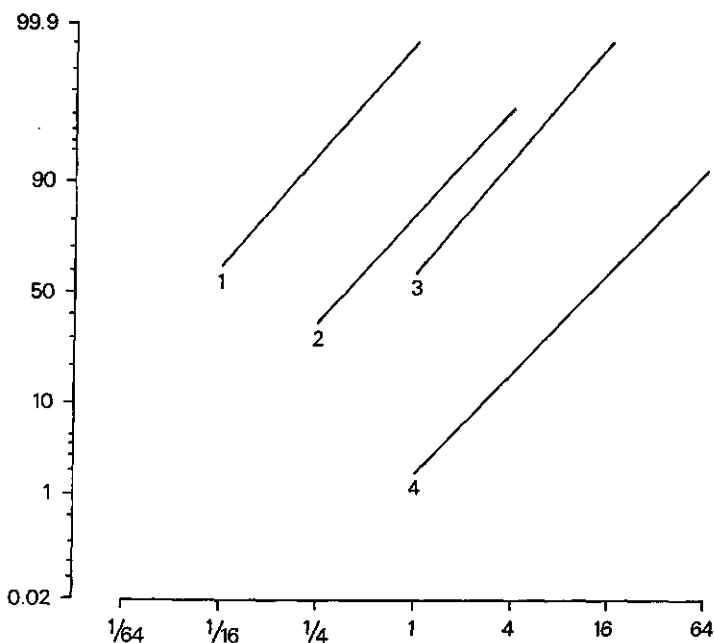


FIG. 8. Results after Table 2 expressed as log-probit lines. Formulae of regression lines:

1 = phenamiphos:  $(y = 1.30x + 2.72)$ ;  $r = 0.97^{ns}$  (EC-50 = 0.05 ppm)

2 = oxamyl:  $(y = 1.26x + 0.88)$ ;  $r = 0.95^{ns}$  (EC-50 = 0.37 ppm)

3 = methamsodium:  $y = 1.35x - 0.18$ ;  $r = 0.98^*$  EC-50 = 0.80 ppm

4 = DBCP:  $y = 1.18x - 1.82$ ;  $r = 0.99^{**}$  EC-50 = 11.80 ppm

Abscissa: concentration of the chemicals. log, scale (= x).

Ordinate: percentage control of *D. dipsaci* (numbers), probit scale (= y).

Cf. Tabel 1 for unexplained symbols.

still influenced the stem tissue at concentrations which already suppressed egg production.

In figure 8 the response of the nematode to treatment is expressed as regression lines, with corresponding formulas, r-values and EC-50's. The T test gives the same sequence of effectiveness as the PI test (3.2.). Phenamiphos was the most effective and the effect was the same in both tests, while oxamyl, methamsodium and DBCP gave rather better results in the T test.

The visual symptoms are recorded in table 3, for 1 and 2 weeks after treatment. In the first sample only 1 (symptoms) or 0 (no symptoms) were recorded, but in the second sample the degree of swelling was indicated by putting 1/2 for light swellings. Assessing nematocidal activity by measuring the degree of swelling appears to be a reliable method. Using 8-10 replicates reliable EC-50 values can be obtained merely by rating of the plant swellings visually one week after planting. In figure 9 the symptoms obtained in the T test are shown.

The influence of soil type on effectiveness of oxamyl and phenamiphos as

TABLE 3. Cf. Table 2 and Figure 8. Numbers of plants with swellings and the estimated EC-50's at 1 and 2 weeks after treatment (cf. text).

A. Visual examination after 1 week.

B. Visual examination after 2 weeks.

C. EC-50 values derived from nematode numbers extracted from the stems after 2 weeks. Cf. Table 1 for further explanation of symbols.

Concentration in ppm a.i. ( $\mu\text{g/ml}$ sand)	Numbers of plants with swellings and EC-50 values											
	oxamyl			phenamiphos			methamsodium			DBCP		
	A	B	C	A	B	C	A	B	C	A	B	C
1/64	4	4		4	4		4	4		4	4	
1/16	4	4		3	2		4	4		4	4	
1/4	3	3		1	0		4	4		4	4	
1	0	0		0	0		3	2		4	4	
4	0	0		0	0		1	0		4	4	
16	0	0		0	0		0	0		4	4	
64	0	0		0	0		0	0		1	2	
EC-50 (ppm a.i.)	.4	.3	.4	.1	.06	.05	2	1	.8	10	16	12

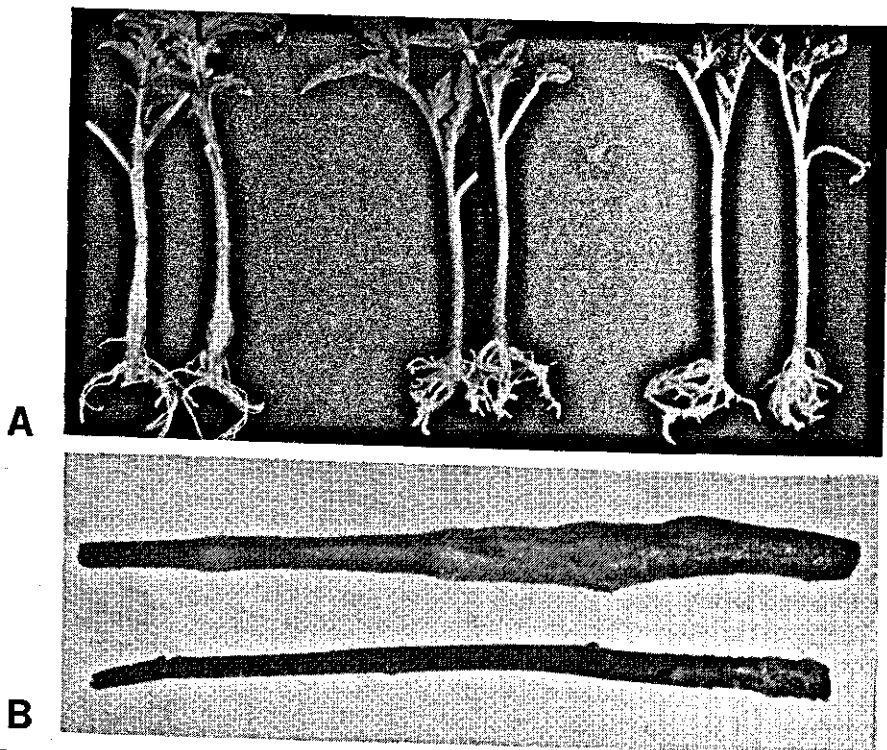


FIG. 9. A. Young tomato plants of the T test, 1 week after treatment. From left to right: control, oxamyl 1 ppm and phenamiphos 1/4 ppm. The stems of the control plants are swollen due to *D. dipsaci* attack. B. Detail of tomato stems of the T test, 3 weeks after treatment. Above: control; below: oxamyl 1 ppm.

determined by the T test are reported under 6.2.2. The T test was also used to study reproduction of *D. dipsaci* after different treatments; cf. 5.4.

### 3.4. THE MODIFIED GALL INDEX TEST ('GI TEST')

In the tropics and regions where *D. dipsaci* is not present, a screen using tropical/sub-tropical nematodes is needed, f.e. the gall index test, which is done with *Meloidogyne* spp. in pots in the greenhouse. A modified method was developed for this work.

#### Description

The gall index or GI test resembles the T test, but *M. incognita* is used instead of *D. dipsaci* and root galling is recorded instead of stem swellings. In figure 10 a scheme of the GI test is given. A 1 ml suspension with about 500 *M. incognita* larvae (B), obtained by placing egg masses upon a nematode extraction filter, and a 1 ml solution of the chemical to be tested (C) are pipetted into a vial containing 5 ml silver sand (A). After incubation at 20 °C for 24 hours a tomato seedling, from which the roots are taken off, is placed few mm into the moist sand (D). Some days later new roots are formed and active *M. incognita* larvae in the sand will penetrate and initiate galling of the roots. After 7 days the roots are washed free from sand and are rated for galling under the dissecting microscope. With the naked eye the degree of galling can be estimated after about 10 instead of 7 days. The results may be given as estimates, but

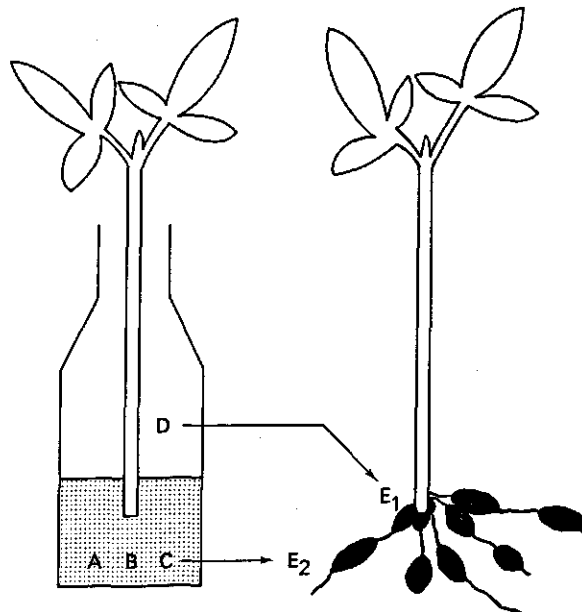


FIG. 10. Scheme of the modified gall index test ('GI-test'). A = 5 ml silver sand in a 10 ml glass vial; B = 1 ml suspension of *Meloidogyne incognita*; C = 1 ml solution of candidate nematicide; D = cutting of tomato plant; E1 = estimate of gall index; E2 = extract of nematodes from the sand. When B and C are added to A, 24 hours is waited before the tomato cutting D is inserted. After 7-10 days the degree of gall formation is estimated or exactly counted.

counted numbers of galls on the root are more exact (E1). Surviving larvae can be extracted by placing the sand on a nematode extraction filter for 24 hours (E2).

The modifications compared to the original gall index test are: a) tomato seedlings without roots can be used; b) the bio-assay takes place in vials of 10 ml comprising 5 ml pure sand; c) less chemical is required; d) the whole test is shortened from 6 weeks to 10 days; e) less labour and greenhouse space is needed.

### Evaluation

The GI test was used to evaluate the four nematicides oxamyl, phenamiphos, methamsodium and DBCP. The description under 3.4. was followed, but in one experiment the nematicides were added 1 day before planting (normal) and in a parallel experiment treatment was done 2 days after planting. Planting dates and nematode inoculations were uniform for the whole experiment; the inoculum rate was 100 larvae per plant. The nematicide concentrations ranged from 1/64 to 64 ppm a.i., as in the T test. Roots sometimes showed phytotoxic effects, which are reported in table 4. Except for galls, roots were normally developed. The untreated plants had 44 and 45 nematode root galls per 4 plants in the series planted 1 day before and 2 days after chemical treatment, respectively.

The results are recorded in table 4 and figure 11. Table 4 gives the numbers of galls together with indications about phytotoxicity. Figure 11 summarizes the statistically analysed results as regression lines: methamsodium results were unusable for statistical calculation.

The following conclusions can be drawn. Firstly, the results of the GI test are similar to those of the two other tests. Secondly the nematicides were less effective if applied after planting and inoculation. Thirdly, the phytotoxicity was slight and about the same as for the T test.

TABLE 4. Index of root galls on tomato plants in the GI test expressed as numbers of galls per 4 plants, 10 days after planting. The nematicides and dosages used are mentioned in the table. A. Nematicide application 1 day before planting the tomato cuttings. B. Nematicide application 2 days after planting the tomato cuttings. Phytotoxicity symptoms: m = moderate root formation; a = root formation absent; ar = air roots formed; b = bad root formation; - = no gall rating is possible for a and ar.

Concentration in ppm a.i. (µg/ml sand)	Numbers of galls per 4 replicated plants after the treatments indicated below:							
	oxamyl		phenamiphos		methamsodium		DBCP	
	A	B	A	B	A	B	A	B
1/64	42	47	26	41	44	46	39	44
1/16	43	50	3	29	39	41	43	46
1/4	20	38	0	4	47	50	46	47
1	14	25	0	0	32	51	22	39
4	0	4	0	0	12m	22m	6	32
16	0	0	0m	0	-ar	-ar	3	16
64	0	0	-a	0b	-a	-a	0m	8m

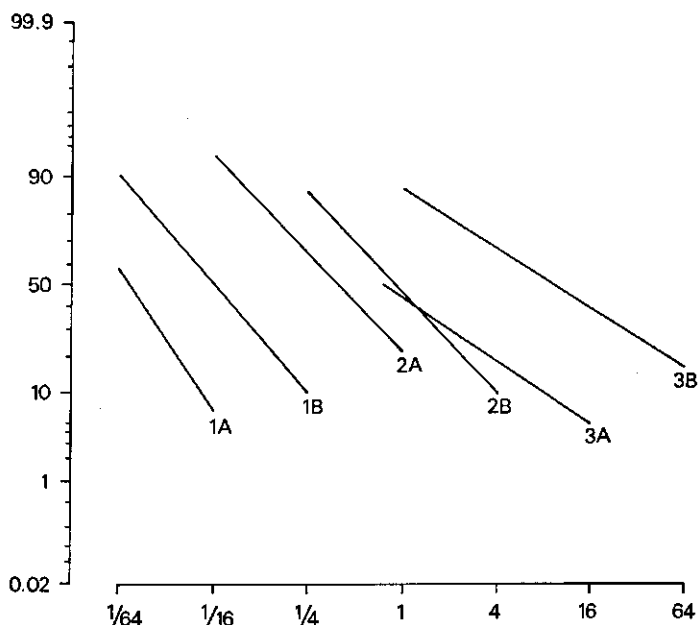


FIG. 11. Results of a GI test, expressed as log-probit lines; cf. Table 4. Indications for the nematicides and treatment times are given in the graphs and stated below: A and B indicate nematicide applications 1 day before and 2 days after planting the tomato cuttings, respectively.

1A = phenamiphos:	no calculation; EC-50 estimated as	0.02 ppm
1B = phenamiphos:	$y = -1.38x + 7.92$ ; $r = -0.99^*$	EC-50 = 0.06 ppm
2A = oxamyl:	$(y = -1.16x + 8.88)$ ; $r = -0.93^{ns}$	(EC-50 = 0.4 ppm)
2B = oxamyl:	$y = -1.20x + 9.75$ ; $r = -0.99^*$	(EC-50 = 0.9 ppm)
3A = DBCP:	$(y = -0.74x + 7.83)$ ; $r = -0.97^{ns}$	(EC-50 = 0.8 ppm)
3B = DBCP:	$y = -0.72x + 9.07$ ; $r = -0.99^{***}$	EC-50 = 10.0 ppm
4A = methamsodium	not mentioned in the graph;	EC-50 estimated as 2.0 ppm
4B = methamsodium	not mentioned in the graph;	EC-50 estimated as 4.0 ppm

Abscissa: concentrations of the chemicals, log. scale (= x).

Ordinate: percentage gall formation on the roots, probit scale (= y).

Cf. Table 1 for unexplained symbols.

### 3.5. DISCUSSION

The PI and T tests appear to be adequate for screening large numbers of chemicals to detect nematicidal activity independent of the mode of action. Each method has advantages and disadvantages, but both tests cover a wider field of nematicidal effects and are more easy to handle than methods in current use. In special cases the GI test may be preferable, e.g. for tropical regions and also if microscopes are not available for evaluation; the last reason also holds for the T test.

As shown in figures 4, 5B and 11, all three tests demonstrate the nematicidal effects of systemic and fumigant nematicides, despite differences in mode of action. Four replicates are usually sufficient to describe the results as regression formulae with corresponding coefficients. The correlation coefficients would have been more significant if more concentrations around the EC-50 values had been chosen initially, f.e. increasing geometrically with a factor 3 instead of 4. Advance knowledge is, of course, necessary to choose the most effective concentrations for obtaining statistically significant correlations.

EC-50 values obtained by the three methods were, apart from small differences, similar except for DBCP. Tables 1-4 and figures 8 and 11 reveal EC-50 values for penetration inhibition, reproduction rate, symptoms of nematode attack and other relevant characteristics of nematode infestation.

The advantage of all these tests is that they are bio-assays; the test plants or plant parts are also used to indicate phytotoxicity of candidate nematicides, namely by blackening of stem parts in the PI test and by various other symptoms in the GI and T tests. A phytotoxic systemic may damage the top leaves of young seedlings and phytotoxic fumigants more often damage roots or lower parts of the stem. We found phenamiphos and methamsodium phytotoxic in concentrations of about 16 ppm, phenamiphos particularly on leaves while methamsodium affected roots and lower parts of the stem. These results confirm the systemic properties of phenamiphos, which gave complete nematode control without phytotoxicity at low concentrations in contrast to the results for methamsodium (cf. table 4). DBCP gave good nematode control but was phytotoxic to tomato seedlings at 64 ppm. Oxamyl gave complete nematode control at 16 ppm, but was not phytotoxic at 64 ppm.

In a large-scale primary screening program it is possible with each of the three methods to test about 250 substances per person per week, f.e. with 2 replicates of 3 concentrations per chemical (1, 10 and 100 or 10, 100 and 1000 ppm a.i.).

From the results discussed here it seems, that systemics inhibit nematode penetration in plant tissue at relatively low concentrations (EC-50 for penetration from 0.01 to 1 ppm a.i.), whereas conventional nematicides are active at much higher dosages (EC-50 roughly between 1 and 50 ppm a.i.). This phenomenon is discussed in more detail in chapter 4, in which the results of testing a wide range of agricultural chemicals on nematicidal activity is described. The biochemical background is discussed in 5.5.

Dosages needed in the field (cf. 1.4.) are often 10 times higher than for laboratory conditions due to adsorption to soils, irregular distribution of chemicals in the soil (1 to 2 million litres per ha for a tilth of 10-20 cm deep) and fluctuation of environmental conditions.

The above-mentioned screening methods have been used to study nematicidal activity of a wide range of agricultural chemicals (4), influence of systemics on nematodes by continued and discontinued exposure (5) and persistence of systemics in soil under various conditions (7), as well as for other investigations.

## 4. GENERAL SCREEN OF AGRICULTURAL CHEMICALS AND CHOICE OF SYSTEMICS FOR FURTHER STUDY

### 4.1. INTRODUCTION

A range of agricultural chemicals, including nematicides, insecticides, fungicides, some herbicides and other substances, were evaluated by the PI test to check their ability to prevent invasion and to choose two systemic nematicides for further detailed investigations.

Table 5 lists relevant data of the chemicals, viz; number, common name, chemical category, trade name or code, formulation, knowledge about biocidal activity and finally also about cholinesterase inhibition. The data are collected from nematological and chemical literature, including industry folders and files. The EC-50 values, although part of the experimental results, are included for completeness and to account for the order in which the chemicals are listed and divided into two groups in the table.

### 4.2. RESULTS AND DISCUSSION

As indicated in table 5, 34 of the 60 compounds showed an EC-50 less than 50 ppm a.i. ( $\mu\text{g/ml}$  sand) and may therefore be considered nematicidal. All 17 compounds with an EC-50 less than 1 ppm appear to be substances with known anti-acetylcholinesterase activity. The statistical analysis of the results for the 34 nematicidal compounds is summarized in table 6. In this table the names of the formulations tested, which are nearly all trade names or codes, are mentioned; the order is the same as for table 5. In figure 12 the results are shown as regression lines, based on the formulae in table 6 and allowing the derivation of EC-50 values for each chemical. The order in which these regression lines are given is not exactly the same as for tables 5 and 6 to avoid clustering of lines and overlap of concentrations tested.

Of the 34 active substances, 17 showed an EC-50 below 1 ppm a.i. Column 5 of table 5 shows, that most of them were known nematicides, but others were only known as insecticides or as fungicides.

The nematicidal effects of the fungicide Hoe-2873 and of the insecticides Dede vap, Mesurol, Dipterex and Folithion is of special interest. They have not been reported as nematicides probably because of: a) the screening methods used; b) their very short life in soil; c) their extremely high adsorption to organic matter in soil; or d) other factors which lessen the activity of the compound or the possibility for application.

The chemicals with an EC-50 between 1 and 50 ppm a.i. are contact or fumi-



TABLE 5. Relevant data of tested chemicals, in order of increasing EC-50 values according to the PI test. The numbers 35-60 were found ineffective, with EC-50 above 100 ppm. Explanations of symbols in columns 2, 4, 5, 6 and 7:

Column 2: o.p. = organophosphate, o.c. = organocarbamate, o.a. = organic acid, h.h. = halogenated hydrocarbon.

Column 4: e.c. = emulsionable concentrate, w.p. = wettable powder, g = granulate, d. = dust, with indications of the percentages active ingredient (100 = pure).

Column 5: a. = acaricide, b. = bactericide, f. = fungicide, g.s. = growth stimulator, g.i. = growth inhibitor, h. = herbicide, i. = insecticide, m. = molluscicide, n. = nematocide.

Column 6: Chol. inh. = acetylcholinesterase inhibition for insects, mammalia or other organisms; + = present, - = absent.

Column 7: EC-50 = median effective concentration: here the concentration in  $\mu\text{g/ml}$  sand which caused 50% inhibition of penetration for *D. dipsaci* in the PI test.

No	1 Common name	2 Chemical category	3 Trade name or code	4 Formulation	5 Biological activity	6 Chol. inh.	7 EC-50
1	dichlorvos	o.p.	Dedevap (DDVP)	e.c.-59	i.	+	0.02
2	phenamiphos	o.p.	Nemacur	e.c.-40	n.i.	+	0.04
3	methiocarb	o.c.	Mesurool	w.p.-50	i.	+	0.06
4	thionazin	o.p.	Nemafos (Cynem)	e.c.-46	n.i.	+	(0.11)
5	-	o.p.	CGA-10576	g.- 5	n.	+	0.12
6	parathion	o.p.	Folidol (E- 605)	e.c.-25	n.i.	+	0.14
7	trichlorphon	o.p.	Dipterex	w.p.-80	i.	+	0.17
8	diazinon	o.p.	Basudine	d.- 2	n.i.	+	(0.27)
9	-	o.c.	Tirpate	g.-10	n.i.	+	(0.29)
10	aldicarb	o.c.	Temik	g.-10	n.i.	+	(0.31)
11	mevinphos	o.p.	Phosdrin	e.c.-25	n.i.	+	0.36
12	carbofuran	o.c.	Furadan (Curater)	w.p.-75	n.i.	+	0.36
13	fensulphothion	o.p.	Terracur	g.-10	n.i.	+	0.38
14	pyrazophos	o.p.	Hoe-2873	e.c.-30	f.	+	0.51
15	fenitrothion	o.p.	Folithion	e.c.-57	i.	+	0.62
16	-	o.p.	AC-92100	g.-10	n.i.	+	0.70
17	oxamyl (thioxamyl)	o.c.	Vydate (D-1410)	e.c.-25	n.i.	+	0.77
18	metham- sodium	o.c.	Monam (Vapam)	e.c.-40	b.h.f.n.i.	-	(1.84)
19	-	o.c.	Bunema (Nema)	e.c.-42	b.h.f.n.i.	-	1.96
20	-	o.p.	SRA-12689	g.- 5	i.	+	2.52
21	thioquinox	o.c.	Eradex	w.p.-50	a. f.	+	3.48
22	phorate	o.p.	Thimet	g.- 5	n.i.	+	3.68
23	lindane	h.h.	$\gamma$ -HCH	w.p.-21	i.	-	3.98
24	trichloronate	o.p.	Phytosol	w.p.-20	n.i.	+	4.30
25	O-methoate	o.p.	Folimat	e.c.-57	i.	+	6.83
26	1, 3-dichloro- propene	h.h.	DD	e.c.-60	h. n.i.	-	9.06
27	mecoprop	o.a.	M CPP	e.c.-56	h.	-	10.91

Continued Table 5

No	1 Common name	2 Chemical category	3 Trade name or code	4 Formula- tion	5 Biological activity	6 Chol. inh.	7 EC-50
28	chlordanifor- methane	-	Imugan	e.c.-27	f.	-	11.31
29	chinome- thionate	-	Morestan	w.p.-25	f.	-	11.41
30	propoxur	o.c.	Undeen	w.p.-50	i.	+	11.63
31	ethiofencarb	o.c.	Hox-1901	e.c.-50	i.	+	12.19
32	azinphos- methyl	o.p.	Gusathion	w.p.-25	i.	+	20.02
33	1, 2-dibromo- 3-chloropro- pane	h.h.	DBCP (Nemagon)	e.c.-75	f.n.i.	-	(28.22)
34	demethon-S- methyl	o.p.	Metaiso- systox	e.c.-51	n.i.	+	49.87
35	$\alpha$ -terthienyl	-	-	100	n.	-	(-)
36	benomyl	o.c.	Benlate	100	f.n.	(-)	(-)
37	bis (p-chloro- phenyl)	h.h.	Arkotine (DDT)	w.p.-25	i.	-	-
38	aldrin	h.h.	-	d.- 2	i.	-	-
39	thiram	-	TMTD	w.p.-80	f.	-	-
40	zineb	o.c.	-	w.p.-70	f.	-	-
41	captan	-	Orthocide	w.p.-50	f.	-	-
42	quintozene	h.h.	Brassicol (PCNB)	d.-20	f.	-	-
43	copperoxy- chloride	-	-	w.p.-50	f.	-	-
44	dichlofluanide	-	Eupareen	w.p.-50	f.	-	-
45	dinitro-o- cresole	-	Delonine	w.p.-46	h. n.i.	-	-
46	methaldehyde	-	Slugit	e.c.-20	m.	-	-
47	simazine	-	Gesatop-50	w.p.-50	h.	-	-
48	chlorpropham	o.c.	Chloro-IPC	w.p.-40	h.	-	-
49	diuron	-	AAkarmex	w.p.-80	h.	-	-
50	lenacil	-	Venzar	w.p.-81	h.	-	-
51	methabenz- thiazuron	-	Tribunil	w.p.-70	h.	-	-
52	paraquat	-	Gramoxone	w.p.-20	h.	-	-
53	pyrazon	-	Pyramin	w.p.-65	h.	-	-
54	metribuzin	-	Sencor	w.p.-70	h.	-	-
55	$\beta$ -indolicace- ticacid	o.a.	Rhizopon-A (IAA)	d.- 1	gs	-	-
56	daminozide	-	B-9	e.c.- 5	gi	-	-
57	chlormequat	-	Cycocel (CCC)	e.c.-40	gi	-	-
58	streptomycine	-	-	100	b.	-	-
59	glucose	-	-	100	-	-	-
60	sodium- chloride	-	-	100	-	-	-

TABLE 6. Detailed results concerning the 34 active compounds in Table 5, ranged according to increasing EC-50 values as derived from the PI test. The results are expressed as percentages of penetration of *D. disseca* in bean tissue, regression formulae and corresponding correlation coefficients, and the derived EC-50 values from the log-probit lines (Fig. 12). Explanation of data in the columns 2, 3, 4, 5 and 6:  
 Column 2: the lowest and the highest concentration as parts per million active ingredient ( $\mu\text{g/ml}$  sand); between brackets the number of concentrations used for statistical calculation (4 replicated).

Column 3: probit percentages for penetration against the control are given corresponding with the concentrations recorded in column 2.  
 Column 4: regression formulae for probit percentage penetration against controls = y on log. concentration of the chemicals as  $\mu\text{g/ml}$  sand = x; between brackets the not-significant regressions.

Column 5: Correlation coefficients (r), with indications of significance.  
 For unexplained symbols cf. Table 1 and Chapter 2.6.

