Studies in the control of *Heterodera rostochiensis* Wollenweber, 1923 and related species, using halogenated hydrocarbon fumigants.

by

A. Omidvar.

A thesis submitted in part fulfilment
of the requirements for the degree of
Doctor of Philosophy
in
the University of London.

Imperial College Field Station,
Ashurst Lodge,
Sunninghill, Ascot,
Berkshire.

1st July 1961.

ABSTRACT.

This thesis is concerned with the fumigation of Heterodera rostochiensis cysts, either in vitro or in soil, under varying conditions of temperature, humidity and dose, using as host plants: potato, tomato, eggplant and black nightshade. The thesis also deals with various types of bioassay applied to these fumigation experiments and finally recommends the final density assay in terms of larvae per gramme of soil. The fumigants used were chlorobromopropene (which is a good contact nematicide but a poor soil fumigant), ethylene dibromide and Nemagon. The latter is interesting for its greatly delayed effect on the eelworm population.

The activity of larvae fumigated with Nemagon, as distinct from their viability, was measured by their vertical movements in a column of sand.

The length of time required for the completion of the hatching process was investigated. The correlation between hatchability and viability of encysted larvae was studied and it was concluded that the emergence of larvae from cysts had no relation to their full viability.

Nematicidal effects of Tagetes plants (African marigold) were sought and it was found that the cultivation of Tagetes in soil infested with potato root eelworm produces a feeble nematicidal effect shown by a slight reduction in eelworm population only over a long period.

The effect of heat on root diffusates was studied. The root diffusates lost their hatching power when they were heated to boiling point.

The host-parasite-fumigant relationship was under observation and attempts were made to investigate any noticeable damage caused either by the parasite or by the phytotoxicity of the fumigants on the host plants.

It was concluded that because of eelworm damage initial infestation for tomato must be lower than for potato. No trace of phytotoxicity under the described conditions was found.

ACKNOWLEDGMENTS.

I should like to express my gratitude to Professor B.G.Peters for supervising this work and for his constant advice, encouragement and help with both nematological and statistical problems.

Thanks are also due to Dr.N.G.M.Hague for advice and for reading the manuscript.

Also I should like to thank Professor O.W.Richards in whose department the work was carried out. I should like to thank Dr.R.E. Blackith for help with statistical problems and Dr.O.F.Lubatti, Dr.F.Call and Mr.A.P.Mainwaring for their help during the chemical assays, and Mr. J.M.Stringer for helping to grow the eggplants and tobacco in his greenhouses.

Again I should like to thank Professor B.G.Peters for his help and advice in photographing illustrations and for his assistance in the preparation of the three dimensional models illustrating nematicidal effects which were exhibited at the Linnean Society of London.

I am grateful to the Iranian Government (Ministry of Agriculture and Seven Years Plan Organisation) for granting leave to pursue these studies and to the Organisers of the Central Treaty Organisation and the British Council for their financial support in the final year of my work.

TABLE OF CONTENTS.

	Page.
GENERAL INTRODUCTION.....	1
PART I. HISTORICAL.....	5
(a) Chemical Control.....	5
i. Shell D-D.....	7
ii. Ethylene dibromide.....	8
iii. Nemagon.....	8
iv. Methyl bromide.....	9
v. Chloropicrin.....	9
vi. Chlorobromopropane.....	9
(b) Methods of assay.....	12
PART II. BIOASSAY TECHNIQUES AND BIOLOGICAL MATERIALS.....	15
(a) Origins of H.rostochiensis material.....	15
(b) Criteria - Methods of assay.....	15
(c) Root diffusate assay.....	16
i. Length of assay.....	17
Experiment No.1.....	18
ii. Vertical movement of H.rostochiensis larvae after various treatment conditions.....	20
Experiment No.2.....	22
Experiment No.3.....	25
Experiment No.4.....	27
iii. Estimation of mortality by root diffusate assay.	28
(d) Root invasion assay.....	32
i. Staining procedure.....	32

Page.

ii. Invasion of eggplant (Experiment No.5)..... 33

iii. Penetration of Heterodera larvae (Experiment No.6)..... 35

iv. Penetration of Heterodera larvae (Experiment No.7)..... 36

(e) Assays based on the final eelworm density..... 37

(f) Soil sterilization..... 38

PART III. FUMIGATION EXPERIMENTS..... 40

General introduction..... 40

Section A - Experiments in vitro..... 41

(a) Introduction..... 41

(b) Experimental procedure..... 42

i. Saturation concentration..... 44

Determination of gas concentration (Experiment No.8)... 44

(c) Methods of assay..... 48

(d) Effect of relative humidity on fumigation..... 51

i. Acclimatisation of cysts (Preliminary Experiment No.9). 51

ii. Effect of R.H. on E.D.B. and Nemagon (Experiment No.10). 52

1. Root diffusate assay..... 52

2. Root invasion assay..... 55

3. Final eelworm population assay..... 56

. Tomato as host (Experiment No.11)..... 56

. Potato as host (Experiment No.12)..... 57

4. Results..... 60

Page.

(e) The effect of temperature on fumigation (Experiment No.13). 62

i. Introduction..... 62

ii. Methods..... 63

iii. Results..... 63

(f) Fumigation of H.rostochiensis cysts at different dosages (Experiment No.14)..... 65

i. Root diffusate..... 65

ii. Root invasion assay..... 66

iii. Final eelworm population assay..... 66

iv. Comparison of methods of assay using Nemagon as fumigant..... 68

(g) Observations on the eelworm population by infecting the planted soil with Heterodera larvae emerged from the fumigated cysts (Experiment No.15)..... 72

i. Results..... 72

(h) Observations on the effect of storage on fumigated cysts (Experiment No.16)..... 73

i. Results..... 74

(i) Discussions of experiments in vitro 76

i. Root diffusate assay..... 76

ii. Root invasion assay..... 77

iii. Final eelworm population..... 80

Page.

Section B. Experiments *in vivo* (Pot experiments)..... 82

(a) Introduction..... 82

(b) Experimental procedure..... 83

(c) Pot experiment in 1959-60 (Experiment No.17)..... 84

i. Methods of fumigation..... 84

ii. Rates of fumigation..... 85

iii. Results..... 87

iv. Conclusion..... 92

(d) Pot experiment in 1960-61 (Experiment No.18)..... 92

i. Introduction..... 92

ii. Rates of fumigation..... 95

iii. Y samples..... 97

iv. Results..... 99

1. Potato as host..... 99

Fumigation with C.B.P..... 99

Fumigation with E.D.B..... 100

Significance charts..... 101

Fumigation with Nemagon..... 101

2. Tomato as host..... 104

Fumigation with C.B.P..... 104

Fumigation with E.D.B..... 105

Fumigation with Nemagon..... 105

3. Eggplant as host..... 107

4. Y samples..... 114

Page.

PART IV. MISCELLANEOUS EXPERIMENTS..... 115

(a) Studies on nematicidal effects of *Tagetes* spp..... 115

i. Observations of nematicidal effect of *Tagetes* (Experiment No.19)..... 116

Results..... 117

ii. Observations of the effect of *Tagetes minuta* (Experiment No.20)..... 118

Results..... 119

(b) Determination of the hatching power of different root diffusates.... 119

i. Introduction..... 119

ii. Root diffusate collection..... 120

iii. The effect of heat on potato root diffusate (Experiment No.21)..... 121

iv. Changes in the dilution curve of root diffusates (Experiment No.22)..... 121

v. Results and Discussion..... 122

(c) Observations on the population build-up of *H.rostochiensis* with different host plants (Expt.No.23).... 124

i. Introduction..... 124

ii. Experimental procedure..... 125

iii. Results..... 126

GENERAL DISCUSSION..... 129

SUMMARY..... 149

REFERENCES..... 151

APPENDIX..... i - xxix

GENERAL INTRODUCTION.

The genus Heterodera of the order Tylenchida, forms a group of about 20 species of cyst-forming root nematodes all of which are parasites on the roots of a wide range of host plants.

Many crops all over the world are seriously affected by these nematodes. The damage caused by one of these species, Heterodera rostochiensis in England has been officially estimated at about two million pounds a year (Peters 1953a), but a more recent appraisal of the situation places the losses even higher.

The susceptibility of different plants listed as hosts, for a given species of Heterodera, differs very widely. A list of host-plants may include many species in which the parasite is able to reproduce very freely, whereas in other hosts the degree of infestation is very low and the females do not mature readily and reproduction is slight.

In most species of Heterodera the larvae are found within brown cysts in the soil and can survive in a dormant condition for several years. It is known that in most species these larvae will be stimulated in the vicinity of the host plant by an excretion from the roots and hatch out from the cysts. The cyst is a tough, brown, inanimate, spherical sac which contain up to several hundred of living eggs and larvae.

The first stage of the life cycle of the parasite occurs in the egg shell and in the second stage invasion of the roots will occur.

Most of the larvae enter, at or near root tips, by their protrusible mouth spear (present in all plant parasitic nematodes). When the larvae have penetrated the root tip, they pass through the epidermis and take up a

position in the endodermis lying along the longitudinal axis of the root. The female worms swell considerably and, the head remaining within the cortex, the typical flask-shaped body bursts out from the root (about eight weeks after penetration). At this stage males leave the root to fertilize females. After fertilization the males presumably die. The cuticle of females gradually becomes tougher and passes from white to golden brown and produce the cysts found in the soil, which vary considerably in size.

Cyst forming nematodes are very difficult to control, because the larvae remain alive for several years, and are also protected by the cyst.

Besides chemical treatments many different methods of control have been suggested, such as crop rotation and searching for resistant varieties of plants, but none of them has been wholly satisfactory. Investigations and different experiments have been carried out in Great Britain to find an active substance similar to root diffusate to apply to infested land and stimulate larvae to hatch in the absence of a host crop, but this method does not seem applicable under field conditions. Fenwick (1956) has shown that the substance will break down rapidly in soil before yielding the expected result.

Cultivation of some plants (Tagetes spp.) has also been advocated as a means of reducing the population of H.rostochiensis (Steiner 1941; Oostenbrink et al. 1957).

Since the damage caused by the genus Heterodera to different crops is of great economic importance it is desirable to find an effective method

for their control. Soil fumigation is reasonably effective against cyst-forming nematodes but because the kill is not complete the residual population will build up very quickly. Various chemicals have been used to kill the encysted larvae and eggs in the soil, some of them have shown considerable effect under favourable conditions.

Bearing in mind the economic importance of H.rostochiensis the present work was designed to investigate the efficacy of some of the better known nematicides against this parasite.

Heterodera rostochiensis attacks the roots of various solanaceous plants. Recently the potato root eelworm has been reported attacking eggplant, Solanum melongena, which is an important crop in Persia (Fassuliotis and Feldmesser 1954). It has also been reported that the weed Solanum nigrum is parasitised by some races of H.rostochiensis.

It was therefore decided to conduct some experiments to measure the degree of infestation of various host plants by using this parasite. The effect of cultivating Tagetes plants and the degree of their root diffusate production has also been investigated.

The problem of testing the nematicidal effect of the fumigants on H.rostochiensis in the presence of different hosts will be considered.

According to the life cycle of H.rostochiensis, three important phases could be distinguished:

a) stimulation of larval hatching by root excretions of the host plant, commonly called "root diffusates",

b) movement in the soil toward the root and penetration into the host plant,

c) completion of the life cycle in the root and forming of new cysts.

Based upon the above mentioned phases three methods of assay could be arranged: (1) by using root diffusate and artificial stimulation of the larvae; (2) by sowing the host plant seed in infested soil and estimating the number of penetrated nematodes; and (3) estimating the number of newly formed cysts in each treatment.

It was decided to compare the three methods of assay, and this thesis is largely concerned with this comparison.

PART I. HISTORICAL.

(a) Chemical Control.

The most destructive of all the cyst forming root eelworms especially in Great Britain is the potato root eelworm.

Although there is not any official report of its occurence in Asian countries including Iran, nevertheless this lack of information does not prove its absence in that part of the world, especially in the temperate regions.

Since these eelworms cause a considerable amount of damage to crops there has been continuous research on various aspects of their biology and control.

Crop rotation, the artificial application of root diffusates which cause larvae to emerge and then die of starvation, cultivation of Tagetes plants in infested soil to suppress the eelworm population, and finally the application of nematicides, have all been tested and advocated by many workers.

Some investigators believe that the use of fumigants alone does not give a satisfactory result unless it is combined with another practical method of control such as crop rotation (Thorne 1951; Mai and Lear 1953). Morgan (1925) tested a number of compounds against H.rostochiensis and claimed that calcium cyanide and carbon disulphide gave a slight reduction in cysts on the roots of host plants. Hurst and Triffit (1935a) found nematicidal effects in an infested potato field from potassium ethyl xanthate and chinosol, and from ferrous sulphate, ferric chloride and ferric oxide.

The use of ferric oxide which gave the best yield, probably neutralized the effect of the root diffusate.

Triffit (1930) and Ellenby (1951) used the root diffusates extracted from some of the crucifers (Brassica hirta) and (B.nigra) in infested soil with H.rostochiensis in presence of the host plant to antagonise the effects of potato root diffusate. They have shown that the root excretions from mustard neutralizes the effect of potato root diffusate and larval emergence will be stopped. In 1937 Hurst and Triffit reported on further small scale tests with ferric oxide and calcium cyanamide; both gave yield increases, but eelworm control was inferior to that in previous pot tests.

Hurst and Franklin (1937) used calcium cyanamide and ferric oxide. The former gave a reasonable increase in potato yield, but the latter gave negative results. Field trials with various forms of ferric oxide and zinc oxide gave disappointing results (Hurst and Franklin 1938b). Hurst (1938b) showed that acetic acid in the form of pyroligneous acid, increased the killing power of cyanamide. Cyanamide was used in powdered and granular forms. He found out that powdered form of cyanamide was more effective than granular one. Edwards (1939a) and Gammell (1944) used calcium chloroacetate in field experiments, but it did not reduce the infestation of the soil. In Germany also various chemicals have been used (Goffart 1943a; Reinmuth 1929) but none of them were of any great practical use.

Many different chemicals such as: sodium hypochlorite, chloroacetates, phenyl, ethyl and n-butyl isothiocyanates have been tried in

different ways against H.rostochiensis but the final results did not show any promise. Some of these compounds were reported to be partially effective by dissolving eelworm cysts or being toxic to eelworms in soil.

In 1939 Smedly reported on the good nematicidal effects of phenyl, ethyl and n-butyl isothiocyanates. She also observed that p-hydroxyphenyl isothiocyanate had no effect and o- and p-tolyl isothiocyanates merely delayed hatching. The phenyl compound has been reported as a good nematicide giving increased yields and reduced eelworm multiplication.

Although resistant to many chemicals, H.rostochiensis like other nematodes is more sensitive to the halogens. Of those that have been tried the most effective compounds are:

i - Shell D-D. (A mixture of 1-3 dichloropropylene, and 1-2 dichloropropane with some other chlorides).

A beneficial effect on pineapples from using D-D mixture soil fumigant was recorded by Carter (1943) against Meloidogyne. In 1944 and 1945 preliminary work was carried out at the Institute of Agricultural Parasitology, St.Albans, under Professor Leiper's direction, using D-D against H.rostochiensis Woll. Some of the results (not yet published) were promising. Field trials with D-D against potato root eelworm went on at seven 2-acre sites (Peters & Fenwick 1949), but the results were not sufficiently satisfactory. In this experiment the highest rate of D-D was 800 lb. per acre which gave a 50% increase in yield and a 50% kill of eelworm as measured four weeks after injection, at only some of the seven sites. However when the injection was followed by a potato crop the eelworm

population increased roughly in proportion to the rate of fumigation.

There are many varying reports as to the efficacy of D-D mixture. Some workers have found it effective against potato root eelworm (Chitwood & Buhrer 1945). This variation in the efficacy of this and other fumigants reported by different investigators could be explained in terms of climate which has been described by Peters (1949).

ii - Ethylene dibromide.

(1-2 dibromoethane), commonly called E.D.B. is another chemical which came into use shortly after D-D (Christie 1950; Parris 1958). Some workers have found it very effective against Heterodera (Chitwood 1951; Peters 1952) whereas unsatisfactory results have been recorded by others (Simon 1949; van den Brande et al. 1951; and Oostenbrink 1954).

Peters (1952) showed that 0.13 ml. of pure E.D.B. per pot (containing about 10 Kg. of soil) gave a 50% kill of H.rostochiensis.

iii - Nemagon.

A trade name for the technical grade of 1-2 dibromo - 3chloropropane is another Shell product which recently has been used by many workers against different nematodes. McBeth (1957) has shown that Nemagon will give good nematode control and yield increases. One of the advantages of Nemagon is that it can be formulated adsorbed on granules. Grainger (1956) investigated the possibilities of mechanical mixing for controlling H.rostochiensis. It might enable the grower to incorporate both nematicide and fertilizer in one operation to save time and difficulty of application of fumigants in other forms.

iv - Methyl bromide

(Bromomethane) (CH_3 Br) is a well-known fumigant and is used widely for killing insects, mites, and nematodes.

Methyl bromide is an effective nematicide and is used for fumigating soil. Feldmesser et al. (1951) reported a variety of responses of H.rostochiensis cysts to treatment with this fumigant. Lear & Mai (1952) reported complete eradication of the H.rostochiensis by fumigation with a dose of 23 lbs. of methyl bromide per 1000 cu.ft. for 16 hours.

In the present work I used this fumigant for soil sterilisation in a large chamber of about 5000 litres capacity. The rate of fumigation was 800 mg.hours per litre (Hague 1958).

v - Chloropicrin.

Known as tear gas (trichloronitromethane). It is applied by injections and leaves the soil somewhat more quickly than D-D or E.D.B.

In many respects chloropicrin amd methyl bromide are excellent soil fumigants, but being very expensive and owing to difficulties in application they are not greatly used.

vi - Bromochloropropene (C.B.P.)

(3-Bromo-1-chloropropene. CHCl = $CH.CH_2Br$).

This chemical was introduced in 1952 by Shell Development as a soil fumigant. The technical product under the common name "CBP-55" contains about 55% 1-chloro-3-bromo-1-propene, together with related brominated and chlorinated C_3 hydrocarbons. It has been used for experimental purposes as a soil fumigant against nematodes but being very

expensive has been replaced by Nemagon and is now off the market. Van den Brande et al. (1955) have shown that H.rostochiensis cysts adhering to Begonia and Gloxinia tubers were completely killed by dipping the infested tubers in Chlorobromopropene emulsions. Concentrations of the emulsion were 0.05% and dipping time was 20, 40, and 60 minutes at room temperature. No phytotoxic effects were reported. Van den Brande et al. (1954) have studied the influence of the factor humidity on C.B.P., C.P., D-D and E.D.B. against the potato root eelworm. The results obtained indicated that C.B.P. gave an excellent nematicidal effect, whereas E.D.B. showed very poor nematicidal action as compared to the other compounds. Hague (unpublished) has shown that C.B.P. may be useful for controlling cysts of H.rostochiensis adhering to seed potatoes which are not damaged at dosages lethal to the nematodes.

Table 1. General properties of some Halogenated Hydrocarbon nematicides.

Common name	Formula and chemical name	Physical State at 20°C.	Specific Gravity of liquid density at ...°C.	Vapour Pressure mm.Hg.at..°C.	Boiling Point at 760mm.	Colour
Chlorobromo-propylene C.B.P.	C_3H_4 Br Cl. (3 Bromo-1-chloro-propene)	Liquid	1.36-1.40	-	130-180°C.	Black
Chloropicrin (tear gas)	CCl_3 No_2 Trichloronitromethane	Liquid	$\underline{d}^{20}$ 1.656	23.8 (25°C)	112.4°C.	Colourless
D-D mixture	1.2 dichloropropene-1.2 dichloropropane, with traces of higher chlorides.	Liquid	$\underline{d}^{20}_{4}$ 1.198	31.3 (20°C.)	(50°C. - 115°C.)	Black (technical) Colourless (pure)
Ethylene dibromide E.D.B.	CH_2 Br - CH_2 Br Dibromoethane	Liquid	d^{25} 2.172	7.69 (20°C.)	131.5°C.	Colourless
Methyl bromide	CH_3 Br Monobromomethane	Gas	$\underline{d}^{o}$ 1.73	1824 (25°C.)	4.4°C.	Colourless
Nemagon (OS 1897)	CH_2Br-$CHBr$-CH_2Cl 1.2 dibromo-3-chloro-propane	Liquid	d^{20} 2.28	0.8 (20°C.)	196° C.	Amber to dark brown

(b) Methods of Assay.

Controlling H.rostochiensis with chemicals involves two problems: First, finding effective nematicides and second, measuring the degree of their efficacy against the parasite. The latter is beset with difficulties.