No	1 Tradename or code of pesticides	2 Concentration range tested	3 Percentages penetration	4 Regression formulae	5 Correlation coefficient (r)	6 EC-50 in ppm
1	Dedevap	1/64- 1 (4)	59.0 2.5	y = -0.73x + 5.95	-0.99**	0.02
2	Nemacur	1/64- 1 (4)	76.0 1.0	y = -1.03x + 6.74	-0.99***	0.04
3	Mesuro	1/16- 4 (4)	50.0 2.5	y = -0.65x + 5.66	-0.98**	0.06
4	Nematof	1/16- 1 (3)	70.0 2.4	(y = -1.25x + 8.01)	-0.98 <sup>ns</sup>	( 0.11)
5	CGA-10576	1/64- 1 (4)	93.0 6.0	y = -1.01x + 7.48	-1.00***	0.12
6	Folidol	1/4 - 4 (3)	75.0 4.3	y = -1.19x + 6.86	-0.99*	0.14
7	Dipterex	1/64- 1 (4)	96.8 9.0	y = -1.07x + 7.93	-1.00***	0.17
8	Basudine	1/16- 4 (4)	83.0 4.2	(y = -0.89x + 6.84)	-0.89 <sup>ns</sup>	( 0.27)
9	Tirpate	1/16- 4 (4)	89.5 1.8	y = -1.12x + 7.38	-0.99***	0.29
10	Temik	1/16- 1 (3)	91.0 16.0	(y = -1.16x + 8.66)	-0.97 <sup>ns</sup>	( 0.31)
11	Phosdrin	1/16- 4 (4)	83.0 9.5	y = -0.75x + 6.97	-0.99**	0.36
12	Furadan	1/16- 4 (4)	87.0 6.4	y = -0.88x + 7.00	-0.99**	0.36
13	Terracur	1/16- 4 (4)	91.5 3.5	y = -1.06x + 7.44	-0.99***	0.38
14	Hoe-2873	1/16- 4 (4)	94.4 6.0	y = -1.05x + 7.64	-0.99***	0.51
15	Folthion	1/16- 4 (4)	83.5 21.5	y = -0.59x + 6.56	-0.99**	0.62
16	AC-92100	1/4 - 4 (3)	81.0 6.5	y = -1.19x + 8.27	-1.00**	0.70
17	Vydate	1/16- 4 (4)	87.5 22.0	y = -0.64x + 6.80	-0.92*	0.77
18	Monam	1/4 - 4 (3)	93.0 28.0	(y = -1.01x + 7.47)	-0.98 <sup>ns</sup>	( 1.84)
19	Bunema	1/4 - 16 (4)	88.5 11.0	y = -0.81x + 7.01	-0.98***	1.96
20	SRA-12689	1/4 - 16 (4)	86.5 19.0	y = -0.66x + 6.77	-0.99***	2.52
21	Eradex	1 - 64 (4)	79.0 3.0	y = -0.90x + 6.70	-0.98**	3.48
22	Thimet	1 - 16 (3)	82.0 15.0	y = -0.97x + 7.86	-1.00**	3.68
23	$\gamma$ -HCH	1 - 64 (4)	83.5 2.6	y = -0.97x + 6.94	-0.99**	3.98
24	Phytosol	1 - 64 (4)	82.0 4.0	y = -0.85x + 7.15	-0.98**	4.30
25	Folimat	4 - 64 (3)	64.0 7.5	y = -0.90x + 7.15	-1.00**	6.83
26	DD	4 - 64 (3)	71.0 9.0	y = -0.94x + 7.45	-1.00**	9.06
27	MCP	4 - 64 (3)	72.0 15.5	y = -0.79x + 7.16	-1.00**	10.91
28	Imugan	4 - 256 (4)	69.0 6.5	y = -0.67x + 6.18	-0.98**	11.31
29	Moresan	4 - 64 (3)	89.3 19.0	y = -0.71x + 6.96	-1.00***	11.41
30	Undeen	4 - 256 (4)	78.0 1.4	y = -1.00x + 6.77	-0.98**	11.63
31	Hox-1901	1 - 64 (4)	77.4 2.0	y = -0.94x + 6.69	-1.00**	12.19
32	Gusathion	4 - 256 (4)	92.7 28.5	y = -0.67x + 7.13	-0.99***	20.02
33	DBCP	4 - 64 (3)	92.0 27.0	(y = -1.01x + 8.45)	-0.95 <sup>ns</sup>	(28.22)
34	Meta-isosystox	4 - 256 (4)	91.6 18.0	y = -0.77x + 7.18	-0.97**	49.87

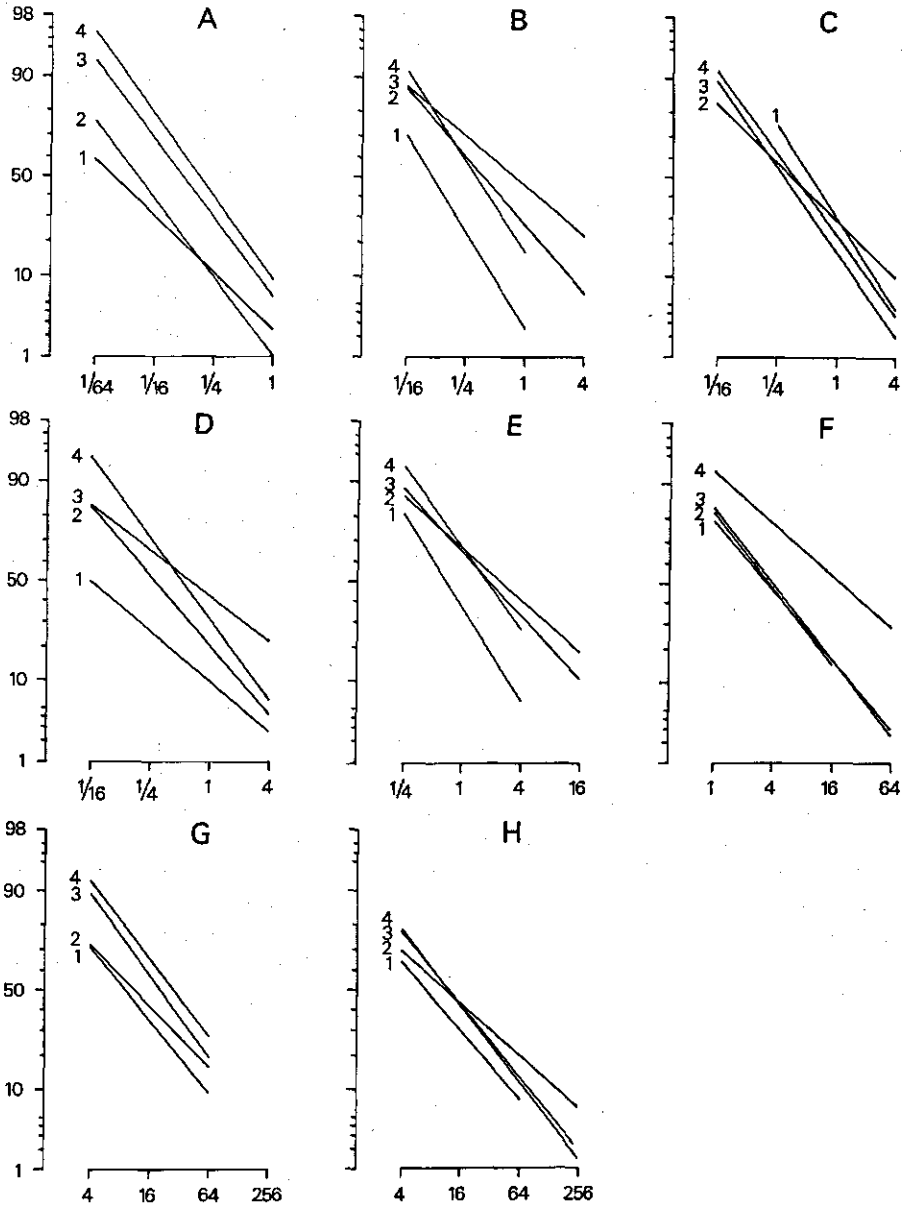


FIG. 12. Results of testing agricultural chemicals with the PI test, expressed as log-probit lines; cf. Table 6 for formulae, EC-50 values and further detailed information about the regression lines of penetration. In this figure 32 compounds showing nematocidal effects are mentioned only. They are indicated in the figure and named at the bottom of p. 46. For unexplained symbols cf. Table 1. ▶

gant nematocides, insecticides, fungicides, sometimes herbicides, or in the case of Eradex an acaricide with fungicidal properties.

It is remarkable that all the nematocides at concentrations below 1 ppm a.i. are known to inhibit acetylcholinesterase in one or more animal groups and they all are organic-phosphate or carbamate compounds. Only half of the 17 compounds active between 1 and 50 ppm a.i. show inhibition of acetylcholinesterase, and none of the 26 inactive compounds has known anti-acetylcholinesterase properties, with some uncertainties regarding benomyl.

The second part of table 5, recording the relatively inactive substances, comprise only few insecticides and only three compounds with nematocidal properties. The rest consists of fungicides, herbicides and some other chemicals. Benomyl may not have been recorded as nematocidal in our test because it has a very low water solubility. A longer incubation period before placing the bean stem parts in the PI test, might have shifted this compound to the group of active chemicals. The nematocidal activity was reported earlier by COOK and YORK, 1972, LAUGHLIN and VARGAS, 1972, MCLEOD, 1972, 1973 and by MILLER, 1969. Benomyl also affects earthworms, *Lumbricus terrestris* (STRINGER and WRIGHT, 1973; WRIGHT and STRINGER, 1973). One of the active compounds from *Tagetes* spp.,  $\alpha$ -terthienyl, was not found nematocidal because we tested the compound in the dark. If tested in the light, the compound has an EC-50 of about 1 ppm, which agrees with the findings of GOMMERS (1972, 1973) and GOMMERS and GEERLIGS (1973).

Some of the ineffective herbicides according to the PI-test could be nematocidal without being traced as effective, because they were so phytotoxic to the stem tissue of field beans that the results were obscured (e.g. Delonine and Chloro-IPC). For Delonine, nematocidal properties have been reported (DECKER, 1969). Other herbicides tested were less toxic to the bean stems than were Delonine and Chloro-IPC. Stem parts treated with glucose and sodiumchloride, were also blackened by phytotoxic reactions at concentrations of 250 ppm. At very high dosages sugar is nematocidal as well as phytotoxic (FEDER, EICHHORN and HUTCHINS, 1963). However, if a compound at 16 or even 64 ppm a.i. is not nematocidal at all, it is unlikely that it will be competitive in practice.

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◀ Abscissae: concentrations of the chemicals, log. scale (= x).  
 Ordinates: percentage of invaded *D. dipsaci* larvae, probit scale = y.  
 Explanation of indications in the figure:

A-1 Dede vap	C-1 Folidol	E-1 AC-92, 100	G-1 DD
2 Nema cur	2 Phos drin	2 SRA-12689	2 MCPP
3 CGA-10576	3 Tir pate	3 Bunema	3 More stan
4 Dipterex	4 Terracur	4 Monam	4 DBCP
B-1 Nema fos	D-1 Mesurol	F-1 Eradex	H-1 Folimat
2 Fura dan	2 Basu dine	2 Thim et	2 Imu gan
3 Vydate	3 Folio thion	3 Lindane	3 Hox-1901
4 Temik	4 Hoe-2873	4 Gusathion	4 Undeen

#### 4.3. CHOICE OF SYSTEMICS

The systemics oxamyl and phenamiphos were chosen for further study.

Oxamyl has already been investigated by the author (BUNT, 1972, 1973). It is a chemical of low phytotoxicity with good systemic and therapeutic effects, and is attractive for further studies, despite contradictions in literature about its effectiveness as a nematicide and about its mode of action. Phenamiphos was chosen from amongst the organic-phosphorus compounds, the second group in which several nematicidal systemics were found during the last decade. Although the dosages recommended for field testing in technical bulletins of the respective companies are the same for phenamiphos and oxamyl, the PI test (cf. 3.2.) indicated that phenamiphos was about 20 times as active as oxamyl (phenamiphos 0.04 ppm and oxamyl 0.77 ppm). Hence phenamiphos was selected for further study with oxamyl.

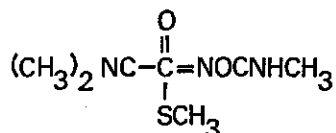
General chemical, physical and biological data for oxamyl and phenamiphos, derived from literature and technical bulletins, are given below.

##### *oxamyl*

Chemical name: methyl N', N'-dimethyl-N-((methylcarbamoyl)oxy)-1-thiooxamidate

Common name: oxamyl (thioxamyl)

Formula:  $C_7H_{13}N_3O_3S$



Trade name/code: Vydate/DuPont-1410

Melting point: 100–102°C; changing to a different crystalline form which melts at 108–110°C

Vapor pressure: practically nonvolatile

Solubility: in water 280,000 ppm (28 gram/100 gram water) at 25°C; also soluble in organic solvents

Formulations: Vydate-L, a 25% liquid formulation that is miscible with water

Vydate-G, a 5 or 10% granular formulation

Toxicology: LD-50 oral: 5.4 mg/kg on non-fasted male rats

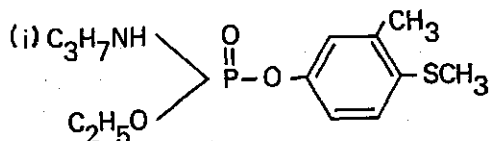
Biological properties: oxamyl, the active ingredient of Vydate, is effective against a wide range of insects, mites and nematodes if applied to plant foliage or soil and is translocated both upwards and downwards in the plant. It is relatively non-phytotoxic to plants.

*Phenamiphos*

Chemical name: ethyl-4-(methylthio)-m-tolylisopropyl-phosphoro-amidate

Common name: phenamiphos

Formula:  $C_{13}H_{22}NO_3PS$



Trade name/code: Nemacur/Bayer 68138

Melting point: 49°C

Vapor pressure: practically nonvolatile

Solubility: in water 700 ppm; well soluble in organic solvents

Formulations: Nemacur-EC, a 40% emulsionable concentrate

Nemacur-G, a 5 or 10% granular formulation

Toxicology: LD-50 oral: 15.3 mg/kg on non-fasted male rats

Biological properties: phenamiphos, the active component in Nemacur, is effective against a wide range of insects, mites and nematodes if applied to plant foliage or soil and is translocated both upwards and downwards in the plant. It has a moderate toxicity to plants.

## 5. DIRECT EFFECTS OF OXAMYL AND PHENAMIPHOS ON NEMATODES IN VITRO

### 5.1. INTRODUCTION

Nematicides which act exclusively as systemics do not affect nematodes *in vitro*<sup>3</sup>. Most present-day systemics, however, have a high oral toxicity to man, mammals and other animal groups, which makes it probable that they affect nematodes also directly. The literature review gives different and contradictory reports. Some workers found little or no effect of systemic carbamates and phosphates on nematodes outside their host (KAAL, 1972; DEN OUDEN, 1971). Others state that these systemics affect plant parasitic nematodes by contact action and that their influence on nematodes inside plants is negligible (HAGUE, 1972; PAIN and HAGUE, 1971a; HAGUE and PAIN, 1973; NELMES et al., 1973).

To understand the mode of action of systemics, *in vitro* experiments to study the effect of oxamyl and phenamiphos were set up. Assessments were as follows:

1. by observation and microphotography;
2. by studying the behaviour of nematodes when continuously exposed to the nematicide;
3. by investigating the recovery of nematodes after temporary poisoning;
4. by studying the reproduction of nematodes removed from the chemical;
5. by measuring the stability of oxamyl in water.

### 5.2. GENERAL OBSERVATIONS AND MICROPHOTOGRAPHS

*D. dipsaci* larvae were exposed to solutions of systemics from 1–10 ppm a.i., and it appeared that movement and behaviour were visibly affected after 10–20 minutes in oxamyl and after 2–4 hours in phenamiphos. Initially nematode movement is stimulated but then gradually slows down; thereafter the nematodes are partially paralysed and the number of body undulations decreases whereas abnormal stylet protrusions are made. All these reactions are concentration-dependant. In high concentrations nematodes become paralysed and seem to be dead.

On close examination under the dissecting microscope, one usually notices light stylet thrusts, pulsations of the median oesophageal bulb and slow, shaky body undulations for several days or even weeks.

The response of *D. dipsaci* larvae exposed to 100–1000 ppm a.i. oxamyl and phenamiphos, differed: oxamyl influenced behaviour of the nematode after 5–10 minutes, but phenamiphos took 2–4 hours to give the same effect. Phenami-

<sup>3</sup> The indication *in vitro* is used here in general for experiments with nematodes in glass vials with water and holds also if plant parts are inserted as bait for the nematodes.



phos, however, killed the nematodes within 1–2 weeks, whereas nematodes treated with oxamyl would recover even though they were in a poor condition.

In oxamyl, their skin became wrinkled, their stylets invisible and the nematodes shortened by 25% due to body contraction. After 3 weeks immersion in 1000 ppm oxamyl some nematodes, however, were still able to resume movement. It appears that oxamyl is less toxic than phenamiphos. If nematodes, treated in 4000 ppm a.i. oxamyl for 3 weeks were transferred to water, some were able to recover completely and in others that part of their body posterior to the intestine resumed normal shape.

In figure 13 suspensions of *D. dipsaci*, untreated and after 24 hours immersion in 10 ppm a.i. phenamiphos, are shown, illustrating the general effects of the treatment on shape and movement of the nematodes. Figure 14 shows *D. dipsaci* 'poisoned' with 4000 ppm oxamyl for three weeks compared to healthy specimens. The wrinkled skins, body contractions and therefore the shortening and thickening of the nematodes are marked. Severe poisoning resulted in invisible stylets, which became visible again if the nematodes were transferred to water. Figure 15 shows a L-4 stage *D. dipsaci* larva exposed for three weeks to 4000 ppm oxamyl, and the same nematode 1, 2, 3 and 4 days after transfer to water, respectively, illustrating the ability of *D. dipsaci* to recover from the poisoning effects of this nematicide. The effects of oxamyl and phenamiphos upon *Pratylenchus penetrans* are shown in figure 16.

Stylet protrusion and crinkling of the body, resulting in shortening and thickening of the nematodes, are the most visible results at 10 ppm a.i. Male specimens of *P. penetrans* often protruded the spicules if immersed in solutions of systemics.

Stylet protrusion, crinkling and paralysis were also observed for *Helicotylenchus* spp., *Paratylenchus* spp., *Tylenchorhynchus dubius*, *Rotylenchus robustus*, *Heterodera rostochiensis* larvae and *Meloidogyne incognita* larvae, but the effects were most pronounced for *P. penetrans* and *D. dipsaci*. *Paratylenchus* spp. showed less visible effects; their movement slowed rapidly down and they became motionless, but in that stage their long stylets were not protruded. The reactions are similar for both chemicals tested and appear to hold for many if not all nematode species, although there are apparently differences in the degree of their reactions.

From these observations, it is clear that systemic nematicides have direct effects upon nematodes, such as kill, change of shape, aberrant behaviour and probably other unknown effects. In concentrations available in the soil water after field application, the main effects are likely to be abnormal behaviour and, therefore, decreasing populations in and around plants.

These chemicals are sometimes indicated as systemics, repellents, nematostats or nematicides. We suppose from our observations, that their main effect on nematodes is acetylcholinesterase inhibition, similar in a general way to their toxic effects on man, mammals, arthropods and other animals. These ideas are discussed further in chapter 5.5.

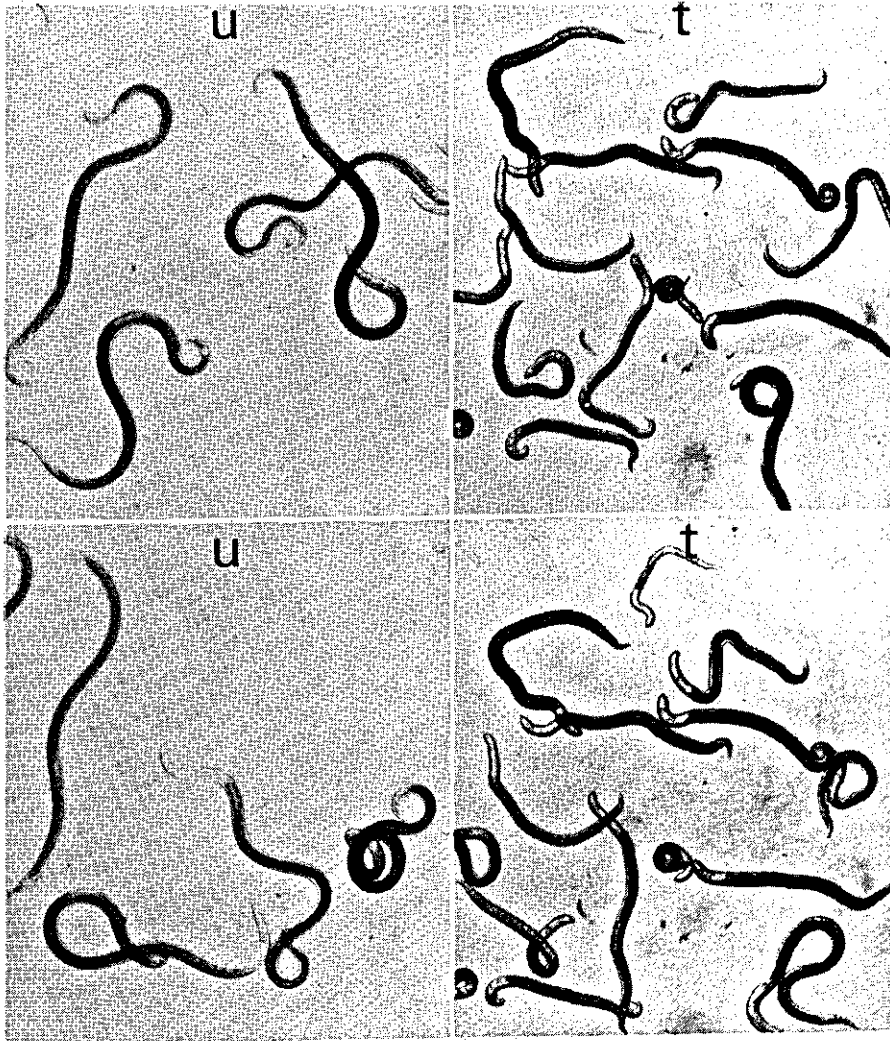


FIG. 13. Microphotographs of suspensions of, mainly, L-4 stage larvae of *D. dipsaci* in water (u) and of *D. dipsaci* immersed in 10 ppm a.i. phenamiphos for 24 hours (t). Bottom row of photographs taken 1 minute after top row, thus illustrating movement patterns of the nematodes. The suspensions have the same nematode density - 25 specimens in a droplet of water on an object-glass - and are all printed at 75 times magnification; less space between treated nematodes indicates less motility and therefore less escape from the photographed microscopic field in which they were originally concentrated.

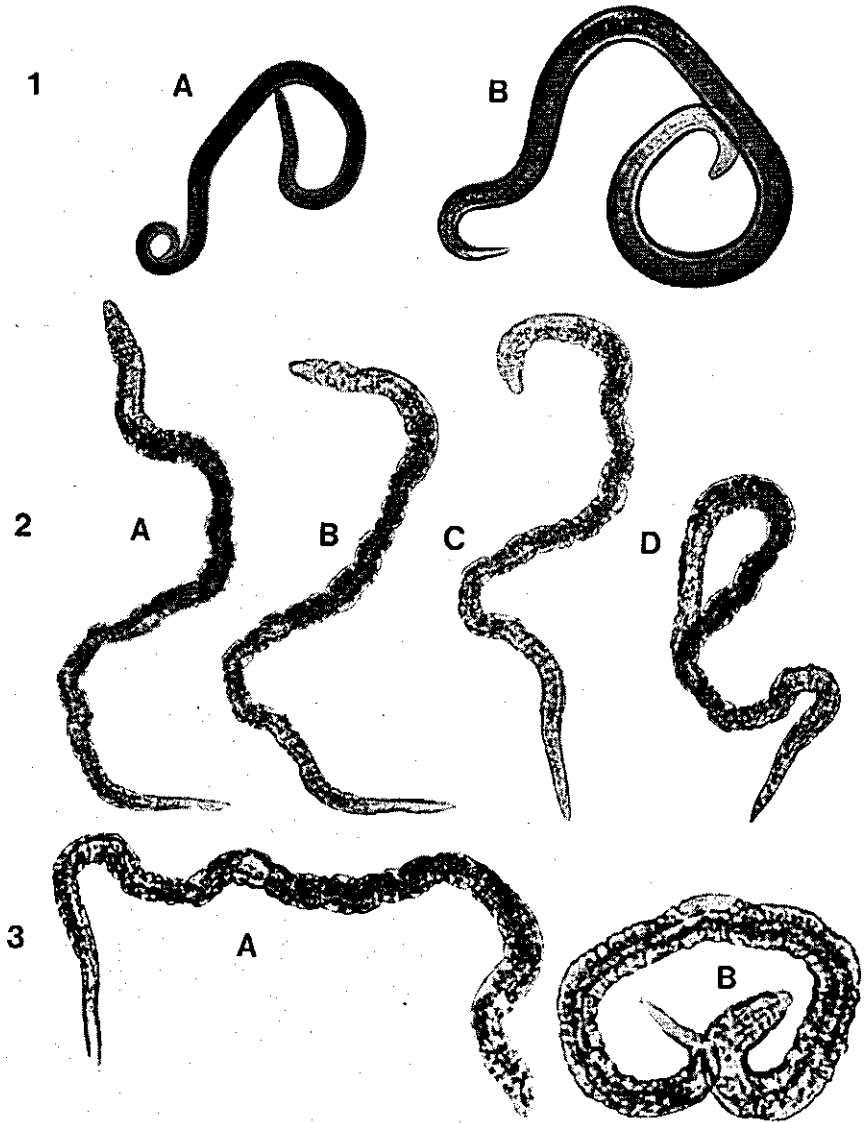


FIG. 14. Microphotographs of an untreated L-4 stage *D. dipsaci* (1) compared to two specimens of L-4 stage *D. dipsaci* dipped in 4000 ppm a.i. oxamyl for 3 weeks (2 and 3). A, B, C and D means that photographs of the same nematode are taken with few minutes intervals; the differences between A-B and between A-B-C-D illustrate that these nematodes still live and move. Magnifications: 1A about 75 times, 1B about 150 times, 2A, B, C, D, about 200 times and 3A, B about 250 times.

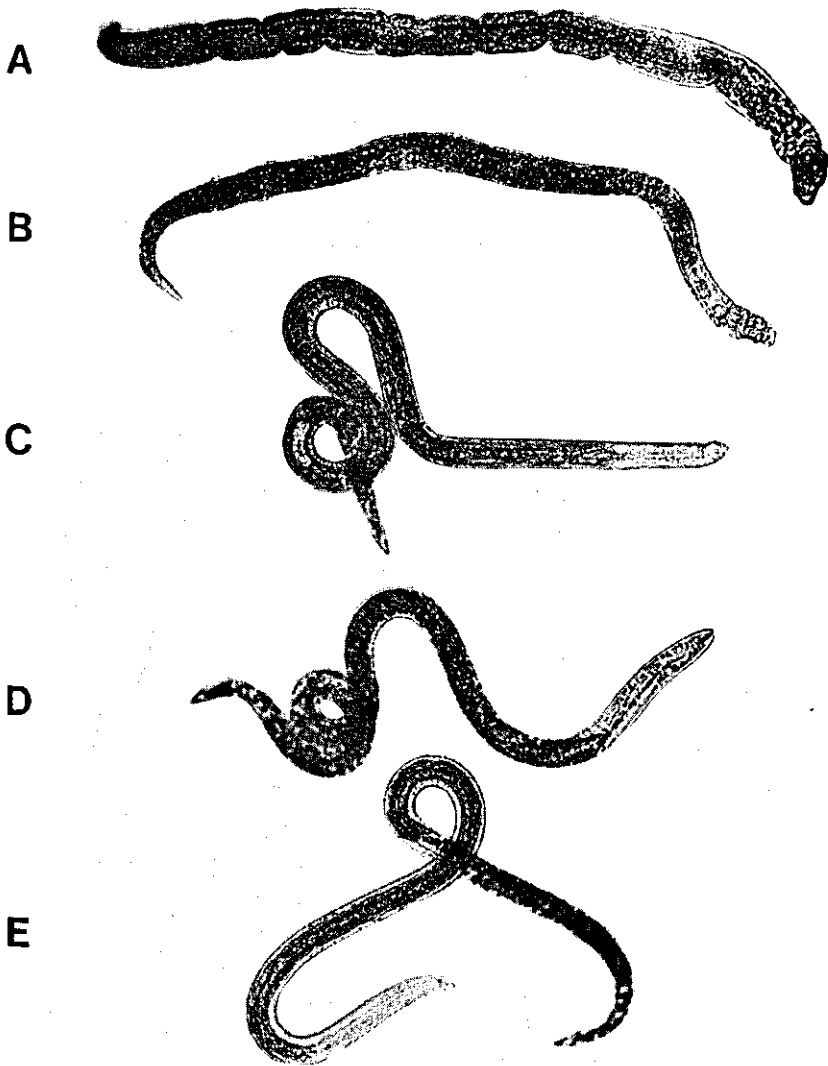
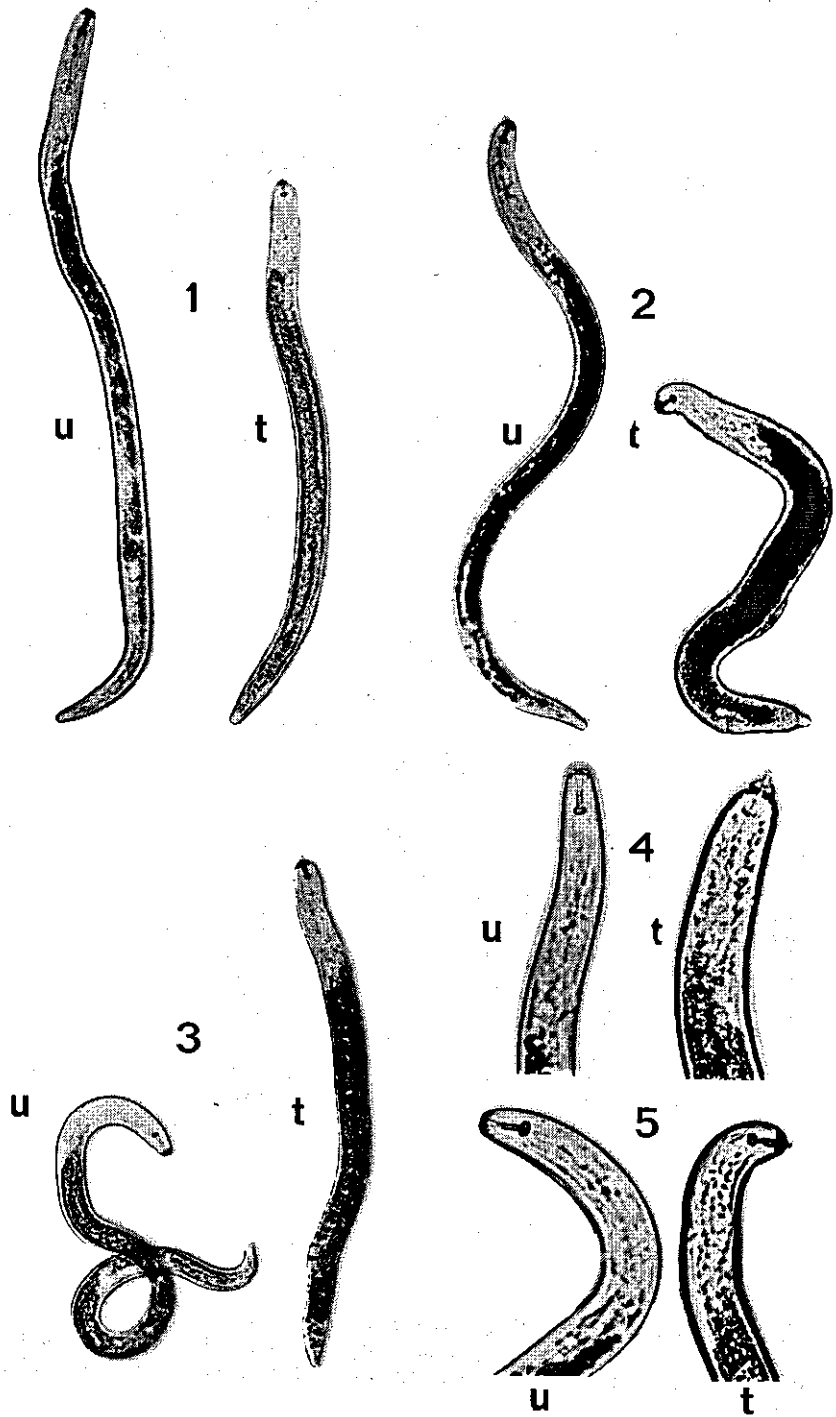


FIG. 15. L-4 stage *Ditylenchus dipsaci* larva which was treated for 3 weeks in 4000 ppm a.i. oxamyl and then transferred to water for recovery. A = before replacement to water, B, C, D and E show the same nematode photographed 1, 2, 3 and 4 days after transfer to water. Magnifications about 200 times.

FIG. 16. Microphotographs of 5 *Pratylenchus penetrans* specimens before treatment (1 to 5, u) and after immersion in 10 ppm a.i. phenamiphos for 24 hours (1, 3 and 5, t) or in 10 ppm a.i. oxamyl for 24 hours (2 and 4, t). Magnifications 1, 2 and 3 about 75 times; 4 and 5 about 150 times.



### 5.3. BEHAVIOUR OF NEMATODES KEPT PERMANENTLY IN SYSTEMICS

L-4 larvae of *D. dipsaci* were kept permanently in oxamyl and phenamiphos 'in vitro', to study the capacity of the nematodes to penetrate plant tissue after different periods of exposure. They were immersed in phenamiphos concentrations of 1/16, 1/4, 1 and 4 ppm a.i. and in oxamyl concentrations of 1, 4, 16 and 64 ppm a.i. ( $\mu\text{g/ml}$  water). On day 1, 3, 7, 10, 14, 17 and 21 after immersion the nematodes' ability to penetrate bean tissue was tested in vials with 2 ml nematicide solution with nematodes + 5 ml sand + a bean stem, therefore as in the PI test (Exp. A).

To determine whether the solutions lost their nematicidal activity during the treatment period, two additional experiments were set up as follows.

Firstly: untreated *D. dipsaci* were dipped in nematicide solutions stored separately at room temperature up till 21 days after the onset of Exp. A, and were then tested after 1 day (Exp. B).

Secondly: the nematicide solutions of Exp. A in which the nematodes had been immersed for 21 days were freed from the nematodes by filtering and were tested again in the PI test with untreated *D. dipsaci* (Exp. C). The Experiments B and C were set up only with the three highest concentrations of the nematicide ranges used in Exp. A.

The results are expressed as regression lines and the derived EC-50 values. Table 7 shows the statistically analysed results of the three Experiments (A, B and C). Figure 17 illustrates the different nematode responses, expressed in penetration power, to oxamyl and phenamiphos if kept permanently in the solutions.

The EC-50 values derived from the regression lines indicate that phenamiphos

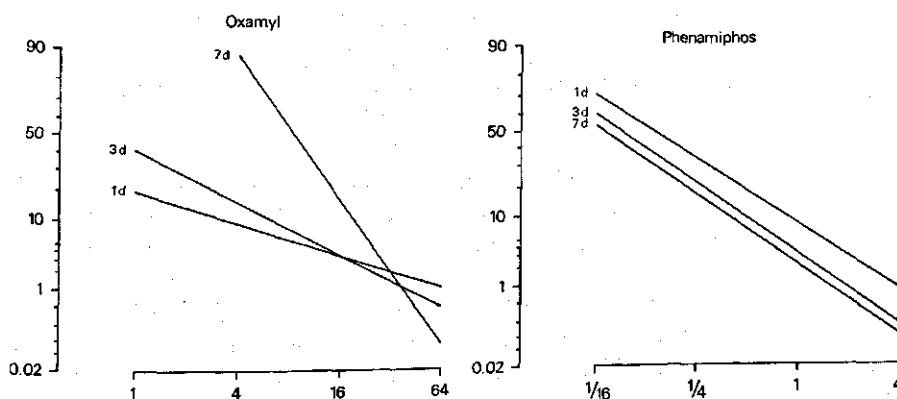


FIG. 17. Dosage-response lines of *D. dipsaci* penetration in *V. faba* stem tissue (PI test), after permanent exposure to solutions of oxamyl and phenamiphos, measured at 1, 3 and 7 days after initiating the treatments in various concentrations as indicated.

Abscissae: concentrations of nematicides in ppm a.i.; log. scale

Ordinates: percentages penetration of *D. dipsaci* against the control; probit scale.

TABLE 7. Effect on penetration of *D. dipsaci* of: 1) dipping in solutions of 1/16, 1/4, 1 and 4 ppm a.i. phenamiphos and 1, 4, 16 and 64 ppm a.i. oxamyl for periods up to 21 days (Experiment A); 2) dipping in nematicide solutions stored separately till 21 days after initiating Experiment A and tested 1 day later (Experiment B); 3) dipping in the 21 days old nematicide solutions of Experiment A after filtering away the old nematodes and tested 1 day later (Experiment C). The effect of the treatments in all three experiments was measured with the PI test; they are expressed as percentages penetration and as the derived regression formulae with corresponding correlation coefficients (r). The EC-50 values read from the log-probit lines are recorded to compare the results. For unexplained symbols cf. Tables 1 and 6. Results of this table are partly illustrated in figure 17.

Examination times in days after treatment	Concentrations usable for statistical analysis	Percentages penetration	Regression formulae	Correlation coefficient (r)	EC-50 in ppm	
<b>PHENAMIPHOS</b>						
Exp. A	1	1/16-4 (4)	70-0.8	$y = -0.98x + 6.49$	-0.98**	0.13
	3	1/16-4 (4)	59-0.2	$y = -1.06x + 6.30$	-0.96**	0.09
	7	1/16-4 (4)	52-0.1	$y = -1.04x + 6.09$	-0.93*	0.07
	10	1/16-4 (4)	81-0.1	$y = -1.43x + 7.29$	-0.95**	0.14
	14	1/16-4 (4)	62-0.3	$y = -1.01x + 6.32$	-0.95*	0.10
	17	1/16-4 (4)	75-0.2	$y = -1.17x + 6.83$	-0.91*	0.14
	21	1/16-4 (4)	27-2.7	$(y = -0.79x + 6.88)$	-0.88 <sup>ns</sup>	(0.13)
Exp. B	21	1/4 -4 (3)	34-2.7	$y = -0.77x + 6.13$	-0.99*	0.12
Exp. C	21	1/4 -4 (3)	45-1.7	$y = -1.00x + 6.88$	-0.99*	0.21
<b>OXAMYL</b>						
Exp. A	1	1-64 (4)	19-1.0	$y = -0.48x + 4.59$	-0.98**	0.08
	3	1-64 (4)	40-0.4	$y = -0.79x + 5.53$	-0.99***	0.64
	7	4-64 (3)	88-0.1	$(y = -2.18x + 10.53)$	-0.97 <sup>ns</sup>	(8.50)
	10	16-64 (2)				(9.25) <sup>1</sup>
	14	64 (1)				(30) <sup>1</sup>
	17	64 (1)				(30) <sup>1</sup>
	21	64 (1)				(36) <sup>1</sup>
Exp. B	21	4-64 (3)	98-6.6	$y = -1.49x + 9.99$	-0.99*	26
Exp. C	21	16-64 (2)				(33) <sup>1</sup>

<sup>1</sup> Estimate, derived only from the concentrations which caused inhibition of penetration.

has strongly inhibited nematode penetration from the first day until at least 21 days, although the effect on day 21 has apparently decreased somewhat, since the EC-50 in Exp. C at 21 days is about twice the EC-50 at the onset of Experiment A.

The EC-50 values for oxamyl increase strongly from day 3 on, which would indicate that oxamyl is unstable in the water or that the nematodes adapted themselves to the nematicide. From Experiments B and C it is clear that oxamyl must have been unstable. Since the PI test registers only the biological activity of the chemicals against the nematodes, the oxamyl concentration in water, at different times after making the solutions, was also determined by gas-liquid chromatography (GLC). Oxamyl (analytical grade) was dissolved in tap water and in demineralised water to 100 ppm concentrations. The solutions were

stored in closed vials at about 2, 12 and 24°C, except for the demineralised water solution which was kept at 24°C only. The samples were analysed in a flame-photometer-detector, after extraction of the oxamyl from the water by chloroform<sup>4</sup>.

The half-life-times (T-1/2) of the chemical in tap water appeared to be 46, 22 and 7 days at 2, 12 and 24°C, respectively; for the solution in demineralised water at 24°C the T-1/2 was 17 days.

It is therefore clear that oxamyl is not very persistent in water, especially not at high temperature. In demineralised water the T-1/2 was more than doubled compared to tap water. This means that less ions, or altered growth conditions for bacteria in the demineralised water, could enhance the stability of oxamyl. Water containing *D. dipsaci*, as in most experiments, may comprise more and also other breakdown-stimulating substances or organisms, which may reduce the T-1/2 of oxamyl to less than a week, as was suggested by the above-mentioned results. This is also roughly in accordance with results of the PI test using *D. dipsaci* kept permanently in oxamyl.

Oxamyl probably breaks down to the sulphoxide and sulphone, and, as NEMES (1971) showed for aldicarb, these breakdown products are probably less toxic for nematodes than the parent compound.

The difference between oxamyl and phenamiphos is also illustrated in figure 17. The slope of the lines reflects the different responses of *D. dipsaci*. As shown in table 7 for oxamyl, the first two examinations of Experiment A delivered statistically significant correlation coefficients for the regression of the penetration only. This means that the regression lines of the 7-days' treatment must be read with reserve, which holds also for the EC-50 values of these and later examinations (10, 14, 17 and 21 days after treatment was initiated). It is, however, clear that these EC-50 values are between 20 and 40 ppm, and it is therefore safe to conclude that oxamyl had disappeared and therefore was unstable in water. The half life span of this chemical in water may be shorter than the two weeks which are reported in literature for oxamyl in soil (ANONYMUS, 1971; BROMILOW, 1973), or *D. dipsaci* is able to escape the poisoning effect of the chemical by breakdown in the nematode itself. The results for phenamiphos are clear-cut and do not suggest loss of effect in the short term and under our conditions.

The regression lines in table 7 show that the PI test gives reliable results. With 8-replicated stem sections of *V. faba*, the EC-50 values are close together (0.07-0.14 for phenamiphos) at the 7 examination times. Rigidly controlled conditions for temperature, light, plant material - stem sections of the same length and from corresponding places of *V. faba* stems - etc. may suppress the variation even further.

<sup>4</sup> The GLC test was kindly carried out by ING. J. SMELT and DR. M. LEISTRA, Laboratory of Insecticide Research (LIO), Wageningen.



#### 5.4. RECOVERY OF TREATED NEMATODES

Leaching of nematicides from plants, soil and nematodes could result in loss of nematocidal activity. Because the concentration of the chemical within nematodes depends on the concentration in the surrounding water (NELMES, 1971), dilution of the chemical in soil by rainfall might reduce the concentration in the nematodes and hence facilitate their recovery.

In four experiments *D. dipsaci* larvae were washed with much water, at different times after treatment with oxamyl and phenamiphos. The effect of washing was tested by measuring penetration of the nematodes in bean stem tissue in the PI test and their reproduction capacity in the T test.

In the experiments *D. dipsaci* larvae were suspended in solutions of oxamyl and phenamiphos. After various exposure times the nematodes were washed free from the nematicides by placing them in a funnel with a 0.02  $\mu$  filter fixed to a suction bottle at a running water tap. The procedure was repeated three times by adding the same amount of water to the nematodes in the funnel as the nematodes were suspended in before washing. The nematodes were sprayed from the filter and stored in erlenmeyer flasks with water closed with cotton-wool plugs at room temperature under normal daylight conditions in the laboratory. Each experiment was set up with enough *D. dipsaci* and chemical solution to allow 8-replicated PI tests for each concentration at each examination time. All the concentrations are expressed as nominal ones in the original nematicide solutions as  $\mu\text{g a.i./ml}$  water. The nematodes were also tested before the chemicals were replaced by water (called 0-day after replacement). The untreated series were handled in the same way, apart for the nematicide treatment. Except for some treatments with oxamyl, nematodes were tested at several times from 0 to 32 or 64 days after washing.

The four experiments were as follows.

1. L-4 larvae of *D. dipsaci* (400/ml) were placed in solutions of oxamyl and phenamiphos at 0, 0.1, 1, 10, 100 and 1000 ppm a.i. for 24 hours. After washing, the oxamyl-treated nematodes were tested up to day 2 and the phenamiphos objects up to day 64 in the PI test. In this experiment the rate of kill at continuous exposure was determined 64 days after the onset of the treatments.
2. A mixed population of L-4 and younger larvae (mostly L-2) of *D. dipsaci* were treated for 24 hours in a 10,000 ppm a.i. solution of oxamyl; then the chemical was replaced by water and the nematodes were tested in the PI test up to 32 days after washing.
3. Phenamiphos 1 and 10 ppm a.i. and oxamyl 1000 and 10,000 ppm a.i., were used to treat a mixture of *D. dipsaci* larvae of the L-4 and younger stages (mostly L-2), for 2 and 4 days, respectively. Then the nematodes were washed and tested for their ability to invade in the PI test up to 32 days after washing.
4. To study the influence of sub-lethal treatments on reproduction, *D. dipsaci* (L-4) was treated with oxamyl at 1000 and 4000 ppm and phenamiphos at 16 and 64 ppm a.i., and after 24 hrs washed thoroughly with water and inoculated onto tomato cuttings as described in the T test, at a density of 500 nema-

todes per plant. After 4 days, the rooted cuttings were washed free from adhering sand and replanted in sterilised soil. At 0, 7, 11 and 16 days after replanting, six replicate plants were examined for stages of *D. dipsaci* inside the stem tissue.

The results are summarised in figures 18 and 19 for Experiment 1, figure 20 for Experiment 2, figure 21 and table 8 for Experiment 3 and in table 9 for Experiment 4.

Penetration of *D. dipsaci* was completely suppressed after 24 hours dip in 1000 ppm oxamyl (Fig. 18). However, if such nematodes were washed and stored in water for 1 day, their penetration power already largely recovered. If the nematodes were washed and stored in water for 2 days, recovery was nearly complete and penetration was similar for all treatments, viz. 75/stem section, as was the case for the untreated control series (not separately shown in Fig. 18).

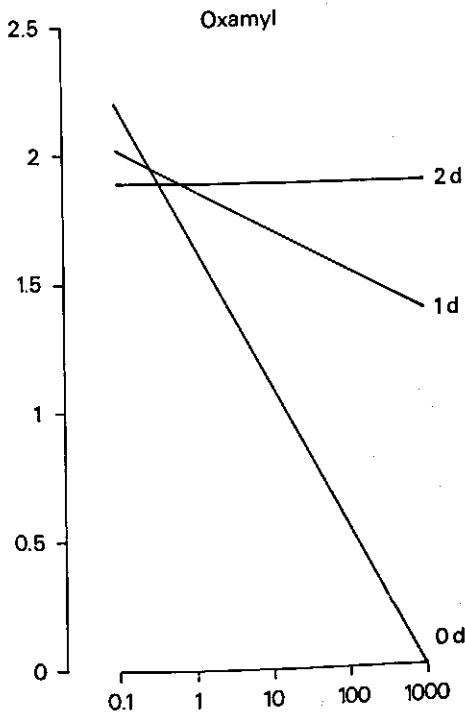


FIG. 18. Regression of the penetration rate of *D. dipsaci* into *V. faba* stems, after 24 hours treatment with oxamyl and subsequent washing with water. Penetration measured at 0, 1 and 2 days after washing as indicated in the graphs. Cf. Table 1 for unexplained symbols.

Formulae of the regression lines:

For oxamyl 0 days:  $y = -0.55x + 2.20$   $r = -0.95^{***}$

For oxamyl 1 day:  $y = -0.16x + 2.02$   $r = -0.77^{***}$

Abscissa: concentration of oxamyl in ppm a.i., log. scale (= x).

Ordinate: number of penetrated *D. dipsaci* per stem section, log. scale (= y).

Phenamiphos, tested upto 64 days after washing the nematicide away, caused different effects (cf. Fig. 19). In this long testing period the untreated nematodes gradually lost their ability to penetrate the host, resulting in a significant decrease in numbers penetrating. Behaviour of the nematodes in 0.1 ppm of phenamiphos was not significantly different from the untreated nematodes; similar results were obtained at 1 ppm phenamiphos treatment 2 days after washing.

On the 8th day after washing, the nematodes treated at 10 ppm were recovered and invaded stem tissue as numerously as the untreated. At 100 and 1000 ppm, however, hardly any recovery occurred.

Concentrations of phenamiphos above 10 ppm obviously cause irreversible poisoning of the nematodes, but oxamyl-treated nematodes recover even at 1000 ppm. In experiment 1, 64 days after washing no kill occurred after 1000 ppm treatment with oxamyl. In the phenamiphos treatments the percentages kill (using the Abbott's correction formula) were 51 %, 73 %, 83 % for 10, 100

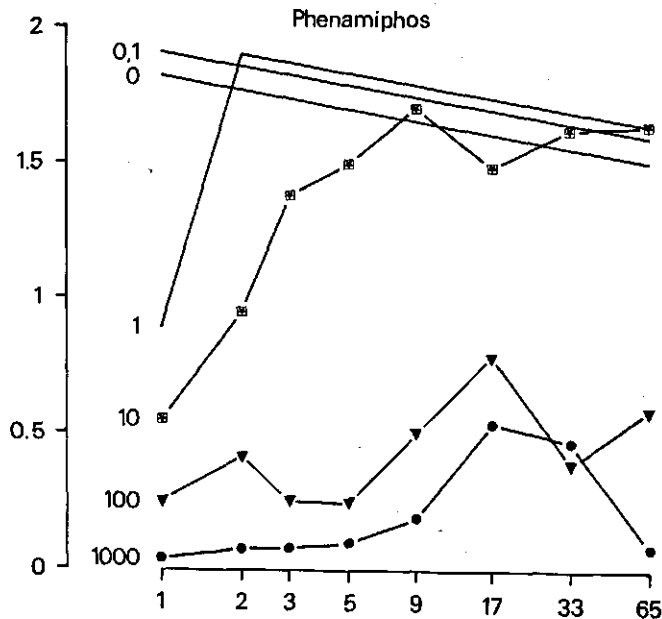


FIG. 19. Regression of the penetration rate of *D. dipsaci* into *V. faba* stems, after 24 hours treatment with phenamiphos and subsequent washing with water. Penetration measured at 0 up to 64 days after washing as indicated on the abscissa. The treatments are indicated in the graphs. Cf. Table 1 for unexplained symbols.

Formulae of the regression lines:

For phenamiphos 0 ppm:  $y = -0.18x + 1.84$   $r = -0.63^*$  ( $n = 8$ ),

For phenamiphos 0.1 ppm:  $y = -0.18x + 1.92$   $r = -0.38^{***}$  ( $n = 56$ ),

For phenamiphos 1.0 ppm:  $y = -0.19x + 1.97$   $r = -0.55^{***}$  ( $n = 48$ ).

Abscissa: time after washing in  $(x + 1)$  days, log. scale ( $= x$ ).

Ordinate: number of penetrated *D. dipsaci* per stem section, log. scale ( $= y$ ).

and 1000 ppm respectively; after permanent exposure for 64 days at 1, 10 and 100 ppm the respective kills were 43%, 71% and 97%. At 64 days exposure with oxamyl the % kills were 29% and 57%, at 100 and 1000 ppm.

Oxamyl, therefore caused only minor irreversible effects, while phenamiphos gave irreversible effects at concentrations above 10 ppm. The quick recovery of *D. dipsaci* from oxamyl treatments is remarkable. In the literature the relatively non-toxic effects of oxamyl to nematodes has been reported, but recovery from such extremely high dosages in a few days after the nematicide had been washed away, has not been previously reported.

The results of Experiment 2, in which *D. dipsaci* was dipped for 24 hours in 10,000 ppm oxamyl, are shown in figure 20 till 32 days after washing the nematodes. Untreated *D. dipsaci* gradually lost penetration power after the 3rd day; this was more pronounced for L-2 than for L-4 larvae. Oxamyl treatment reduced immediate penetration power to zero, but both L-4 and L-2 stage larvae recovered; L-4 faster than L-2. After 2 days there was considerable recovery, indicating that oxamyl has little or no lethal effect on *D. dipsaci* at 24 hours exposure.

The inhibition of penetration caused by oxamyl is really reversible up to very high concentrations of the nematicide. This underlines the problem involved in

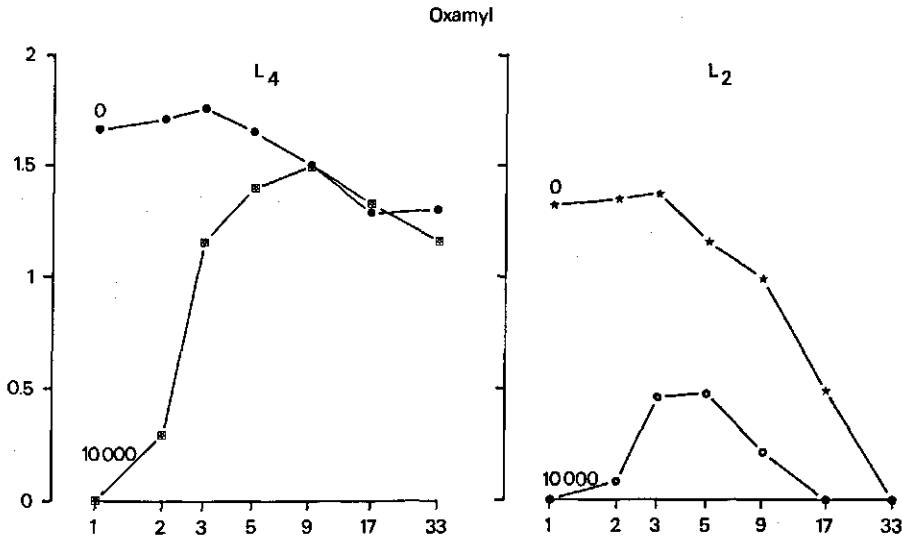


FIG. 20. Penetration rate of *D. dipsaci* larvae into *V. faba* stems, after 24 hours treatment with 10,000 ppm a.i. oxamyl and subsequent washing with water. Penetration measured at 0 up to 32 days after washing as indicated on the abscissae. Left L-4 and right L-2 stage larvae as indicated.

Abscissae: time after washing in  $(x + 1)$  days, log. scale.

Ordinates: number of penetrated *D. dipsaci* larvae per stem section, log. scale.

detecting systemics with tests based on contact effects – resulting in kill of nematodes – only.

The results of Experiment 3 are shown in figure 21 and table 8. Nematodes

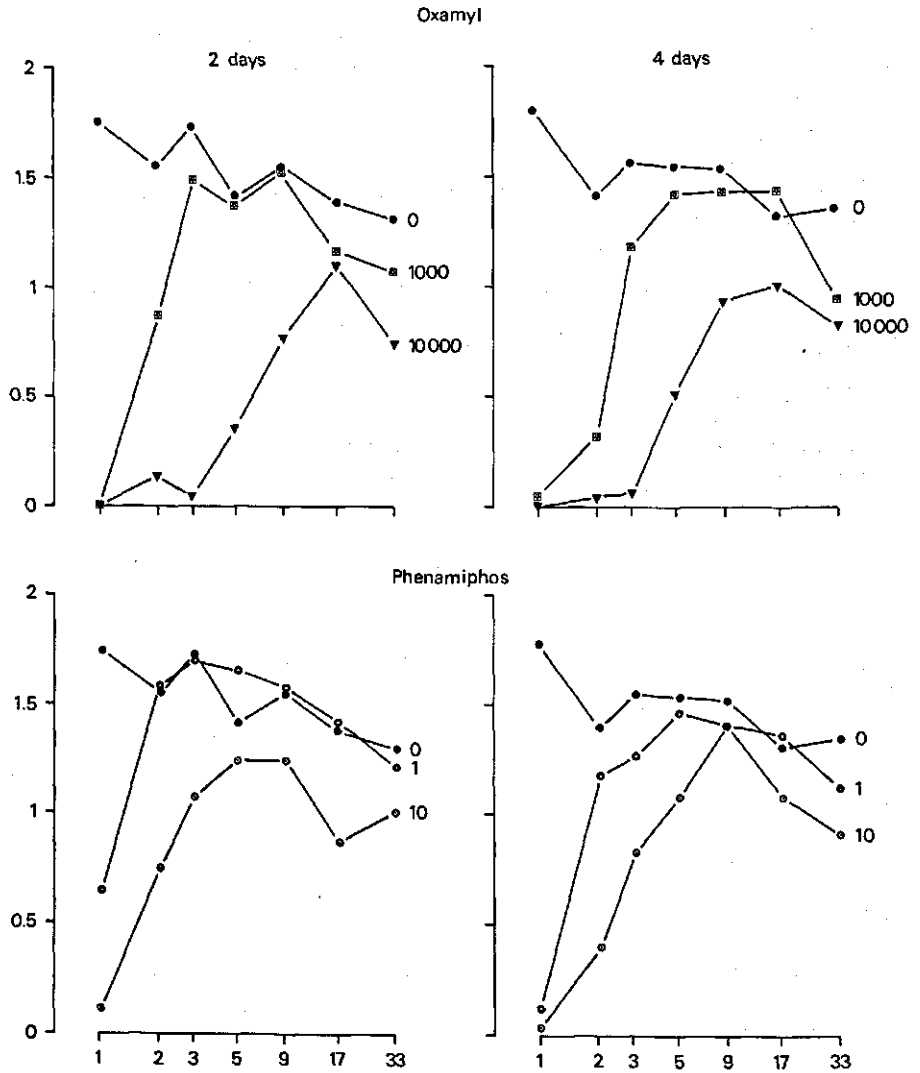


FIG. 21. Penetration rate of *D. dipsaci* L-4 stage larvae into *V. faba* stems, after 2 and 4 days treatments in oxamyl and phenamiphos as indicated in the figures and subsequent washing with water. Penetration measured at 0 up to 32 days after washing as indicated on the abscissae.