The establishing of various technical procedures to obtain the treated cysts and/or larvae should not go unnoticed. Recovering the cysts from soil before and after applying nematicides, counting the number of viable larvae per g. of soil, duration of the effect of the nematicide in soil and finding the right time for measurement of efficacy of the fumigant, are some of the obvious problems which have attracted the investigators for many years.

Morgan (1925) devised the method of recovering cysts from soil by floatation in water which was adapted afterwards by other workers as a standard method (Fenwick 1940). The method of cyst counting was studied quantitatively by Morgan and Peters (1929) who showed that cyst counts from air-dried soil measured by weight were the least variable. Samples weighing 200g. (Fenwick 1940) are nowadays in routine use.

The counting of white cysts exposed on the roots of potted plants when carefully turned out is another method of investigation of the efficacy of fumigants which was devised by Triffit (1929a).

"Timed count Technique" which is based on the mean number of white females observed on the root system of living potato plants in one minute, was proposed by Chitwood and Feldmesser (1948), as a method of assay.

This method gives no approximation of the actual numbers of the

parasite and requires experienced personnel and is considered too subjective by most workers (Fenwick & Reid 1951a). Estimation of the number of larvae per cyst in each treatment is another method of measuring the degree of efficacy of the fumigant used.

Following Baunacke's (1922) discovery of root-stimulated hatching, Triffitt (1930a) found that the root excretion of the growing potato plants stimulated the larvae to hatch. Morgan (1925) tested the root diffusate from mustard, which is a host of H.schachtii, to stimulate potato root eelworm but failed. The reason for the inhibitory effect of mustard was studied by Triffitt (1930). She found that the diffusate from mustard has the effect of antagonizing the potato diffusate. This was confirmed by Smedley (1939) who found that sub-lethal dilutions of certain isothiocyanates delayed hatching in H.rostochiensis larvae. In 1932 Triffitt showed that besides root diffusate extracted from host plants, excretions from certain grasses also stimulated the hatching of potato root eelworm larvae.

Fenwick (1942b) described methods for counting eelworm larvae artificially "hatched" from cysts by the use of Calcium hypochlorite solution, and stimulated to hatch by dilute picric acid (1943b).

The artificial stimulation of H.rostochiensis larvae to hatch as a method of estimation of the efficacy of nematicides has been studied and improved. Fenwick (1949) has shown that the numbers of larvae hatching from individual cysts are so variable that it is desirable to use a batch of 100 cysts in any one test. He has also investigated (1951) the ideal conditions of a hatching test such as temperature, darkness and volume of

diffusate used.

Finally the penetration of treated cysts into the roots of the host plant has been advocated by some investigators. Peters (1949) has used the method as a criterion of efficacy of different fumigants against H.rostochiensis. By this method the number of nematodes which have penetrated to the plant roots could be estimated by staining them in the roots (McBeth et al. 1941; Goodey, T. 1937; Goodey, J. 1957).

PART II. BIOASSAY TECHNIQUES AND BIOLOGICAL MATERIALS.

(a) Origins of H.rostochiensis material.

The cysts used in the experiments described in this thesis were obtained mainly from three different sources:

i. Pot-raised cysts. These cysts were produced in 1956 and in 1958 in pot experiments and supplied by Dr.N.G.M.Hague.

ii. Field cysts. The cysts were obtained from Ashurst fields and were used for the preliminary experiments (1958).

iii. "Cysts of Solanum nigrum type." These were obtained from a local infestation in Sunninghill where a potato crop failed in 1959. Cysts were found on the roots of Solanum nigrum growing in the same field, which is a common weed in this area. Hence these cysts were designated "Solanum nigrum type".

The pot-raised cysts produced in 1956 were only used in the preliminary experiments (1958-1959). Since their rate of hatch was very low (25-20% of the viable contents), cysts raised in 1958, whose rate of hatching was about 90%, were used in the rest of the experiments.

(b) Criteria. Methods of assay.

Estimation of the effectiveness of a fumigant on potato root eelworm was based on three methods. The first method was a more or less artificial one and could be done by stimulating the hatching of second stage larvae in fumigated cysts with root diffusate extracted from host plants.

The second method was based on the number of larvae penetrating

the host plant roots from treated cysts, compared with those from untreated cysts. The third method was to measure the final eelworm population in the soil after growing the host plant.

(c) Root diffusate assays.

The experiments in this section were mainly concerned with:

i. The determination of the length of time taken satisfactorily to complete hatching tests.

ii. Vertical movement of larvae of H.rostochiensis after various treatment conditions.

iii. Estimation of mortality by root diffusate assay.

General procedure for setting up batches of cysts.

Batches of 100 cysts were counted out on perspex slides (Fenwick 1940) and picked up with the Hesling aspirator (Hesling 1952) and soaked in tap water in solid watchglasses for one week (Fenwick 1951b).

The water was removed and replaced by about 20 drops of root diffusate, the root diffusate being changed at 8-day intervals, the emerged larvae were then counted.

The hatching test experiments were carried out in incubators at 25°C. $\pm$ 0.5°C. To prevent the contents of the watchglasses from drying out the rims were moistened with water and covered with glass lids.

The relative humidity of the incubator was maintained at about 60-70% by placing two pyrex dishes full of water in the incubator.

The emerged larvae in each watchglass were removed with a pipette

and poured into a measuring cylinder. Sufficient water was added to make a suspension with approximately two hundred larvae per ml. The suspension was then agitated with a stirrer and small aliquots were pipetted out and the larvae were counted in a three-chamber counting slide, each of 1 ml. capacity (Fenwick 1951).

The final count was made by multiplying the total volume of the solution by the number of larvae per ml.

In cases where only the total hatch was needed the emerged larvae were removed at 8-day intervals and stored together in 5% formalin solution in 3" × 1" glass tubes. The final count was made by the method described above.

i. The determination of the length of time required to complete the hatching process.

In nematicidal experiments with H.rostochiensis it is common practice for the efficacy to be measured by stimulating the larvae of the treated cysts to emerge under the influence of root diffusate. In this kind of assay it has been the custom to terminate the process after three weeks, after which the total number of the emerged larvae in each treatment has been estimated and used as a criterion. However, it has been shown (Fenwick 1957) that fumigation of cysts with ethylene dibromide, in addition to reducing their hatchability, also affects the form of the cumulative hatching curve by increasing the time necessary to attain the point of inflection. Consequently a longer period than three weeks was required in order to be able to compare the hatches from fumigated and control cysts.

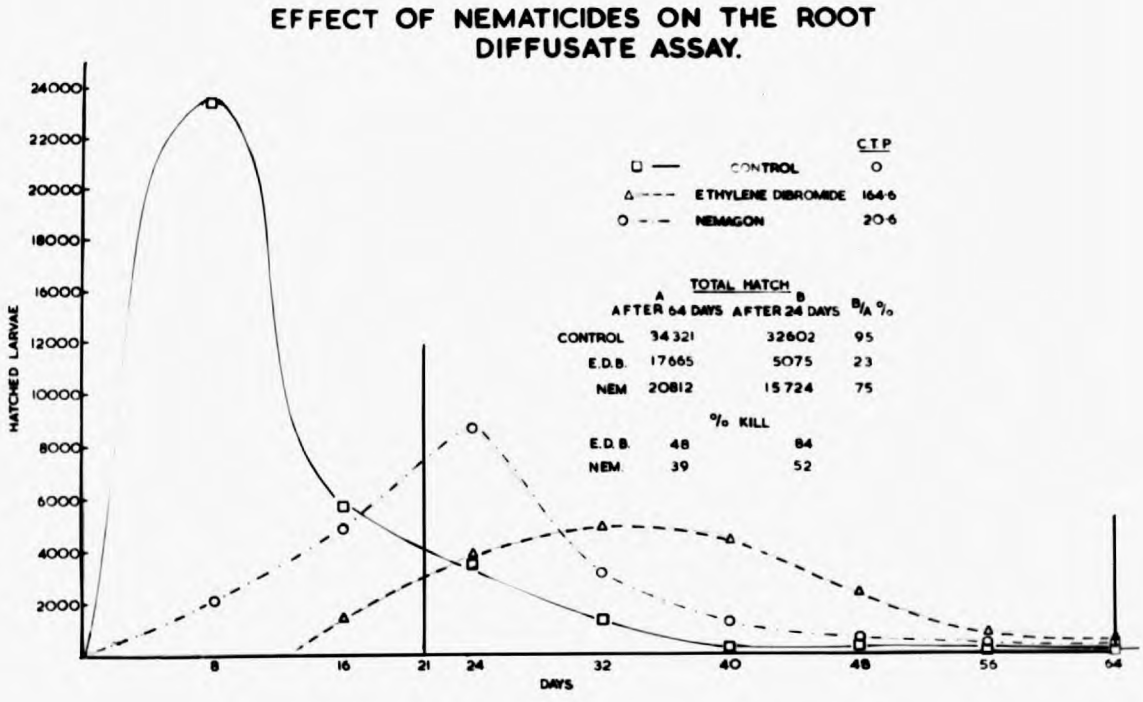


Fig 1 Absolute hatch at 8 day intervals

The following problems also remained unsolved, which I decided to investigate.

1. Whether, in the treated cysts, the reduction of larval hatch was due to kill or to the delaying action of the fumigant.

2. Whether there were any biological differences between the larvae hatching from fumigated and control cysts.

3. The effect of other fumigants (Nemagon and Chlorobromopropylene) on the reduction of hatch and the form of the hatching curves.

To determine the required length of time for the hatching process of fumigated cysts as well as investigating the possibility of biological differences in the emerging larvae at different periods of hatch the following experiments were carried out:

Experiment No.1.

Replicated batches of 100 cysts fumigated with C.B.P., Nemagon and ethylene dibromide at different C × T products and at 100% relative humidity were soaked in tap water for one week followed by potato root diffusate. The number of emerged larvae was counted at the end of successive 8-day intervals for 64 days, until the number of larvae emerging from both treated and control cysts had reached a very low figure.

The total hatch from the control series represented 85 to 93% of the total viable cyst content. The data are shown in Fig.1 and Table 1.

Results.

Assuming there is a positive correlation between hatchability and viability of emerged larvae one can arrive at the following conclusions:

Table 1. Comparison of 24 day and 64 day hatch of H.rostochiensis (fumigated with EDB and Nemagon at different rates and at 8°C.) (Mean of two).

Fumigant	Period of exposure	Emergence total larvae per 100 cysts	
		After 24 days	After 64 days
EDB	30 minutes	20693	30913
"	120 "	5075	17664
"	480 "	-	-
Nemagon	30 "	31424	33242
"	120 "	18359	31412
"	480 "	15724	20812
Control	-	32602	34321

1. A fixed time for comparing the treated and untreated cysts in the hatching test may be misleading. Consequently the experiment should be continued until the number of larvae emerging has decreased to a very low level, when each treatment will give a reasonable comparison of the effect of different fumigants.

2. It is clear from the data obtained that the shape of the hatching curve differs with different fumigants.

The vertical lines drawn on the graphs (Fig.1) corresponding to 21 and 64 days reveal that a misleading result would be obtained if the three weeks hatch alone were used. The curves of Fig.1 are shown in terms of absolute hatch per 8-day interval, rather than as customary cumulative curves, in order to emphasize the differences of form.

ii. Vertical movement of H.rostochiensis larvae, down a column of sand, after various treatment conditions.

In the previous experiment the delaying action of the fumigant on the hatching process was demonstrated. In some treatments there was evidence (see table below) that after 3 weeks very little hatching had occured in treated cysts whereas after 9 weeks the number of larvae emerged from these cysts was much greater.

Table 2. Comparison of 24-day with 64-day hatch (after 120 minutes treatment). (Extreme examples).

Fumigant	Emergence total larvae per 100 cysts		Temperature at fumigation time
	After 24 days	After 64 days	
EDB	6318	16147	8°C.
EDB	3832	19181	8°C.
Nemagon	3892	22370	20°C.
Nemagon	3280	25451	20°C.

However, when these fumigated cysts were introduced into the soil for an assay using an estimation of the final cyst density, the number of new cysts was far less than that produced by untreated ones. It was, therefore, desirable to investigate the general metabolic depression of the larvae leading to the failure to form new cysts. The lethargy of fumigated larvae might be due to failure of their activity, which could be measured by their movements.

Movement of Heterodera larvae has three phases, emergence from the cysts, movement in the soil when the larvae probably are attracted by substances leached from the roots of the host plants, and finally invasion of the host through the cells of the root.

The vertical and horizontal migration of Heterodera larvae have been investigated before (Peters 1953; Wallace 1955).

Comparisons of vertical movement of hatched larvae were made in the following two ways: comparison of different weeks' hatch from cohtrol cysts, and comparison of hatch from fumigated and unfumigated cysts. These comparisons are described in the following three experiments (Nos. 2 to 4).

Experiment No.2.

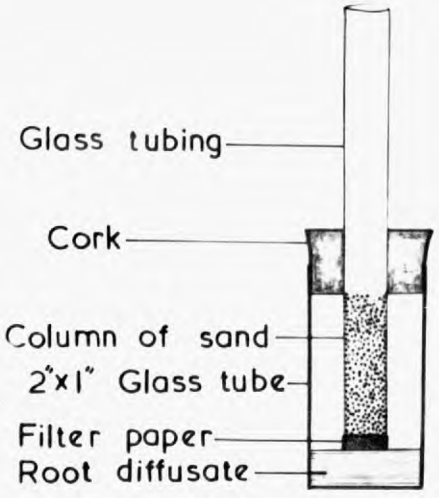
In this preliminary experiment it was decided to compare the downward migrations of Heterodera larvae in a column of sand by using water and root diffusate.

Twenty lengths of glass tubing (10 cm. length and 1 cm. bore) were covered at one end with filter paper and filled up to 3 cm. with sand of particle size, 150 - 250 μ (Wallace 1958). The sand in each tube was saturated with tap water and connected to a suction pump for 10 seconds to aërate the sand. By this means excess water, which would tend to impede eelworm movement was removed (Peters 1953), about 20 - 30% of the moisture content being taken out.

A known number of larvae activated by the technique of Wallace (1955) was introduced into the open end of each column of sand with a fine pipette.

The use of the suction pump caused excess water to be removed but the larvae remained in the first half cm. of the sand column, the sand presumably preventing the passive transportation of the larvae with the water.

Ten flat-bottomed glass tubes (2" × 1") were filled with water and



Fig

Fig.2. Flat-bottomed glass tube for eelworm movement.

ten others with root diffusate up to a depth of 1 cm. Ten of the glass tubes containing sand were corked into the tubes containing water and the others into the tubes containing root diffusate (Fig.2).

The bottom of each was in contact with the liquid contents i.e. root diffusate and water. The tubes were left in the dark at 25°C. for a period of 5 days, by which time the larvae which migrated through the sand and filter paper were recovered and counted. The percentage of larvae which migrated was calculated (see table below). This experiment was repeated and the results confirmed each other. Replication in each treatment was tenfold.

Table 3. Migration of Heterodera larvae in column of sand with root diffusate and water.

Fluid	No. of larvae migrated (10 replications)	Remained in sand	Total
Water	1478	7022	8500
Root Diffusate	2109	6391	8500

Results.

A χ^2 test revealed that there was a significant difference (at 5% level) between the two treatments, which means Heterodera larvae showed a stronger tendency to move towards root diffusate than water. The experiment was repeated but this time the results were assessed after 7 days. Replication was tenfold in each treatment. Consequently in other experiments concerned with larval movement root diffusate was used.

The data were analysed statistically by analysis of variance, which showed a significant difference at the 5% level of probability.

Table 4. Analysis of variance for determination of significant difference between the number of larvae migrated towards root diffusate and water in column of sand.

Number of Replication	No. of larvae migrated		Total no. of larvae introduced to the column
	Water	Root diffusate	
1	118	266	1030
2	180	235	1030
3	134	263	930
4	115	236	930
5	155	253	930
6	191	180	730
7	159	118	730
8	135	299	730
9	119	115	730
10	172	144	730
Total	1478	2109	8500

Analysis of Variance.

Items	D.F.	S.S.	M.S.	F.
Root diffusate	1	19908.1	19908.1	8.123 *
Residual	19	46566.5	2450.8	
Total	20	66474.6		

* See explanation on Page 31.

Table 5. χ^2 test for larval movement in column of sand towards root diffusate and water.

Fluid (Observed)	Total larvae migrated 10 replicants	Non-migrated	Grand Total
Water	1478	7022	8500
Root diffusate	2109	6391	8500
	3587	13413	17000
(Expected)			
Water	1793	6706	8500
Root diffusate	1793	6706	8500

$$\Sigma\left[\frac{(O - E)^2}{E}\right] = 141.41 \ ***$$

To measure the activity of larvae which emerged in the first, second and third weeks respectively (Experiment No.3).

The delayed action of the hatching process caused by three of the fumigants that I used led me to suppose that there might be some difference between the larvae emerging early and those of later emergence. To investigate this, the following experiment was carried out: three batches of 100 cysts were picked up at random and placed in dry watchglasses.

The first batch was soaked in water for one week and then exposed to root diffusate. The second batch was then similarly soaked in tap water followed by root diffusate and a third batch was treated yet another week later. Thus at the end of the fourth week larvae which had emerged at the third, second and first weeks were available at the same time.

A known number of emerged larvae of each type was introduced into a column of sand by the method described previously (see above page 22). The root diffusate from each glass tube was replaced at the end of the 4th, 8th and 16th days. Replication was tenfold in each treatment. The number of migrated larvae in each treatment was counted, the results being shown in the table below.

Table 6. The migration through a column of sand of larvae emerging in the first, second and third weeks.

Time of emergence	Total larvae introduced	Migrated larvae			Total
		4th day	8th day	16th day	
1st week	4300	2324	144	53	2521
2nd week	4700	2967	142	73	3182
3rd week	4500	1084	160	74	1318

Results.

With two degrees of freedom the data obtained showed that there was a significant difference between the number of larvae emerging at each week. The larvae which emerged in the 2nd week were the most active and

those emerging in the 3rd week were the least active. Details are in Appendix Table 7A.

Observations on the difference in activity between fumigated and non-fumigated Heterodera larvae (Experiment No.4).

In this experiment a known number of larvae which had emerged from cysts fumigated *in vitro* with Nemagon at concentration × time products of 2.1 and 10.4 mg.h./l., were compared with unfumigated larvae by the method used in Experiment No.2. The data are shown in the table below.

Table 8. Comparison of the activity of larvae emerged from cysts fumigated for a period of 30 and 120 minutes and controls.

Treatment	Total larvae introduced (10 replications)	Migrated downwards	Non-migrated
Nemagon at C.T.P. 2.1 mg.h./l.	8250	3287	4963
Control	7050	3808	3242
Nemagon at C.T.P. 10.4 mg.h./l.	6400	1765	4635
Control	5600	3278	2322

iii. Estimation of mortality by root diffusate assay.

Hatching tests were carried out on fumigated cysts which had been stored for at least one month or more according to the type of experiment. Batches of 100 cysts at different treatments were soaked in water for a week followed by root diffusate for a period of 9 weeks. The total number of emerged larvae was then counted by the method described before. The per cent. mortality was calculated by using the following equation:

$$M\,\% = \frac{LC - LT}{LC} \times 100$$

Where LC = the number of larvae emerged in the controls.

LT = the number of larvae emerged in the treatments.

There are other methods of calculation to obtain per cent. mortality. To investigate the degree of accuracy in each formula, they were tested statistically as follows:

Estimation of the per cent. mortality of H.rostochiensis by hatching with root diffusate, and using different equations.

Estimation of the per cent. mortality of H.rostochiensis larvae due to the effect of nematicides in hatching tests could be obtained by using one of the following three formula.

1. $$\frac{\text{Number of non-hatched larvae}}{\text{Number of the total cyst content}} \times 100$$

2. $$\frac{L(\text{control}) - L\ (\text{treated})}{L\ (\text{control})} \times 100$$

3. $$\frac{(\text{Treated} - \text{Control}) \text{ mortality}}{100 - \text{Control mortality}} \times 100$$

In the first equation the actual number of hatched and non-hatched larvae would be considered, whereas in the second equation there are assumptions that the number of cyst contents is the same and equal to the control. The number of non-hatched larvae is also disregarded. In the third equation, it is assumed that the control had a 100% hatch.

It is obvious that less accuracy in estimation can be expected by the assumption of one or two hypotheses, as in the 2nd and 3rd formulae, instead of actual numbers. However, the advantage of equation No.2 is that the method is quicker and less laborious, i.e. no crushing of cysts or counting of non-hatched larvae is necessary.

To investigate the degree of accuracy and the possibility of using the second method of estimation the data of experiment No.14 (Part III) were subjected to analysis using the three equations mentioned above.

Analysis of variance produced the table below and showed that there was no significant difference between the results by using either No.1 or No.2 equation. Consequently in all experiments where the per cent. mortality was to be estimated the No.2 equation was used.