Abscissae: time after washing in  $(x + 1)$  days, log. scale.

Ordinates: number of penetrated *D. dipsaci* larvae per stem section, log. scale.

from all treatments were able to recover nearly completely, except at 10,000 ppm a.i. oxamyl when nematodes had not recovered to the level of untreated on the 32-nd day after washing. The time of exposure of the nematodes to the chemicals, 2 or 4 days, was not important, although the effect for the first 2 days (0 and 1 day after washing) was more pronounced if the nematodes were kept in the nematicides longer. Ten ppm a.i. phenamiphos is about as effective as 1000 ppm a.i. oxamyl. Oxamyl at 10,000 ppm caused the most pronounced inhibition and the first signs of recovery were seen 4 days after washing. Comparison of the results with those of experiment 2 (24 hours pretreatment with 10,000 ppm oxamyl and subsequent washing the chemical away by water), indicates that the time of exposure to the chemical in this high concentration is important; if exposed for 1 day the recovery started on the first day after washing and if exposed 4 days (Exp. 3) recovery started on the fourth day.

The response of the L-2 larvae is given in table 8. These young stages of *D. dipsaci* apparently were not able to recover from the 2 and 4 days' treatments with 10,000 ppm oxamyl and hardly so from treatments with 10 ppm phenamiphos and 1000 ppm oxamyl. As mentioned before (Fig. 20) L-2 larvae of *D. dipsaci* recovered partly from a 24 hours' pretreatment with 10,000 ppm oxamyl.

Because nematodes are killed directly by high concentrations of inorganic salts like NaCl, KI, Na<sub>2</sub>SO<sub>4</sub> and others, it seems likely that 10,000 ppm oxamyl kill *D. dipsaci*, especially L-2 stage larvae, by the osmotic pressure of the nematicide rather than by a specific nematicidal effect. SIMONS, 1973, found *Tylenchorhynchus dubius* more sensitive to exposure to 0.05 M solutions of NaCl than *Rotylenchus robustus*. BLAKE, 1961, found a 36 hrs incubation of *D. dipsaci* (L-4)

TABLE 8. Penetration rate of second stage larvae of *D. dipsaci* into *V. faba* stems, after 2 and 4 days treatment with oxamyl and phenamiphos and subsequent washing with water as indicated in the table. Penetration was measured at 0 up to 16 days after washing. Results are expressed as the log. transformed numbers of second stage larvae penetrated per stem section in the PI test. A - indicates that no larvae had penetrated; ox = oxamyl; phen = phenamiphos; concentrations in ppm a.i.

Examination time in days after washing	Exposure time in days before washing; concentrations of nematicides									
	2 days					4 days				
	Untreated	phen-1	phen-10	ox-1000	ox-10,000	Untreated	phen-1	phen-10	ox-1000	ox-10,000
0	1.37	0.14	-	-	-	1.39	-	-	-	-
1	1.03	0.80	0.19	0.19	-	0.88	0.21	-	-	-
2	1.17	0.87	0.21	0.46	-	1.11	0.38	0.14	0.04	-
4	1.01	0.76	0.17	0.66	-	1.19	0.21	0.04	0.20	-
8	0.79	0.40	0.10	0.17	-	0.72	-	-	-	-
16	-	-	-	-	-	-	-	-	-	-

in 1 M urea lethal for the nematodes, which is about comparable to the osmotic strength of a lethal concentration of oxamyl for all stages of *D. dipsaci* after 24 hrs incubation.

The results of Experiment 4 are described in table 9. The nematode counts indicate that the reproduction capacity was not destroyed by the treatments with the systemics. The significantly lower numbers of larvae after treatment in the highest concentrations of the two nematicides are probably due to losses of penetrative power and not to loss of reproductive capacity of penetrated nematodes.

TABLE 9. Reproduction of *D. dipsaci* in tomato plants after non-lethal treatments with oxamyl and phenamiphos and subsequent washing of the nematodes in water. Figures represent the numbers of adults and larvae per plant as means of 6 replicates. Numbers within the same column labelled by the same letter do not differ significantly at the 1% level, according to Tuckey's multiple range test. Mentioned are the mean counts over the 4 examinations.

Treatments	Adult nematodes	larvae
Untreated	134 a	8 a
oxamyl 1000 ppm	100 a	7 a
oxamyl 4000 ppm	33 b	3 b
phenamiphos 16 ppm	76 ab	6 a
phenamiphos 64 ppm	23 b	3 b

Summarising we can conclude that very high concentrations of oxamyl are not directly lethal to *D. dipsaci*, because nematodes recover after several days exposure to concentrations of the chemical even at 10,000 ppm a.i. (or possible more), and the effects caused by oxamyl are at any rate reversible. At concentrations of oxamyl killing *D. dipsaci*, osmotic effects are more likely to be important.

The effects of phenamiphos on *D. dipsaci* are clearly different and are less reversible at the relatively low concentrations (above 10 ppm). Although nematodes are not killed directly if exposed to 100 ppm phenamiphos, they could not recover and invade plant tissue after washing with water, as was the case for the 10 ppm concentration.

Our results indicate that both chemicals do not interfere with reproduction of *D. dipsaci* treated with sub-lethal concentrations.

Finally, the results obtained in the recovery experiments agree with earlier results (5.3.) in which *D. dipsaci* recovered if left permanently in solutions of 1-64 ppm oxamyl, whereas phenamiphos suppressed penetration into plant tissue if the nematodes were kept permanently in solutions of 1/16-4 ppm. This indicates, that oxamyl has a short life in water as well as within the nematodes.

## 5.5. DISCUSSION

The *in vitro* experiments clearly show that oxamyl and phenamiphos directly affect on nematodes as seen under the dissecting microscope (5.2., Figures 13, 14, 15 and 16). *D. dipsaci* can however recover from permanent immersion in oxamyl concentrations up to 16 ppm and perhaps even higher, which can be explained by short life of oxamyl in water and by recovery of the initially poisoned nematodes (5.3. and 5.4.). The same phenomenon could be seen when nematodes were exposed to oxamyl under the dissecting microscope; initially stylet protrusions were observed and normal movement stopped, but behaviour becomes normal again later on, namely at concentrations below 1 ppm after a few days. Nematodes dipped permanently in phenamiphos did not recover, which can be explained by long life of the chemical in water combined with irreversible poisoning of the nematodes.

The recovery experiments indicate, that the effects of short exposures (for 1-4 days) in phenamiphos are reversible for concentrations up to 10 ppm, while *D. dipsaci* can recover from 1-4 days' immersion in oxamyl concentrations up to 10,000 ppm (which may cause osmotic damage). It was demonstrated that recovered *D. dipsaci* were able to penetrate *V. faba* or *L. esculentum* stem tissue, and to reproduce in the normal way, even after exposure to 4000 ppm.

It is not quite certain that the data described in this chapter, are valid for other nematodes and for other organophosphates and organo(oxim)carbamates. They, however, serve as a basis for a hypothesis about the mode of action of the systemic nematicides.

Much is already known about the poisoning effects and the chemical and biochemical background of organophosphates and carbamates in insects and in man and mammals. A recent review by ELSKAMP, MEETER and BERENDS, 1974 about the 'toxicology of organophosphates', indicates that the effects of those pesticides upon vertebrata is disruption of the transport signal in the neurosystem by inhibition of acetylcholinesterase, an enzyme essential in the breakdown of acetylcholin, which is a transmitter substance for the signals to the muscular system. The result is that acetylcholin accumulates, which results in convulsions, paralysis and finally death. For vertebrates the most feared symptom is paralysis of the respiratory muscles and subsequent oxygen shortage to the brain, leading to a quick death. In insects, which have a respiratory system based on diffusion in trachea, the death cause is unknown, as stated by O'BRIEN (1967) as follows: 'Acetylcholine levels in poisoned insects rise sharply in organophosphate poisoning, ... This and the above facts have convinced the majority that cholinesterase inhibition is a biochemical lesion in insects. But whereas for the vertebrates one can trace the physiological consequences of the lesion, no such consequence has been traced in insects. The immediate cause of death is completely unknown'. O'BRIEN (1967) also concluded: 'carbamates react with acetylcholinesterase in a way precisely analogous to the reactions of organophosphates and acetylcholin'. The reactions involved are: first a complex



between acetylcholin and the phosphate or carbamate is formed (1); secondly, this complex reacts to deliver the phosphorylated or carbamylated enzyme (2) and thirdly, the original enzyme could be formed by a hydrolysis reaction called dephosphorylation or decarbamylation (3). The rate constants for these reactions,  $k_1$ ,  $k_2$  and  $k_3$ , respectively, determine the amounts of complex, phosphorylated or carbamylated enzyme and for recovered enzyme. The rate constant for complex formation, a reversible reaction, is  $k-1$ . In general, for organophosphates as well as for carbamates, the affinity constant,  $K_a = k-1/k_1$ , is quite small and therefore complex formation is favoured.

For organophosphates,  $k_2$  is relatively fast and  $k_3$  extremely slow, resulting in an accumulation of the phosphorylated (inhibited) enzyme. In this case the rate constants result in near-complete absence of the complex. From this it follows that organophosphates in general cause irreversible enzyme inhibition, which can only be counteracted by the use of therapeutic agents, directly or shortly after initiation of the poisoning, as described for man by ELSKAMP, MEETER and BERENDS (1974). These therapeutic agents are reagents which react better with the phosphorylated enzyme than with hydroxyl for which the  $k_3$  is too small as already mentioned, and their reaction may lead to induced recovery of the enzyme.

For organocarbamates,  $k_2$  is slower than for organophosphates and  $k_3$ , although smaller than  $k_2$ , is still significant, while  $K_a$  is very low. From this it follows that small amounts of the complex between acetylcholinesterase and the carbamate are present and large amounts of the carbamylated enzyme. If one removes the inhibitor (in our cases by dilution with water or by the quick breakdown of oxamyl itself), the enzyme recovers activity partly by the effect of the  $k_3$  and also by reversal of the complex. A typical value for carbamates of the  $k_3$  is  $0.05 \text{ min}^{-1}$ , from which O'BRIEN, 1967 calculated that the half life of decarbamylation is about 40 minutes, so that fully carbamylated enzyme would be almost fully restored by a few hours of dialysis. Chemically, these 'reversible' reactions are in fact not reversible, because the carbamate is cleaved in the process, but only from the point of view of the enzyme the reaction appears to be reversible. Because in fact the carbamate chemical is steadily destroyed, the enzyme will recover totally if the reactions are allowed to proceed long enough (which is the case in our experiments with nematodes, because they are – even at high concentrations – still alive after many weeks).

From our experiments on permanent exposure of *D. dipsaci* to oxamyl and phenamiphos and on recovery after temporary exposure to these chemicals, the following conclusions may be drawn; taking relevant literature into account:

1. Cholinesterases are found in many nematode species, including *D. dipsaci*, (LEE, 1965; KÄMPFE and DIETZE, 1972; WELLE and BIJLOO, 1965).
2. KÄMPFE and DIETZE, 1972 were able to show the inhibition of cholinesterase with systemic nematicides in nematode sections histochemically and found cholinesterase located in the central nervous system.
3. From our experiments it follows that the poisoning with phenamiphos was

irreversible compared to that of oxamyl, which was reversible for treatment concentrations up to 10,000 ppm a.i. within an exposure period of 24 hours.

4. For both nematicides, we found a slow 'killing effect' on *D. dipsaci*; this was marked for oxamyl even at high concentrations up to 1000 ppm a.i. At rates used under field conditions, kill was achieved rather in weeks than in days (ch. 6).

5. Muscular contractions, paralysis and other symptoms indicating poisoning of nerve systems, were observed in all nematodes examined (5.2.).

Our experiments do not give exact evidence for the biochemical mode of action of systemics in nematodes. The many similarities with their effects in insects and mammals, however, support the hypothesis that they act upon the enzymes involved in the transmission signal to muscular tissues by transmitter substances, such as acetylcholinesterase.

It seems, however, that the enzymes involved and/or the muscular tissue they act upon, are less essential in nematodes than in insects and mammals, because nematodes are killed much more slowly by organophosphates and carbamates. This, however, could perhaps be due to the lack of a circulatory system in nematodes. Oxygen is taken up by nematodes from the environment by diffusion through the body wall and transported to the internal organs and tissues through the pseudocoelomic fluid. Besides this, soil and plant nematodes can withstand low oxygen concentrations (LEE, 1965). Other toxic mechanisms cannot, however, be excluded; more exact information about the chemical and biochemical processes in poisoned and healthy nematodes are needed. Perhaps other enzymes than those studied in insects and mammals may be involved, because organophosphate and carbamate systemics inhibit a wide range of enzymes (OOMS, 1961).

## 6. EFFECTS OF OXAMYL AND PHENAMIPHOS ON NEMATODES AND OTHER METAZOA IN SOIL AND IN PLANTS

### 6.1. INTRODUCTION

The *in vitro* experiments indicated that the chemicals oxamyl and phenamiphos directly affect nematodes. Oxamyl is unstable and phenamiphos relatively stable in water solutions and oxamyl causes reversible and phenamiphos irreversible poisoning of *D. dipsaci* (5.2., 5.3. and 5.4.).

Systemic nematicides are applied in various ways, although basic data about their mode of action and transport in the soil and in the plant are still scarce, incomplete and often contradictory.

In this chapter some short- and long-term experiments are described studying the effects of various methods of application of oxamyl and phenamiphos, namely soil drenches around plants with established nematodes in roots and stems (6.2.), root dips against *P. penetrans* (6.3.), foliage treatment (6.4.), treatments of microplots with and without a test plant against nematodes and other *Metazoa* (6.5.), and a field trial including repeated applications (6.6.). The results are discussed under 6.7., referring also to earlier *in vitro* studies (Ch. 5.) and to more thorough observations of some important side effects in chapter 7.

### 6.2. SOIL DRENCH

An extensive literature review on application of systemic nematicides to the soil and to the plants, is given under chapter 1.4. From the results reported in literature about their effectiveness as soil drenches, it is clear that they offer possibilities of eradicating established nematode populations in roots. The effectiveness of oxamyl and phenamiphos applied as a soil drench against nematodes inside plants was investigated at different temperatures and on different soils.

#### 6.2.1. *Treatment of Pratylenchus penetrans at different temperatures*

##### Experiment

Winschoten soil, infested with a high density of *P. penetrans*, was drenched with oxamyl and phenamiphos to control the nematodes in the roots of *Ligustrum ovalifolium*. The experiment was conducted at 10, 15, 20 and 25°C.

Young plants of *L. ovalifolium* were obtained by rooting top-cuttings in the greenhouse following normal procedures. After four weeks uniform, well-rooted plants were transplanted in plastic pots containing 1 litre Winschoten soil with *P. penetrans* (cf. 2.3.) and placed at 15°C. One month later the young

plants had started to grow and were used for 5 replicates of 7 treatments (untreated control and 6 nematicide objects), at the 4 temperatures and evaluated at 4 examination dates (4, 12, 20 and 28 weeks after treatment). The plants were placed as a randomised block design in each of the four temperature compartments and precautions were taken to avoid unwanted variability. Directly after transfer to the temperature compartments, the planted soil was drenched with 100 ml per pot of the nematicide concentrations to reach 1, 4 and 16 ppm a.i. (mg/litre soil). A saucer under the pots prevented loss of nematicides by leaching. After the drench, the soil was allowed to dry before watering was resumed. Control plants received water only. The number of *P. penetrans* per root system at the time of the drench treatment varied from 400–600 and was about 500 as average per 5 replicate plants. On 4, 12, 20 and 28 weeks after the drench treatment, 1/4 of the plants were uprooted and evaluated. The weights of plant shoots (leaves and stems) and roots were determined as well as plant height. *P. penetrans* were extracted from the roots and the nematode population in the soil determined. It should, of course, be realised that in this experiment curative treatments were applied to plants which had grown in infested soil already for 1 month.

## Results

### A. *P. penetrans* in roots

Because the nematicide treatments hardly influenced root growth, the numbers of *P. penetrans* are shown per root system in figure 22. The following conclusions can already be drawn:

- a. Oxamyl and phenamiphos both reduce the numbers of *P. penetrans* in roots of *L. ovalifolium* initially, more so at higher concentrations. The effect of concentration is greater for phenamiphos than for oxamyl, particularly if the lowest and highest concentrations of both nematicides are compared, but all concentrations except 1 ppm are effective for at least 6 months.
- b. The highest concentration of both nematicides (16 ppm) gives a high degree of control; phenamiphos seems to be slightly more effective than oxamyl.
- c. The effect of temperature was marked but complex, as was the case in earlier experiments by the author (BUNT, 1973). At 10°C nematode numbers rise with time, although 4 weeks after soil treatment the numbers at 10°C were somewhat lower than at higher temperatures. Degree of control, nematode reproduction rate and plant growth may be expected to influence nematode density with time. The results at low temperature, however, are remarkable and may be important in practical application. Phenamiphos is more effective than oxamyl at 20 and 25°C and at 16 ppm phenamiphos may give long-lasting, highly effective control. Complete control would be particularly important for cleaning the roots of transplant crops.
- d. The effectiveness of oxamyl and phenamiphos appear to vary with temperature and time of sampling. In table 10 a summary is given where oxamyl was superior (1) or inferior (0) to phenamiphos. A certain regularity can be traced:

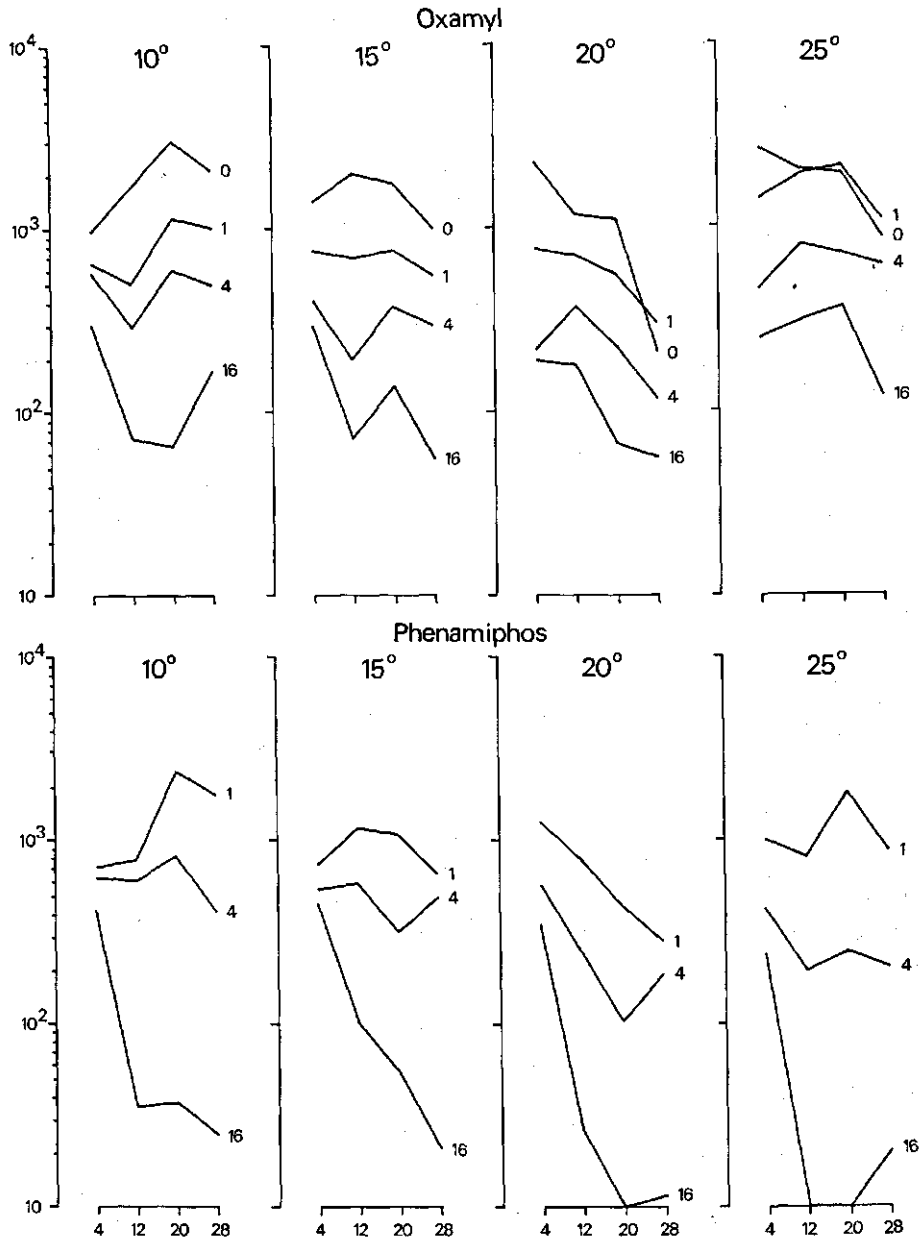


FIG. 22. Density of *P. penetrans* inside the roots of *Ligustrum ovalifolium* plants after a soil drench with 0, 1, 4 and 16 ppm oxamyl and phenamiphos with time and temperature as indicated in the figure.

Abscissae: Time in weeks after soil drench.

Ordinates: Number of *P. penetrans* per plant as mean of 5 replicates; log. scale.

TABLE 10. Frequency that oxamyl (1, 4 and 16 ppm) was superior (1) or inferior (0) to phenamiphos (1, 4 and 16 ppm) against *P. penetrans* in roots of *L. ovalifolium* at 4, 12, 20 and 28 weeks after a soil drench was given to infested plants at 10, 15, 20 and 25°C.  $\Sigma$  = sum.

Temperatures	10°C				15°C				20°C				25°C								
	4	12	20	28	4	12	20	28	4	12	20	28	4	12	20	28					
Evaluation time																					
ppm a.i.				$\Sigma$				$\Sigma$				$\Sigma$				$\Sigma$					
1	1	1	1	1	4	0	1	1	1	3	1	1	0	0	2	0	0	0	0	0	
4	1	1	1	0	3	1	1	0	1	3	1	0	0	1	2	0	0	0	0	0	
16	1	0	0	0	1	1	1	0	0	2	1	0	0	0	1	0	0	0	0	0	
	$\Sigma$	3	2	2	1	8	2	3	1	2	8	3	1	0	1	5	0	0	0	0	0

at 10 and 15°C oxamyl was superior to phenamiphos for at least 12 weeks after treatment, but from then on phenamiphos was usually more effective. At 20°C oxamyl was superior for 4 weeks only. At 25°C phenamiphos was superior to oxamyl in all respects. At 16 ppm the results with phenamiphos were similar to those with oxamyl at the lower temperatures, but phenamiphos was certainly superior at 20 and 25°C. Oxamyl, therefore, is superior to phenamiphos at lower dosages, at lower temperatures and for the shorter times after treatment. At higher dosages, at higher temperatures and longer periods after treatment phenamiphos was superior to oxamyl.

The statistical analysis shows that all main effects and all interactions are highly significant for numbers of *P. penetrans* per plant as well as for numbers per gram of root. The influence of concentration on effect was more pronounced for phenamiphos than for oxamyl.

An additional experiment with the same treatments and with 5 replicates per treatment, was done to study the effect of transferring treated plants from 10 to 20°C. Plants were grown for 12 weeks at 10°C and then 16 weeks at 20°C. The results are comparable to those at 28 weeks in figure 22, which indicates that permanent low temperature may promote good nematode control by oxamyl, but that this beneficial effect does not appear when the plants are transferred to 20°C after 12 weeks.

#### B. Nematode community in soil

In the soil the densities of *P. penetrans* were relatively low during the experiment (100–300/100 ml of soil); the same was true for the other plant parasitic nematodes, namely *Tylenchorhynchus* spp. (50–100), *Helicotylenchus* spp. (50–200) and *Trichodorus* spp. (50–100). There were numbers of other tylenchid (*Tylenchus* spp. and *Psilenchus* spp.) and saprozoic nematodes. Table 11 gives total nematode numbers (plant parasites and saprozoic); table 11A gives the frequency that oxamyl was superior or inferior to phenamiphos. None of the

TABLE 11. Total nematode numbers (plant parasites + saprozoic) per 100 ml soil under *L. ovalifolium*, after soil drenches with 1, 4 and 16 ppm oxamyl (ox.) and phenamiphos (ph.) with time (4, 12, 20 and 28 weeks after treatment). Cf. table 11A.

Temperature	10°C				15°C				20°C				25°C			
	4	12	20	28	4	12	20	28	4	12	20	28	4	12	20	28
ppm a.i.																
Untreated	2210	2290	4060	6140	1940	4870	5000	6600	3300	3480	3865	5000	1830	1880	2010	700
Ox. 1	1320	1010	2190	3220	1320	2110	5255	7680	1380	3870	4365	5000	1660	1260	2390	840
Ox. 4	620	630	1380	1730	740	770	4220	5260	840	2970	3560	5080	740	960	2200	840
Ox. 16	690	320	570	1340	580	450	1400	8180	360	2360	4280	3620	770	760	1750	860
Ph. 1	1470	1560	1470	2040	1760	2040	3220	5460	1250	2300	4055	12640*	980	970	1370	500
Ph. 4	1230	760	800	830	1340	950	1470	2460	1020	1260	2135	2740	1120	490	730	740
Ph. 16	980	530	620	430	880	370	320	320	500	300	75	680	420	180	270	310

a. Community with extremely high number of saprozoic nematodes

treatments was very effective against soil populations at any of the temperatures. There was originally some effect, increasing with dosage, but hardly influenced by temperature except for 25°C, at which temperature soil nematode populations fell. The unfavourable effect of 25°C increased with time. There were also differences between nematode groups; at low temperature saprozoic and at high temperature plant parasitic species were more susceptible to the nematicide treatments.

TABLE 11A. Frequency that oxamyl (1, 4 and 16 ppm) was superior (1) or inferior (0) to phenamiphos (1, 4 and 16 ppm) against the nematode community in total (plant parasites + saprozoic) in soil under *L. ovalifolium* at 4, 12, 20 and 28 weeks after a soil drench was given at 10, 15, 20 and 25°C. Cf. table 11.  $\Sigma$  = sum.

Temperatures	10°C				15°C				20°C				25°C								
	4	12	20	28	4	12	20	28	4	12	20	28	4	12	20	28					
ppm a.i.					$\Sigma$				$\Sigma$				$\Sigma$				$\Sigma$				
1	1	1	0	0	2	1	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0
4	1	1	0	0	2	1	1	0	0	2	1	0	0	0	1	1	0	0	0	0	1
16	1	1	1	0	3	1	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0
	$\Sigma$	3	3	1	0	7	3	1	0	0	4	2	0	0	1	3	1	0	0	0	1

Table 11 and 11A are based on mean samples (1 or 2 per sampling date). Although these results must be taken with reserve, the following observations were made:

- a. Oxamyl and phenamiphos, both, have a direct effect on nematodes in soil, and saprozoic nematodes are at least as susceptible as plant parasites. The percentage reduction compared with untreated is about 25–75%, depending on concentration and time of sampling; this is low compared with the 90–99% control given by fumigants.
- b. The response of nematodes in soil seems to follow the same pattern as for *P. penetrans* in the roots. Oxamyl seems to be somewhat superior to phenamiphos at 10°C up to 12 weeks and at 15°C up to 4 weeks after treatment, but phenamiphos becomes superior to oxamyl with increasing time and at high temperatures.
- c. If the three main groups of nematodes (known plant parasites, other *Tylenchida* and saprozoic nematodes) are considered separately, the concentration effect is significant for all groups in all objects, except for plant parasites on the 4 and 28 weeks evaluation dates. These results are not recorded separately in the table.

### C. Growth response of *L. ovalifolium*

Time and temperature had much influence on seedling growth, as could be expected. Shoot length and weight differed significantly between objects and also the interaction between time and temperature was significant (cf. Fig. 23).

The influence of the nematicides was also significant; but was not great. The statistical analyses of some interesting criteria are summarised in table 12. Oxamyl significantly influenced plant height, whereas phenamiphos did not. Because the 16 ppm dose of oxamyl showed some leaf scorching at 20 and 25°C, the effect of oxamyl on growth may be masked. Phenamiphos showed no visible leaf scorching, but its good nematode control combined with lack of growth stimulation suggests that phenamiphos must have had some phytotoxic effect. Earlier work by EISSA (1971) indicates that *Ligustrum* is relatively sensitive to several soil disinfectants.

The influences of the nematicides on shoot weight was significant; but small considering that a much lower density of *P. penetrans* in the soil can damage plants (OOSTENBRINK et al., 1957). *L. ovalifolium* has apparently not been very susceptible to damage by nematodes and was not a very suitable host for *P. penetrans* under the conditions of the experiment – see the low density of *P. penetrans* in the roots of untreated plants at 28 weeks (cf. Fig. 22). The same soil planted with apple seedlings (*Pyrus malus*) indicated that apple was very susceptible to damage by this nematode and that oxamyl enhanced seedling growth much at 20 and 25°C (BUNT, 1973).

Root weight was also stimulated by the nematicidal treatment (Table 12) but this stimulation was slight and there was no significant difference between oxamyl and phenamiphos.



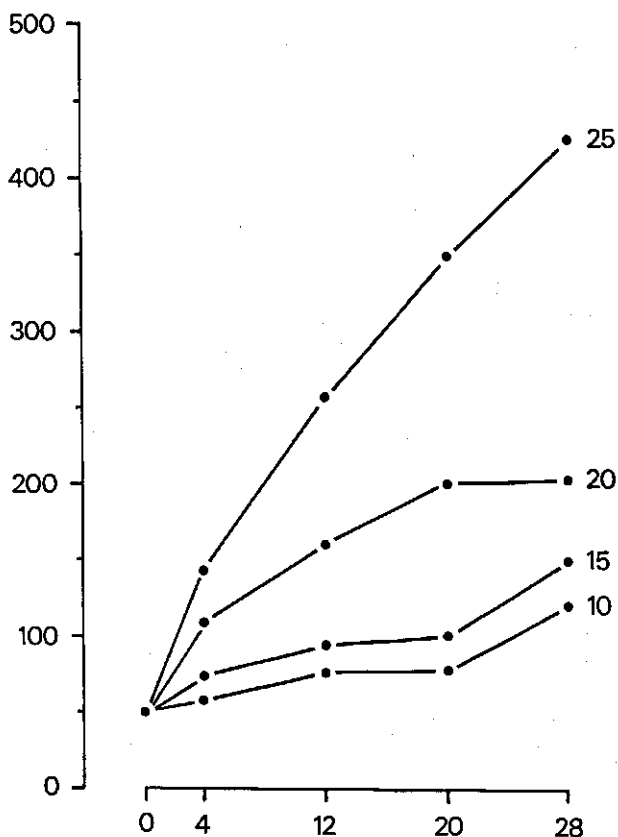


FIG. 23. Weight of *Ligustrum ovalifolium* plants with time at different temperatures (indicated).

Abscissa: Time in weeks after soil drench.

Ordinate: Aerial plant weight in grams per 5 plants; means over all treatments.

TABLE 12. Summary of growth responses of *L. ovalifolium* to soil drenches with 1, 4 and 16 ppm oxamyl and phenamiphos, with indications of statistical significance.

Treatments	Average height increase in cm per plant per 4 weeks	Average shoot weight in g per plant over 28 weeks	Average shoot weight in g per plant over 28 weeks
Untreated	3.28 a <sup>1</sup>	4.23 d	5.56 g
Oxamyl 1	3.87	4.72 e	5.94 h
Oxamyl 4	3.87 } b		
Oxamyl 16	4.00		
Phenamiphos 1	3.66		
Phenamiphos 4	3.65 } c	4.55 f	6.03 i
Phenamiphos 16	3.66	4.23/4.64***	5.56/5.99***
Oxamyl 1, 4, 16 ppm			
Phenamiphos 1, 4, 16 ppm		ns	1/16**
Control/nematicides			
Concentrations (1/4/16 ppm)	ns		

1. Cf. table 1. a/b\*\*, a/c<sup>ns</sup>, b/c<sup>ns</sup>, d/e\*\*\*, d/f\*\*, e/f\*\*\*, g/h<sup>ns</sup>, g/i<sup>ns</sup>, h/i<sup>ns</sup>.

Comparison of the results in figure 23 and table 11 indicate that 25°C is very favourable for *L. ovalifolium* and unfavourable for the nematode populations as a whole, including *P. penetrans*.

### 6.2.2. Treatment of *Ditylenchus dipsaci* in different soils

In two experiments, on different soils with respect to organic matter content, the effectiveness of oxamyl and phenamiphos to eradicate *D. dipsaci* from tomato seedlings, *L. esculentum*, was investigated.

#### Experiment 1

Three weeks old tomato seedlings, heavily infested with *D. dipsaci* by the T test procedure (3.3), were replanted to 100 ml pots with sand or soils containing 12.5%, 25% and 50% organic matter. After replanting, the soil was drenched with oxamyl 10 ppm, phenamiphos 10 ppm or water (untreated); replication was 3-fold. At the time of treatment the plants showed severe symptoms of *D. dipsaci* attack, such as swellings and discoloration at the base of the stem, every plant having about 500 specimens of *D. dipsaci*. A fortnight after treatment plants were uprooted and washed free from soil. Nematodes were extracted by placing the longitudinally cut stems in water.

#### Results

The results are reported in table 13 and figure 24. The effect of treatments differed significantly from each other (\*\*\*). Both nematicides caused a high degree of nematode control inside the plant tissue, higher for oxamyl than for phenamiphos. The interaction between nematicides and soil type was also significant (\*\*). The effect of the soil type on nematode numbers inside the stem tissue as average for all treatments is given in figure 24. The significant correlation coefficient of this regression line shows much higher nematode densities in soils with high content of organic matter.

#### Experiment 2

In this experiment about 15 *D. dipsaci* were inoculated into the stem of every plant in a T test and 5 days after inoculation the first symptoms of attack were visible on some stems. From the inoculated plants 10 were picked out at random

TABLE 13. Numbers of *D. dipsaci* extracted from stems of *L. esculentum* seedlings 2 weeks after a soil drench with 10 ppm oxamyl or phenamiphos. Between brackets log. transformed numbers and LSD-values. Figures are numbers per plant; means of 3 replicates.

Treatments	<i>D. dipsaci</i>
Untreated	3316 (3.52)
Oxamyl 10 ppm	192 (2.28)
Phenamiphos 10 ppm	355 (2.55)
LSD .05	(0.14)
.01	(0.19)

FIG. 24. Density of *D. dipsaci* inside the stems of *L. esculentum* at different organic matter levels of the soil, expressed as plant average for all treatments, i.e. 9 plants. Abscissa: Organic matter percentages of the soil (v/v). Ordinate: Numbers of *D. dipsaci* per plant; log. scale.

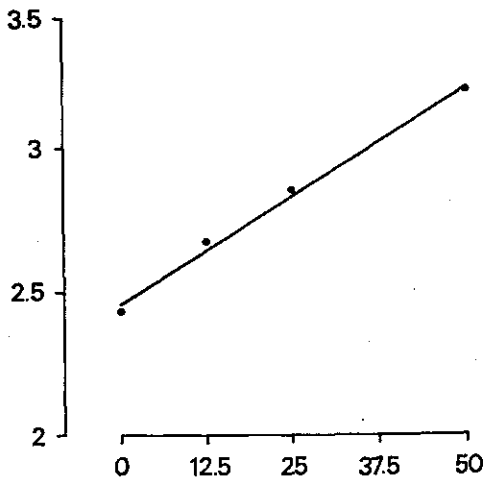


TABLE 14. Numbers of different *D. dipsaci* stages (eggs, larvae and adults), from *L. esculentum* seedlings grown in two soils, extracted two weeks after soil drenches with 1, 4 and 16 ppm oxamyl and phenamiphos, expressed as total numbers per 10 plants. Plant weight and symptoms caused by *D. dipsaci* are also recorded.

Treatments in ppm a.i.	Plants		Nematodes			
	Weight/plant	Symp- toms <sup>1</sup>	Eggs	Larvae	Adults	Total
<i>River sand</i>						
Untreated	4.23	27	377	1797	241	2415
Oxamyl 1	4.37	16	24	340	55	419
Oxamyl 4	4.11	10	2	4	44	50
Oxamyl 16	3.62	5	0	2	29	31
Phenamiphos 1	4.24	7	0	0	25	25
Phenamiphos 4	4.81	6	0	0	22	22
Phenamiphos 16	3.66	9	0	0	15	15
<i>Potting soil</i>						
Untreated	16.13	21	102	808	158	1068
Oxamyl 1	18.60	15	16	51	54	121
Oxamyl 4	17.83	4	0	5	46	51
Oxamyl 16	16.37	3	0	1	44	45
Phenamiphos 1	18.12	18	8	93	58	159
Phenamiphos 4	17.66	11	0	7	37	44
Phenamiphos 16	16.40	5	0	0	33	33

1. In fact symptoms were divided into four groups: 0 = no symptoms; 1 = local swellings of small size; 2 = local swellings of moderate size; and 3 = swellings covering a great part of the stem bases. The maximum number for 10 plants is thus 30.

for every treatment. Two soils were used, and seven drench treatments were applied to each. The treatments are indicated in table 14, which summarizes also the results.

### Results

Symptoms, number of nematode eggs, larvae and adults, as totals of the 10 replicates are recorded in table 14. The eggs were mainly produced by egg-laying females during extraction, which took a 48 hours period.

The results indicate that plant growth was strongly influenced by soil type; the best growth being obtained from potting soil. Both nematicides were phytotoxic in sand at 16 ppm, but in potting soil treatment did not influence plant growth significantly. There was some evidence that symptoms could be related to the total numbers of nematodes, number of larvae and number of adults in that order of importance.

The numbers of eggs and larvae per plant were too low in most treatments to permit statistical analysis, but the influence of the different treatments was obvious. Oxamyl as well as phenamiphos were very effective in both soils, although somewhat more effective in sand, especially phenamiphos. Phenamiphos was perhaps superior to oxamyl against adults and total numbers in sand, but this was not so in potting soil. On both soils the nematicides were less effective at 1 ppm dose than at 4 and 16 ppm (\*\*\*) .

From these two experiments it appears that the effectiveness of oxamyl is only slightly affected by the organic matter content of the soil, whereas it reduces the nematicidal effect of phenamiphos treatments. BROMILOW (1973) states that the partition coefficient of a chemical in soil influences its effectiveness in soils with different contents of organic matter. The partition coefficient ( $Q$ ), gives an indication for the quantitative distribution of a chemical in the soil organic matter and the soil water:  $Q = \text{chemical concentration in the soil organic matter} / \text{chemical concentration in the soil water}$ . For phenamiphos this  $Q$ -value is relatively high, viz. 130, and for oxamyl low, viz. 2. The facts that nematodes live in the soil water and that plants take up chemicals via the soil water makes this  $Q$ -value important and explains why phenamiphos lost its relatively higher effectiveness over oxamyl in the potting soil compared to the sandy soil.

### 6.3. ROOT DIP

As already discussed under 1.4.2., systemic nematicides can be used to eradicate plant parasitic nematodes from infested plant material, such as roots, bulbs, tubers and other transplants. An experiment was set up to study the effect of oxamyl and phenamiphos against *P. penetrans* inside the roots of *L. ovalifolium*.

### 6.3.1. Treatment of *P. penetrans*

#### Experiment

Roots of *L. ovalifolium* seedlings with about 1000 *P. penetrans* per root system, were placed in solutions of 10 ppm a.i. oxamyl, 10 ppm a.i. phenamiphos or water (control) for 1 week. Nematodes leaving the roots during this week were collected and counted (A). The roots were then removed from the stems, weighed, cut to 1 cm pieces and put in a funnel-spray apparatus. One week later the nematode catch was again collected and counted (B). The total numbers extracted ( $A + B = C$ ) indicate the response of the nematodes to the nematicide treatments in general.

#### Results

The results are summarised in table 15. Oxamyl has inhibited the nematodes from leaving the roots as long as oxamyl was present (A) but this inhibition was reversible (B). Phenamiphos, however, caused an irreversible inhibition; the nematodes did not leave the roots during the treatment and they did not recover from the inhibition in water in the funnel-spray extraction during the next week. From experiments described under 5.3. and 5.4. it is likely that phenamiphos has irreversibly inhibited locomotion rather than killed the nematodes. This inhibition, however, must finally result in death of the animals. The results, for both nematicides, are in agreement with those of the recovery experiment described under 5.4.

TABLE 15. Numbers of *P. penetrans* extracted per plant from root systems of *L. ovalifolium* seedlings during one week exposure to 10 ppm oxamyl and phenamiphos (A), and numbers of *P. penetrans* extracted subsequently from the same root systems after they had been cut to pieces and placed for one week in a funnel-spray apparatus (B); the last column gives the total numbers extracted ( $A + B = C$ ). Between brackets log. transformations and LSD-values.

Treatments	From undamaged roots during treatment, first week (A)	From roots in funnel-spray apparatus, second week (B)	Total (C)
Untreated	420 (2.62)	783 (2.89)	1210 (3.08)
Oxamyl 10 ppm	55 (1.74)	964 (2.99)	1030 (3.01)
Phenamiphos 10 ppm	88 (1.94)	244 (2.39)	370 (2.57)
LSD .05	(0.27)	(0.35)	(0.29)
.01	(0.36)	(0.48)	(0.40)

### 6.4. FOLIAGE TREATMENT

Besides acropetal transport by the transpiration stream of a plant (apoplastic transport), which is important for nematode control inside roots, stems and/

or leaves after soil application of systemics, it has been reported that both oxamyl and phenamiphos can be transported from the leaves to the roots or even to the soil around the roots in quantities sufficient to eradicate nematodes there. This basipetal or symplastic transport, via phloem tissue, was studied by CRISP (1972) who concluded that apoplastic transport was common, but symplastic transport was rare for organophosphate and for organocarbamate insecticides-nematicides, certainly in concentrations sufficient to eradicate root parasites.

For oxamyl, basipetal transport was reported by the manufacturer as well as by TAYLOR and ALPHEY, 1973 and others (cf. 1.4.). TAYLOR and ALPHEY got evidence for basipetal transport of oxamyl by spraying plants and by showing that later on the soil from around these plants was nematicidal. If this soil was mixed with untreated soil it controlled the nematodes *Longidorus elongatus* and *Xiphinema diversicaudatum* and also the virus transmission which these nematodes otherwise caused. Because the roots of the sprayed plants had been removed, they concluded, "that the 'transferred' nematicidal effect lies in the production of a nematicidal root exudate".

For phenamiphos the manufacturer also report basipetal transport.

In reports by ZECK, 1971 and HOMEYER, 1971a 85-100% control of *Rotylenchulus reniformis* was obtained after leaf application to pine-apple, in and around the roots.

To get insight into this problem a special experiment with *D. dipsaci* and *P. penetrans* was set up.

### Experiment

Four *V. faba* plants were grown together in a plastic bag containing 500 ml silver sand and placed into a pot; there were 3 replicates per treatment. When the plants were 20 cm high, the whole foliage 5 cm above soil level was immersed for 10 seconds in oxamyl at 3000 and 9000 ppm a.i. and in phenamiphos at 1000 and 3000 ppm a.i. A wetting agent was added to the solutions (1 ml Agral, containing 25% nonylfenolpolyglycoether, per litre). The water control received Agral only. To prevent chemical contamination of the soil several precautions were taken. The plants were held completely turned over for 12 hours till they were dry. Besides this a layer of vaseline had been brushed as a 1 cm high collar around the stem foots just above soil level before the plants were dipped and after dip treatment the plastic bags were also wrapped around the stem foots. If plants had to be watered the bags were loosened and fixed again after watering. It was estimated that about 2 ml dip solution was used to wet every single plant, i.e. 8 ml/pot, and that about half of it had stayed on the leaf surface after the plants had been dried in reversed position. The treated plants were used to study various problems, namely:

A. The nematicidal effect of the ethanol extract obtained by washing the outside of the plants.

For A, the plants of 3 pots (4 plants/pot) were dipped for 10 seconds in 100

ml ethanol and the ethanol extract was evaporated, the residue being dissolved in 12 ml water and used in the PI test to determine nematicidal effectiveness. About 75 ml of the original 100 ml ethanol was available for evaporation, the rest being lost during the dip treatment. The residue was used to make a dilution range in water of 7 treatments, eg. 1 = undiluted, 2 = 1/4, 3 = 1/16, 4 = 1/64, 5 = 1/256 and 6 = 1/1024 times diluted, respectively, and 0 = water control, containing about the same concentration in the undiluted treatment as originally used on the plants.

B. The nematicidal effect of the ethanol extract obtained by washing the sand adhering to the roots of the lifted plants.

For B, plants as under A were removed from the soil in the plastic bags. The moist sand sticking to the roots was allowed to dry and then collected. Then this sand from the rhizosphere was washed with ethanol, the ethanol from the extract was evaporated and the residue was dissolved in water and used in a PI test to determine the nematicidal effectiveness against *D. dipsaci* penetration in *V. faba* stems. The extracts tested were the whole quantity washed from 5 ml rhizosphere sand per test plant stem.

C. The direct nematicidal effect of the sand taken from the roots of the lifted plants.

For C, the dried rhizosphere sand was directly tested in the PI test by placing 5 ml sand in the test vials and incubating 2 ml *D. dipsaci* suspension for 24 hours.

D. The direct nematicidal effect of the sand-mixture of the whole bags.

For D, a mixture of the sand of 4 pots was used to fill PI test vials with 5 ml sand and to determine the effect on penetration of *D. dipsaci*.

E. The effect of the treatments upon penetration into the originally treated plants by *D. dipsaci* inoculated into the sand.

F. The effect of the treatments upon penetration into the originally treated plants by *P. penetrans* inoculated into the sand.

For E and F, each pot had been inoculated with 10,000 *D. dipsaci* or 10,000 *P. penetrans* at 1 week after dipping the leaves, and the nematodes were extracted from the stems and from the roots, respectively, at 1 week after inoculation.

#### Results and discussion

The results are summarised in table 16. It appears that only the residues from the leaves are nematicidal. The ethanol extract from the rhizosphere sand, from the directly tested rhizosphere- and the mixed sand were not nematicidal. It is obvious that the dips did not influence penetration into dipped plants by *D. dipsaci* and *P. penetrans* inoculated into the potting sand one week after dipping these plants.

Thus there is no evidence to confirm the reports on systemic nematode control in soil and roots by basipetal transfer of nematicidal principles after application of oxamyl and phenamiphos to the foliage. The fact that residues from

TABLE 16. Summary of results of *V. faba* foliage dip treatment in 1000 and 3000 ppm phenamiphos or 3000 and 9000 ppm oxamyl: cf. text.

- A. Number of penetrated *D. dipsaci* in *V. faba* stems in a PI test with dilutions of the extract obtained by washing the outside of the plant foliage in alcohol 10 days after the foliage had been dipped in the systemics.
- B. As under A, for the alcohol extract of sand from the rhizosphere of *V. faba* plants 10 days after foliage had been dipped in the systemics. The extracts added were the quantities washed out of 5 ml rhizosphere sand.
- C. Effect of 5 ml rhizosphere sand if directly tested in the PI test.
- D. Effect of 5 ml sand mixture of the whole pots if directly tested in the PI test.
- E. Average number of extracted *D. dipsaci* per plant, 2 weeks after dipping the foliage in systemics and 1 week after inoculation the nematodes in the sand.
- F. Average number of extracted *P. penetrans* per plant, 2 weeks after dipping the foliage in systemics and 1 week after inoculation the nematodes in the sand.

Tested objects (cf. text)	Untreated	phen. 1000	phen. 3000	oxam. 3000	oxam. 9000	
Original nematicide dips in ppm a.i.						
A. 0 = water (control)	230	-	-	-	-	
1 = not diluted	57a	3 ↑	0 ↑	2 ↑	3 ↑	
2 = 4 × diluted	106a	5 ↑	2 ↑	4 ↑	1 ↑	
3 = 16 × diluted	163a	3 ↑	1 ↑	8 ↑	3 ↑	
4 = 64 × diluted	241	12 b	4 ↑	25 ↑	7 ↑	
5 = 256 × diluted	225	44 ↓	3 ↓	111 ↓	19 ↓	
6 = 1024 × diluted	251	115 ↓	25 ↓	214 ↓	88 ↓	
B. Rhizosphere/alcohol	229	245	226	254	235	ns
C. Rhizosphere/direct	237	221	239	215	242	ns
D. Sand mixture/direct	242	218	231	229	251	ns
E. <i>D. dipsaci</i> /plant	700	720	644	820	756	ns
F. <i>P. penetrans</i> /plant	352	408	386	297	403	ns

- a. These three concentrations of the leaf surface residues may have caused penetration inhibition by nematicidal properties or due to visible phytotoxic effects on the *V. faba* stem parts.
- b. Objects connected by arrows are all significantly different from the control.

the outside of the leaves were still nematicidal even 10 days after dipping suggests that the residues dripping from leaves or moving along stems after spraying or dipping, or after watering the plants, account for the reports in literature on basipetal transport in nematicidal effective quantities. Experiments with labeled chemicals could definitely solve the question whether basipetal transport of these nematicides does or does not occur in treated plants. Unfortunately, they were not available in the author's experiments.



## 6.5. TREATMENTS OF MICROPLOTS

To determine contact and systemic properties of oxamyl and phenamiphos under near-field conditions some experiments were done in microplots with fallow soil (6.5.2.) and the same soil under ryegrass, *Lolium perenne* (6.5.1.). Nematode as well as micro-arthropod communities were studied in these long-term experiments.

### 6.5.1. Soil under *Lolium perenne*

#### Experiment

In autumn of 1972, 1.5 m<sup>3</sup> sandy loam was collected from a fallow field near Ellecom on which maize had been grown the previous season.

The soil was dried in the greenhouse for sieving, mixing and sampling. The numbers of nematodes and microarthropods as well as pH, organic matter, clay content and some other characteristics were determined (cf. 2.3.1.). The numbers of nematodes per 100 ml of soil were as follows: *Pratylenchus crenatus* 1580 + *Tylenchorhynchus dubius* 380 + *Paratylenchus* sp. 30 + *Helicotylenchus* spp. < 10 + other tylenchids (mainly *Tylenchus* and *Psilenchus* spp.) 170 + saprozoic 2920.

After thorough mixing, soil lots of 70 litres were treated with the nematicides by mixing; untreated soil was handled in the same way without adding chemicals. The five treatments were:

- 10 and 50 ppm a.i. oxamyl (Vydate-5G formulation);
- 10 and 50 ppm a.i. phenamiphos (Nemacur-5G formulation);
- Untreated, without chemical.

Directly after treatment, the soil was poured in 50 × 90 cm microplots well-separated on a glass-house bench, as illustrated in figure 25.

At the same time, 3.0 grams seed of *L. perenne* cv. Pelo was spread on the soil surface of each plot and covered with a 1 cm thick layer of sterile river sand. The temperature in the greenhouse varied with season between 20 and 30°C, which was rather high for the grass and for some nematode species (e.g. *Pratylenchus crenatus*).

The microplots (cf. Fig. 25) were sampled several times in a special way; sampling dates and methods are indicated in figures 26 and 27. Every sample was collected with a 7 cm ø borer to a depth of 5 cm, and was subdivided into two halves for microarthropod and nematode extraction (Fig. 27). Every sample therefore furnished about 65 ml for nematode and 65 ml for microarthropod extraction.

There were three replicate plots and three sample cores were taken per plot, thus delivering 9 individual sample cores per treatment or 45 in total for nematode extraction and also for microarthropod extraction at each sampling date. For little over one year samples were taken every 6 weeks, except for the last sampling interval which was 12 weeks.

7	Nemacur - 10 ppm	Vydate - 50 ppm	8
6	Untreated	Vydate - 10 ppm	9
5	Vydate - 50 ppm	Nemacur - 50 ppm	10
4	Nemacur - 10 ppm	Vydate - 10 ppm	11
3	Vydate - 10 ppm	Untreated	12
2	Nemacur - 50 ppm	Vydate - 50 ppm	13
1	Untreated	Nemacur - 10 ppm	14
0	Untreated - extra	Nemacur - 50 ppm	15

FIG. 25. Scheme of greenhouse experiment with oxamyl and phenamiphos against nematodes and microarthropods under *Lolium perenne*. Plot number 15 shows the nett plot size for sampling and yield determination. Cf. also figures 26 and 27.

Before each soil sampling, the grass was cut and weighed, fresh and oven-dry. Yield was used to measure plant growth; reproduction of root parasites is related to growth and saprozoic animals in the soil also depend on the organic matter produced by the plant roots. Besides this, plant yield reflects phytotoxic effects of the pesticides.

*Lolium perenne* was chosen as a test plant because it grows continuously and

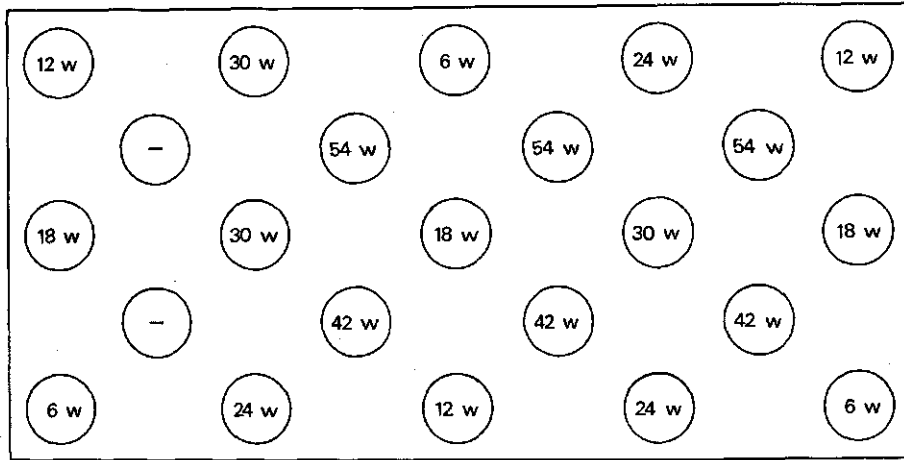


FIG. 26. Scheme of sampling dates (6, 12, 18, 24, 30, 42 and 54 weeks after treatment) and sampling spots for every nett plot.

because samples can be taken from a cut grass plot without damaging the rest of the plot. The sandy loam soil was chosen because it harboured several nematode species parasitic to grass, and because it was fertile and easy to handle for sampling and extraction. Fertilizer was added to obtain relatively good grass growth, but the experimental conditions of a high temperature and relatively low

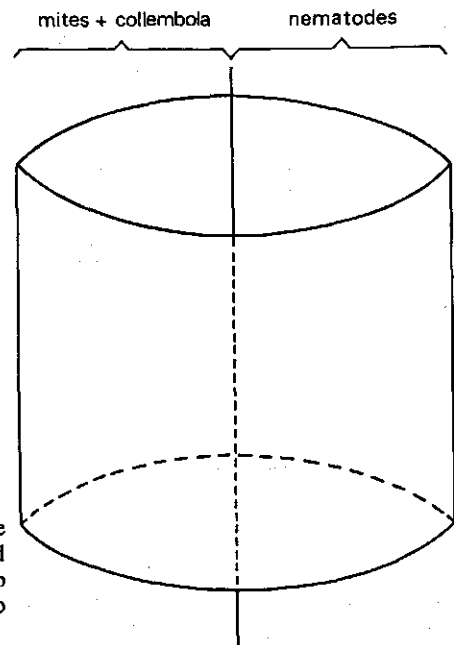


FIG. 27. Size, shape and subdivision of sample cores for nematode and for microarthropod extractions. Each core was about 5 cm deep and furnished 65 ml soil for each of the two different extraction procedures.

light intensity were not favourable enough to allow high nitrogen application throughout the experimental period.