Table 9. The effect of fumigating Heterodera rostochiensis cysts with Nemagon in glasschambers. (Experiment No.14. Part III).

Time of exposure in minutes (Saturation-Concentration)	Emergence Total larvae per 100 cysts	% Mortality by using Equation No.			Angular transformation Ø		
		1	2	3	1	2	3
64	23427	18.007	13.200	12.101	25.10	21.30	20.36
128	20123	38.676	25.443	34.258	38.47	30.26	35.85
178	17539	46.511	35.017	42.658	42.99	36.27	40.80
256	5027	83.311	81.137	82.109	65.88	64.23	64.97
512	7	99.999	99.974	99.999	89.43	89.01	89.43
Control	26990	6.719	0	0			

Analysis of variance between 1 and 2.

Source of Variance	D.F.	S.S.	M.S.	F (V.R.)
Regression line	1	5233.42	5233.42	
Deviation from regression (slope)	1	10.05	10.05	< 1 n.s.
Difference in intercepts	1	43.19	43.19	<1 n.s.
Residual (Error)	6	447.60	74.60	
Total	9	5734.26		

In most experiments an analysis of variance has been carried out in which the variance ratio (F) was calculated for each factor and tested for significance at a given level of probability.

In this thesis levels of significance are represented by:

n.s. = not significant.

* = significant at 5% level.

** = " " 1% level.

*** = " " 0.1% level.

The differences between treatments, when necessary have been compared with the corresponding critical difference by listing totals in descending order of magnitude.

The critical difference is the product of the Student's t. at the 5% level and the standard error of the difference between any two totals, using the following equation:

$$C.D. = t \times Sn$$

$$Sn = \sqrt{s^2 \times 2 \times n}$$

where: C.D. = Critical difference.

Sn = Standard error of difference.

s^2 = Error variance.

n = number of observations in each total.

The other abbreviated symbols which have been used throughout the analysis of variance tables are:

D.F. = Degrees of freedom.

S.S. = Sums of squares.

M.S. = Mean squares.

F. = Variance ratio

ϕ = Angular transformation.

(d) Root invasion assay.

(Estimation of mortality by measuring the infectivity of host plant seedlings).

Invasion of the root by the parasite was examined by introducing the fumigated cysts or larvae into the soil in which seeds of tomato and eggplant (host plants) were sown.

At the 2nd or 4th leaf stage the seedlings were lifted and the roots carefully washed free of soil. They were then gently dried between blotting paper and chopped up and weighed. According to the type of experiment one quarter, one half or the total weight of the roots was stained in acid-fuchsin/lactophenol (Goodey 1957). The number of nematodes in each root sample was then estimated.

i. Staining procedure.

In order to estimate the number of nematodes penetrated into the roots, samples of the roots were stained with Acid fuchsin in lacto-phenol (J.B.Goodey 1957). The roots were placed in 0.05% hot Acid fuchsin-lactophenol, in a test tube, the whole being heated in a water bath. The latter was used to adjust the necessary time of heating to get a reasonable

stain according to the thickness and the age of the root. The period of heating for the roots of second leaf stage seedlings was 15 minutes and for four leaf stage host plants, 30 minutes. Heating the root by a water bath, instead of direct boiling for two minutes, has another distinct advantage. When the bulk of the population reaches the fourth or pre-adult stage, especially in controls and at the fourth leaf stage of seedlings, most of these nematodes become dislodged in direct boiling, and therefore in the washing process will get lost, whereas in a water bath a steady heating will prevent the nematodes from being dislodged in the staining process. Staining was followed by washing the roots with slightly acidified water then they were left in plain lactophenol overnight.

ii. Invasion of eggplants "Aubergine" (Solanum melongina) by Heterodera rostochiensis. (Experiment No.5).

It has been shown by previous experiments (Fassuliotis and Feldmesser 1954) that eggplants are an important factor in the biology of the potato root eelworm and that they can be attacked by this nematode. They also showed that Heterodera larvae were stimulated to hatch from cysts by diffusates produced by the roots of growing eggplants. However it seemed important to investigate the degree of invasion of potato root eelworm into the roots of the host and compare it with potato and tomato.

Three sterilized seed boxes were filled with 5 Kg. of sterilized and artificially infested soil. The initial eelworm density was about 60 eggs per gramme of soil. A hundred seeds of the following plants were sown at equal distances apart in the soil of three seed boxes.

Tomato ("Leader") in the first box.

Eggplant ("Dolmei") Persian variety, in the second box.

Eggplant ("Long purple") English variety, in the third box.

They were left until the 4th leaf stage (35 days) after which 20 seedlings were removed at random from each box and the adhering soil gently washed off. The number of nematodes in one half of the sample was then estimated. The data are shown in the table below:

Table 10. The number of penetrated larvae per g. of root in tomato (Leader) and two different varieties of eggplants.

Host plant	No. of plant seedlings	Wt. of roots g.	Nematodes found		
			per sample	per 20 plants	per g. of root
Tomato	20	0.810	1019	2038	2516
Eggplant Dolmei	20	0.394	304	608	1543
Eggplant Long purple	20	0.304	323	646	2125

Results.

1. Two varieties of eggplants could be considered as a host and they were attacked by H.rostochiensis.
2. The degree of infestation was lower than tomato.
3. The growth of eggplant was very slow.

iii. Penetration of first, second and third week emerging larvae into the host plant. (Experiment No.6).

In the movement experiments on H.rostochiensis described previously it was shown that larvae which emerge in the third week are slower in movement and consequently are less active.

To confirm this observation the following experiment was carried out, using the same type of larvae but measuring their activity in relation to their invasion of the host.

First, second and third week emerging larvae were obtained as already described (page 26). A known number of each was introduced into the soil through a fine syringe at about one inch depth in small clay pots (60's) planted with tomatoes (21 days old). Ten replicates were used for each treatment, the total number of pots being 30. One month later the plants were removed and the roots were washed and stained. The larvae which had penetrated were estimated (table below).

Table 11. Penetration of the larvae into the tomato plants.

Type of larvae	No.of root	Initial infestation Total of 10	Wt. of root	Nematodes found	%	Penetration per g. of root
First week	10	6400	4.570	2780	43.4	584
Second week	10	5870	4.220	2912	49.6	690
Third week	10	6000	5.400	2416	40.2	447

iv. Experiment No.7.

The previous experiment was repeated using a slightly different method as follows.

Thirty tomato plants were grown in such a way that on a given day, (0), 10 of them were 21 days old; 10 were 14 days old; and 10 were 7 days old. Larvae were hatched from a single batch of cysts.

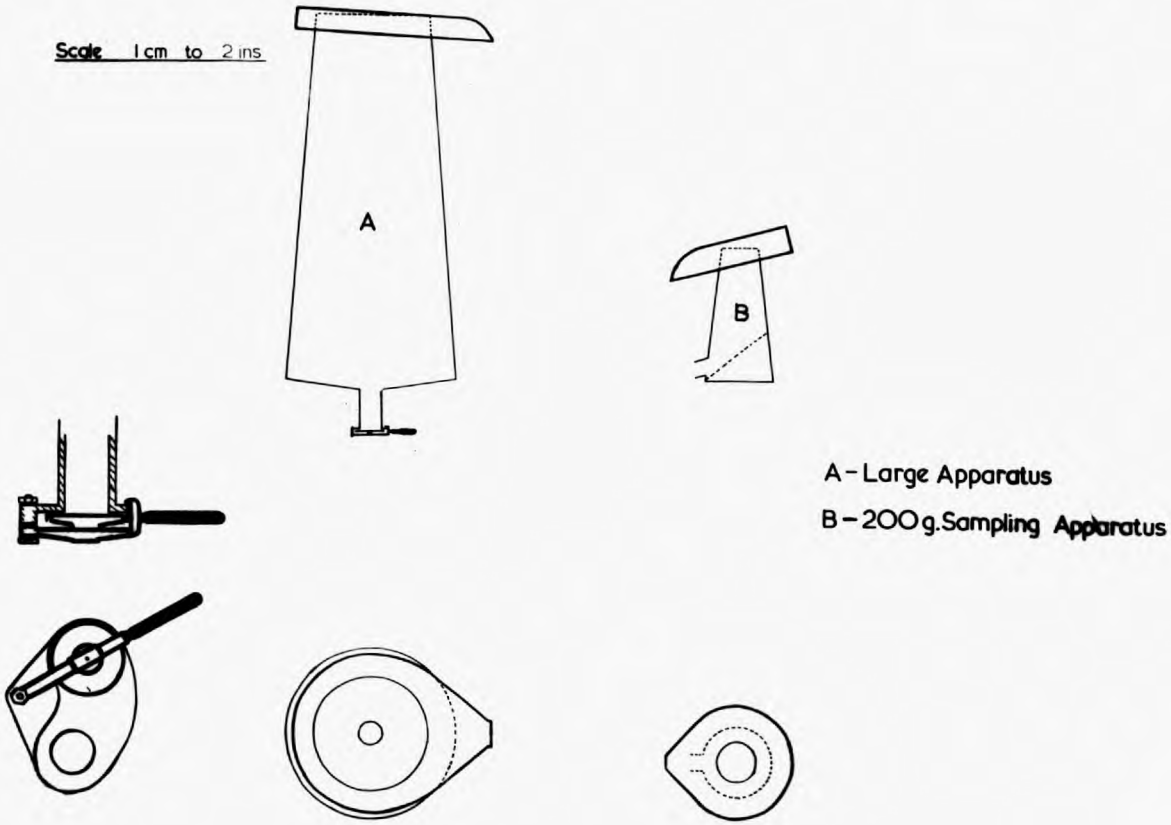
At day 0 the 21 day old plants were inoculated with a known number of larvae hatched in the first week. At day 7 the known number of larvae hatched in the second week were inoculated into the second batch of 10 plants, which were now also 21 days old. In a similar way larvae hatched in the third week were inoculated into 21 day old plants at day 14.

Root examination was carried out at day 20, 27 and 34 respectively, thus allowing 20 days for invasion to occur in each of the 3 groups of 10 plants. The numbers of larvae at the various stages of development in the whole root system were estimated by staining the roots in acid fuchsin and macerating them in a Waring blender (Goodey, 1957). Aliquots of the resulting suspension were counted. The results are shown in the table below.

Table 12. Penetration of tomato roots by larvae emerging in the 1st, 2nd and 3rd weeks.

Type of larvae	No. of root	Initial infestation Total of 10	Nematodes found	%
1st week	10	11250	2554	22.7
2nd week	10	9350	3279	34
3rd week	10	9260	1241	13.4

Fig 3 Cyst recovery apparatus



Results.

The difference between the number of the first, second and third week larvae which penetrated the host plant roots was statistically significant at 0.01 probability level (see Appendix Tables 13A and 14A). The highest rate of penetration was observed in the larvae emerging in the second week and the lowest rate in the third week larvae. This supports the results of the experiments in which larval movement in sand was measured (see page 26).

(e) Assays based on the final eelworm density.

The final eelworm density was estimated in terms of the number of newly formed cysts and in terms of the number of eggs per gramme of soil.

Estimation of soil populations was determined by the Fenwick can apparatus (Fig.3). Samples of soil were air-dried and then sieved. To obtain a reasonable estimation five or four hundred grammes of soil were weighed (Jones 1955) and washed in a Fenwick can (Fenwick 1940). In all pot experiments only one 400 g. sample was taken, this action being justified because the dispersal of cysts within a pot of soil, thoroughly mixed before sampling, should fit the Poisson distribution. In washing process the dried cysts floated up with some debris. Both were collected on a 50 mesh/inch sieve and were dried. The dried "float" was placed on the top of a sheet of white board inclined onto another sheet of board. The round cysts rolled down and were collected with an aspirator (Hesling 1952). These cysts were then sieved into 3 size groups: $> 599\mu$, from 599 to 422μ, $< 422\mu$. Initial infestation was always made from cysts of size grade

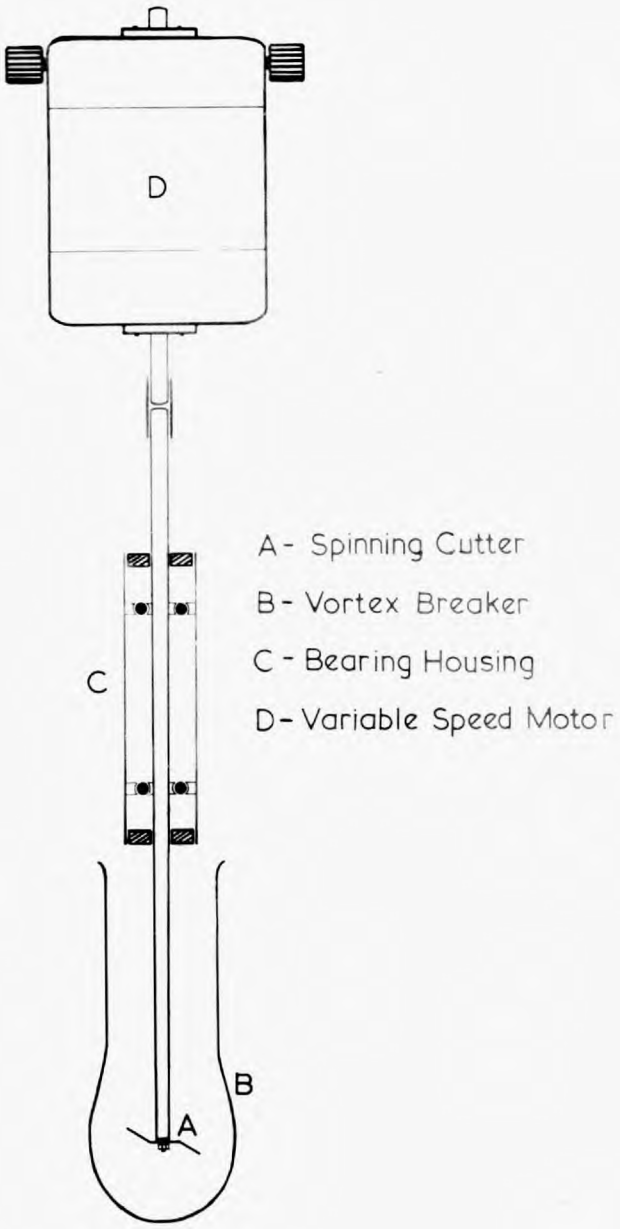


Fig. 4 Homogeniser

599 to 422μ, thus it was possible to determine the number of newly formed cysts of different sizes in all treatments.

The homogenizer technique was used to estimate the number of eggs (and/or larvae) per cyst (Bijloo 1954).

A hundred cysts were crushed on a chanelled metal slide (6" × 2") by means of a glass rod and washed into a homogenizer tube in which agitation was carried out by the blades of the homogenizer (Fig.4).

After 30 seconds the suspension was made up to a known volume and the eggs and larvae were counted in 1 ml. in a 3-chamber counting slide (Goodey 1957).

By this method the number of eggs (and larvae) per cyst could be obtained. The product of this number and the number of cysts per g. of soil gives the number of eggs per g. of soil.

(f) Soil Sterilization.

It is necessary that the soil used in the different experiments should be free from viable nematodes (especially cyst forming species). For this reason the soil was treated either by steam sterilization (1959 experiments) or by fumigation with methyl bromide (1960 experiments).

Steam sterilization was carried out by passing steam through the soil until the temperature reached about 80°C. at which it was kept for two hours. The sterilized soil was then allowed to cool by spreading it on a cemented floor.

To get rid of ammonia which was formed during the sterilization, the soil was turned with a shovel frequently for a month and exposed to the

atmosphere. It was then mixed with clean sand in the proportion (3 : 1) by volume.

In experiments 1960-61 the soil was sterilized by fumigation with methyl bromide at a C.T.P. of 800 mg.h. per litre which C.T.P. should control "all" nematodes in soil, (Hague 1958).

After a week's exposure to the open air (Christie 1959) the soil was thoroughly mixed with sand (3 : 1 by volume).

PART III. FUMIGATION EXPERIMENTS.

General Introduction.

The control of cyst forming nematodes with chemicals involves several problems. The efficacy of potentially useful nematicides must be estimated and their method of application worked out. Economic considerations have to be taken into account with particular reference to production costs, i.e. a good nematicide may be too expensive while a much poorer one may be cheap to produce because it is a by-product of some other industry. Also mammalian toxicity must be investigated.

Several useful nematicides have been developed in the last 20 years. D-D mixture (1-3 dichloropropene-1.2 dichloropropane) was introduced by the Shell Chemical Corporation in 1944. Ethylene dibromide came into the market shortly afterwards. Methyl bromide (bromomethane) was also found to be a good nematicide under restricted circumstances.

Nemagon (1-2 dibromo-3 chloropropane) formerly called Shell O.S. 1897 was introduced in 1954 as a new and cheap soil fumigant, while Chlorobromopropane (brominated D-D) was introduced about the same time but was taken off the market because of its excessive cost.

The efficacy of soil fumigants is known to depend on the temperature and the moisture content (Peters 1954; Call 1957). Nemagon appears to function more effectively than other fumigants at high temperatures, probably because of its high boiling point. The present series of experiments were carried out to determine the relative efficacy of the three following

fumigants using H.rostochiensis as test organism, and also to establish the most satisfactory method of assay.

The fumigants were Nemagon, E.D.B. and C.B.P. Two types of experiments were carried out.

1. Experiments in vitro using specially designed desiccators as fumigation chambers.
2. Experiments in vivo (pot experiments).

Section A : Experiments in vitro.

(a) Introduction.

Hitherto little work has been undertaken on the effects of nematicides on H.rostochiensis in vitro. Usually the effect of nematicides has been estimated by the number of larvae hatching from treated cysts exposed to root diffusate, or the number of nematodes invading the roots of the host plant (Peters and Fenwick 1949; Peters 1952; Fenwick 1957; Hague 1958). A comparison of the number of hatched larvae in treated cysts and the control was used as the criterion. But the relation between hatchability and viability of encysted larvae is not yet clear and also there is a possibility that some treated larvae can penetrate the plant host but cannot produce new cysts.

In this section preliminary observations on the behaviour of Heterodera cysts exposed to different fumigants under different conditions are described.



Fig.5. Specially designed desiccators used as glass chambers equipped with two fans run by a small motor. The air-tight jar with the same conditions as the desiccator was used for control.

(b) Experimental Procedure.

The glass chambers used were ordinary desiccators (capacity 2.3 litres) which were set in different constant temperature rooms to obtain the required temperature during the fumigation process.

To ensure even distribution of fumigants prior to and during the fumigation all desiccators were equipped with two fans run by small motors (Fig.5).

Constant relative humidities in desiccators were obtained by using pure water for 100% R.H. and KOH solutions for other humidities (Solomon 1951).

The cysts were put in desiccators for 2 to 3 days before fumigation, to acclimatise them to the conditions involved. (Acclimatisation of cysts will be described later on in this section).

Fumigation was carried out by introducing the fumigants into the desiccators in an open solid watchglass, in which some strips of filter paper had been fixed to allow a better evaporation of the fumigant.

For chamber fumigations it is customary in the Parasitology Department at Ashurst Lodge to represent the effective dose as a product of the concentration of the fumigant and the number of hours of exposure to the fumigant. The product is known as the Concentration-time product (C.T.P.) and is measured in milligramme-hours per litre which for practical purposes is equivalent to ounce-hours per thousand cubic feet.

Fumigation of the cysts in glass chambers at a high C.T.P. at saturation concentration of the gas was achieved by removing the lid of the desiccator and placing the acclimatised cysts on the upper layer of the

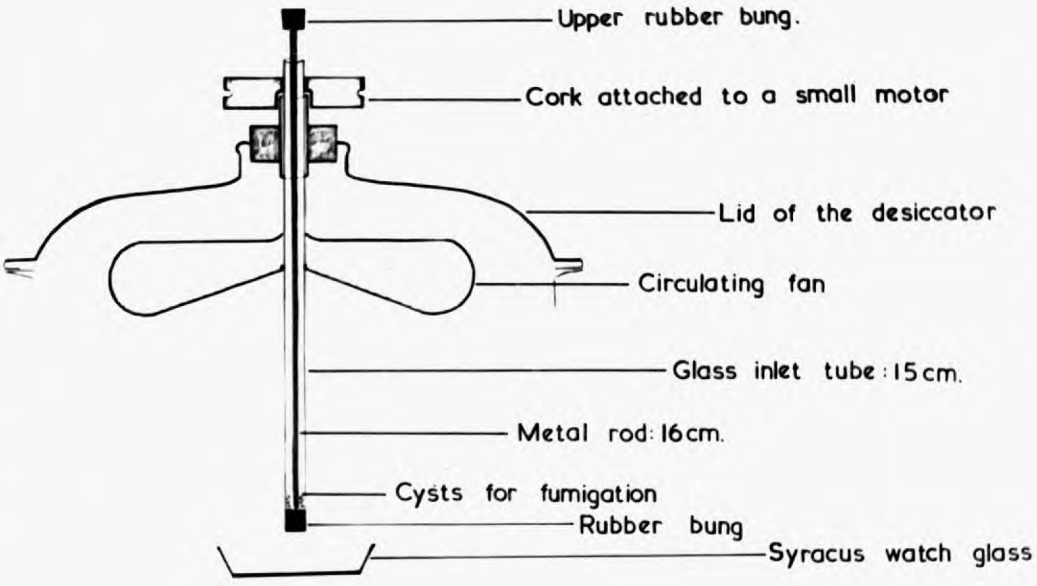


Fig. 6 Special designed desiccator for short time fumigation

chamber.

Removing the lid to introduce the cysts caused an immediate change in the saturation concentration of the fumigant which however, would soon be restored due to the presence of liquid fumigant in the chamber. The time taken for the saturation concentration to be restored was only a matter of minutes and in a long exposure was unimportant. At a low C.T.P. (i.e. at a short exposure period) however, this time lag formed a major part of the fumigation time.

To obviate the effect of opening the desiccator lid, the length of glass inlet tube (0.4 cm. bore) inserted into the bung at the top of the desiccator for holding the fans was extended about 15 cm. so as to become also a means of introducing the cysts. Both ends of this glass tube could be closed by means of small rubber bungs (Figs. 5 and 6).

A metal rod, 16 cm. long, was inserted through the upper rubber bung so that when this was replaced the bung at the other end of the glass tubing was pushed out. The required numbers of cysts were passed into this tube through a funnel. When the upper stopper was fitted, the other one holding the cysts was pushed out. The cysts were poured into a syracuse watchglass which was placed under the tube on the upper layer of the desiccator. Thus the cysts were introduced into the glass chambers without removing the lid and without changing the conditions.

Counted batches of cysts were also placed in air-tight jars, with the same conditions as desiccators but not fumigated, and these were used as controls (Fig.5).

i. Saturation Concentrations.

The required amount of fumigant to reach the saturation concentration in each desiccator was obtained by using Avogadro's theorem.

$$F = \frac{M \times 273 \times 1000 \times P}{293 \times 22.4 \times 760}$$

Where M = molecular weight of fumigant.

273 = absolute temperature at 0°C.

293 = 20°C. (or any other temperature used by adding it to 273)

P = vapour pressure in mm. of Hg.

760 = normal atmospheric pressure.

1000 = millilitres per litre.

In the case of C.B.P. the formula was unknown and it was impossible to estimate the saturation concentration of the fumigant to which the cysts were exposed.

In order to test Avogadro's theorem the following experiments were carried out to estimate the saturation concentration of Nemagon and ethylene dibromide in the atmosphere of glass chambers during fumigation, by decomposing samples through a heated platinum wire by catalytic oxidation (Lubatti 1949; Lubatti and Blackith 1958).

Determination of gas concentration. (Experiment No.8).

When fumigants are introduced into glass chambers and in the presence of R.H. solutions, some physical or chemical absorbtion of the gas usually occurs. In order to overcome the sorption of fumigant and sampling

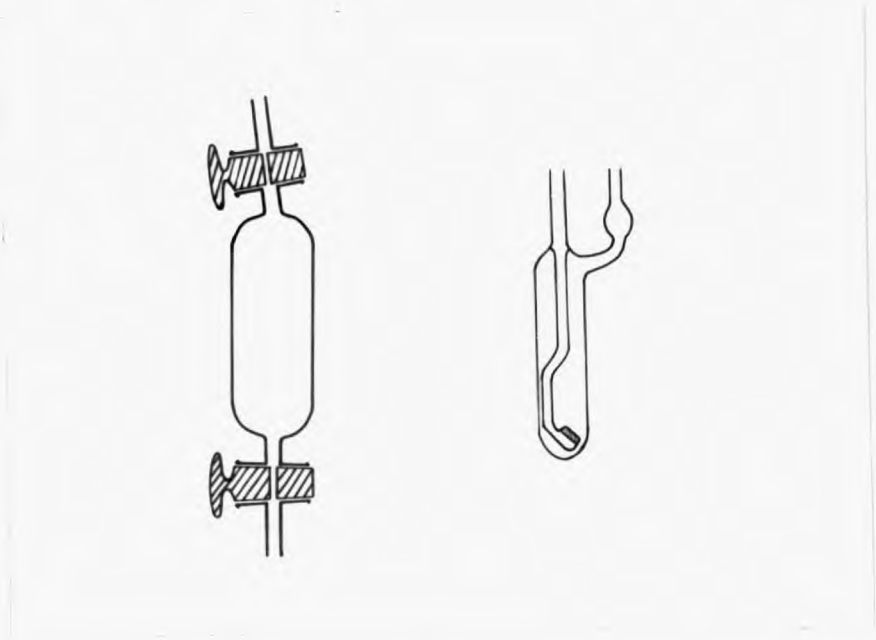


Fig.7. Bubbler and Calibrated sampling tube.

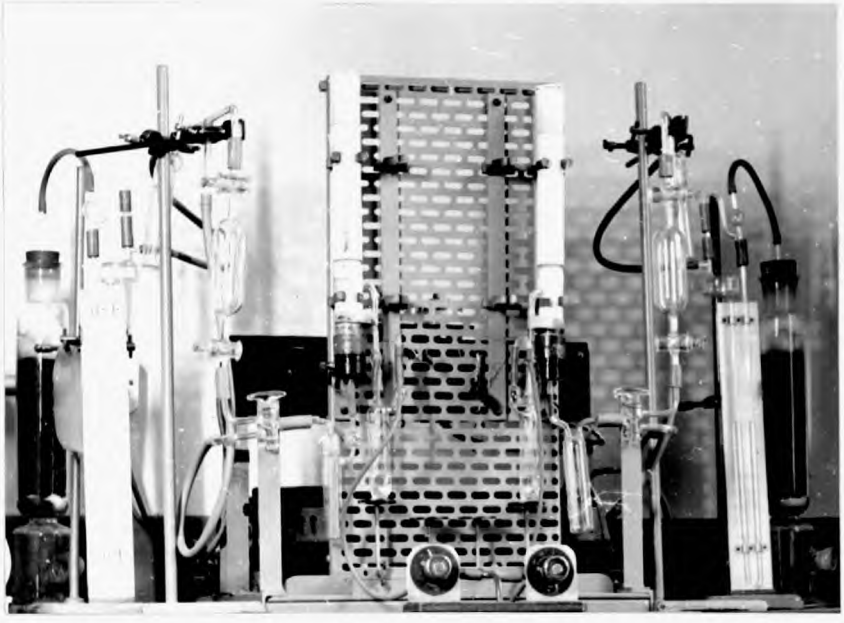


Fig.8. Combustion apparatus for decomposing halogenated hydrocarbon fumigants.

losses, excess fumigant was placed into desiccators to obtain saturation concentrations.

The concentration of gas within the chamber was determined by taking two samples 24 hours after the introduction of the fumigant.

The volume of the sampling tubes (Fig.7) was determined by weighing the tube empty and full of distilled water at a known temperature.

The dry sampling tubes were evacuated by means of a vacuum pump for three minutes and then connected to the glass tube passing through the desiccator at the top (Fig.5).

Nemagon and ethylene dibromide were determined by burning the fumigant to bromine or chlorine which was estimated potentiometrically or colorimetrically.

The sampling tubes were connected to the apparatus designed by Lubatti for decomposing the samples (Fig.8).

The fumigants were drawn in a stream of air already purified by passing through a column of charcoal, to pass over the heated platinum, and the liberated bromine or chlorine was passed through bubblers (Figs. 7 and 8), containing a 5 ml. solution of a mixture of 30 ml. of 0.1 N Sodium hydroxide and 1 ml. of 100 volume hydrogen peroxide. The sodium bromide and sodium chloride formed were determined by titration against silver nitrate (approximately $\frac{N}{50}$).

The calculation of the concentration of fumigants in mg./l. was made by using the following formula.

$$R \times F \times 1000 / V$$

where

R = no. c.c. silver nitrate used.

F = a "factor" for the silver nitrate. This is the product of the normality of the silver nitrate and the molecular weight of the fumigant.

V = volume of the sampling flask.

Series of samples taken from desiccators saturated with Nemagon and ethylene dibromide were anslysed (Tables 15 and 16).

The results showed that the saturation concentration of Nemagon was 5.2 mg./litre (a mean of six replications). This was confirmed by Lubatti (private communication).

Table 15. Determination at 20°C. of saturation concentration of samples of "NEMAGON" at 100% relative humidity.

Molecular weight	236.24	
Vapour pressure	0.8	(at 20°C.)
mg./l. saturation concentration using Avogadro's theorem	10.33	

Sample	mg./l.
1	5.1
2	5.7
3	4.8
4	5.2
5	4.8
6	5.6
Mean	5.2

Table 16. Determination at 20°C. of saturation concentration of samples of "Ethylene dibromide" at 100% relative humidity.

Molecular weight	187.9
Vapour pressure	8
mg./l. saturation concentration using Avogadro's theorem	82.2

Catalytic thermal decomposition apparatus (Lubatti and Blackith 1956).

Sample	mg./l.
1	72.8
2	71.8
3	65.2
4	70.8
5	72.1
6	74.78
Mean	71.41

However, from Avogadro's theorem 10.33 mg./l. Nemagon would be expected. These different values for Nemagon were due to a low vapour pressure and the impurity of the material, whereas in ethylene dibromide which was much purer, the difference in results obtained by the two methods was not as great.

In experiments in vitro different C.T. products were obtained by changing the duration of exposure. When the necessary time for each fumigation had elapsed the desiccator was opened and the cysts transferred to open watchglasses or small tubes and exposed to a gentle current of air from a small fan to dispel any fumigant left on them. They were then kept

uncovered in the dark until the next procedure.

(c) Methods of Assay.

The efficacy of treatment was determined in three ways as described in detail in Part II.

The percentage mortalities for the three methods of assay were calculated as follows:

1. Root diffusate assays.

The number of larvae emerging from batches of 100 cysts in the treated $(L/100c)_T$ and the number of larvae emerging per 100 cysts from the control $(L/100c)_C$ were counted after a 9 weeks exposure to the root diffusate.

Percentage mortality was estimated by using the following formula.

$$\frac{(L/100c)_C - (L/100c)_T}{(L/100c)_C} \times 100$$

2. By introducing treated and untreated cysts into soil and then comparing the invasion of host seedlings.

The number of nematodes invading the roots of the plant hosts were estimated and the above mentioned equation was used to obtain percentage/ mortality.

3. By introducing cysts into soil in which a host was grown, and comparing final eelworm populations in different treatments.

The final eelworm population was estimated in two ways.

1. The total number of cysts was estimated. Percentage mortality was obtained by using the following equation (Hague 1960):

$$\text{Mortality} = \frac{[(c/g)\ \text{Control} - (c/g)\ \text{initial}] - [(c/g)\ \text{treated} - (c/g)\ \text{initial}]}{(c/g)\ \text{Control} - (c/g)\ \text{initial}}$$

$$\% \text{ Mortality} = \frac{(c/g)\ \text{Control} - (c/g)\ \text{Treatment}}{(c/g)\ \text{Control} - (c/g)\ \text{initial}} \times 100$$

2. In terms of viable eggs per gramme of soil, given by the number of eggs and larvae per cyst in each treatment, multiplied by the number of new cysts per gramme. Percentage mortality was estimated as follows:

$$\% \text{ Mortality} = \frac{(L/g.)\ \text{Control} - (L/g.)\ \text{Treated}}{(L/g.)\ \text{Control}} \times 100$$

The percentage mortalities at the different treatments were transformed to angles and plotted against the log transformation of the exposure time.

The data obtained from the fumigation experiments have been used to compare the efficacy of different fumigants and different rates in each fumigant against H.rostochiensis. Several parameters have been considered.

1. The slope of the regression line is inversely proportional to the standard deviation of the tolerance distribution which may be supposed to exist in the population of nematodes. The slope may reflect quantities which in general cannot wholly be dissociated in experimental work. Thus it can be shown that the slope also varies with the availability of the fumigant to the site of action within the nematodes. In practice not only is it difficult to distinguish changes in the availability from changes in the underlying tolerance distribution but it is also arguable that these two concepts describe different aspects of the same bio-physical process.

Availability is defined by the fraction of the applied fumigant which is being taken up by the nematodes.

2. The position of the regression line is epitomised by the median response dose. This dose is by definition the amount of fumigant required to induce the observed response in 50% of the organisms and is consequently a direct measure of the nematicidal effect of the fumigant. This "median lethal dose" (L.D. × 50) is the most sensitive measure for comparing efficacies of fumigants.

In experiments where plants were grown for the assay the following additional criteria were used to detect the effects of eelworm or fumigant.

1. The height of the host plant after 50 to 75 days.
2. The weight and the number of tomatoes or potatoes in each pot.

This section of the thesis has been divided into the following divisions:

1. The effect of relative humidity on the fumigation of H.rostochiensis (labelled: d).
2. The effect of temperature on the fumigation of H.rostochiensis (labelled: e).
3. The effect of the fumigation of H.rostochiensis with different rates of Nemagon, C.B.P. and E.D.B. at 20°C. and 100% relative humidity (labelled: f).
4. Observations on the final eelworm population by injecting the planted soil with Heterodera larvae emerged from the fumigated cysts (labelled: g).

5. Observations on the effect of storage on fumigated cysts (labelled: h).

(d) Effect of relative humidity on fumigation of H.rostochiensis.

i. Acclimatisation of cysts (Preliminary Experiment No.9).

Before starting the main experiments it was desirable to find out the time required to acclimatise the cysts to the environmental conditions involved, i.e. in glass chambers prior to fumigation.

Batches of 1000 cleaned cysts were soaked in water for 3 days, then the excess water was removed by means of a pipette, and by rolling the cysts over filter paper. Then they were weighed on an analytical balance and exposed in three desiccators with relative humidities of 100, 80 and 60% at 20°C. Batches of 1000 air-dried cysts were also weighed and placed in the same desiccators at the same time without preliminary soaking. After 20 and 48 hours all the batches of dried and presoaked cysts were taken out and weighed again. The results of this experiment are set out in Tables 17 and 18. Replication in each treatment was two-fold.

Table 17. Mean weights of 1000 cysts (size 599 to 422μ).

Air-dried weight	Weight after 3 days soaking in water	Weight after 20 hours in desiccator	% R.H.
0.0430	0.0741	0.0599	100
0.0432	-	0.0549	100
0.0431	0.0699	0.0468	80
0.0431	-	0.0447	80
0.0433	0.0811	0.0443	60
0.0431	-	0.0432	60

Table 18. Mean weights of 1000 cysts (size 599 to 422μ).

Original air-dried weight	Weight after 3 days soaked in water	Weight after 48 hrs. in desiccator	% R.H. in desiccator
0.0432	0.0731	0.0575	100
0.0433	-	0.0570	100
0.0431	0.0752	0.0450	80
0.0430	-	0.0450	80
0.0430	0.0670	0.0431	60
0.0433	-	0.0435	60

It will be seen from the tables that after 20 hours there were still some differences in weight between the presoaked and air-dried cysts, whereas in 48 hours time there was no significant difference in weight between the different treated cysts. Therefore in all experiments herein described the cysts which were fumigated *in vitro* were left in glass chambers for a period of 48 hours prior to fumigation.

ii. The effect of relative humidity in the fumigation of H.rostochiensis with E.D.B. and Nemagon. (Experiment No.10).

1. Root diffusate assay.

Batches of 100 cysts in watchglasses were placed in 3 desiccators at 100, 80 and 60% relative humidity respectively. Fumigation was carried out at 20°C. by the method described before (page 42).

The period of fumigation was 4, 8, 16 and 32 hours for each

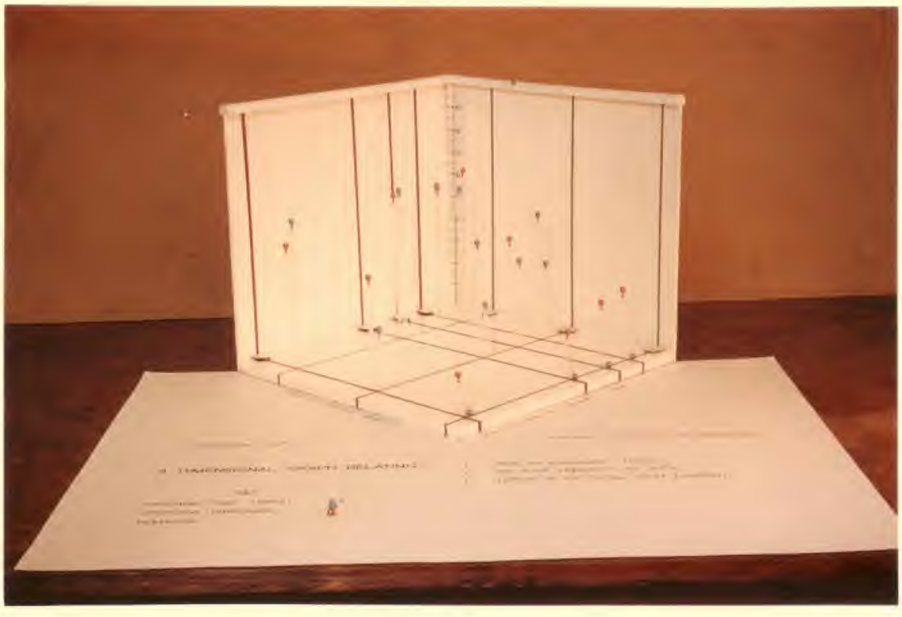


Fig.9. Three dimensional graph relating

x : time of exposure (hours)

y : relative humidity at 20°C.

z : hatch % of total cyst content.

KEY:

Hatching time 64 days

E.D.B.

Nemagon

fumigant. Treated cysts were removed and stored for a month, after which a root diffusate assay was carried out. Replication was two-fold and the hatching process was continued for 64 days.

The results are presented in Appendix Table 19A, Tables 20 and 21 and Fig.9. As it is shown in Table 21 the degree of control increased the higher the relative humidity; the best result was obtained at 100% R.H. So in all other experiments the fumigations were carried out at 100% R.H. In E.D.B. no hatch occured at 100% R.H. even after only 4 hours. (See Appendix Table 19A).

Table 20. Fumigation of H.rostochiensis with EDB and Nemagon (Experiment in vitro)

Analysis of variance (effect of R.H. at the time of fumigation).

Log of (hatched larvae + 1) [1].

	Sources	D.F.	S.S.	M.S.	V.R.	
a	Nematicides (N)	1	15.4406	15.4406	a/h 3151	***
b	Period of exposure (P)	3	0.0729	0.0243	b/h 4.96	**
c	Relative humidity (R)	2	40.1293	20.0646	c/h 4094	***
d	N × P	3	0.0232	0.0074	d/h 1.51	n.s.
e	R × P	6	0.0205	0.0034	e/h 0.69	n.s.
f	N × R	2	25.3828	12.6914	f/h 2590	***
g	N × P × R	6	0.0871	0.0145	g/h 2.96	*
h	Residual	24	0.1166	0.0049		
	Total	47	81.2730			

1. In the cysts treated with EDB at 100% R.H. no hatch occured therefore log (x + 1) was used in all treatments.

Table 21. Fumigation of H.rostochiensis with EDB and Nemagon (Experiment in vitro).

Treatment totals, differences and critical differences. Root diffusate assay.

1. Period of exposure (saturation concentration of fumigants).

Period	Totals	Difference.	
4 hours	35.051		
		0.063	n.s.
8 hours	34.988		
		0.785	*
16 hours	34.203		
		0.211	n.s.
32 hours	33.992		
Critical difference	0.708		

2. Nematicides, Relative humidities and Interactions.

Log (L + 1)

	Nematicides		
	E.D.B.	Nemagon	Σ. R.H.
R.H. 60%	29.125	29.034	58.159
R.H. 80%	26.380	28.203	54.583
R.H. 100%	0.000	25.492	25.492
Σ. N.	55.505	82.729	

Critical differences { 0.578 | 0.817 ; 1.001 | - }

Further batches of cysts fumigated at 100% relative humidity in this experiment were kept for further observations.

Two other methods of assay were used to determine the efficacy of Nemagon and EDB in treated cysts.

2. Root invasion assay.

Batches of 200 cysts which had been fumigated with Nemagon and E.D.B. at 4 different rates (see above) were stored in open tubes for a period of 4 months, after which they were mixed with the soil of 24 small pots. Six pots inoculated with untreated cysts were used as a control. This gave three replications for the 10 treatments. The pots were sown with tomato seeds and left in the greenhouse and growth allowed to proceed, watering being carried out when necessary.