#### Results on the nematode populations

The data (Table 17) comprise the total populations of *P. crenatus* + *T. dubius* + *Paratylenchus* sp. + *Helicotylenchus* spp. (which are the main nematode species in this soil for which *L. perenne* is known to be a host), the data for *Helicotylenchus* alone, and the data for the 'other tylenchids' for which *L. perenne* is a doubtful host. These figures are given as mean numbers of nematodes per 300 ml soil, calculated after log-transformation of the original numbers per 3 replicate plots.

The results for four single species or groups, viz. *Paratylenchus* sp., *P. crenatus*, *T. dubius* and the saprozoic nematodes, are illustrated in figure 28 and seem to reflect the results for residual effect of these nematicides in the same soil (see chapter 7.2. Fig. 33).

The effects of treatment, time and interaction of treatment and time were statistically significant (Table 17) (\*\*\*) for all nematode categories except the 'other tylenchids' for which only treatments differed significantly (\*\*\*). The order of nematode densities was: control > oxamyl 10 ppm > oxamyl 50 ppm > phenamiphos 10 ppm > phenamiphos 50 ppm, with a few exceptions at some sampling dates. The reduction of nematode species was generally significant up to about 6 weeks for oxamyl at 10 ppm and 18 weeks for oxamyl at 50 ppm. For phenamiphos this period was longer than 54 weeks, except perhaps for the saprozoic nematodes which at least started to increase again after 18 weeks. Even 10 ppm phenamiphos caused complete or near-complete eradication of plant parasitic and other nematodes for about 30 weeks after treatments.

The possibility of re-establishing nematode populations depends on whether nematicides are persistent in the soil and the animals, and also on the reproduction rate of the surviving nematodes. Differences between plant parasitic species are visible in the oxamyl treatments (Fig. 28). The order of increase was generally *P. crenatus* < *T. dubius* < *Paratylenchus* sp. The final numbers may also be determined by the equilibrium density which also varies with species and by possible interspecific competition. *Paratylenchus* and *Tylenchorhynchus* sp., for example, reached a high density in short time and may afterwards have suppressed the rate of increase of *Paratylenchus* or *Helicotylenchus* sp. This may lead to a change in composition of the original community, as reported earlier for *Paratylenchus* spp. after fumigation with DD (ANONYMOUS, 1973) and for *Criconemoides* spp. following aldicarb soil treatment (JOHNSON and BURTON, 1971). In these cases the nematode populations were found to be suppressed shortly after the nematicide treatment. For *P. penetrans*, living inside plant roots free from competing nematodes higher numbers were found on treated than on untreated plants 12 weeks after treatments with oxamyl (OVERMAN, 1971; BUNT, 1973). But such results may depend on nematicide dosage, temperature and plant species (BUNT, 1973; cf. also 6.2.1.). The density of a species at a certain date after

TABLE 17A. Average log. numbers of nematodes per 300 ml of soil treated with 10 and 50 ppm a.i. oxamyl (ox.) and phenamiphos (ph.) after the periods indicated in the table. Data on single species are illustrated in figure 28.

Time in weeks after treatment	Untreated	ox. 10 ppm	ox. 50 ppm	ph. 10 ppm	ph. 50 ppm
	<i>P. crenatus</i> + <i>T. dubius</i> + <i>Paratylenchus</i> sp. + <i>Helicotylenchus</i> spp.				
6	3.86	3.03	2.86	2.52	2.41
12	4.07	3.31	2.24	1.68	1.99
18	4.20	4.15	2.03	1.56	1.69
24	4.16	4.24	2.42	.80	—
30	4.28	4.32	2.50	—	—
42	4.28	4.15	4.19	1.61	.66
54	4.18	4.13	4.16	.59	1.20
	<i>Helicotylenchus</i> spp.				
6	—	—	—	—	—
12	1.01	.80	.40	—	—
18	.50	—	—	—	—
24	2.06	1.36	.50	—	—
30	2.60	1.94	.50	—	—
42	3.28	2.41	—	—	.40
54	3.47	2.89	2.29	—	.43
	"Other tylenchids"				
6	2.94	1.77	1.57	1.46	1.16
12	3.14	1.29	1.50	.40	—
18	3.01	1.31	1.40	.40	.80
24	2.97	2.07	.96	—	—
30	2.97	1.61	1.11	—	—
42	3.13	2.07	1.34	.40	—
54	3.37	2.09	2.62	.68	.43
Average with time for 'Other tylenchids'	3.07	1.74	1.50	.48	.34

B. Statistical analysis according to Tuckey's multiple range test

Objects	All known plant parasites	<i>Helicotylenchus</i> spp.	'Other tylenchids'
	LSD	LSD	LSD
Treatments	*** ) .05 = 1.83	*** ) .05 = 1.69	*** .05 = .61
Time	*** ) .01 = 2.07	*** ) .01 = 1.91	ns
Treatment × Time	***	***	ns

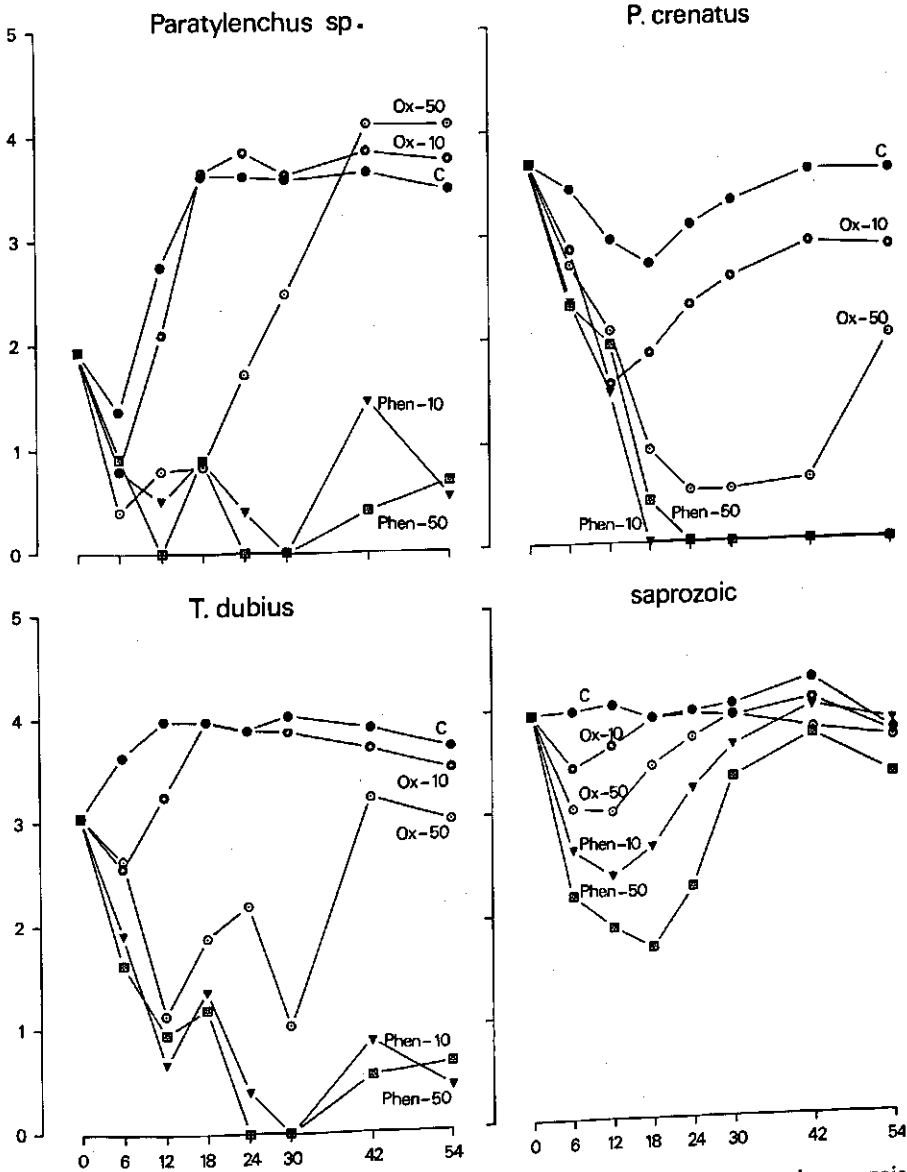


FIG. 28. Response with time of *Paratylenchus* sp., *P. crenatus*, *T. dubius* and saprozoic nematodes to soil treatments of 10 and 50 ppm a.i. oxamyl (ox) and phenamiphos (phen) in soil under *L. perenne* (indicated). Each point is the after log. transformation averaged number of nematodes per 300 ml of soil of 3 replicate plots (i.e. of 9 soil samples).  
 Abscissae: Time in weeks after treatment.  
 Ordinates: Numbers of nematodes per 300 ml soil; log. scale.

treatment is thus not always related to the effectiveness of the chemical. Effectiveness of a nematicide, therefore, can only be judged if evaluations are made over a long period following treatment.

Temporary protection of young plants from nematode attack with an efficient nematicide may increase plant yield, as found in this experiment for grass yield (cf. Table 19).

#### Results on *Acarina* and *Collembola* populations

All species of *Acarina* and *Collembola* in the test soil reacted in a similar way to the nematicides except for the saprozoic *Acarina*, *Rhodacarellus sileciacus*, a mite predatory to nematodes, restored its density to that of the untreated plots about 24 weeks after treatment. In the phenamiphos treated soil this density level was not yet reached even at 54 weeks (Fig. 29). The springtail *Tullbergia*

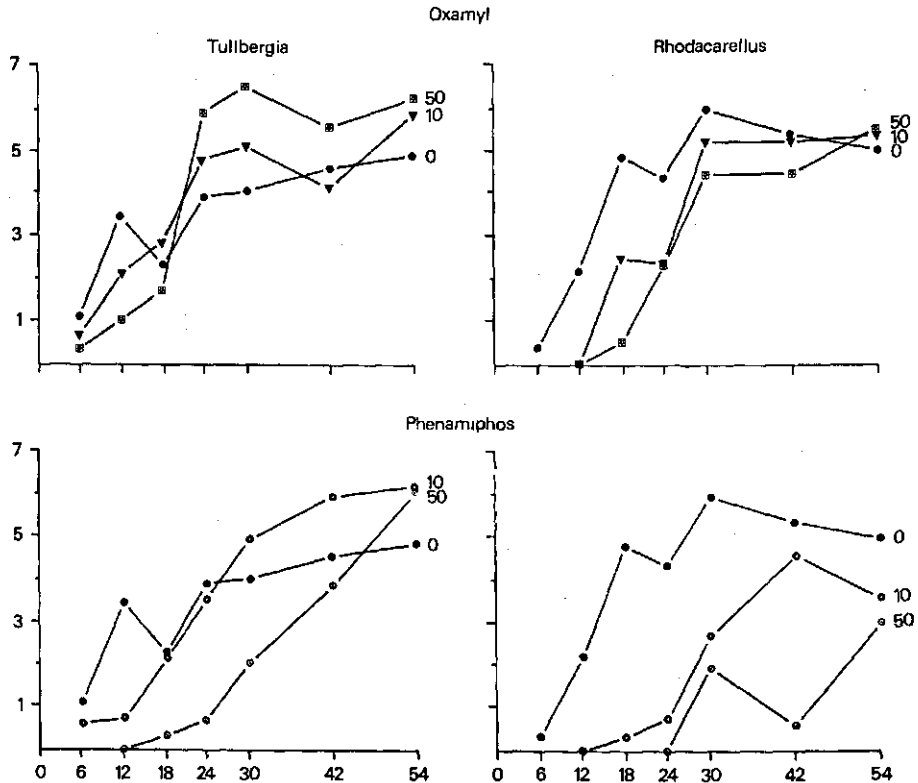


FIG. 29. Response with time of *Rhodacarellus sileciacus* and *Tullbergia krausbaueri* to soil treatments of 10 and 50 ppm a.i. oxamyl and phenamiphos in soil under *L. perenne* (indicated). Each point is the after log. transformation averaged number of microarthropods per 300 ml of soil of 3 replicate plots (i.e. of 9 soil samples).

Abscissae: Time in weeks after treatment.

Ordinates: Numbers of microarthropods per 300 ml soil; log. scale.

*krausbaueri* was apparently less susceptible to the treatments than the mite *Rhodacarellus sileciacus*. The density of *Tullbergia krausbaueri* rose above that of untreated soil after 24 weeks for both oxamyl dosages and after 54 weeks for both phenamiphos concentrations (Fig. 29).

As stated for nematodes competition appears to play a role in the re-establishment of different mite and springtail species after a soil treatment. Re-establishment of *Rhodacarellus sileciacus* not only depends on the density of nematode prey, but it may also depend on the numbers of the springtails which are also prey. Microarthropod densities were very low at the start of the experiment (Fig. 29), and it is unlikely that they would have a significant influence in regulating the population of nematodes.

There was a significant correlation between the density of soil nematodes and *Rhodacarellus* spp. (Fig. 30). It is remarkable that the numbers of saprozoic mites are not measurably influenced by any of the treatments (Table 18), which were, after all, fairly high.

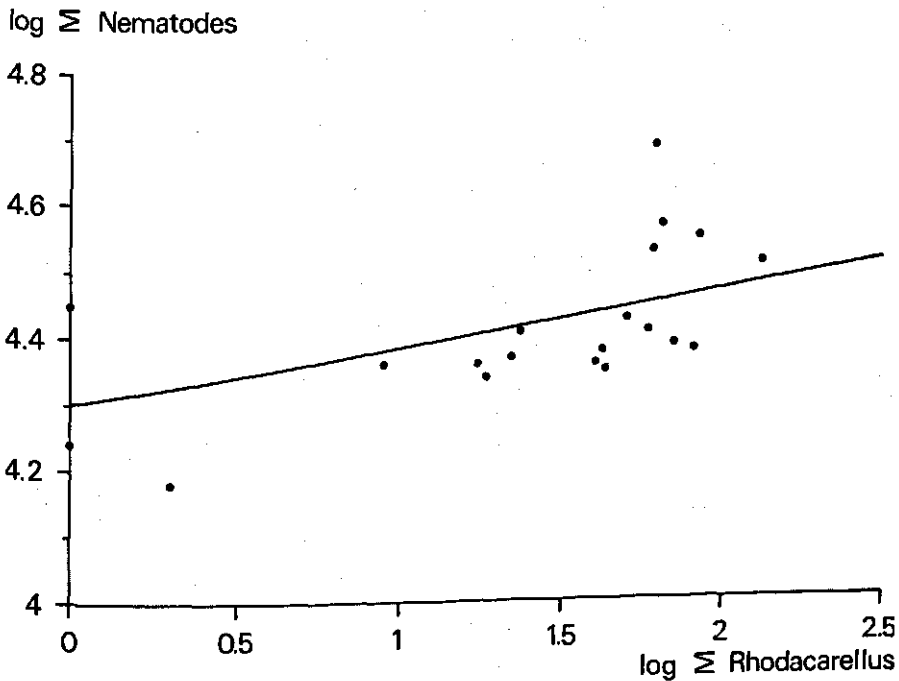


FIG. 30. Relation between nematode density and density of *Rhodacarellus sileciacus*.  
 Abscissa: Numbers of *Rhodacarellus sileciacus* per 9 samples; log. scale.  
 Ordinate: Numbers of nematodes per 9 samples; log. scale.  
 Formula:  $y = 0.10x + 4.27$   $r = 0.60^{***}$ .



TABLE 18. Log. numbers of saprozoic *Acarina* per 300 ml of soil treated with 10 and 50 ppm a.i. oxamyl (ox.) and phenamiphos (ph.) after the periods indicated in the table. Data on other species of *Acarina* and of *Collembola* are illustrated in figure 29. Only time had a significant influence on the numbers of saprozoic mites (\*\*\*) , with LSD-values for .05 = .27 and for .01 = .32, according to Tuckey's multiple range test.

Time in weeks after treatment	Untreated	ox. 10 ppm	ox. 50 ppm	ph. 10 ppm	ph. 50 ppm	Average
6	.39	.36	—	.10	.30	.23
12	1.73	1.79	1.79	1.73	1.30	1.67
18	1.99	1.89	1.98	1.89	1.74	1.90
24	1.67	1.41	1.91	1.72	1.40	1.62
30	1.55	1.64	1.69	1.62	1.63	1.62
42	1.55	1.42	1.31	1.73	1.52	1.51
54	.95	.88	1.27	1.59	1.35	1.21

#### Yield of the host plant *Lolium perenne*

At the sampling dates yields in terms of grams fresh and dry materials per plot are summarised in Table 19. The last two cuttings, at 48 and 54 weeks after treatment, are not considered reliable and are not taken into account because of poor light conditions in winter and because the many samples already bored by that time may have increased variability.

TABLE 19A. Grass yield, after treatment with 10 and 50 ppm oxamyl (ox.) and phenamiphos (ph.) with time as indicated in the table. Figures are the mean yields in grams fresh materials per plot, with dry weights mentioned between brackets. Cutting time in weeks after treatment.

Cutting time	untreated	ox. 10 ppm	ox. 50 ppm	ph. 10 ppm	ph. 50 ppm	Average
6	340 (27)	428 (34)	395 (32)	350 (28)	257 (23)	354 (28.6)
12	324 (36)	335 (38)	378 (39)	315 (35)	243 (30)	319 (35.3)
18	153 (20)	143 (19)	196 (23)	142 (18)	130 (17)	153 (19.5)
24	205 (28)	214 (29)	198 (28)	196 (27)	170 (25)	197 (27.3)
30	165 (20)	155 (19)	185 (22)	180 (23)	138 (18)	165 (20.5)
36	360 (40)	332 (37)	354 (40)	381 (42)	330 (38)	352 (39.4)
42	207 (26)	196 (25)	197 (25)	216 (27)	200 (26)	203 (25.9)
Average	251 (28.3)	258 (28.6)	272 (29.8)	254 (28.4)	210 (25.3)	— —

#### B. Statistical analysis according to Tuckey's multiple range test

Objects	LSD .05	LSD .01	Significance
Fresh weights: Treatments	40	48	**
Time	51	60	**
T × T	—	—	ns
Dry weights: Treatments	3.70	—	*
Time	4.71	5.60	**
T × T	—	—	ns

At the first sampling date, oxamyl caused significantly higher yields than in phenamiphos and untreated plots. Phenamiphos at 50 ppm a.i. was obviously phytotoxic; this was visible also from burned tips of the leaves. Oxamyl did not appear to be phytotoxic even at the highest dose of 50 ppm. The 50 ppm dose of oxamyl caused significant long-lasting increase in yield higher than all other plots, including oxamyl at 10 ppm, probably due to better control of plant parasitic nematodes by oxamyl at 50 ppm: from these results it seems that nematodes did not cause marked damage here. Phenamiphos at 10 ppm caused a marked reduction in plant parasitic nematodes combined with non-significant yield increases which could be explained by phytotoxicity at the lowest dose of this compound.

### 6.5.2. Fallow soil

#### Experiment

Parallel with the previous experiment in the same greenhouse compartment, soil from the same source was split into ten-litre portions and mixed with 10 and 50 ppm a.i. of oxamyl and phenamiphos including untreated and then placed in eternite trays of  $80 \times 10 \times 15$  cm. In this experiment no grass was grown, the soil being left fallow. In the same way as in the previous experiment samples were taken for nematode counts.

#### Results on the nematode populations

The results are given in figure 31 as regression lines for the four nematode species or groups; results of the statistical analysis are summarised in table 20.

There was a marked effect of the treatments on the nematodes in fallow soil. The nematode densities decreased significantly throughout the year. The result was similar in the untreated soil, but the numbers in the treated soils were always significantly lower, except for the saprozoites at the highest doses of oxamyl and phenamiphos (cf. Fig. 31), where numbers increased with time until 54 weeks after treatment, when they were not significantly different from the untreated. This is a remarkable result which cannot easily be explained.

The slopes of the regression lines for most oxamyl treatments were similar to the untreated, due almost certainly to short residual life of the compound combined with recovery of the nematodes (cf. Fig. 31 for *P. crenatus* and 'total plant parasites'). Phenamiphos gave steeper regression lines, indicating again the longer residual life of the compound and or persistent poisoning of the nematodes. Although nematode densities for all oxamyl treatments were significantly lower than for the untreated, the regression lines of oxamyl 10 ppm were not significantly steeper for any of the nematode groups, and the same held for oxamyl at 50 ppm for *P. crenatus*. This means that the reduction caused by oxamyl occurred within about 6 weeks after treatment, followed by natural decline of the nematode populations as in the untreated soil. This agrees with the result of the previous experiment, indicating that 6 weeks after treatment of the soil oxamyl is no more effective. For all treatments with phenamiphos, the re-

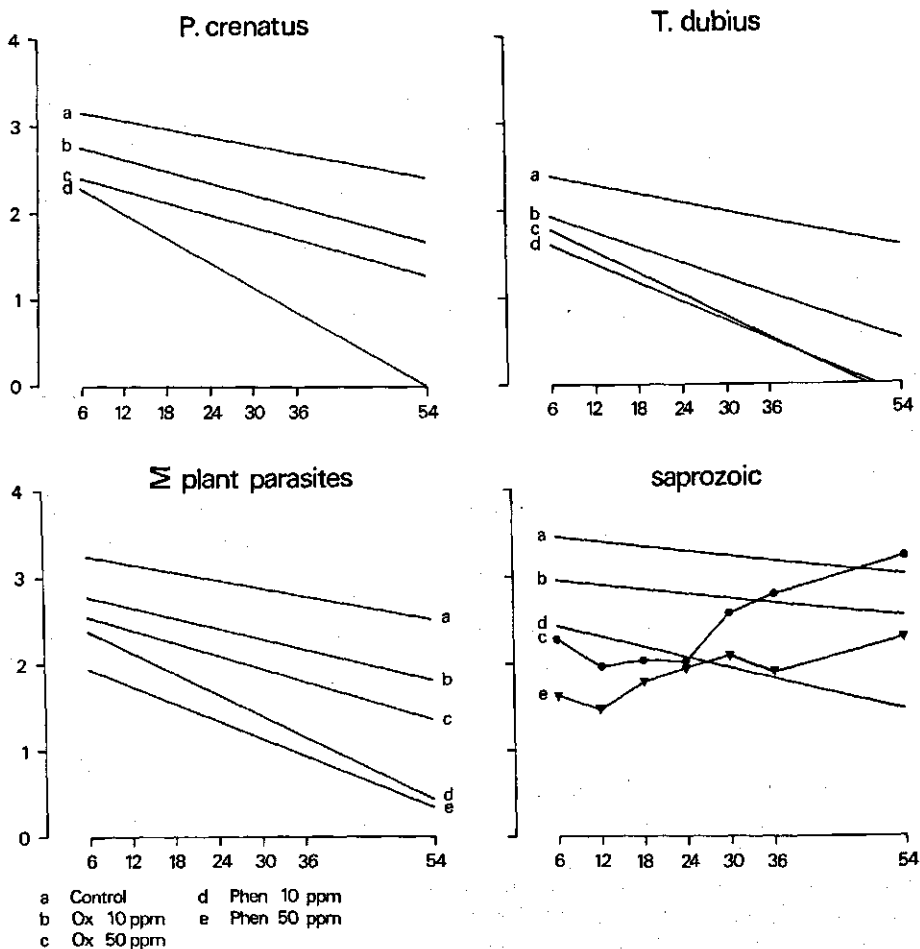


FIG. 31. Regression lines for the numbers of different nematode species with time in fallow soil after treatments with 10 and 50 ppm a.i. oxamyl (ox) and phenamiphos (phen); indications in the figure. For statistical analyses cf. table 20.  
 Abscissae: Time in weeks after treatment.  
 Ordinates: Numbers of nematodes per 100 ml of soil; log. scale.

duction of the nematodes continued to be greater than for the untreated throughout the whole year of observation.

In an additional experiment grass was grown in the pots filled with the treated soils of the aforementioned 'fallow' experiment, and nematode populations were studied. Their response is summarised in table 21. For both oxamyl treatments complete re-establishment of saprozoic nematodes takes place; for 10 ppm the numbers increased to 3 times above the control soil. Tylenchid nema-

Table 20. Response of nematodes to 10 and 50 ppm a.i. oxamyl (ox.) and phenamiphos (ph.) in fallow soil, compared to fallow soil (untreated), expressed as regression formulae derived from the log. transformed nematode numbers per 100 ml soil, ( $=y$ ), together with lowest and highest values of the log. transformed nematode numbers, correlation coefficients ( $r$ ), and indications of significance of  $r$ . Periods of soil sampling were: 6, 12, 18, 24, 30, 36 and 54 weeks after treatment ( $=x$ ). Only treatments which delivered significant correlation coefficients are mentioned.

Treatments in ppm a.i.	Log. values		Formulae	Correlation coefficients ( $r$ )
	Highest	Lowest		
<i>P. crenatus</i>				
Untreated	3.16	2.39	$y = -0.10x + 3.26$	-0.96***
Ox. 10	2.75	1.64	$y = -0.14x + 2.89$	-0.94***
Ox. 50	2.40	1.28	$y = -0.14x + 2.54$	-0.95***
Ph. 10	2.29	0.01	$y = -0.28x + 2.57$	-0.91***
<i>T. dubius</i>				
Ox. 10	1.95	0.50	$y = -0.18x + 2.13$	-0.81**
Ox. 50	1.77	0.54	$y = -0.25x + 2.01$	-0.78**
Ph. 10	1.61	0.53	$y = -0.22x + 1.82$	-0.77**
All known plant parasitic nematodes				
Untreated	3.25	2.50	$y = -0.09x + 3.34$	-0.96***
Ox. 10	2.80	1.80	$y = -0.13x + 2.93$	-0.98***
Ox. 50	2.52	1.35	$y = -0.15x + 2.67$	-0.96***
Ph. 10	2.40	0.41	$y = -0.25x + 2.65$	-0.97***
Ph. 50	1.96	0.31	$y = -0.21x + 2.17$	-0.81**
Saprozoic nematodes				
Untreated	3.46	3.01	$y = -0.06x + 3.51$	-0.90***
Ox. 10	2.96	2.53	$y = -0.05x + 3.02$	-0.96***
Ph. 10	2.42	1.44	$y = -0.12x + 2.54$	-0.93***
All nematodes (total)				
Untreated	3.68	3.18	$y = -0.06x + 3.74$	-0.94***
Ox. 10	3.19	2.62	$y = -0.07x + 3.26$	-0.94***
Ph. 10	2.72	1.43	$y = -0.16x + 2.88$	-0.96***

TABLE 21. Re-establishment of nematode populations in soil treated with 10 and 50 ppm a.i. oxamyl and phenamiphos and kept fallow for 30 weeks, followed by 18 weeks growth of *Lolium perenne*. Figures are numbers of nematodes per 100 ml soil.

Treatments in ppm a.i.	<i>P. crenatus</i>	<i>T. dubius</i>	<i>Paratylenchus</i> spp.	Other tylenchids	saprozoic nematodes
Original Untreated (fallow)	260	80	10	180	1760
New Untreated (fallow + <i>L. perenne</i> )	235	4930	2880	490	3835
Oxamyl 10	145	2150	3950	1490	11095
Oxamyl 50	10	455	15	215	4165
Phenamiphos 10	5	0	0	55	1500
Phenamiphos 50	0	0	0	30	3660

todes practically re-established their densities in the soil treated with 10 ppm oxamyl, but not at 50 ppm, although *T. dubius* almost reached the initial level. In phenamiphos-treated soil only saprozoic nematodes increased, *P. crenatus*, *Paratylenchus* sp. and *T. dubius* being kept on very low levels.