After 28 days (about the 2nd leaf stage of tomatoes) two replicates of each treatment were examined for root invasion (as described on page 32). The third replicate was examined after 42 days (4th leaf stage). The results are shown in Tables 22 and 23.

Table 22. The number of nematodes which had penetrated the host plant roots at different treatments, after 28 days.

Fumigant	Period of fumigation	No. of nematodes per g. of root	No.of nematodes (mean of 2) per pot.	Wt. of the root (mean)
Nemagon	4 hours	211	484	2.290
"	8 hours	175	276	1.575
"	16 hours	75	223	2.990
"	32 hours	7	9	1.205
Control	-	251	211	0.840

Table 23. The number of nematodes which had penetrated the host plant roots at different treatments, after 42 days.

Fumigant	Period of fumigation	No.of nematodes per g. of root	Nematodes per pot	Wt. of the root
Nemagon	4 hours	87	743	8.55
"	8 hours	85	722	8.5
"	16 hours	15	120	8.04
"	32 hours	-	-	7.30
Control		156	616	3.95

3. Final eelworm population assay.

Two different sets of experiments were carried out to determine the efficacy of Nemagon and E.D.B. on H.rostochiensis cysts fumigated at 4 different rates and at 100% R.H.

Potato tubers and tomato seedlings were used as hosts.

∝. Determination of the number of newly formed cysts by using fumigated cysts and tomato plants (Experiment No.11).

Clay pots (size 24) were filled with about 3.5 Kg. of air-dried soil, artificially infested with 1000 cysts previously fumigated with Nemagon and E.D.B. at 4 different rates (Experiment No.10).

The soil was a mixture of 3 parts loam, 2 parts peat and one part sand. Pots inoculated with non-treated cysts were used as controls.

Tomato seedlings were transplanted to these pots and left in the greenhouse until the end of the growing season. They were watered when

necessary by an equal amount. The weight of the fruit in each treatment was noted and used as a criterion to estimate the efficacy of the fumigant. Since treated cysts but untreated soil were used, there is no question of phytotoxicity. At the end of the season the haulms were lifted and 500 g. of the soil sample was washed. The number of newly formed cysts was estimated. (See Table 24).

β. Determination of final eelworm population by using potato plants as a host (Experiment No.12).

Batches of 1100 cysts fumigated with E.D.B. and Nemagon at 4 different rates (Experiment 10) were mixed with the soil from clay pots (7.0 Kg. soil with 15% moisture content in each pot).

The initial level of infestation was about 50 to 60 larvae per gramme of soil. An Arran Banner seed potato was planted in each pot. The pots were sunk in the sand outside and left for the complete growth of the potato plants. Watering was carried out when necessary.

At the end of the season the soil in each pot was air-dried and 500 g. was washed through the Fenwick Can.

The number of cysts was counted. See Table 25.

Table 24. The final cyst population of the cysts treated with Nemagon at different rates (Experiment No.10) at 100% R.H., and at 20°C. in vitro. Tomato.

Period of exposure	No. of cysts recovered from 500g. of soil sample			
	>599μ	599-422μ	<422μ	Total
4 hours	-	177	189	366
"	4	181	166	351
8 hours	3	169	32	204
"	-	186	29	219
16 hours	-	170	36	206
"	-	182	25	207
32 hours	-	123	6	129
"	-	119	-	119
Control	5	324	620	949
"	6	312	773	1091

Initial infestation 145 cysts per 500 g. of soil, about 100 eggs per g. of soil.

Table 25. The final cyst population of the cysts treated with Nemagon at different rates (Experiment No.10) at 100% relative humidity and at 20°C. in vitro. Potato.

Period of exposure.	No. of cysts recovered from 500g. of soil sample			
	>599μ	599-422μ	<422μ	Total
4 hours	50	302	84	436
"	12	234	121	367
8 hours	18	184	53	255
"	7	183	35	225
16 hours	0	75	10	85
"	6	74	15	95
32 hours	0	68	0	68
"	0	77	1	78
Control	556	941	368	1865
"	339	1192	376	1907

Initial infestation 90 cysts per 500 g. of soil, about 60 eggs per g. of soil.

Table 26. Comparison of three methods of assay after fumigation of cysts with Nemagon.

Experiment in vitro (1959).

Time of exposure	Root Diffusate		Invasion to the root				Final Density. Tomato.	
			2nd leaf		4th leaf			
	Hatch	% Kill	L/g	% Kill	L/g	% Kill	cyst/500g.	% Kill
4 hours	2056		211		87		358	
		71.81		15.93		44.23		75.66
8 hours	1900		175		85		211	
		73.95		30.28		45.51		92.46
16 hours	1261		75		15		206	
		82.71		70.12		90.38		93.03
32 hours	1165		7		-		124	
		84.03		97.21		100		100
Control	7295		251		156		1020	

4. Results.

Root diffusate assay: analysis of variance (Tables 20 and 21) showed that relative humidity had a great effect on the hatching process. Appendix Table 19A shows a significant increase in the effect of the fumigant with an increase in relative humidity.

The effect of the duration of exposure was only significant for the difference between the results at 8 hours or fewer and 16 hours or more.

However this result cannot be considered very dependable, as in the case of ethylene dibromide at 100% relative humidity no hatch occured, and in all cases the total hatch was very low (20 to 25%) even in the controls.

The data also suggested that fumigation of cysts with Nemagon and ethylene dibromide, in addition to reducing their hatchability, had a delaying action on the time of emergence, i.e. the number of emerged larvae in the controls at the first week was significantly higher than the number of emerged larvae from all other treated cysts: this situation changed in later weeks.

Root invasion assay: Tables 22 and 23 show the number of nematodes per gramme of root and also the number of nematodes per root system at 2 different times of examination (the second and fourth leaf stages of tomato plants). It can be seen that the number of nematodes penetrating the roots from the cysts treated with Nemagon at 8 hours and 16 hours duration of exposure, is higher than in the controls, largely because the latter have a smaller root system.

The data from the hatching process have shown that the emergence of the larvae in treated cysts will be delayed compared with controls and it is possible that a similar effect will be found in invasion assays. This possibility will be discussed later in the thesis.

Final eelworm population: Tables 24 and 25 show that the total number and also the size of new cysts in potato and tomato were significantly different in all treatments, the parasite being much more successful in the potato.

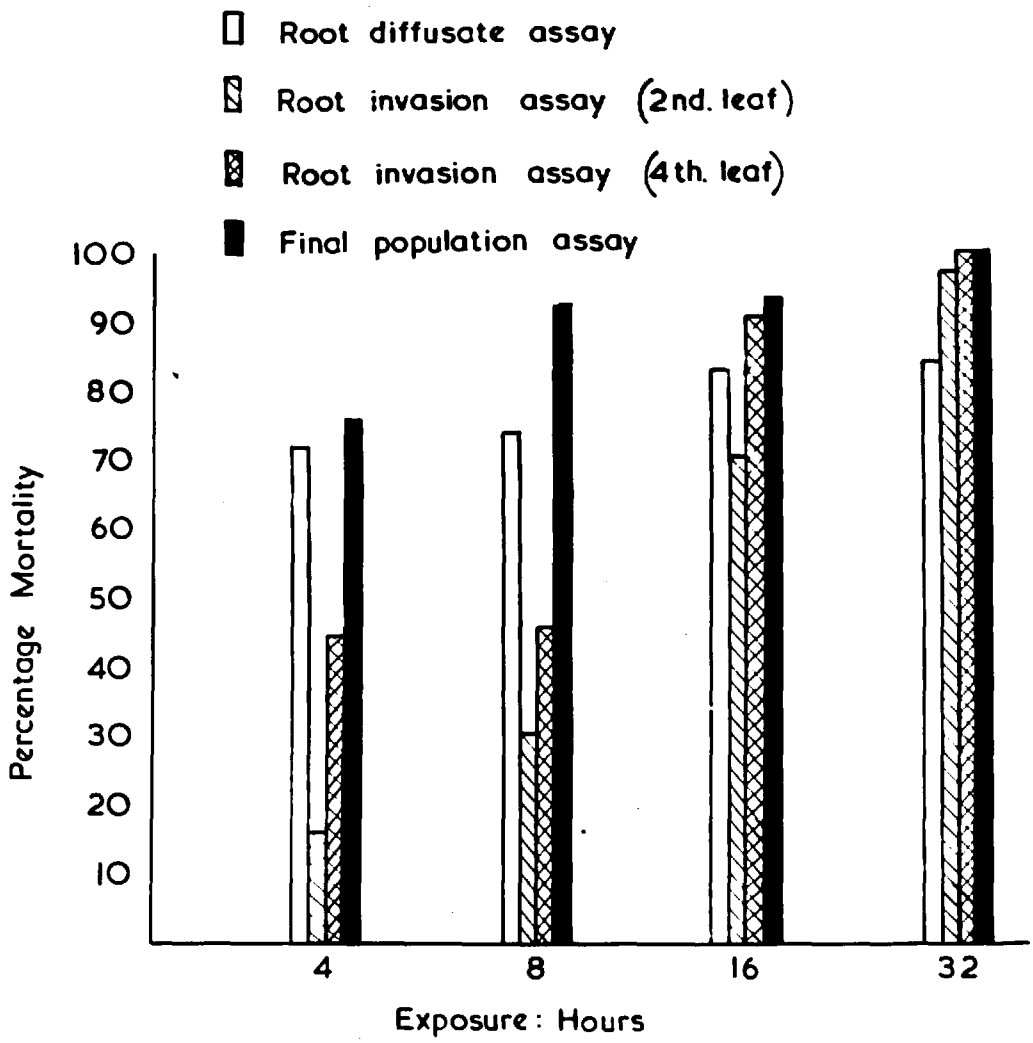


Fig.10 Comparison of 4 methods of assay

The height of the plants and the weights of the tomato fruits and potato tubers were measured, but no significant differences were found in relation to the degree of infestation due in turn to the effect of the different rates of fumigation.

Table 26 and histogram Fig.10 show the difference of the three methods of assay:-

The real efficacy of the fumigant was revealed only in the final density assay in which the highest kill was recorded, probably because sufficient time had elapsed both to allow for any delayed action of the fumigant and also to disregard those penetrated larvae which could not reach maturity due to the later effect of the fumigants.

(e) The effect of temperature on fumigation of H.rostochiensis (Experiment No.13).

i. Introduction.

According to Harrison (1956), temperature has no effect in glass fumigation chambers, when the cysts of H.rostochiensis are exposed to the vapour of fumigants. This was not accepted by Fenwick (1957), who suggested that fumigants have a greater effect at high temperatures.

Consequently it was desirable to test the claims of the above mentioned workers. This was done by studying the reaction of cysts of H.rostochiensis to the vapour of 3 fumigants, C.B.P., E.D.B. and Nemagon, at different temperatures. In addition the effect of different doses of these fumigants was studied.

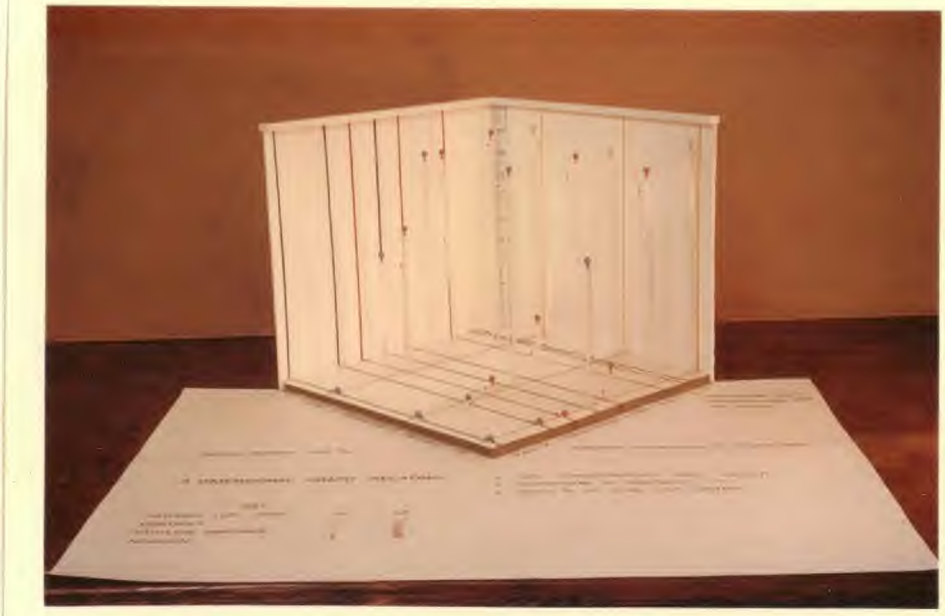


Fig.11. Three dimensional graph relating

x : Log (concentration x time) mg.hrs./l.

y : Temperature at treatment : T°C.

z : Hatch % of total cyst content.

KEY.

Hatching time (days)	24	64
Controls		
E.D.B.		
Nemagon.		

ii. Methods.

Nine fumigation chambers (modified desiccators) were set up in 3 different constant temperature rooms at 30°, 20° and 8°C.

Because it had been learnt from previous experiments that fumigation at 100% relative humidity gave the greatest kill, both prior to and during the fumigation, 100% R.H. was used and was achieved by pouring a 2 cm. layer of tap water into the bottom of each desiccator. Fumigation was carried out as described previously. The period of exposure in saturated concentration of each nematicide was $\frac{1}{2}$, 2 and 4 hours. Fumigated cysts were stored for one month, after which the hatching test was carried out. Replication was twofold. The cumulative emergence at 24 and 64 days for each treatment is shown in Table 27 and Fig.11.

iii. Results.

The data obtained show that a greater effect of fumigation took place at higher temperatures. In all cases the greatest kill was obtained at 30°C. which seems to be a lethal temperature for the parasite. At 8°C. the total number of emerged larvae was very high. Consequently in this type of experiment a temperature of 20°C. would be more suitable.

In the case of C.B.P. in all treatments no hatch occured. This shows that the period of exposure to this fumigant was too high so it was adjusted in later experiments.

The data resulting from this experiment, and previous experiments dealing with relative humidity, suggest that environmental conditions have a great effect when Heterodera cysts are exposed to the vapour of different

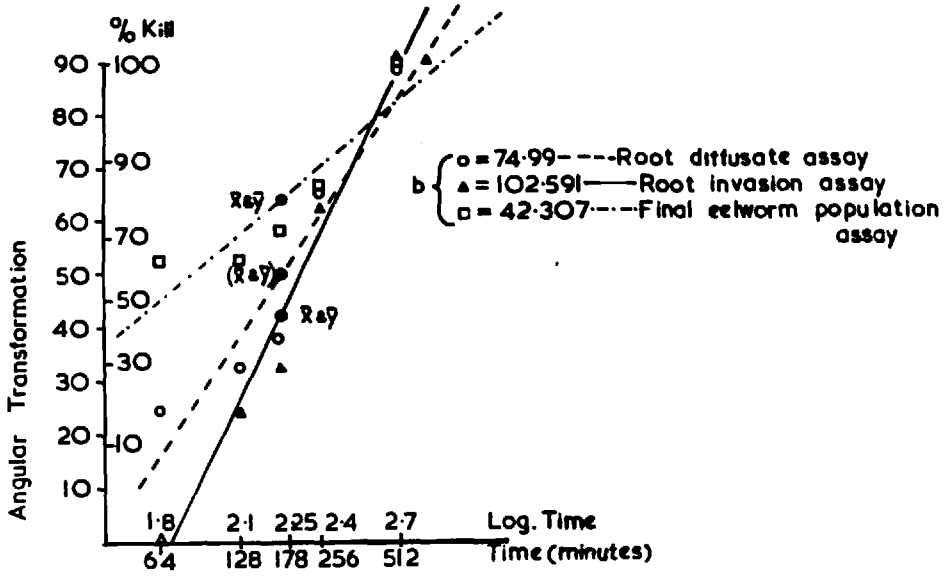


Fig. 12 Comparison of 3 methods of assay

Experiment in vitro

Table 27. The cumulative emergence of batches of 100 cysts at 24 and 64 days and at different temperatures (Experiment No.10).

Fumigant	Time of Exposure (mins.)	Total No.of Hatched larvae (24 days)			Total No.of Hatched larvae (64 days)		
		8°C.	20°C.	30°C.	8°C.	20°C.	30°C.
EDB	30	24012	28141	4625	30556	30294	15394
"	30	17375	23973	5047	31270	26657	16116
	120	3832	-	-	16147	-	-
	120	6318	-	-	19181	-	-
	480	-	-	-	-	-	-
	480	-	-	-	-	-	-
Nemagon	30	32528	23746	22460	34452	26632	24839
	30	30320	28926	22242	32033	30787	24718
	120	19688	3892	374	33724	22370	3970
	120	17030	3280	187	29100	25451	4084
	480	16324	953	-	19776	2427	-
	480	15124	1171	-	21847	2139	-
Control		30379	27733	28566	32726	28139	28961

fumigants, which is in agreement with the observations of many other workers (e.g. Fenwick 1957; Van den Brande et al 1956).

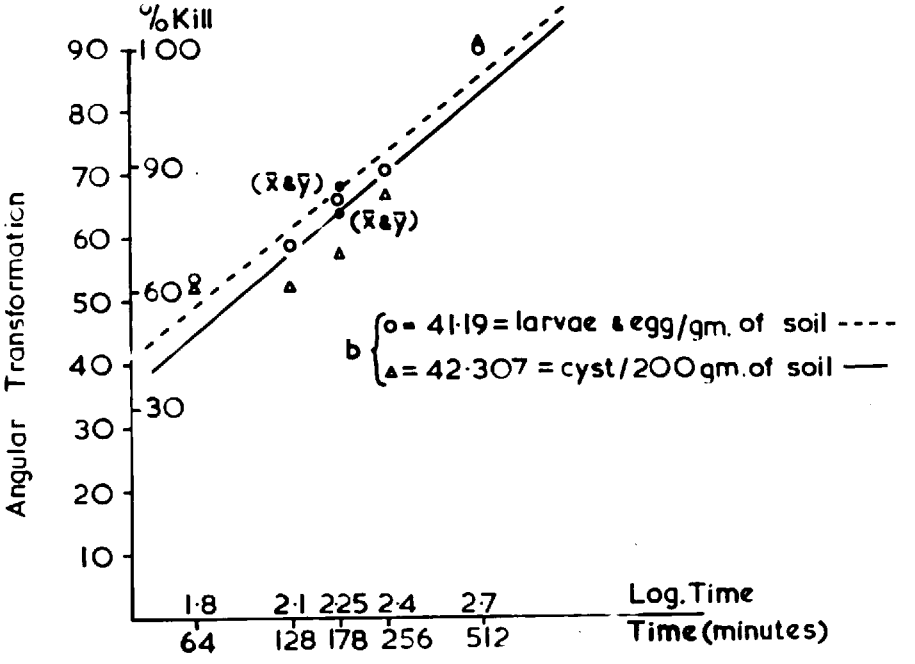


Fig. 13 The response of H.Rostochiensis fumigated with Nemagon. Experiment in vitro. Final Density Assay

(f) Fumigation of H.rostochiensis cysts at different dosages and at 20°C. and 100% relative humidity (Experiment No.14).

Resulting from the experience gained in the experiments discussed under (d) and (e) above, cysts were treated as in the previous experiment but for a different period of exposure as described below:

1. Nemagon: 64, 128, 178, 256 and 512 minutes.
2. E.D.B.: 32, 45, 64 and 89 minutes.
3. C.B.P.: 2, 4, 8 and 16 minutes.

Fumigation was carried out at 20°C. and at 100% R.H. Fumigated cysts were stored for 45 days after which they were tested in three ways:

1. Hatching test with root diffusate (root diffusate assay).
2. Root invasion of the host plants.
3. The final population of new cysts and larvae per gramme of soil.

i. Root diffusate assay.

Batches of 100 treated cysts were hatched as described previously and the results are shown in full in Appendix Table 28A. When the angular transformation of the percentage mortality is plotted against dose for Nemagon the L.D.50 is shown to be between exposures of 178 and 256 minutes at saturation concentration (see Fig.12).

The C.B.P. and E.D.B. results could not be shown graphically because there were not enough points to draw a graph but rough estimates gave L.D.50 of 2 and 30 minutes respectively. Thus C.B.P. is much more effective than E.D.B. and Nemagon when the root diffusate assay is used to measure efficacy, and when fumigation is in vitro.



Fig.14. Eggplant seedlings in soil infested with 50 H.rostochiensis cysts fumigated with Nemagon.



Fig.15. Tomato seedlings in soil infested with 50 H.rostochiensis cysts fumigated with Nemagon.



Fig.16. Tomato seedlings in soil infested with 50 H.rostochiensis cysts fumigated with E.D.B.



Fig.17. Tomato seedlings in soil infested with 50 H.rostochiensis cysts fumigated with C.B.P.

ii. Root Invasion Assay.

After storage for 45 days each batch of 50 cysts per treatment was mixed with 200g. of sterilised soil. The soil was a mixture of 3 parts loam and one part sand. Infested soil was placed in clay pots (size 60).

Each treatment was replicated 4 times, two replicates being planted with tomatoes and two with eggplants. Sixteen tomato seeds or eggplant seeds were planted at equal distances apart in each pot. After about 35 days the roots of each plant were washed free of soil and stained: the nematodes were counted as described in Part II.

The results are shown in Appendix Table 29A and Fig.12, and show that the percentage mortality in all treatments was less than by the root diffusate assay.

iii. The final eelworm population assay.

Each batch of 50 cysts per treatment was mixed with 200g. of sterilised soil and placed in clay pots (size 60). Potato chits were planted in each pot and left for 15 weeks, after which the haulms of the plants were lifted and the new cysts in each pot were recovered to estimate the cyst density in the soil. The estimates of eggs per cyst for the criterion (L/g) were obtained by using the homogeniser technique. Percentage mortality for (L/g) and new (c/g) was estimated as described in Part II. The results are shown in Appendix Table 30A, Table 31 and Figs.12 and 13, where the means of the percentage mortalities have been calculated for the two forms of assay.

Although analysis of variance (Appendix Table 32A) showed that the variance ratio "F" was significant, the regression line in Fig.12 for the final density assay (cyst/g. of soil), did not seem to be the best fitted line for the data. In an attempt to obtain a better idea of the pattern presented by the efficacy of the fumigant against the parasite, the method of orthogonal polynomials was used based on logarithmic transformations of four doses, i.e.: 64, 128, 256 and 512 minutes of saturation concentration of the fumigant, leaving out the odd dosage of 178 minutes which was not of equal interval with the others. (This method of analysis requires the x values to be at equal intervals).

The orthogonal polynomials and the corresponding sums of squares were calculated (see Appendix Tables 35A and 36A). The analysis of variance was then carried out as below:

Analysis of Variance (Orthogonal polynomials).

Fumigation of H.rostochiensis with Nemagon in vitro.

(Final density assay cyst/200g. of soil. Experiment No.14).

	Items	D.F.	S.S.	M.S.		F.	
a	Linear	1	1612.9	1612.9	a/(b +c)	11.98	n.s.
b	Quadratic	1	266.8	266.8	a/d	33.95	**
c	Cubic	1	2.5	2.5	(a +b)/d	39.56	**
d	Error	4	190.03	47.51	b/d	5.61	n.s.
e	Total	7	2072.23				

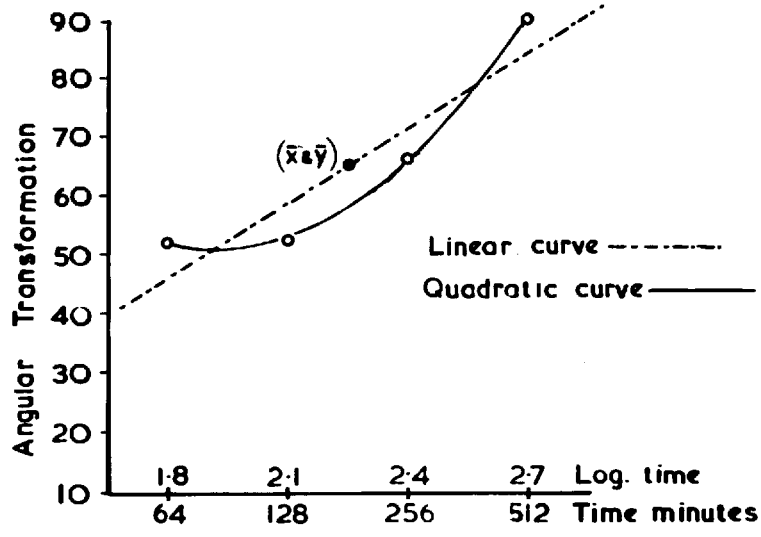


Fig.18 Relation between log dose and % kill
Fumigation of H. Rostochiensis with Nemagon
Experiment in vitro
Final Density Assay Cysts/gm. of soil

Having only four dosages the analysis of variance could be applied for three degrees of curvature. It can be seen that the mean square for the linear and quadratic terms is significantly greater than that of error and therefore a curve corresponding to a quadratic equation would best fit the data (Fig.18). I am unable to explain in biological terms this lack of rectilinearity between log dose and the angular transformation of percentage kill.

iv. Comparison of methods of assay using Nemagon as fumigant.

Since there were a sufficient number of dosage rates giving kills spread over the range 0 - 100% mortality, comparison between the regression lines obtained could be made.

First a test was made between the two methods of estimating the final density assay e.g. (L/g) and new (c/g), no significant difference was found between the regression lines for the two forms of the final density assay (see Fig.13 and Appendix Tables 32A and 34A). However an analysis of variance (Table 37) showed that there were significant differences between the number of larvae per cyst in different treatments.

The final density assay of (new cysts per 200g.) gives a value of 64 minutes of exposure to Nemagon at saturation concentration which is equivalent to 5.55 mg.hrs. per litre for the median lethal dose (L.D.50) whereas this value for C.B.P. is less than two minutes.

The results obtained support the results of previous experiments in revealing that the final eelworm population assay is more reliable than the other two methods and gives a higher kill.

The median lethal dose of H.rostochiensis by using Nemagon has been estimated. The results are summarized below in terms of mg.h./l. (See Fig. 12 and 13).

Nemagon L.D.50 (mg.h./l.)

Methods of assay			
Root diffusate	Root invasion	Final density assay	
		Cyst/g. of soil	eggs/g. of soil
13.43	16.90	5.55	4.33

Table 31. The effect of fumigating H.rostochiensis cysts with Nemagon, EDB and CBP in glass chambers at 20°C. and 100% R.H. Experiment in vitro. (Comparison of different methods of assay).

	% Kill.				
Fumigant and time of exposure (mins.)	Root Diffusate	Invasion to tomato roots	Invasion to eggplant roots	Final eelworm density, new cysts	Final eelworm density L/g of soil
Nemagon 64	16.71	-	8.2	62.5	64.48
" 128	28.46	16.69	27.13	62.89	72.58
" 178	37.65	29.14	45.51	71.88	82.46
" 256	82.13	77.76	47.33	83.98	88.79
" 512	99.90	100	100	100	100
C.B.P. 2	57.22	54.13	57.95	83.72	82.35
" 4	95.56	99.04	90.97	96.87	93.12
" 8	97.81	100	100	100	-
" 16	100	100	100	100	-
E.D.B. 32	50.23	94.13	84.86	99.47	-
" 45	94.69	100	100	100	-
" 64	100	100	100	100	-

Table 37. The effect of Nemagon on the number of larvae/cysts at different treatments. Experiment No.14 in vitro.

Treatments	Number larvae/cyst	Log	Totals	Difference		
Control	224	2.350				
"	217	2.336	4.686			
64 minutes	188	2.274		0.129 n.s.		
"	192	2.283	4.557		0.350 *	
128 minutes	138	2.140		0.221 n.s.		
"	157	2.196	4.336			0.418*
178 minutes	99	1.996		0.197 n.s.		
"	139	2.143	4.139			
256 minutes	101	2.004		0.144 n.s.		
"	98	1.991	3.995			

Critical difference = 0.257

Analysis of variance (log L/cyst).

	Items	D.F.	S.S.	M.S.	F.
a	Treatments	4	0.163106	0.0407765	a/b 16.186 **
b	Residual	5	0.012596	0.002519	
c	Total	9	0.175702		

(g) Observation on the final eelworm population by injecting the planted soil with Heterodera larvae emerged from the fumigated cysts. Experiment No.15.

The larvae which hatched from the batches of the cysts treated with Nemagon and the controls (Experiment No.14 and Root diffusate assay) were estimated at 8 day intervals and then were introduced into the soil of small pots planted with tomato seedlings. Replication was two-fold.

The tomato pots were left in the greenhouse in a randomised pattern for 15 weeks, after which the plants were lifted, the soil was air-dried and washed in the Fenwick can to estimate the cyst density in the soil. The main purpose of this experiment was to throw light on the difference between "hatchability" and "viability".

i. Results.

The data are shown in Table 38 and support the previous experiment which had been set up by infesting the soil with fumigated cysts.

The final numbers of new cysts in all treatments are far less than in the control. The numbers of larvae initially added to the controls and to the lowest treatment (Nemagon 64 minutes) did not greatly differ, but the newly formed cysts from treated larvae were about 1/3 of those from the controls. This suggests that most of the treated larvae are unable to complete their life cycle and die before maturity. Thus, over 5% of the control larvae formed cysts whereas less than 2% of the treated larvae did so.

Table 38. Infestation of soil with larvae emerging from 100 fumigated cysts treated with Nemagon.

Treatment Period of Exposure	Mean number of emerged larvae injected to soil of each pot. a	Final eelworm population new cysts per pot. b	100 b/a c	% Mortality d
64	20655	391	1.89	68.96
128	17571	303	1.72	75.87
178	13898	142	1.02	88.73
256	4744	75	1.58	94.04
Control	22859	1260	5.51	0

(h) Observations on the effect of storage on cysts which had been previously fumigated with E.D.B., Nemagon and C.B.P. Experiment No.16.

Fenwick (1957) has shown that when cysts fumigated with E.D.B. were tested three months after treatment, a greater decrease in hatchability was observed than in cysts tested immediately after fumigation. Based on this, the cysts fumigated with Nemagon in glass chambers (Experiment No.10) were stored in open tubes for three months, before being used for the hatching test. Duplicate batches of 100 cysts which were soaked in water were exposed to root diffusate, and larvae emerging at the end of 8 day intervals were collected. At the end of 64 days exposure the total number of emerged larvae was estimated. The result is set out in Table 39.

Another test was carried out the following year using cysts treated with C.B.P., E.D.B. and Nemagon (Experiment No.14). The time of storage

was extended to 6 months after which the total larval emergence from each treatment was estimated. The data are presented in Table 40.

i. Results.

The data from cysts treated with Nemagon and stored for three and six months respectively suggest that fumigation of cysts with this fumigant only reduces their hatchability. The action of the fumigant in effecting larval emergence was almost the same both shortly after and a long time after treatment. But the two other fumigants in addition to reducing the hatchability, produced increasing effects long after treatment. The data obtained from these experiments in addition to the data obtained from final eelworm population experiments (see Appendix Table 30A and Table 38) where similarly treated cysts and larvae were used, produce reasonable evidence that there is no direct correlation between hatchability and viability of larvae.

Table 39. The effect of storage on cysts which had been previously fumigated with Nemagon (time of storage 32 and 90 days).

(Root diffusate assay).

Time of Exposure (hours) (Saturation concentration)	Emergence total larvae per 100 cysts	
	After 32 days (mean of 2)	After 90 days (mean of 2)
4	2056	2034
8	1900	1866
16	1261	1311
32	1165	875
Control	6795	6162

Table 40. The effect of storage on cysts which had been fumigated with different fumigants (time of storage 45 days and 6 months) (Root diffusate assay).

Fumigant (Saturation concentration)	Time of exposure (minutes)	Larval emergence (100 cysts)(mean of 2)		% Reduction
		45 days	6 months	
Nemagon	64	23427	19125	18
"	128	20123	18253	9
"	178	17539	23502	-
"	256	5027	144	71
"	512	7	0	100
C.B.P.	2	12032	1020	91
"	4	1250	111	92
"	8	611	21	97
"	16	0	0	-
E.D.B.	32	13999	6	100
"	45	1493	0	100
"	64	12	0	100
Control	-	28128	24750	

(i) Discussions on experiments in vitro.

i. Root diffusate assay.

The root diffusate assay which has been used as a method of determination of the efficacy of a nematicide is based on the general assumption that about 80 to 90% of the viable larvae in an ordinary cyst will hatch in an interval of three weeks, therefore in treated cysts any reduction in the larval emergence can be considered as a sign of the nematicidal effect of the fumigant.

In fumigation in vitro I have shown first that a three weeks hatch can be used only for untreated cysts and is not in my experience applicable to fumigated cysts, which are affected by delayed hatching. Secondly, although larval emergence is a good indication of the viability of the parasite, it does not necessarily mean that the emerged larvae can survive and produce a new generation even if they penetrate the host plant (see Appendix Tables 28A, 29A, 30A).

Thirdly, the selection of a suitable time for the hatching test is another problem. Fenwick (1957) fumigated cysts of H.rostochiensis with E.D.B. in vitro. He found that unless the cysts are stored for several months before the hatching tests are conducted, the estimates of % kill will vary with the length of time between fumigation and the hatching test. Observations on the effect of storage which have been described in this thesis gave similar results. When cysts were treated with ultimately lethal dosages of E.D.B. and C.B.P. the larvae could not emerge, but in the cysts fumigated with ultimately lethal dosages of Nemagon most of the larvae

continued to hatch and their appearance was very similar to the untreated larvae.

Cysts treated with E.D.B. and C.B.P. which were stored for six months gave a very low larval hatch even after 64 days exposure to the action of potato root diffusate, although a considerable hatch was observed from similarly treated cysts one month after fumigation.

When the number of emerged larvae from cysts treated with Nemagon and stored for one month was compared with that of cysts stored for six months the hatch was almost identical (with one exception). However, most of these emerged larvae (regardless of the storage time) were not capable of completing their life cycle in the host plant roots and they seemed to be very inactive (see experiment No.4, page 27 and No.14, page 65). This phenomenon has been very misleading and in addition to the other difficulties in the hatching test, throws some doubt on the validity of root diffusate assay in nematicidal experiments.

ii. Root invasion assay.

By comparing the roots of tomatoes and eggplants from soil containing either untreated or treated cysts the following differences were clearly distinguished:

In the controls and especially in the tomatoes the roots showed considerable necrosis and reduction, and swellings were very marked. All the control plants were damaged but stunting was less in the eggplants than in the tomatoes (see Figs. 14 and 15). Both hosts showed all treatments better looking than the controls and without definite swellings on the

roots.

The weight of the root system of the tomato plants in each treatment was about twice that of the eggplants, but the total number of nematodes present in the roots of the tomato almost exceeded three times that of the eggplants (see Appendix Table 29A). This suggests that the tomato is a more suitable host than the eggplant.

In the lower treatments with Nemagon and C.B.P. nematodes in the roots could be seen at different stages including even premature males and females, but in the higher treatments and in all treatments with ethylene dibromide almost all nematodes were in the second and third stages of their development. Most of the nematodes in the treatments had little or no food reserves in the intestinal region and even some of the nematodes which had developed to the fourth stage looked relatively empty. It seems likely that these individuals would be unable to complete their life cycle and form new cysts.

The data on the hatching process have shown that the emergence of the larvae in treated cysts will be delayed, and a similar effect was also found in invasion assays. Thus the following course of events probably takes place:

1. At a very early stage in the growth of the plant, about two weeks after germination, the number of nematodes in the root system of the controls will be greater than in the roots of plants growing in soil infested with treated cysts.

2. Somewhat later, larvae begin to hatch from the treated cysts, hatching

being delayed by fumigation, and from there onwards the number of these larvae in the roots gradually increases, and in the lower treatments may exceed the number in the controls, where nematodes may have begun to leave the root (i.e. adult males).

3. If at this stage one takes the number of nematodes in the whole root system, in some treatments the number of eelworms will be greater than in the controls. If however one takes the number of nematodes per gramme of root the position will be reversed, i.e. the number of larvae from treated cysts per g. of root will be less than in the control. This may be due to the plant in the control becoming stunted at an early stage as a result of early invasion by larvae, and the consequent cessation of normal root growth. At the time of root invasion, however, in the lower treatments the root system will be better established and more extensive and the chance of a larva penetrating a root will then be increased (see Tables 22 and 23, and Appendix Table 29A).

4. Even though the plants were kept until the end of the growing season most of the larvae from treated cysts failed to complete their life cycles. By the fourth stage these larvae had no visible contents and appeared to be dead, probably due to the effect of the fumigant.

The two criteria; the number of nematodes per g. of root and the number of nematodes in the whole root system, give different estimates of percentage mortality neither of which is in close agreement with the percentage mortality obtained from the final eelworm population (Tables 26 and 31).

5. The delayed hatching from old cysts (Franklin 1938) is another factor which should be considered in both root diffusate and root invasion assay. Franklin has shown that the hatching larvae from new cysts invaded potato roots more rapidly than was the case with older cysts. In such a case the root would be less likely to be invaded at the second leaf stage, though it might be infested later in the growing season.

In root diffusate assay, when old cysts were used, irregularity of hatching was unavoidable and confused the results.

iii. Final eelworm population.

Experiment No.14 which was mainly carried out to compare the best of three methods of assay, once again showed that the number of larvae per gramme of soil based on the final cyst population will be the best method and most reliable assay, and supports the results of the experiments which were carried out in 1959 (see Experiment No.10, page 52).

Appendix Tables 28A and 30A show that although the number of hatched larvae in some treatments (E.D.B. 32 minutes, Nemagon 64, 128, 178, 256 minutes, Chlorobromopropane 2 minutes) was rather high, the final eelworm population was nil or very small, which proves that there is no relation between hatchability and viability of the cyst contents and supports the results obtained in Experiment No.15.

In some of the treatments with Nemagon most of the newly formed cysts were more or less shrunk and although they looked to be new by their size and brightness, they were almost empty and deeply dimpled. It could be concluded that most probably some of the fumigants have three different

kinds of effects:

a. general reduction of the number of newly formed cysts.

b. reduction in the size of the cysts.

c. reduction of the number of eggs and larvae per cyst.

The effect "b" simultaneously produced a reduction in cyst content. Nevertheless categories "b" and "c" have been classified separately because two cysts of exactly the same size could have different cyst contents due to differences in shape. These differences result from malformation of fumigated cysts and their dimpling.

The criterion new cysts per 200 g. shows clear treatment differences (see Appendix Table 30A).

The estimations of larvae per cyst in Nemagon treated cysts were obtained and have been shown in Table 37. Analysis of variance showed that there were significant differences between the number of cyst contents in different treatments. An increase of the C.T.P. of the fumigant resulted in a significant decrease of the number of eggs per cyst. This suggests that although the criterion of new cysts per g. of soil shows clear treatment differences, the larval count method is preferable as being more precise.

The percentage mortalities estimated in cyst and larval counts were transformed to the angular scale and plotted against the logarithm of the time of exposure (Fig.13).

Although no significant difference was found between the regression lines for the cyst and larval counts, nevertheless since in cyst counts two different effects of the efficacy of the fumigant (viz. size of the

cysts and number of larvae per cyst) will be concealed, the criterion would be only partially satisfactory. This strongly suggests that in this comparison the slope and position of regression lines are not a good criterion for judgment, probably because the sampling system is not efficient. Otherwise one would expect the larvae/g. criterion to show itself superior.

Section B. Experiments in vivo.

Pot Experiments.

(a) Introduction.

Many workers (Peters & Fenwick 1949; Harrison, 1957; Hague 1958) have assessed the efficacy of fumigants by observing the natural multiplication of H.rostochiensis in the presence of a host plant.

The search for new nematicides is in progress, but estimations of their efficacy against the parasite should be measured by a convenient and reliable method before being used on a field scale. In pot experiment the soil can be infested at a desirable level and mixed before sampling and many other factors can be controlled. Although higher kills would be expected in nematicidal experiments carried out in pots (Peters 1952), Pot tests are still a practical and valuable way of comparing different nematicides. The possibility of relating the nematode response to a given dosage of nematicide and thus of comparing the efficacy of various fumigants led me to carry out both the previous "in vitro" and the present "in vivo"

experiments. The soil was either naturally or artificially infested with H.rostochiensis and various criteria were employed to measure the effect of the fumigants used, but all eelworm criteria (unless otherwise shown) relate to a final density assay.

(b) Experimental procedure.

The fumigation experiments were carried out in pots using 3 fumigants: E.D.B., Nemagon and C.B.P. For the first year, 1959, two hosts (tomato and potato) and two types of soil suitable to each plant, were used. But in 1960 it was decided to use only one type of soil. This was a mixture of 3 parts loam and one part sand. The soil was artificially infested with the appropriate cysts (graded to size 422 to 599μ for all experiments).

Using the same type of soil meant that the fumigants would be acting under the same conditions. During fumigation in each year soil temperature and moisture content were measured. The pots of soil were fumigated and left for about 1 to 2 months, after which the soil from each pot was removed and thoroughly mixed with 11 g. of fertilizer, a mixture of 5 g. superphosphate, 4 g. amonium sulphate and 2 g. of potassium sulphate.

Since the concentration of the fumigant was greater round the inoculation point and fell off towards the edge of the pot (Call 1955) it was important to thoroughly mix the cysts which had received the different concentrations. This was accomplished by mixing the fertilizer with the soil which also assisted the escape of residual fumigant and reduced the possibility of phytotoxic effects. Clay pots, size 12, with a capacity

of about 8 Kg. soil and 25 cm. high were used. One Arran Banner seed potato was planted in each pot.