### 6.6. FIELD TRIAL WITH REPEATED TREATMENTS

From a trial under grass at the 'Institute of Biological and Chemical Research on Field Crops and Herbage' by Ir. G. C. Ennik, soil samples for nematode studies were taken on plots which were repeatedly drenched with oxamyl and phenamiphos. The soil was treated once before and once after the first cutting and once after the second cutting, i.e. at intervals of about 6 weeks. Each treatment was a drench of 2 kg a.i. per hectare given to the soil surface. At 4 sampling dates the average nematode numbers in the plots were determined as percentages of the control and are reported in figure 32. The first treatment and

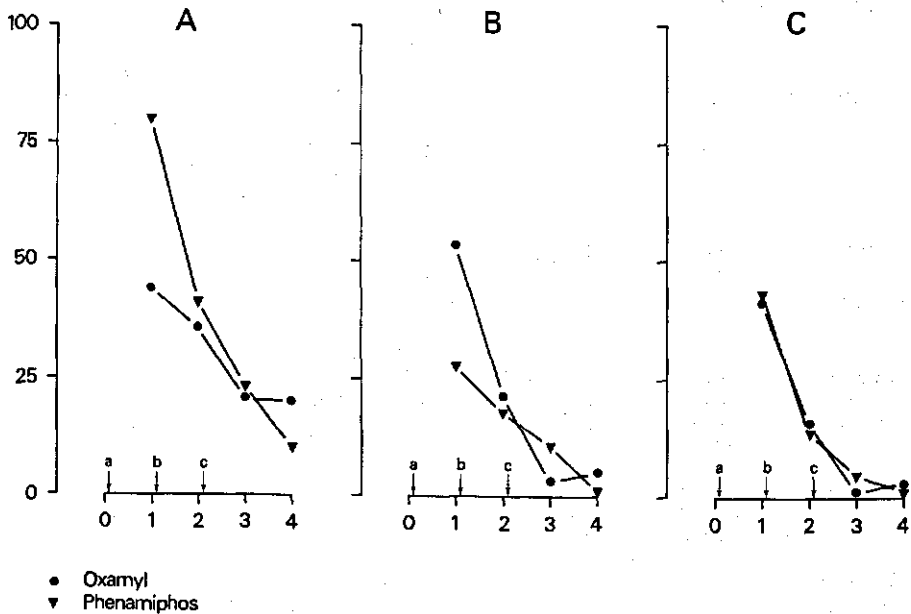


FIG. 32. Nematode densities with time when 3 soil drenches were given with 6 weeks intervals to a field under grass with oxamyl and phenamiphos, 2 kg a.i./ha per drench. Saprozoic nematodes (A), other tylenchids (B) and total of plant parasitic nematodes (C) are recorded separately. Indications a, b and c, with arrows on the abscissae means first treatment (10 April before grass growth), second treatment (end of May) and third treatment (medio July), respectively.

Abscissae: Time of grass cutting and soil sampling for nematodes; intervals between periods of about 6 weeks.

Ordinates: Percentages of the numbers of nematodes against the control.

soil sampling was on the 10th April in the year 1973. In figure 32 only the effects of the treatments with time are recorded.

## Results

Oxamyl reduced nematode numbers as effectively as phenamiphos in this case, when treatment was repeated every 6 weeks. Nematode densities started to increase or stopped decreasing as soon as the oxamyl treatment was stopped, whereas they continued to decrease in the phenamiphos plots.

This experiment confirms that the persistence of the chemicals, perhaps together with the persistence of the poisoning effects, largely determines the response of the nematode populations with time. It demonstrates also, that oxamyl with its low persistence may give the same result in the field as the more persistent phenamiphos even at the low dose of 2 kg/ha if the treatment is repeated at proper intervals.

## 6.7. DISCUSSION

The long-term experiments in soil with and without a host plant (6.5., 6.6.) indicate that the chemicals used affect nematodes in soil directly, although also nematodes in roots and stems can be eradicated by soil drenches with oxamyl and phenamiphos (6.2.1., 6.2.2.). This direct effect seems to be more important than the systemic effect via the host, for the reduction of nematodes on fallow soil was as good as on soil with a host; saprozoic nematodes were influenced almost as much as plant parasites.

The results confirm our earlier finding that the systemics differ essentially. In soil and water oxamyl has a short life, whereas phenamiphos has a long life combined with more persistent poisoning effect. We know from other experiments, that there are organophosphate systemics, f.e. the experimental CGA-10576, which combine a relatively short life in soil (cf. oxamyl) with a long-term effect against freeliving nematodes in soil (cf. phenamiphos) which must be due to irreversible poisoning or quick kill of the nematodes (BUNT, unpublished).

With respect to recovery of a treated nematode population, persistence of the chemical itself and persistence of the poisoning effect (irreversible or reversible) appear to be important factors which act independently. Data in this chapter support the early hypothesis (ch. 5.), 'that the high oral toxicity to mammals of most present-day systemic nematicides alone makes it probable that they affect nematodes also directly, and not only via their host'. A nematicide with irreversible poisoning effects may cause a marked, long-lasting reduction of the population despite its short persistence, whereas a nematicide with reversible poisoning effect and short persistence may easily result in a high final density: see also the discussion of the *in vitro* experiments (5.5.) and figure 33 about persistence of oxamyl and phenamiphos in soil. Whether such recovering populations can still damage the host plant is uncertain, although *D. dipsaci* treated with sublethal concentrations of oxamyl and phenamiphos reproduced nor-

mally after recovery. For other nematode species this was not investigated directly; in practice it is important to know whether damage occurs only if young plants are heavily attacked.

The final densities of nematode populations after treatment with systemics sometimes differ, probably due to different reproduction rates rather than to differential sensitivity of the nematodes.

Except for some species of saprozoic mites, microarthropod populations appear to react to treatments with systemics much the same way as nematodes. For their reproduction predacious mites probably depend on the total densities of nematodes but they do not seem to play a great role in the regulation of nematode densities in soil under our experimental conditions.

The significant increase in grass yield (6.5.1.), shortly after treatment with oxamyl, could be explained by the protection of the young plants against nematode attack; for phenamiphos this was obscured by the phytotoxic effects on the grass; lower doses of phenamiphos may result in poorer nematode control but better growth of grass.

## 7. PERSISTENCE, PHYTOTOXICITY AND OTHER SPECIFIC EFFECTS OF OXAMYL AND PHENAMIPHOS

### 7.1. INTRODUCTION

Besides the effects of systemic nematicides to nematodes, several specific effects could be important for the total effect of these chemicals on plant growth. From these specific effects we studied persistence, phytotoxicity to higher plants, fungitoxicity, bactericidal effect and finally we made an attempt to induce resistance to oxamyl and phenamiphos in a *D. dipsaci* population. The results of each section are directly discussed, which makes a general discussion of this chapter superfluous.

### 7.2. PERSISTENCE

Systemics are mostly incorporated in the soil and it is for several reasons necessary to know their persistence in soil. For phenamiphos this was several months according to HOMEYER (1971). No documented information on persistence of granulated oxamyl is available from literature, but there are indications that oxamyl is rather unstable. It is also important to know the influence of temperature and other environmental factors on loss of activity of systemics in soil. Therefore we did the following experiments with oxamyl and phenamiphos.

#### Experiments

A long-term experiment with the two nematicides in soil at various temperatures was set up<sup>5</sup>. Dosages of 10 ppm a.i., as 5% granules, were thoroughly mixed with Ellecom soil (15% moisture). Each batch of soil (10 litres per treatment per temperature) was stored in closed plastic bags at 5, 15 and 25°C. Every 6 weeks during one year, 100 ml samples of each treatment were taken after mixing the soil and analysed, using an ethanol extraction technique by adding 50 ml ethanol (96%) to the soil samples in 200 ml erlenmeyer flasks. The flasks were closed with parafilm and stored for 2 days at room temperature. Then the ethanol extract was filtered off on a millipore filter bottle and a 20 ml sample, of about 25-30 ml recovered, was used for further analyses. The 20 ml ethanol sample was evaporated at 35°C in about 24 hours. The residue was taken up in 10 ml water and used in the PI test to determine the residual nematicidal activity. With this method only relative values are obtained, but it is an advantage that the method registers only biologically active substances against nematodes. The soil residue itself gave no nematicidal effect after extraction with ethanol.

<sup>5</sup> In fact a third, experimental nematicide was included in this and some other experiments and calculations. It was left out because it did not furnish new insight.



From each residue sample of 10 ml, 4 replicated vials of the PI test were each furnished with 1 ml of solution and the remaining 6 ml was diluted with water to 12 ml. Then again 4 replicated vials of the test were each furnished with 1 ml. The remaining 8 ml was diluted to 16 ml, from which also 4 replicated vials obtained 1 ml each. The dosages were indicated as N(ormal), 1/2N and 1/4N respectively. Therefore representing the nematicide residues from maximal 1/25, 1/50 and 1/100 of the original sample of 100 ml treated soil. Because in the procedure of the PI test (3.2.) 5 ml sand is used, the maximal concentration in the sand was 8, 4 and 2 ppm a.i., respectively, assuming that 100% extraction was achieved and that the ethanol contained the same amount of residue as the ethanol left in the soil sample.

From the bulk of soil in the plastic bags a 100 ml soil sample of each treatment was examined for surviving nematodes, 30 weeks after treating the soil, to measure the fate of the original soil populations in relation to the various treatments and temperatures.

### Results and discussion

The regression of the activity of the nematicides with time are shown in figure 33 as % of penetrated *D. dipsaci* in the bean tissue of the PI test, thus indicating the reverse of residual activity. Table 22 summarised the statistical analysis together with the lowest and highest percentages of penetration.

Phenamiphos was more residual than oxamyl (Fig. 33). The concentrations (1, 1/2 and 1/4N) had a distinct influence in all cases. The effect of both chemicals disappeared more rapidly at the higher temperatures. At 25°C only phenamiphos could be detected for more than 1 year. For oxamyl this was only the case at 5°C. As shown in figure 33, the nematicidal effect of the extracted residues at 5°C at 6 weeks after treatment was nearly complete for both nematicides. Oxamyl was originally about as active as phenamiphos, but the effect was reduced greatly with increasing temperature as well as with time. At 25°C the residual effect of 1N oxamyl was practically lost after 12 weeks, whereas phenamiphos still had effective residues after 54 weeks (cf. Fig. 33 and Table 22).

Table 23 summarizes the nematicidal effect of the two nematicides directly upon the nematodes in the soil at the different temperatures, expressed as percentages of the control. It is clear that oxamyl had killed fewer nematodes than phenamiphos, and also that it was less effective at high temperature. For phenamiphos the reverse was true; higher temperatures resulted in an increased effect on the saprozoic nematodes. Oxamyl showed a relatively greater effect on saprozoic nematodes than on the plant parasite *Pratylenchus crenatus*, whereas phenamiphos was equally effective on saprozoic and plant-parasitic nematodes.

Longevity or persistence at one hand and reversibility of the poisoning effect on the other, determine to a great extent the effectiveness of a nematicide in

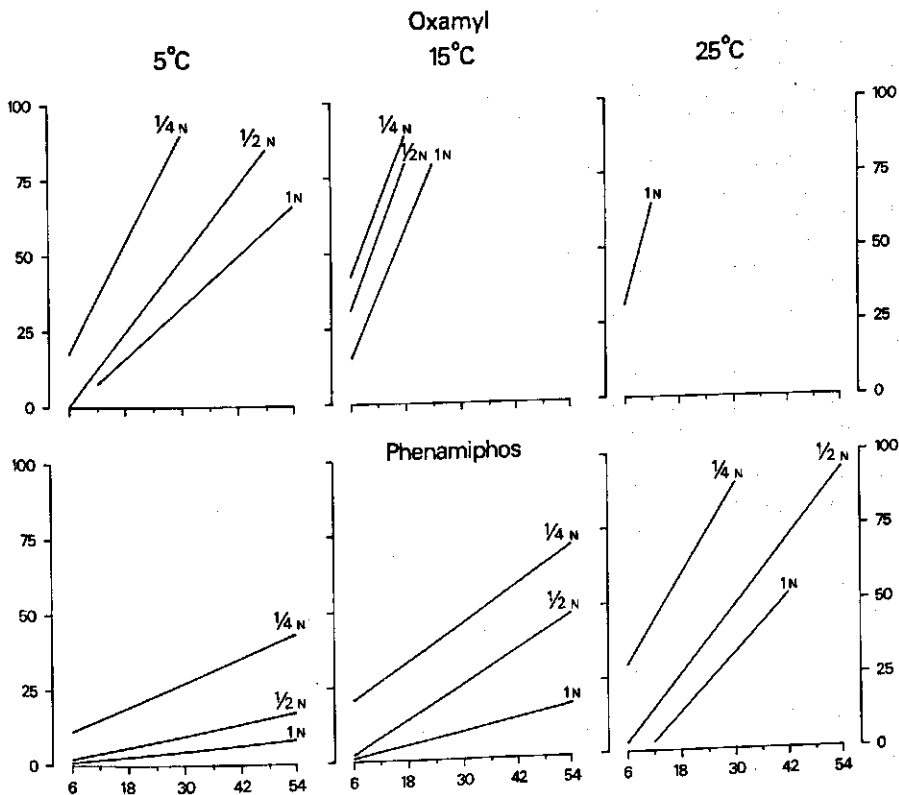


FIG. 33. Regression lines of the residual activity of the nematicides oxamyl and phenamiphos in Ellecom soil treated with 10 ppm a.i. of the chemicals as granule formulations, and measured by the PI test. The ethanol-extractable residues were measured in three concentrations (1, 1/2 and 1/4 N (normal)) and from three soil-store temperatures (5, 15 and 25°C). The statistical analyses are summarised in table 22.

Abscissae: Examination times in weeks after treatment.

Ordinates: Percentage invasion of *D. dipsaci* in *V. faba* tissue against the control, thus indicating the reverse of residual activity.

soil, as well as the danger for residues. Both aspects are important and each of them may determine the possibilities of a nematicide for use in agriculture. As mentioned in chapter 5, the poisoning effect of oxamyl to *D. dipsaci* was reversible up to 10,000 ppm and that of phenamiphos not reversible above 10 ppm. This is in agreement with the nematode counts in the soil of the present experiment. Oxamyl gives a decreasing kill of nematodes at increasing temperatures while phenamiphos gives the reverse effect. Also in this experiment nematodes seem to overcome the poisoning effect of oxamyl better than of phenamiphos. Phenamiphos appears to combine a long life in soil with irreversible poisoning effect on the nematodes. The fact that both chemicals are most stable at low temperature (Fig. 33) is understandable, because biological and che-

TABLE 22. Persistence of oxamyl and phenamiphos in Ellecom soil, at three temperatures, assessed at maximal 9 times at 6-weeks' intervals, every time at three concentrations of the ethanol-extracted residues (1N(normal), 1/2N and 1/4N), in PI tests. The chemicals were originally applied as granulated formulations at 10 ppm a.i. Cf. text and figure 33.

1. Treatments: chemicals, storage temperature (5, 15 or 25°C) and relative final residue doses (1N, 1/2N and 1/4N)	2. Assessment periods with measurable and calculable residue effects	3. Percentages penetration corresponding with periods in column 2	4. Formulae of the regression lines	5. Correlation coefficients (r) and significance
<i>1 N series</i>				
Oxamyl 5°C	2-9	7- 66	$y = 8.50x - 10.69$	0.96***
Oxamyl 15°C	1-5	16-102	$y = 21.70x - 6.42$	0.98***
Oxamyl 25°C	1-3	32- 99	$y = 33.60x - 1.97$	0.99*
Phenamiphos 5°C	1-9	1- 9	$y = 1.01x - 0.53$	0.79**
Phenamiphos 15°C	1-9	1- 19	$y = 2.22x - 1.45$	0.76**
Phenamiphos 25°C	2-7	3- 52	$y = 5.63x - 17.04$	0.92**
<i>1/2 N series</i>				
Oxamyl 5°C	1-9	1- 97	$y = 12.06x - 11.42$	0.97***
Oxamyl 15°C	1-4	32-104	$y = 24.15x - 7.35$	0.92*
Oxamyl 25°C	1-2	52- 96		ns
Phenamiphos 5°C	1-9	2- 17	$y = 1.86x + 0.14$	0.76**
Phenamiphos 15°C	1-9	2- 48	$y = 5.74x - 4.14$	0.80***
Phenamiphos 25°C	1-9	3- 94	$y = 11.33x - 8.20$	0.95***
<i>1/4 N series</i>				
Oxamyl 5°C	1-5	18- 90	$y = 17.92x + 0.26$	0.92**
Oxamyl 15°C	1-4	42-113	$y = 23.53x + 18.45$	0.90**
Oxamyl 25°C	1-2	85-102		ns
Phenamiphos 5°C	1-9	12- 43	$y = 3.90x + 7.40$	0.63*
Phenamiphos 15°C	1-9	20- 71	$y = 6.43x + 13.35$	0.77**
Phenamiphos 25°C	1-6	29-105	$y = 15.04x + 14.06$	0.83**

mical reactions are generally slowed down at low temperatures.

The influence of temperature could be important in practical application, because at low temperatures residues in soil and plant will usually last longer. On the other hand, the temperature may influence the degree of nematode control. Oxamyl may give better nematode control at low than at high temperatures, but this would not be an advantage for this chemical with a reversible poisoning effect, because surviving nematodes which recover will be able to infest plants and to multiply. It is only just after application of oxamyl that nematodes are unable to damage plants or to multiply.

The relatively high nematode kill obtained in this experiment (cf. Table 23),



### 7.3.1. Effects on higher plants

#### Experiment

Eleven plant species, of which some relevant data are reported in table 24, were used to determine phytotoxicity of oxamyl and phenamiphos 200, 800 and 3200 ppm a.i., when used as foliar sprays. Young plants of about 4 weeks after seeding were planted in eternite trays of  $80 \times 10 \times 15$  cm. In every tray 2 plants of every plant species were planted and there were 2 replicated trays for each treatment. Every treatment had, in this way, 4 replicated plants per species. Plants were allowed to recover from transplanting for about 1 week and were then treated. The nematicide solutions in 5% acetone were applied with a paintsprayer with a fine nozzle. Plants were sprayed till the spray liquid started to run off. The eternite trays with treated plants were placed in a greenhouse compartment of 22-25°C. Evaluation took place 10 days after treatment by weighing the above-ground parts. Plants sprayed with 5% acetone in water served as control.

TABLE 24. Relevant data on the eleven plant species tested for their phytotoxicity by spraying the foliage with oxamyl and phenamiphos 200, 800 and 3200 ppm a.i. (w/v) in the spray liquid. The plant species are recorded in the very order of an decreasing sensitivity to phenamiphos 3200 ppm. Potato was planted as pre-germinated eye cuttings, and apple seed was vernalised during 6 weeks in moist sand at 3°C in a refrigerator, before seeding.

No.	Plant species	Cultivar	Sowing date	Transplanting date	Plant height in cm at spraying
1	Wheat	Stella	1972-10-16	1972-11-1	10
2	Maize	Caldera	1972-10-16	1972-11-1	12
3	Potato	Eigenheimer	1972-10-16	1972-11-1	15
4	Pea	Mansholt	1972-10-16	1972-11-1	5
5	Cucumber	Lange gele tros	1972-10-16	1972-11-1	5
6	Tobacco	Glutinosa	1972- 9-16	1972-11-1	3
7	Tomato	Moneymaker	1972-10- 9	1972-11-1	5
8	Beet	Corona	1972-10-16	1972-11-1	3
9	French bean	Processor	1972- 9-16	1972-11-1	10
10	Apple	Bittenfelder	1972- 9-16	1972-11-1	3
11	Broad bean	Dubbel wit	1972-10-16	1972-11-1	15

#### Results and discussion

The results for the 3200 ppm a.i. concentration of the systemics is reported in figure 34; whereas the results of the other two dosages are discussed below. As shown in figure 34, phenamiphos 3200 ppm killed the mono-cotyle plant species wheat and maize completely and was very toxic to potato, pea and cucumber (all statistically significant\*\*\*). Plants which were not killed within a few days after treatment, mostly recovered to a certain extent before the experiment was terminated. Although the 3200 ppm dose of oxamyl yielded less than the lower

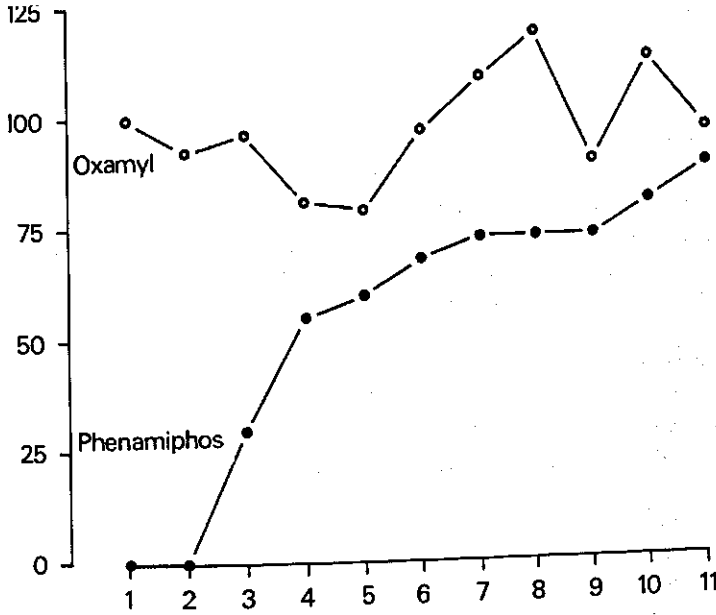


FIG. 34. Response of eleven plant species recorded in tabel 24, in their juvenile stage, to foliar sprays of 3200 ppm a.i. oxamyl and phenamiphos. Plants are placed in the order of decreasing response to 3200 ppm phenamiphos.

Abscissa: Indications for plant species according to tabel 24.

Ordinate: Percentage weight of aerial plant parts against control as average of 4 replicate plants.

doses of the chemical and the control in several cases, the differences were never significant.

At the 800 ppm dose, only phenamiphos caused a significant reduction in fresh plant weights of wheat, maize, pea and cucumber; however none of the plants were killed and all recovered more or less within 10 days after treatment. The 200 ppm dose was not phytotoxic to any of the plant species.

Although some reductions of fresh weight of above-ground parts occurred for tobacco, tomato, beet, French bean, apple and broad beans at the 3200 ppm dose of phenamiphos, these were not significant. Phenamiphos was more or less toxic to all plant species tested (Fig. 34).

The symptoms of phytotoxicity were not different for the two systemics. Slight toxic effects were small necrotic spots on the leaves, especially at leaf edges and tips where droplets dried. When toxicity was severe whole leaves were sometimes burned and dropped off within a few days after spraying; this reaction was only seen for phenamiphos at 3200 ppm, in pea, cucumber and potato. Oxamyl showed only slight effects on potato, tomato and pea and in no case was plant weight significantly decreased.

### 7.3.2. Fungitoxicity

#### Experiment

A test range of 28 isolates of soil fungi belonging to *Oomycetes*, *Zygomycetes*, *Ascomycetes/Deuteromycetes* and *Basidiomycetes*, including two yeasts (*Rhodotorula rubra* and *Candida albicans*), were tested *in vitro* with a concentration range of oxamyl and phenamiphos. They are listed in table 25. The media on which the growth of the microorganisms was measured were: potato dextrose agar (pH 5.6), for numbers 1-2; potato dextrose agar + 50 ppm vendarcine (pH 5.2-5.6), for numbers 3-19, 24 and 26-31, and malt agar (pH 5.2) for numbers 20-23.

Criteria for growth were colony diameter, except for the yeasts for which the numbers of cells per ml suspension was used as criterium of growth. Both nematicides were tested at 1, 10 and 100 ppm a.i. ( $\mu\text{g/ml}$  substrate), and with two replicated agar plates in 10 cm diameter petri discs.

#### Results and discussion

The results summarised in table 25 are expressed as relative EC-50 values, i.e. the concentrations of the systemics in ppm a.i. which caused 50% inhibition of radial growth as derived from the average growth of the two replicates. The mean deviation was always lower than 10%.

The numbers 20, 21, 22 and 23 are fungi predatory to nematodes (nematode-trapping) in soil. From these fungi, the numbers 22 and 23 were not tested for, and the two other nematode-trapping fungi were very sensitive to phenamiphos. Oxamyl had no effect on fungi in general, except for one saprozoic species (*Gelasinospora cerealis*) which had an EC-50 of 10 ppm. Phenamiphos was generally toxic to soil fungi, with only slight differences amongst the various groups of fungi tested.

These results generally agree with those of CAYROL, CUANY and B'CHIR (1972) who found several soil isolates of nematode-trapping fungi sensitive to Nemafofos (an organophosphate systemic nematicide comprising thionazin), whereas Dupont 1410 (= oxamyl) hardly had any fungitoxic effect. They found abnormal growth of some fungi after treatment by oxamyl, whereas we did not; this may be due to the fact that they tested liquid cultures and we tested on agar plates, which could lessen the toxicity of compounds in our case.

It is obvious from these results that phenamiphos suppresses fungi in soil which may indirectly increase persistence of phenamiphos in soil. Suppression of plant pathogenic fungi may be beneficial, but the elimination of fungi predatory to nematodes and the partial elimination of the soil flora in general could be negative points for the application of phenamiphos.

Oxamyl gave some stimulation of the numbers 1 and 2 (*Oomycetes*) and 4 (*Zygomycetes*). The doses of oxamyl showing this effect, however, are higher than those recommended for nematode control in the field, and an effect of this nematicide on soil fungi at practical doses is therefore unlikely. For phenamiphos the concentrations showing strong inhibition of fungal growth *in vitro*

TABLE 25. Sensitivity of soil fungi to oxamyl and phenamiphos, expressed as EC-50 values derived from a test range of the concentrations 1, 10 and 100 ppm a.i. Fungi were measured according to radial growth on agar plates, and yeasts as numbers of cells per ml suspension.

Test organisms <sup>a</sup>	Incubation time in days	EC-50 values in ppm a.i.	
		oxamyl	phenamiphos
<i>Oomycetes</i>			
1 <i>Phytophthora cryptogea</i>	7	>100	<1
2 <i>Pythium irregulare</i>	3	>100	<1
<i>Zygomycetes</i>			
3 <i>Mortierella elongata</i>	4	>100	1- 10
4 <i>Mucor racemosus</i>	3	>100	10-100
<i>Ascomycetes/Deuteromycetes</i>			
5 <i>Chaetomium globosum</i>	7	>100	ca 1
6 <i>Gelasinospora cerealis</i>	6	ca 10	1- 10
7 <i>Phoma herbarum</i>	7	>100	1- 10
8 <i>Phoma betae</i>	7	>100	ca 1
9 <i>Ascochyta lycopersici</i>	7	>100	ca 1
10 <i>Botrytis cinerea</i> (BS) <sup>b</sup>	7	>100	10-100
11 <i>Botrytis cinerea</i> (BR) <sup>c</sup>	7	>100	ca 10
12 <i>Cladosporium herbarum</i>	6	>100	1
13 <i>Aspergillus flavus</i>	6	>100	>1
14 <i>Penicillium notatum</i>	6	>100	>1
15 <i>Talaromyces vermiculatum</i>	6	>100	1- 10
16 <i>Cylindrocarpon destructans</i>	6	>100	1- 10
17 <i>Fusarium redolens</i>	6	>100	1- 10
18 <i>F. oxysporum</i> f. <i>lycopersici</i>	6	not tested	1- 10
19 <i>Trichoderma</i> sp.	6	>100	ca 10
20 <i>Harposporium anguillulae</i> (PN) <sup>d</sup>	11	>100	not tested
21 <i>Nematocnonus robustus</i> (PN) <sup>d</sup>	11	>100	not tested
22 <i>Arthrobotrys oligospora</i> (PN) <sup>d</sup>	6	>100	ca 1
23 <i>Dactylaria candida</i> (PN) <sup>d</sup>	11	>100	ca 1
24 <i>Alternaria</i> sp.	6	>100	1- 10
25 <i>Gilmaniella humicola</i>	6	>100	1- 10
26 <i>Doratomyces microspora</i>	6	>100	1- 10
<i>Basidiomycetes</i>			
27 <i>Rhizoctonia solani</i>	6	>100	ca 1
28 <i>Fomes annosus</i>	6	>100	< 1
<i>Yeasts</i>			
29 <i>Rhodotorula rubra</i>	6	>100	10-100
30 <i>Candida albicans</i>	6	>100	10-100

a. Isolates obtained from soil, roots, seeds or encapsulated nematodes

b. Benomyl-sensitive strain

c. Benomyl-resistant strain

d. Predacious to nematodes



are about the same as recommended for nematode control in the field. The nematicide phenamiphos must therefore be considered a soil fungicide as well.