These potato tubers were a final selection of some potatoes which had been kept in the greenhouse to chit. All the chits but one were removed from each of the selected tubers. The pots were sunk in sand in which positions were allocated by randomisation and they were watered when necessary.

About September the pots were removed and left to dry. The soil from each pot was thoroughly mixed and the final cyst population was estimated by the usual technique (see Part II).

(c) Pot experiment in 1959-1960. (Experiment No.17).

In 1959 a preliminary experiment, using ethylene dibromide and Nemagon was carried out. The soil used was sterilised by steam heating (see Part II). During fumigation the pots were placed in a cold house at the environmental temperature.

The moisture content of the soil before fumigation was measured and adjusted to about 15 to 17 per cent. To determine the moisture content of the soil, samples were taken and their moisture content estimated by heating in an oven at 104°C. for 24 hours.

i. Methods of fumigation.

Fumigation was done in 9" whalehide pots lined with polythene tubing of 0.005" thickness. The polythene tubing was cut to a length of 17" and placed inside the pots to prevent the escape of the fumigant. The

lower part of the tubing inside the pot was folded so that only a small aperture was left for drainage. The pots were filled with 7.5 Kg. of soil. The soil in the pots was hand compressed, especially at the surface to prevent the escape of the fumigant after application.

The fumigant solution was poured through a hollow glass tube with a glass rod inside to prevent it from being filled with soil when inserting it in the centre of the pot (Peters 1952).

To inoculate the pot, the glass tube was inserted into the soil to a depth of 3.5 inches. The rod was removed and the liquid fumigant poured down through a funnel. The tube was then taken out and the hole was filled with soil. For each rate the fumigant was made up to 10 ml. with acetone and after each inoculation about 1 to 2 c.c. of acetone were poured into the tube to wash it and were then poured into the pot.

ii. Rates of fumigation.

Peters (1952) fumigated H.rostochiensis with E.D.B. in pots holding about 10 Kg. soil. Potatoes were planted seven weeks after fumigation. The median lethal dose of E.D.B. was found to be about 0.13 ml. per pot. When H.gottingiana was used as a test organism in pot experiments, a 50% kill was achieved with a dose of about 0.07 ml. of E.D.B. (Clements 1959).

Based upon the above data the range of dosages of E.D.B. used in (1959) was a geometric series, with a factor of two from 0.016 to 0.256 ml. per pot.

For Nemagon there was no such information, therefore a wider range

of dosages was designed to include a 50% kill, the factor being 4 and 2 as follows:

Ethylene dibromide	Nemagon
1. Untreated control (10 ml. acetone only)	1. Untreated control (10 ml. acetone only)
2. 0.016 ml.	2. 0.001 ml.
3. 0.032 ml.	3. 0.004 ml.
4. 0.064 ml.	4. 0.016 ml.
5. 0.128 ml.	5. 0.064 ml.
6. 0.256 ml.	6. 0.128 ml.
	7. 0.256 ml.
	8. 0.512 ml.

Soil temperature during fumigation 8.2°C.

Average : 10.3°C.

Air temperature : 11.3°C.

Moisture content : 15.22%

All but control pots were fumigated in early April and left for one month, after which the soil from each pot was emptied into a large tray and mixed. The soil was replaced in clay pots and potato tubers (Arran Banner) were planted. Tubers were selected for even size (mean weight 80 $\pm$ 5 g.). Pots were then plunged in sand in a randomized design.

During the growing season some of the potato plants were attacked by a combination of virus x and y. Although all possible precautions were taken, some of the plants had to be removed earlier than others. Consequently the scheme in Nemagon treatments had to be divided into two blocks.

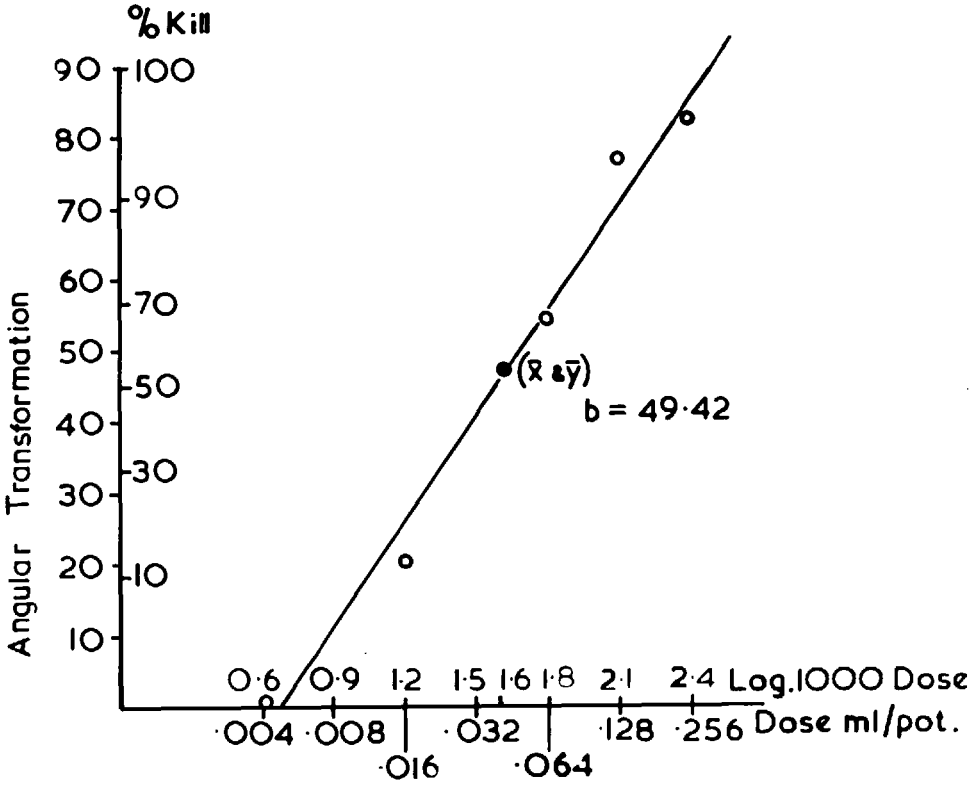


Fig.19 The response of H.Rostochiensis fumigated with Nemagon. Host potato. Pot experiment 1959. Final Density Assay Cyst/gm. of soil

Fortunately the attack of the disease was in such a position that one complete replication of each treatment remained until the end of the season whereas the other replicates were removed. As a result there were two blocks of the pots each with a complete replication of each treatment. The first block (B) was removed on August 25, and the second (A) on September 15.

iii. Results.

The results are summarized in Appendix Tables 41A and 45A and Table 48. As the data in an untransformed state were not suitable for analysis of variance, they were transformed to the logarithmic scale, which has been shown to be a satisfactory transformation (Peters 1948 and 1952).

The analysis of variance (Tables 42 and 43) shows that the number of cysts produced in Block (A) was higher and significantly different from that in Block (B). Consequently the efficacy of the fumigant against the parasite was considered by using Block A data. As a sufficient number of dosage levels and significantly different percentage mortalities (cyst/g. of soil) were available the latter was plotted against the former and a linear relationship was obtained (Fig.19).

Plant height and the weight of the tubers were measured but no significant differences were found, which is in agreement with other workers (Hague 1959; Hague & Hesling 1958).

The results presented in Appendix Table 45A for E.D.B. were not very fruitful, because in the two lower doses (0.016 and 0.032 ml.) no kill occured and in other doses the percentage kill was very high. In lower

doses both in Nemagon (0.001 and 0.004 ml. per pot) and in E.D.B. treatments (0.016 and 0.032) the population levels were higher than controls, but analysis has shown that they are not significantly different from controls (Tables 44 and 47).

Table 42. Analysis of variance for two Blocks (A and B) of potato pots fumigated with Nemagon at different rates (Experiment No.17)

Log cysts/500 g. of soil.

	Items	D.F.	S.S.	M.S.		F.	
a	Doses	7	6.04663	0.8638	a/c	63.985	***
b	Blocks	1	0.10488	0.1048	b/c	7.762	*
c	Interaction	7	0.09516	0.0135			
d	Pots	15	6.24667	0.4164			
e	Samples per pot	16	0.06095	0.0038			
f	Total	31	6.30762				

Table 43. (Experiment No.17). Treatment totals differences and critical differences.

Treatment.	Totals.	Difference.
Block A	41.443	1.832 *
Block B	39.611	

Critical difference = 1.55

Table 44. Fumigation of H.rostochiensis with Nemagon (Experiment No.17). Treatment totals, differences and critical differences.

cyst/500 g.

Treatment	Totals	Differences
0.004 ml.	12.154	
		0.282 n.s.
Control	11.872	
		0.001 n.s.
0.001 ml.	11.871	
		0.882 *
0.016 ml.	10.989	
		0.909 *
0.064 ml.	10.080	
		1.878 *
0.128 ml.	8.202	
		0.239 n.s.
0.256 ml.	7.963	
		0.040 n.s.
0.512 ml.	7.923	

Critical difference = 0.777

Table 46. Analysis of variance of H.rostochiensis fumigated with E.D.B. at different rates. (Experiment No.17).

Log cysts/500 g. of soil.

	Items	D.F.	S.S.	M.S.		F.	
a	Doses	5	0.771030	0.1542	a/b	140	***
b	Error	6	0.007074	0.0011			
c	Totals	11	0.778104				

Table 47. Fumigation of H.rostochiensis with E.D.B. (Experiment No.17).
Treatment totals, differences and critical difference.
cyst/500g.

Treatment	Totals	Differences	
E. 0.0016 ml.	6.047		
		0.025	n.s.
E. 0.0032 ml.	6.022		
		0.071	n.s.
Control	5.951		
		0.601	*
E. 0.064 ml.	5.350		
		0.285	*
E. 0.128 ml.	5.065		
		0.314	*
E. 0.256 ml.	4.751		

Critical difference = 0.396

Table 48. Fumigation of H.rostochiensis with E.D.B. and Nemagon Experiment No.17).

Initial infestation.

cyst/1000g.	Larvae/cyst	Larvae/g. of soil
180	335	60.3

The final population of cysts. (Nemagon)

Treatment	Cyst/1000g.	% Kill
Control	1961	0
0.001 ml.	2074	0
0.004 ml.	2339	0
0.016 ml.	1749	11.90
0.064 ml.	768	66.98
0.128 ml.	265	95.23
0.256 ml.	205	98.60
0.512 ml.	206	98.54

(E.D.B.)

Treatment	Cyst/1000g.	% Kill
Control	1893	0
0.016 ml.	2113	0
0.032 ml.	2052	0
0.064 ml.	954	54.82
0.128 ml.	684	70.58
0.256	475	82.78

iv. Conclusions.

The results of the in vivo fumigation experiments were somewhat unreliable for the following reasons:

1. The cysts were very old and gave a low hatch.
2. The Nemagon used was obtained from an old stock.
3. Due to the attack of the virus x disease only one replicate for Nemagon treatments was used.

Points (1) and (2) apply also to the early in vitro experiments.

Where any conclusions could be drawn, the results indicated that first: the range of doses of both fumigants was too high to include the L.D.50. The experiment using Nemagon showed slight indication that 50% kill occured between 0.016 ml. and 0.064 ml. Nemagon (Appendix Table 41A and Fig.19).

Secondly it emerged that a large increase in eelworm population during the season depended on the presence of viable larvae and eggs which must be allowed sufficient time to develop, especially after the use of fumigants. The final eelworm population in the soil of potato plants in Block B which were lifted at too early a date was significantly lower than Block A in which the plants were allowed to grow for a longer period of time.

(d) Pot experiment in 1960-61. (Experiment No.18).

i. Introduction.

The procedure was basically the same as for the experiments in 1959 (Experiment No.17), but three fumigants and three host plants were used, eggplant being the third host.

In in vitro experiments it was shown that the efficacy of chlorobromopropene against H.rostochiensis was promising. Therefore I decided to investigate the effect of this fumigant against the parasite in pot experiments.

The eggplant is an economic crop in the U.S.A. and in the middle East, especially in Persia. Fassuliotis and Feldmesser (1954) have shown that potato root eelworm can attack and complete its life cycle on this plant. Although there is no evidence as yet in Iran that H.rostochiensis attacks eggplants or other crops, it is nevertheless important to investigate the degree of infection of potato root eelworm in this plant and compare it with potato and tomato. Consequently in 1960 a series of pot experiments was designed using 3 fumigants (C.B.P., E.D.B. and Nemagon) with 3 host plants (tomato, potato and eggplant). The rates of fumigation for Nemagon and E.D.B. were adjusted according to the information gained from the 1959 experiments. For C.B.P. a wider range was used since L.D.50 had not been ascertained. In the case of E.D.B. some indication was obtained that the L.D.50 occured between .064 ml. and .128 ml. For this reason a new range of rates was employed.

The Nemagon used in 1960 was from a new stock 95% technical, whereas in 1959 Nemagon used was 55% pure and from an old stock. The cysts used in 1960 were from a 1958 stock with about 90% hatchability compared with the 20 to 25% hatchability of those used in 1959.

Another change in procedure was the replacement of steam sterilisation of the soil by fumigation with methyl bromide. In steam sterilisation,

besides the liberation of ammonia, which is known to inhibit the hatching of larvae from the cysts (Johnson and Townsend 1949), other effects follow such as serious alterations in the microflora of the soil. For such a soil to revert to its original condition requires a long period.

The disadvantages associated with the use of methyl bromide are not so great. The concentration × time product for methyl bromide was 800 mg.h./l. (Hague 1958). After sterilisation, the soil was left for about 10 days to ensure the escape of the methyl bromide (Christie 1959).

In the experiments the moisture content and temperature of the soil was measured before and during fumigation as previously described.

Pots of soil already mixed with 1000 cysts were fumigated on February 1 and left for 8 weeks. On March 25 the soil from each pot was mixed with fertilizer and put into clay pots (10" diameter) and at the beginning of April, seven-week old tomato seedlings were transferred to the pots which were left in the greenhouse in a randomized design.

Similar experiments were set up using eggplant but since the rate of growth of the seedlings was so slow they were kept a few weeks longer before transplantation and liquid manure was applied to encourage their growth. They were planted in the 10" pots in early May.

Replication in each treatment was twofold for tomato and eggplant.

The tomato plants were lifted at the beginning of September and the eggplants at the end of September.

About 300 potato tubers (Arran Banner) were left to sprout on the surface of a layer of peat in boxes.

When the biggest chit in each potato was about 1 cm. long, 75 tubers of approximately the same size were selected. All but the biggest chit were removed and one potato tuber was planted about 2" deep in each pot on May 1. The pots were plunged in the soil in a randomized design, and all possible precautions were taken to prevent the plants from being attacked by fungi or other harmful organisms. Replication was threefold. The pots were watered when necessary.

ii. Rates of fumigation (1960).

On the basis of pot experiments in 1959 a new range of rates for Nemagon and E.D.B. was designed to cover a 50% kill in both fumigants. Based on the information gained in in vitro experiments, C.B.P. showed a promising result. Therefore a range of dosages was designed which did not exceed 0.128 ml. per pot.

Where potato was used as host plant, there were seven rates of fumigation for Nemagon and ethylene dibromide and eight rates for C.B.P. as follows:

	Nemagon	Ethylene dibromide	Chlorobromopropene
1.	0.016 ml.	0.032 ml.	0.004 ml.
2.	0.0224 ml.	0.045 ml.	0.008 ml.
3.	0.032 ml.	0.064 ml.	0.016 ml.
4.	0.045 ml.	0.089 ml.	0.032 ml.
5.	0.064 ml.	0.128 ml.	0.045 ml.
6.	0.089 ml.	0.178 ml.	0.064 ml.
7.	0.128 ml.	0.256 ml.	0.089 ml.
			0.128 ml.

Soil temperature at the time of fumigation : 8.5°C.

Air temperature : 9.4°C.

Mean air temperature during the fumigation : 8.0°C.

Range : 4.5 - 13.3°C.

Mean soil temperature at 2" below the surface : 7.0°C.

Range : 5.0 - 11.0°C.

Moisture content (% dry soil) : 17.1%

Soil mixture : 3 parts loam, 1 part sand.

When tomato and eggplants were hosts the following rates of fumigation were used:

	Nemagon	Ethylene dibromide	Chlorobromopropene
1.	0.032 ml.	0.032 ml.	0.032 ml.
2.	0.045 ml.	0.045 ml.	0.045 ml.
3.	0.064 ml.	0.064 ml.	0.064 ml.
4.	0.089 ml.	0.089 ml.	0.089 ml.
5.	0.128 ml.	0.128 ml.	0.128 ml.

Although precautions were taken to prevent the potato plants from being attacked by different sources of disease, unfortunately some of them were infected by virus x and y.

Insecticide was applied to control the few aphids present which may have been responsible for transmitting the virus y disease but little could be done about the presence of virus x, and it was thought that the seed potatoes might have been infected when planted.

Because of the infection the triplicate potato pots were divided

into two sets A and B. Set A contained the diseased replication and was lifted on August 30. Set B, however, contained the remaining two replications which were allowed to continue their growth until the end of the season. The efficacy of each fumigant was measured by the final eelworm population and the results of the treatments for each nematicide are shown in a series of tables for each host plant and include:

1. An analysis of variance of the data for all treatments.
2. Treatment totals and their critical differences.
3. The number of cysts for 400 g. of soil, larvae per g. of soil and percent. kills.

All these data relate to Set B: Set A was used only to compare cyst populations in Sets A and B.

iii. y sample as criterion.

In pot experiments in 1960 in addition to final density assay it was desirable to investigate the immediate effect of Nemagon on treated cysts at the time when the fumigated soil in whalehide pots was transferred to the clay pots, i.e.: 8 weeks after injection and before planting the host plant. For this reason four extra pots with the same conditions as the others were fumigated with 0.032, 0.045, 0.064, 0.089 ml. of Nemagon.

On March 25 the soil of these pots was removed, samples were taken and washed, and fumigated cysts were extracted by the method described in Part II. Batches of 50 cysts from each treatment were soaked in water for a week followed by the exposure to root diffusate. The period of hatching

was 64 days and the total number of hatched larvae were collected at 8-day intervals. At the end of 64 days the cysts were crushed and the number of unhatched eggs and larvae was estimated by Bijloo's homogenizer. The results are set out in Table 49. These correspond to the so-called Y samples of Peters and Fenwick (1949).

Table 49. The number of hatched larvae from batches of 50 cysts fumigated with Nemagon by soil injection and recovered from treated soil after 8 weeks.

Y samples.

Treatment	Total larval emergence 64 days	Non-hatched eggs and larvae	Total number of eggs and larvae per 50 cysts	% Kill Y sample (R.D. assay)
0.032 ml.	12800	4260	17060	24.97
"	10560	3885	14445	26.89
0.045 ml.	11300	4504	15804	28.50
"	9800	4523	14323	31.58
0.064 ml.	12000	6240	18240	34.21
"	7450	5880	13330	44.11
0.089 ml.	9200	8390	17590	47.70
"	8550	5719	14269	40.01

Comparison of 2 different methods (Y and Z Samples).

Treatment	% Kill	
	Y samples	Z samples
0.032 ml.	25.85	52.90
0.045 ml.	29.96	68.64
0.064 ml.	38.39	82.08
0.089 ml.	44.29	92.26

iv. Results.

1. Potato as host.

Appendix Tables 50A, 51A and 52A show the number of new cysts per 400 g. of soil, number of larvae per cyst, number of larvae per g. of soil and the percentage mortalities calculated in terms of new cysts per g. of soil and also in terms of larvae per g. of soil.

Height of haulms and weight of tubers shown by Peters (1948) to be fairly sensitive as criteria, were also recorded but no significant differences were found between different treatments. This suggests that the infestation level even in the controls was unable to cause measurable damage, and also that there were no phytotoxic effects.

Tables 53, 54, 59 and 62 show treatments to be significant for all data (cysts/400 g. and larvae/g. of soil), but only interaction of hosts and doses (Tables 59 and 62) was not significantly different. The reason for this will be discussed later on.

Fumigation with C.B.P.

The results of application of C.B.P. varied very much in the presence of different host plants (see Appendix Table 50A).