### 7.3.3. *Effects on Rhizobium trifolii*

It is known from literature, that nematode infestations (OOSTENBRINK et al., 1957), as well as soil treatments with nematicides (LIN, FUNKE and SCHULZ, 1972) may sometimes reduce the number of nitrogen root nodules on Leguminosae. Therefore the effect of oxamyl and phenamiphos on nodulation was studied in an experiment.

#### Experiment

Four weeks old red clover seedlings were transplanted to plastic pots containing 150 ml of sterilized potting soil with 60% organic matter and placed in constant temperature cabinets of 15 and 20°C. One week later each plant was inoculated with 10 ml suspension with about 250 *P. penetrans* derived from apple seedlings; the inoculum was not sterilized against fungi and bacteria. One week later the soil was drenched with oxamyl and phenamiphos to give 1, 4 and 16 ppm a.i. in the soil (w/v). Controls were: 1. + nematodes and - chemical; 2. - nematodes and - chemical; 3. - nematodes + 16 ppm oxamyl, and 4. - nematodes + 16 ppm phenamiphos.

Two months after treatments the plants were uprooted and washed free from adhering soil. Nematodes were extracted by the cottonwool-filter method. Estimates were made for root occupation by *Rh. trifolii* nodules in the scale 0-5 (0 = no nodules present; 5 = all roots occupied by nodules).

#### Results and discussion

The treatments with systemics unexpectedly delivered hardly an effect on the nematode densities in the soil and the roots. This may be due to the fact that we used soil with much organic matter. The influence on the occupation of the clover roots by *Rh. trifolii* nodules however was marked and significant. This is illustrated as a regression line in figure 35. Because oxamyl and phenamiphos gave exactly the same regression values, only the line of oxamyl is drawn. Both nematicides induced a significant decrease in root nodulation at increasing dosages, but only in the plant series which were inoculated with nematodes.

The treatments 2, 3 and 4 which received no nematodes showed root nodulation, but to a lesser extent than treatments with nematodes. Here no influence of the two systemics was traced. In *P. penetrans* inoculated pots we found an average nodule index of 1.98 compared to 1.08 in pots without nematodes. If the highest dose of the nematicides was omitted the nodule indexes were 2.23 and 1.08, respectively, the differences being significant.

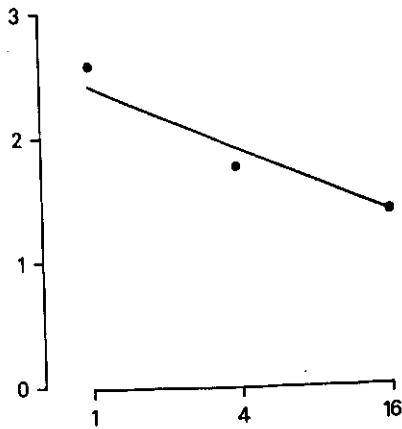


FIG. 35. Nodulation of *P. penetrans*-infested red clover roots by *Rh. trifolii* ( $= y$ ) upon treatments with 1, 4 and 16 ppm a.i. oxamyl and phenamiphos ( $= x$ ); the regression lines were practically the same for both nematicides.

Abscissa: Concentration of the systemics in ppm a.i.; log. scale.

Ordinate: Relative rates of nodulation.

Formulae: For oxamyl:  $y = -0.5x + 2.88$   $r = -0.44^{**}$ .

For phenamiphos:  $y = -0.5x + 2.88$   $r = -0.46^{**}$ .

It is clear, that the chemicals reduced bacterial nodulation in nematode-infested red clover seedlings. That this influence did not occur on nematode-free plants can only be explained if we accept that the nematode inoculum increased nodulation, as appeared to be the case, and at the same time promoted nodulation by transport of bacteria or in some other way. This result is in itself interesting. Transmission of bacteria by nematodes was also reported by HAWN (1971) for *Corynebacterium insidiosum* by *D. dipsaci* on alfalfa. LIN, FUNKE and SCHULZ, 1972, reported the inhibition of the growth of *Rhizobium* bacteria *in vitro* by organophosphorus pesticides including phenamiphos. Although the oximcarbamate aldicarb reduced the growth of such bacteria *in vitro* the effect was less pronounced. Most sensitive in their *in vitro* tests were *Rh. leguminosarum* and the physiological related *Rh. trifolii*. More experiments are needed to study whether systemics influence nodulation noticeably and thus cause damage under field conditions.

#### 7.4. RESISTANCE OF NEMATODES

In other organisms, especially fungi and insects, several mechanisms to overcome a treatment with biocides are known, e.g. breakdown rate, penetration inhibition, altered enzyme patterns which prevent the biocide from reaching its site of action, and others (Busvine, 1968; Dekker, 1972, 1973; Oppenorth and Houx, 1968).

Although physiological specialisation with respect to food or host ranges and also with respect to temperature is common in various nematode species, no nematode populations have yet been found which have acquired resistance to nematicides of any type. The only record, by CASTRO and THOMASON (1971), is not decisive. They obtained an *Aphelenchus avenae* population with perhaps some resistance against ethylene dibromide (EDB), if the nematodes were grown on a fungus culture, but the possibility that the host fungus caused more rapid breakdown of the chemical was not excluded. The only conventional nematicides which have been used intensively for some decades already are dichloropropene (DD), ethylenedibromide (EDB), dibromochloropropane (DBCP) and methamsodium (Monam). They all seem to have an unspecific, broadspectrum affect on all kind of animals, which may not leave much room for developing resistance mechanisms. The systemics, which affect nematodes by a more specific action (probably acetylcholinesterase inhibition as indicated under 5.5.), may select more rapidly those specimens that have acquired resistance, and thus some experiments were done to study this aspect.

### Experiments

*D. dipsaci* populations were exposed to sub-lethal concentrations of oxamyl and phenamiphos via the plant for a period of 18 months, indicated hereafter as 'permanent exposure'. Other lots of *D. dipsaci* were treated for 24 hours with high, but also sub-lethal, concentrations of the same systemics, which was repeated once per 10-12 weeks. This was indicated as 'semi-permanent exposure'. The *D. dipsaci* from the semi-permanent exposure treatment were cultured in fresh potato tubers after filtering the nematicide solutions without any further washing of the nematodes with water. The initial number of *D. dipsaci* at each nematicide concentration was about 100,000 per batch. The untreated batches were of 1000 nematodes only, because test plants would have been unable to survive attack by a higher number of *D. dipsaci*. The treatments are indicated below in detail.

A. For the permanent exposure *D. dipsaci* was inoculated onto *V. faba* and onto *L. esculentum* plants, which were drenched with oxamyl and phenamiphos in 0, 1/4, 1, 4 and 16 ppm a.i. doses each time that the plant series were renewed and re-inoculated with the original nematodes under observation. Plants were grown in sand instead of potting soil to prevent loss of chemicals by adsorption and some fertilizer was added during the growing period. To enhance penetration by *D. dipsaci* young plants of about 3-4 weeks were used after reducing the root systems below soil level. Every 6-8 weeks nematodes were extracted from the plants and inoculated onto new plants which were re-treated with the systemics. If only very few nematodes could be recovered from certain treatments the nematicide concentrations in these treatments were lowered to prevent complete loss of the populations. In this way all populations could be kept in breeding conditions.

B. For the intermittent exposure *D. dipsaci* was treated with 0, 4, 16 and 64 ppm a.i. phenamiphos and 0, 1000, 4000 and 16,000 ppm a.i. oxamyl during 24 hours. Then the nematicides were filtered off and the nematodes together with the filter paper used for filtering the nematicides away, were placed into fresh potato tubers. Every 2-3 months nematodes were extracted from the tubers and treated again, after which the procedure was repeated. In the phenamiphos treatments only the two lower dosages (4 and 16 ppm) allowed the nematode populations to breed. All oxamyl treatments were kept in breeding condition during 18 months. In some treatments the potato tubers showed symptoms of *D. dipsaci* rot before tubers of other treatments gave symptoms; they were then stored at 4°C to prevent complete decay of the tubers and corresponding loss of the nematodes.

For both series A en B nematodes were disinfected at inoculation against fungi and bacteria by Aretan and Streptomycine, respectively, to prevent attack of plants or tubers by micro-organisms (cf. 2.2.)

### Results and discussion

After 18 months populations were tested in the PI test to see to what extent they had acquired resistance to treatment with oxamyl and phenamiphos. By then about 10 successive plant series had been infested (A) and in the intermittent exposure test about 6 lots of potato tubers had been inoculated (B).

The EC-50 values for both series A and B varied between 0.04 to 0.07 ppm for phenamiphos doses, except for the 16 ppm dose of the permanent exposition series which had an EC-50 of about 2.0, which is 3-5 times the normal EC-50 value for phenamiphos. Unfortunately, there were only a few nematodes in the surviving population, so that only 2 replicates could be set up in this PI test, which reduces the reliability of this result.

The EC-50 values for oxamyl for all objects of both series A and B were between 0.6 and 1.1 ppm, which leaves no room to suggest any degree of resistance in the *D. dipsaci* populations treated with this nematicide throughout the 18 months' period of the experiment.

The results indicate, that marked resistance against the organophosphate and organocarbamate systemics cannot readily be selected out in *D. dipsaci*. This may hold for nematodes in general. It was concluded earlier (5.5) that the effect of these chemicals on nematodes is less drastic than the effect on insects, which are known to develop resistance against this group of chemicals rather readily. The fact that soil treatment with systemics in the field leaves a large part of the nematodes alive and often recoverable, supports this point. The stress on populations in the field is never so high as in the experiments above and the chance that resistance would develop under field conditions must therefore be smaller.

One experiment, however, indicated that phenamiphos induced slight resistance in *D. dipsaci*. We cannot neglect this result, despite the fact that too few nematodes survived to make this result clear-cut. It is considered significant, that this indication was obtained with the marginal dose of the most stable

compound with specific mode of action, therefore under the circumstances which could be expected most favourable to induce resistance or to select less susceptible nematodes. More results, however, are needed before it can be stated with confidence that phenamiphos can develop resistant nematode strains.

## SUMMARY

In this study, nematicidal effects, mode of action and specific characters of some systemic nematicides were studied, in search of substitutes for the widely used soil fumigants that require high dosages. The thesis comprises:

- a review of literature,
- development of techniques,
- a test for nematicidal effectiveness of agricultural chemicals,
- a detailed study of two systemic nematicides in vitro and in vivo,
- a study of some important side-effects of the two systemic nematicides.

### *Review of literature*

The main groups of test animals (nematodes and arthropods) and the systemic and fumigant categories of nematicides are briefly reviewed, and a scheme of the principal interactions between nematicide, fauna, flora and soil is given (Fig. 1). The literature review stresses:

- the great increase in crop yields possible by soil disinfection,
- the desirability of replacing currently used nematicides applied at dosages of 100-1000 kg a.i. per ha,
- the effect of systemic nematicides on the relation plant/nematodes at dosages of 1-10 kg a.i. per ha,
- the controversial opinions on mode of action of systemic nematicides,
- the lack of information on residues and side-effects,
- the selectivity of techniques for testing nematicides, which thus may not detect effective chemicals.

### *Development of techniques*

Materials and methods are briefly indicated. Special attention was given to the development of new screen techniques. Three biological assays, called 'penetration inhibition' test (PI test), 'therapeutic' test (T test) and 'gall index' test (GI test) were developed or modified to meet the requirements for effective and rapid screening of all known types of nematicides, with emphasis on systemics (Fig. 2, 7 and 10).

The PI test measures inhibition of invasion by *Ditylenchus dipsaci* in stem sections of *Vicia faba* (Fig. 3-6; Table 1). The T test can also be used like the PI test - to measure inhibition of invasion by *D. dipsaci* - but can be also used to measure the therapeutic effect of substances in *Lycopersicum esculentum* infested by *D. dipsaci* (Fig. 8 and 9; Tables 2 and 3). The GI test measures the effect on gall formation of *L. esculentum* by *Meloidogyne incognita* (Fig. 11; Table 4).

### *Nematicidal effectiveness of some biocides*

This general screen as a basis for appraisal and choice between compounds

for further study was made with the PI test. Tables 5 and 6 and Fig. 12 summarize details and results.

Thirtyfour of 60 preparations tested were effective with an EC-50 (median effective concentration) of 50 mg/litre (ppm) or less. The very active materials were organophosphates and organocarbamates and are known to inhibit acetylcholinesterase; they comprise a group of 17 compounds with an EC-50 of 1 ppm or less. Of those materials, the following had not previously been recorded as nematicidal: dichlorvos, methiocarb, trichlorphon, pyrazophos, fenitrothion. However, three known nematicidal active compounds showed an EC-50 above 50 ppm:  $\alpha$ -terthienyl, benomyl and dinitro-o-cresol (Table 5).

Oxamyl, an organocarbamate, and phenamiphos, an organophosphate, were chosen on the basis of these results and of other properties for further study on their effects and mode of action.

#### *Effects of oxamyl and phenamiphos in vitro*

Both chemicals had direct effects on nematodes (contact action), including protrusion of stylets, and shortening, swelling and wrinkling of their body, resulting in aberrant undulations and reduced mobility (Fig. 13-16). The symptoms of poisoning, however, were reversible for oxamyl, but less so for phenamiphos.

When *D. dipsaci* was permanently exposed to solutions of the preparations, the nematode was initially affected but recovered gradually in oxamyl concentrations up to 64 ppm. Phenamiphos did not allow recovery – not even at 0.1 ppm – during 21 days' exposure (Fig. 17; Table 7).

When *D. dipsaci* was washed with water, nematodes treated with 1000 ppm a.i. oxamyl for 24 hours recovered in 2 days; even after 4 days' treatment with oxamyl solutions at 10000 ppm a.i. nematodes in the L-4 stage could recover. The effects of phenamiphos were less reversible; after 24 hours' treatment in 100 ppm a.i. the nematodes did not regain normal behaviour, but nematodes did recover after treatment with 10 ppm a.i. Recovered nematodes could reproduce normally (Fig. 18-21; Tables 8 and 9).

The effects observed and those noted in the literature indicate that the effects were caused by inhibition of acetylcholinesterase or other neuro-enzymes.

#### *Effects of oxamyl and phenamiphos in soil and plants*

Soil drenches with aqueous solutions of both preparations reduced *Pratylenchus penetrans* in plant roots or even eradicated them, and also reduced nematode densities in soil. Phenamiphos, particularly, is less effective in organic soils. Root dips and foliage sprays (without preventing soil contamination) were effective, but basipetal transport of the substances could not be demonstrated. Also within plants, the effects of oxamyl on nematodes were reversible and of phenamiphos irreversible.

At low temperatures, at low dosages and in the first weeks after soil was drenched, oxamyl was superior as a nematicide to phenamiphos, either to

control nematodes inside or outside the roots of the test plants. At high temperatures, for high dosages and longer periods, the reverse was true (Fig. 22-24; Tables 10-14).

The effect of the two substances on nematodes in microplots sown with *Lolium perenne* (Fig. 25-27) were generally similar to those obtained *in vitro* and in drench treatments: this was true for several species of nematodes and microarthropods, although saprozoic mites were less susceptible (Fig. 28 and 29; Tables 17 and 18). Phenamiphos caused a greater and longer effect than oxamyl, although oxamyl too greatly reduced populations for several weeks after treatment. Plant growth was best on oxamyl plots as phenamiphos was apparently phytotoxic to *L. perenne*.

The same treatments on fallow soil confirmed that the substances had an almost equal, direct effect on the nematodes as for nematodes in soil in which *L. perenne* was growing (Fig. 31; Table 20).

#### *Some specific effects of oxamyl and phenamiphos*

Oxamyl persisted less in soil than phenamiphos (Fig. 33; Table 22); in fact oxamyl was so transient that low-temperature application increased the nematicidal effect markedly.

Experiments *in vivo* with sublethal concentrations of oxamyl and phenamiphos suggest that hardly any resistance could be expected with these systemics. About 9 successive generations of *D. dipsaci* were tested for resistance in one year.

Sprays with oxamyl were not toxic to plants while phenamiphos was relatively toxic (Fig. 34), with differences from species to species of plant.

A test *in vitro* on toxicity to fungi showed that oxamyl has no fungitoxic properties and phenamiphos has (Table 25).

Both substances reduced *Rhizobium trifolii* nodulation on red clover plants infested by *P. penetrans* (Fig. 35).

#### *General conclusions*

Systemic nematicides are useful for preventing nematodes from attacking crops, by preventing penetration of nematodes or even by eradicating nematodes that have already entered roots, stems or leaves. The nematicidal effects and persistence is somewhat greater for phenamiphos than for oxamyl.

Apparently they not only influence nematodes through the plant (by systemic action), but also in the soil (by contact).

Systemic nematicides may also prevent damage to plants by microarthropods.

As to residues, oxamyl seems less dangerous to the environment than phenamiphos, because oxamyl is quickly broken down to biologically inactive substances. The combination of short persistence and reversibility of the poisoning effect makes oxamyl – in contrary to phenamiphos – nematostatic rather than nematicidal.



# SAMENVATTING

## EFFECT EN WERKINGSWIJZE VAN ENKELE SYSTEMISCHE NEMATICIDEN

In een onderzoek naar substituënten voor de veel en in hoge doseringen toegepaste bodemfumigantia, werden de nematicide effecten, de werkwijze en andere eigenschappen van enkele systemische nematiciden bestudeerd. Het proefschrift omvat:

- een literatuuroverzicht,
- ontwikkeling van technieken,
- een onderzoek naar de nematicide werking van een aantal gewasbeschermingsmiddelen,
- een gedetailleerd onderzoek aan twee systemische nematiciden *in vitro* en *in vivo*,
- een onderzoek naar enkele belangrijke neveneffecten van de twee systemische nematiciden.

### *Literatuuroverzicht*

De belangrijkste groepen van proefdieren (nematoden en arthropoden) en de categorieën van de in de gasfase werkzame en van de systemische nematiciden, zijn kort aangegeven. Figuur 1 toont de belangrijkste interacties tussen nematiciden, de fauna, de flora en de grond. Het literatuuroverzicht onderstreept:

- de mogelijkheden om door grondontsmetting de gewasopbrengst te verbeteren,
- de wenselijkheid om de tot nu toe gebruikte nematiciden, in doseringen van 100-1000 kg actieve stof per hectare, te vervangen,
- het effect van systemische nematiciden op de relatie plant/nematoden, in doseringen van 1-10 kg actieve stof per hectare,
- de tegenstrijdige meningen over de werkwijze van systemische nematiciden,
- het gemis aan goede informatie over residuen en neveneffecten,
- dat de technieken voor het toetsen op nematicide werking selectief zijn en aanleiding kunnen zijn voor verlies van potentieel goede middelen.

### *Ontwikkeling van technieken*

De gebruikte materialen en methoden zijn kort vermeld. Speciale aandacht werd geschonken aan de ontwikkeling van nieuwe toetstechnieken. Drie bio-toetsen, aangeduid als 'penetration inhibition' toets (PI toets), 'therapeutic' toets (T toets) en 'gall index' toets (GI toets), werden ontwikkeld of aangepast om te voldoen aan het vereiste van effectieve toetsing op de nematicide werking van bekende typen nematiciden, met de nadruk op de systemische (Fig. 2, 7 en 10).

Als maat voor de werkzaamheid van middelen in de PI toets, dient de penetratieremming van *Ditylenchus dipsaci* in stengelstukjes van *Vicia faba* (Fig. 3-6; Tabel 1). De T toets kan worden gebruikt als de PI toets – om de penetratieremming van *D. dipsaci* te bepalen – maar kan bovendien worden aangewend om het therapeutisch effect op met *D. dipsaci* besmette *Lycopersicon esculentum* te onderzoeken (Fig. 8 en 9; Tabellen 2 en 3). De GI toets kan worden gebruikt om het effect van middelen op de mate van galvorming door *Meloidogyne incognita* bij *L. esculentum* te bepalen. (Fig. 11; Tabel 4).

#### *Nematicide effect van enkele biociden*

Deze algemene toets diende als basis voor de beoordeling op nematicide werking van een aantal biociden, alsook tot selectie van enkele systemische nematiciden voor meer gedetailleerd onderzoek. Gegevens en resultaten zijn samengevat in de Tabellen 5 en 6 en in Figuur 12.

Uit 60 middelen waren er 34 effectief ( $EC_{50} < 50$  dpm) en 17 zeer effectief ( $EC_{50} < 1$  dpm). Deze zeer effectieve biociden behoren tot de organofosfaten en organocarbamaten en zijn bekend als remmers van het enzym acetylcholinesterase. Tot deze verbindingen behoren er waarvan de nematicide of nematostatische werking niet bekend was, namelijk dichloorfos, methiocarb, trichloorfon, pyrazofos en fenitrothion. Drie verbindingen waarvan nematicide activiteit gerapporteerd werd,  $\alpha$ -terthienyl, benomyl en dinitro-o-cresol, vertoonden geringe werking.

Oxamyl als een organocarbamaat en phenamiphos als een organofosfaat werden op grond van de resultaten en andere criteria gekozen voor meer gedetailleerd onderzoek over effect en werkingswijze.

#### *Effecten van oxamyl en phenamiphos in vitro*

Beide middelen hadden een direct effect op nematoden (contactwerking), zoals uitstekende mondstekel, en verkorting, verdikking en rimpeling van het lichaam, resulterend in een verkrampde lichaamshouding en verminderde mobiliteit (Fig. 13-16). De symptomen van vergiftiging waren reversibel voor oxamyl maar nauwelijks voor phenamiphos.

Als *D. dipsaci* permanent wordt gedompeld in oplossingen van de beide middelen, kan geleidelijk herstel optreden in oxamyl concentraties tot 64 dpm. Phenamiphos laat geen herstel toe – zelfs niet in 0.1 dpm – gedurende 21 dagen dompeling (Fig. 17; Tabel 7).

Tijdelijke blootstelling (1-4 dagen) aan oxamyloplösungen tot 10.000 dpm gevolgd door uitspoeling van het nematicide, resulteerde voor de L-4 stadia van *D. dipsaci* tot volledig herstel. De effecten van phenamiphos waren minder reversibel: herstel was slechts mogelijk na tijdelijke dompeling in concentraties beneden 100 dpm. Herstelde dieren vertoonden een normale reproductie (Fig. 18-21; Tabellen 8 en 9).

De waargenomen effecten in combinatie met literatuurgegevens, waren aanleiding tot de hypothese dat deze effecten toegeschreven dienen te worden aan de remming van acetylcholinesterase of andere neuro-enzymen.

### *Effecten van oxamyl en phenamiphos in grond en planten*

Begietingen van grond met waterige oplossingen van de beide middelen reduceerden aantallen nematoden in (*Pratylenchus penetrans*, *D. dipsaci*) en buiten de wortels (diverse tylenchide en saprofage soorten). In gronden met een hoog organische stof gehalte was phenamiphos minder effectief dan oxamyl. Beide middelen waren effectief als bladbespuitingen (zonder preventie van grondcontaminatie) of indien de wortels van besmet plantgoed werden gedompeld in oplossingen ervan: basipetaal transport kon niet worden aangetoond. Ook in planten was de werking op nematoden van oxamyl reversibel en van phenamiphos irreversibel. Bij lage temperatuur, in lage doseringen en op relatief korte termijn na de begieting van de grond, was oxamyl effectiever dan phenamiphos, zowel in de grond alsook binnen de plant. Bij hoge temperatuur, in hogere doseringen en op latere tijdstippen na de behandeling trad het omgekeerde effect op (Fig. 22-24; Tabellen 10-14).

Het effect van beide nematiciden op nematoden in microveldjes begroeid met *Lolium perenne* was vergelijkbaar met dat van de in vitro en de grondbegietingsproeven. Dit geldt voor de nematoden- en arthropodenpopulaties. Saprofage mijten waren minder gevoelig (Fig. 25-29; Tabellen 17 en 18). Phenamiphos veroorzaakte een sterker en langduriger effect dan oxamyl. Het gras reageerde gunstig op de behandelingen met oxamyl. Phenamiphos was in de gebruikte doseringen fytotoxisch.

Vergelijkbare behandelingen op braakliggende grond bevestigden het vermoeden dat directe effecten van oxamyl en phenamiphos op nematoden van groot belang moeten worden geacht (Fig. 31; Tabel 20).

### *Specifieke effecten van oxamyl en phenamiphos*

Oxamyl was minder persistent dan phenamiphos (Fig. 33; Tabel 22). De instabiliteit van oxamyl verklaart waarom een verlaging van de temperatuur de nematicide werking verlengt.

*In vivo* proeven met oxamyl en phenamiphos in sublethale concentraties voor *D. dipsaci* toonden, dat de kans op resistentie daartegen gering moet worden geacht. Er werden 9 opeenvolgende generaties van *D. dipsaci* gekweekt in een jaar voor toetsing op resistentie.

Oxamyl was, bij bespuiting van planten, minder fytotoxisch dan phenamiphos, met verschillen in gevoeligheid tussen plantsoorten (Fig. 34). Alleen phenamiphos vertoonde duidelijk fungicide eigenschappen *in vitro* (Tabel 25). Beide middelen reduceerden de nodulatie door *Rhizobium trifolii* op rode klaver in aanwezigheid van *P. penetrans* (Fig. 35).

### *Algemene conclusies*

Systemische nematiciden lijken aantrekkelijke middelen om gewassen te beschermen tegen aantastingen van nematoden, door het tegengaan van penetratie of door uitschakeling van reeds binnengedrongen nematoden. Het nematicide effect en de persistentie van phenamiphos waren meestal groter dan van oxamyl.

Er werd aangetoond dat nematoden niet alleen via de plant (systemische werking) maar ook in de grond (contactwerking) worden beïnvloed.

Systemische nematiciden kunnen ook worden gebruikt ter voorkoming van schade aan planten door microarthropoden.

Gezien de geringe persistentie lijkt oxamyl minder gevaarlijk voor het milieu dan phenamiphos. Oxamyl wordt kennelijk snel afgebroken tot biologisch inactieve componenten. De combinatie van geringe persistentie met een reversibel vergiftigingseffect op nematoden maakt, dat oxamyl – in tegenstelling tot phenamiphos – eerder tot de nematostatische dan tot de nematicide verbindingen gerekend dient te worden.

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## CURRICULUM VITAE

Johannes Ardinus Bunt, geboren 24 januari 1938 te Kesteren.

Lagere Landbouwschool te Kesteren, 1950-1954.

Medewerker op landbouwbedrijf, 1954-1958.

Middelbare Landbouwschool te Andelst, 1958-1959.

Gastmedewerker aan de Afdeling Nematologie der Landbouwhogeschool, 1959-1963.

Gastmedewerker aan het Instituut voor Planteziektenkundig Onderzoek, 1963-1966.

Opleiding tot botanisch analist te Wageningen (STOVA), 1960-1965.

Basisopleiding voor technisch chemicus te Arnhem (PBNA), 1965-1966.

Landbouwhogeschool te Wageningen, hoofdrichting Fytopathologie, keuzevakken: de Nematologie, de Entomologie en de Organische Chemie. De praktijktijd werd doorgebracht aan het Woodstock Agricultural Research Centre (Shell) te Sittingbourne, Kent, Engeland. De studie werd aangevangen in september 1966 (via een bijzondere toelating verleend door het M. v. L. & V.) en voltooid in januari 1972.

Onderzoek over systemische nematociden aan de Landbouwhogeschool te Wageningen, Afdeling Nematologie, onder leiding van Dr. M. Oostenbrink, februari 1972-februari 1975.

Vanaf 1 januari 1975 werkzaam als wetenschappelijk medewerker aan de Landbouwkundige Afdeling van B.V. Chemische Pharmaceutische Industrie 'LUXAN', te Elst (Gld.), ten behoeve van de ontwikkeling van gewasbeschermingsmiddelen en andere landbouwchemicaliën.