In potato plants the efficacy in all rates was very poor and variable. Analysis of variance of the data showed no significant differences at different rates. The results suggested that although C.B.P. was an excellent contact nematicide and has shown a promising effect in vitro against H.rostochiensis, which is in agreement with many other workers (van den Brande et al. 1954 and 1955; Hague private communication), in pot experiments it showed a very poor result. One can compare these

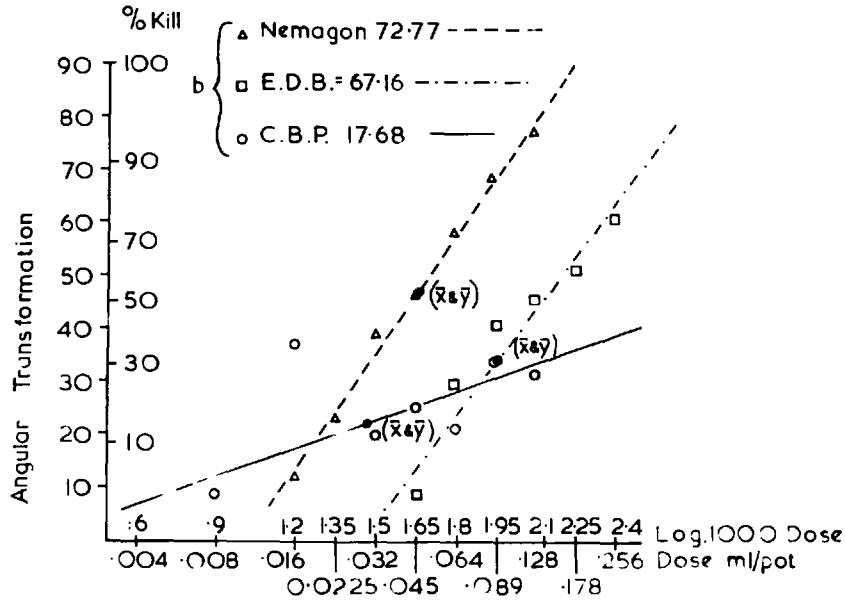


Fig. **20** The response of H. Rostochiensis fumigated with C.B.P. E.D.B. & Nemagon
Host potato (pot experiment 1960)
Final Density Assay (Cysts/gm. of soil)

results with those of in vitro experiments. It can be seen that in many cases the results obtained under laboratory conditions cannot be found in a field scale.

The percentage mortalities (cyst and larvae/g. of soil) were transformed to angles and plotted against Log dose. The regression lines were calculated and are shown in Figs.20 and 21;.

Fumigation with E.D.B.

The criteria: new cysts, and number of larvae per gramme of soil, show clear treatment differences. At the dose 0.032 ml. the number of newly formed cysts was higher than in the control. This might possibly be a stimulatory effect of a low dose producing a higher final population. A similar result was obtained in experiment No.17 (1959). Analysis of variance in both cases revealed that although the number of newly formed cysts at the dose 0.032 ml. was slightly higher than in the controls the differences were not significant. However 9.29 per cent. mortality was obtained when the number of larvae per g. was used as criterion (see Appendix Table 51A).

Linear relationships were shown (Figs.20 and 21) between the angular transformation of the percentage mortalities and the log dose. The L.D.50 according to the regression lines obtained for both criteria (cyst/g. of soil or larvae/g. of soil) was 0.135 ml. per pot which is in close agreement with Peters (1952).

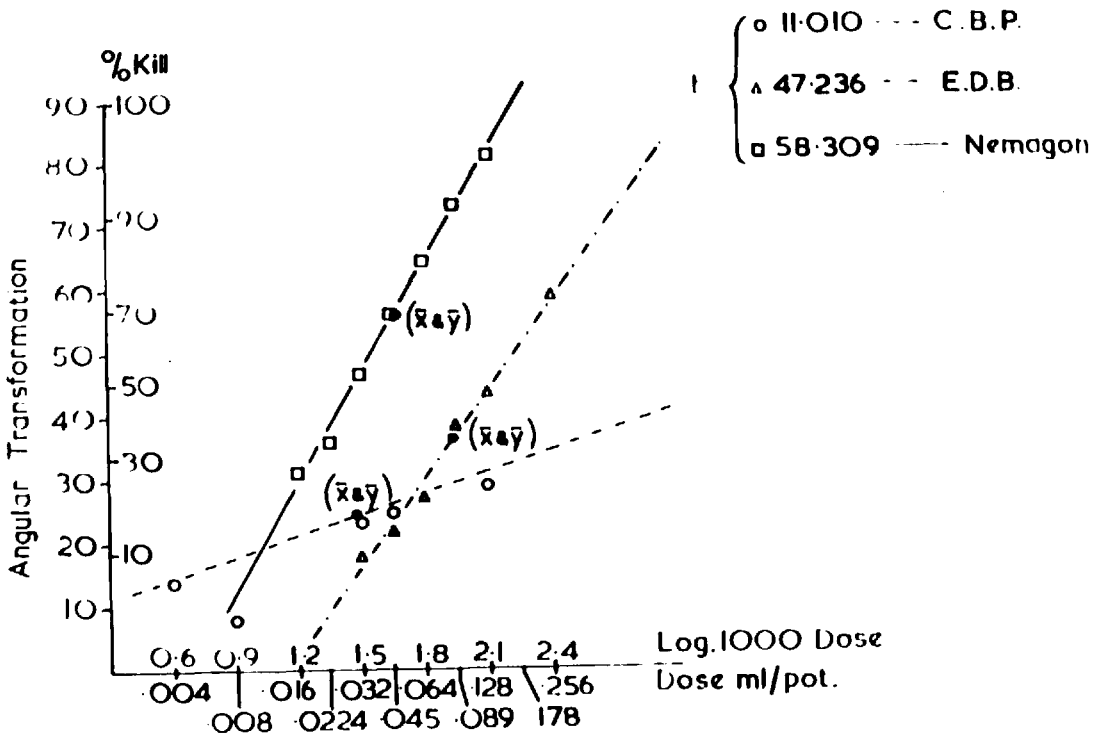


Fig.21 The response of H.Rostochiensis fumigated with C.B.P. E.D.B. & Nemagon. Host potato. Pot experiment 1960 Final Density Assay larvae/gm. of soil.

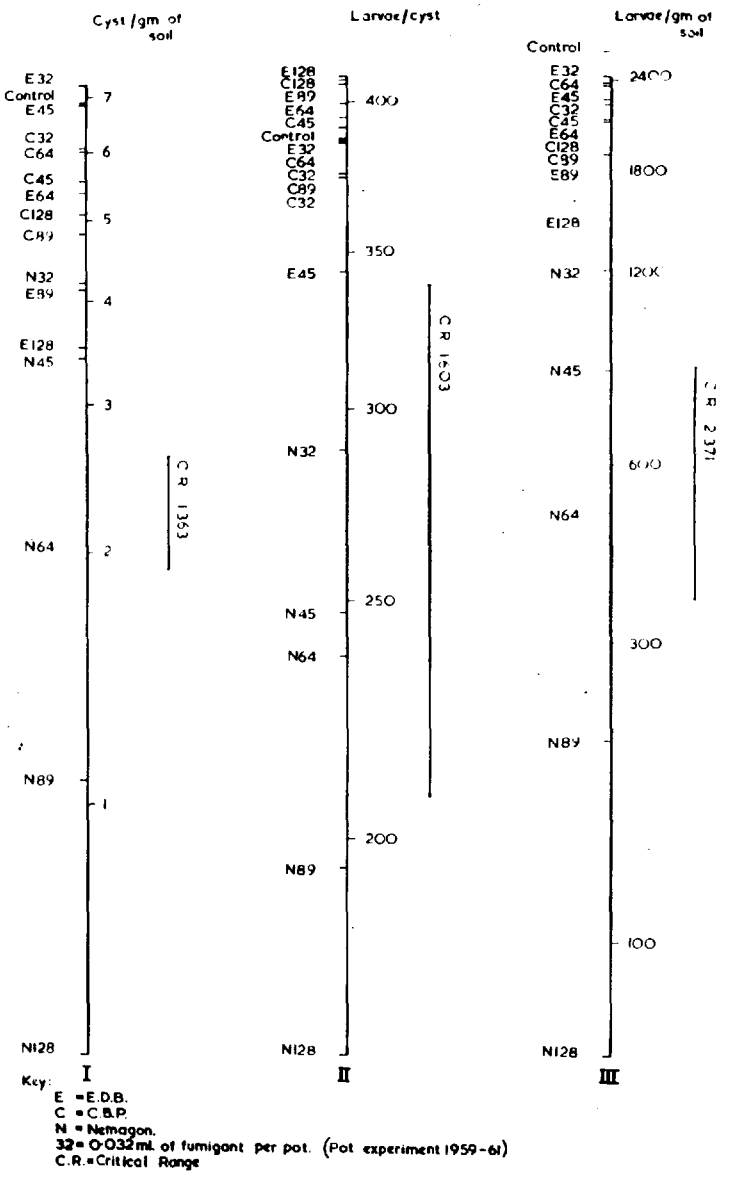


Fig. 22 Charts for finding significant treatment differences. Pot Experiment 1959 – 61.

Significance Charts.

In order to convey at first glance a clear impression of the differences between treatments and their significance, three charts have been prepared (Fig.22). The geometric mean values of the cysts per g. of soil, larvae per cyst, and larvae per g. of soil in each treatment have been plotted on logarithmic scales. The shorter line "critical range" represents the critical difference between means and is labelled with its antilogarithm, which is thus a "critical ratio"; any two points on the diagram separated by more than this line are significantly different at the 5% level of probability. The chart can be used by transferring the length of the critical range on to the edge of a piece of paper which is then slid along the line in each case. In chart no.I each point shown is the mean value of the two treatments treated alike. In chart no.II each point shown is the number of eggs per cyst in each treatment. In chart no.III each point is the product of the numbers of eggs per cyst and the mean value of the number of cysts per g. of soil in each treatment. Standard errors for charts II and III were based on interactions.

Fumigation with Nemagon.

Percentage kill measured by the number of cysts per 400 g. of soil and the number of larvae per g. of soil is calculated and shown in Appendix Table 52A.

The number of larvae per cyst which was obtained in all treatments, and did not significantly differ in cysts fumigated with C.B.P. and E.D.B. was significantly different in cysts fumigated with Nemagon. The number of eggs and larvae per cyst gradually decreased as the rate of fumigant

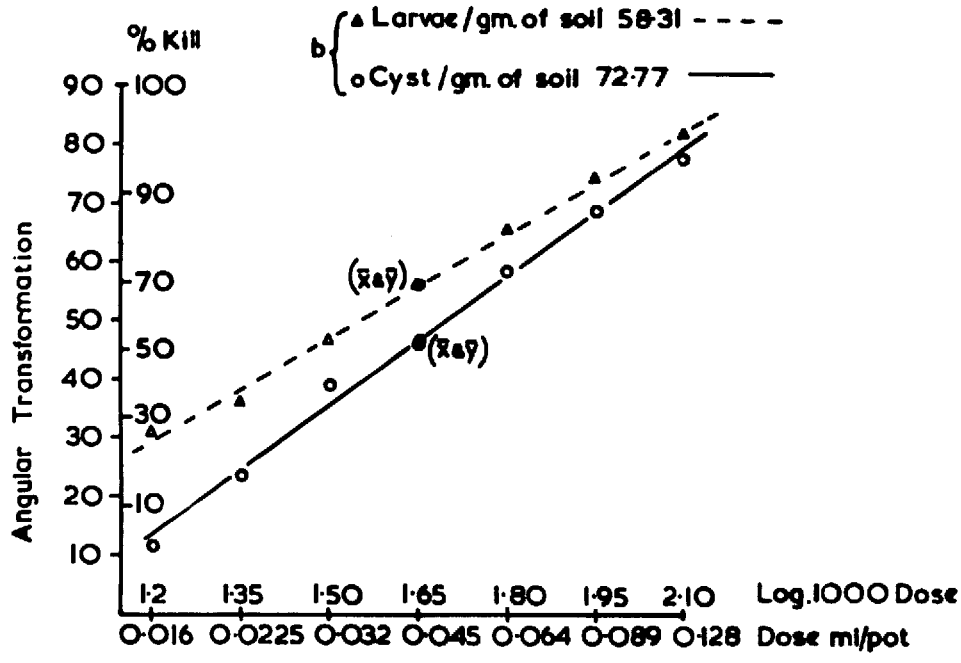


Fig. 23 The response of H. Rostochiensis fumigated with Nemagon
Host potato Final Density Assay

increased (see Chart II/Fig.22). Consequently a higher kill was expected by using the number of larvae per g. of soil. An angular transformation of percentage kill (cyst/g. of soil and larvae/g. of soil) was obtained and plotted against log dose of rates. The L.D.50 in each criterion was calculated (Fig.23). It should be emphasized that all cysts were graded for size and the above reduction in larvae per cyst is therefore not due to the cysts being smaller (though this latter effect is also found, as described later).

Table 53. Fumigation of H.rostochiensis 1960. Pot experiment No.18.

Analysis of variance of final infestation data.

Log : (Cyst/400g. of soil).

Fumigants : C.B.P., E.D.B. and Nemagon.

Host : Potato

Doses : 0.032, 0.045, 0.064, 0.089 and 0.128 ml./pot.

Items	D.F.	S.S.	M.S.	F.
a Nematicides	2	1.600880	0.800440	a/c12.63** a/e942.80***
b Doses	4	0.807110	0.201778	b/c 3.18n.s. b/c237.66***
c Interaction	8	0.507080	0.063385	c/e 74.66***
d Treatments	14	2.915070	0.208219	
e Error	15	0.012739	0.000849	
f Total	29	2.927809		

Treatment totals, differences and critical differences.

Nematicides	Totals	Differences	Doses	Totals	Differences
C.B.P.	33.447	0.251 n.s.	0.032 ml.	20.155	0.300 *
E.D.B.	33.196	4.570 *	0.045 ml.	19.855	0.597 *
Nemagon	28.426		0.064 ml.	19.262	0.987 *
			0.089 ml.	18.275	0.753 *
			0.128 ml.	17.522	

Critical difference = 0.278 Critical difference = 0.215

Table 54. Fumigation of H.rostochiensis (1960). Pot Experiment No.18.

Host : Potato.

Mean log (cyst/400g.). Interaction.

		C.B.P.	E.D.B.	Nemagon	∑ Doses
Doses µl	32	3.387	3.466	3.225	3.359
	45	3.356	3.433	3.138	3.309
	64	3.384	3.333	2.913	3.210
	89	3.285	3.216	2.636	3.046
	128	3.310	3.149	2.301	2.920
∑ Nematicides		3.345	3.320	2.843	

Standard error of mean : 0.0206 | 0.0119 / 0.0092 | 0.0053

2. Tomato as host.

Fumigation with C.B.P.

The results are shown in Appendix Table 55A where the mean number of cysts, the number of larvae per cyst, larvae per g. of soil, and the percentage mortalities have been calculated. The number of new cysts per 400g. and the number of larvae per g. of soil exceeded those of the controls. The reason for these ambiguous results (probably due to a high initial infestation) has been described below in the discussion.

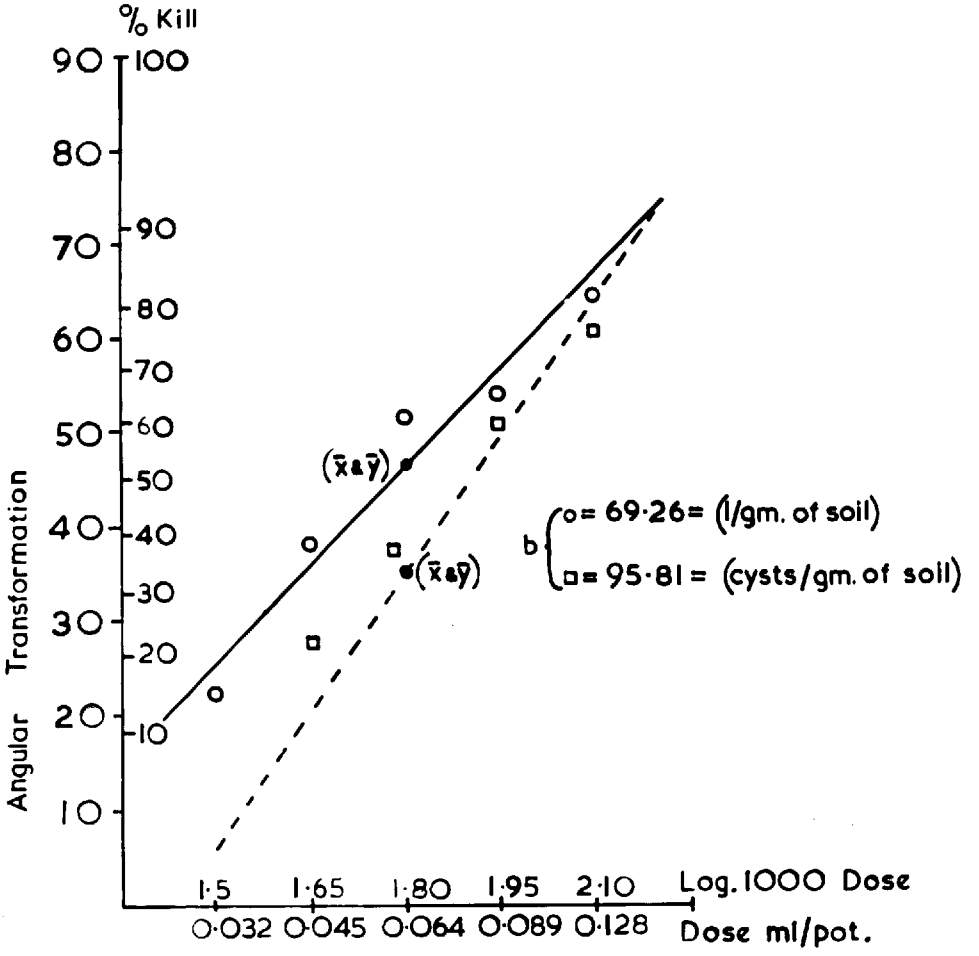


Fig. 24. The response of H. Rostochiensis fumigated with Nemagon. Host tomato.

Final Density Assay.

Fumigation with E.D.B.

Similar ambiguous results were obtained as described for C.B.P., resulting in a greater eelworm population than in the control at 0.032 and 0.045 ml. per pot. The higher dosage rates had a nematicidal effect on the eelworms. The data, being now limited to only three different rates, could not yield any further information.

Fumigation with Nemagon.

The dosage rate of 0.032 ml. per pot also produced an increase in the number of cysts compared with untreated controls. But taking the number of eggs per cyst into account, a 14.54% kill was obtained in terms of L/g. Similar reduction of eggs and larvae per cyst was obtained at increased rates of application, which resulted in a higher kill in terms of larvae than of cysts/g. As a sufficient number of dosage levels and significantly different percentage mortalities (cyst/g. of soil and L/g. of soil) was available, the latter were plotted against the former and two regression lines (Fig.24) obtained, a goodness-of-fit test was applied to them: the deviations from linearity were estimated. There was no significant departure from linearity. The analysis of variance arising from this test are given in Table 56.

Table 56. Analysis of variance of final infestation data.

Fumigation of H.rostochiensis 1960 with Nemagon.

Host : Tomato. (Goodness-of-fit test for data of Fig.24 and Appendix Table 55A).

1. Larvae/g. of soil.

	Items	D.F.	S.S.	M.S.	F.
a	Variance attributable to regression	1	1079.31321	1079.31321	a/b 69.57 **
b	Residual	3	46.54129	15.51376	
c	Total	4	1125.85450		

2. Cyst/g. of soil.

	Items	D.F.	S.S.	M.S.	F.
a	Variance attributable to regression	1	2065.5366	2065.5366	a/b 59.95 **
b	Residual	3	103.3492	34.449	
c	Total	4	2168.8858		

The final density assay (new cysts per 200g.) gave a value for the median lethal dose between 0.064 ml. and 0.089 ml. of the fumigant per pot, whereas this value in terms of larvae per g. of soil is about 0.060 per pot. However, none of these results can really be considered very dependable as in all cases, the final eelworm population was influenced by the initial level of infestation, and this was higher than the optimum, thus yielding ambiguous results.

3. Eggplant as host.

The results for eggplant in almost all treatments are not so reliable and suggest that even at the lowest rates applied, considerable reduction was obtained. In Appendix Table 57A the mean number of cysts per 400 g. of soil, number of eggs per cyst and larvae per g. of soil have been shown.

Considering the number of cysts per 400g. of soil the final density assay shows an increase in the population of about twice the initial infestation, but using the number of larvae per g. of soil as a means of estimating the final population, and comparing it with the initial infestation, a general reduction in almost all treatments resulted: this is another reason why the number of larvae per g. of soil would be a more reliable criterion. In all treated pots the population levels were considerably below the initial infestation level. The rates of fumigation varied a little among themselves, but no definite trends emerged.

The results of nematicides were confused but a general reduction of the infestation was evident at any dose level (see Appendix Table 57A).

Table 58. Analysis of variance of final infestation data.

Fumigation of H.rostochiensis (Pot Experiment No.18).

Fumigants: C.B.P. E.D.B. Nemagon.

Host plant : Potato Tomato Eggplant.

Log (larvae/cyst).

	Items	D.F.	S.S.	M.S.	F.
a	Hosts	2	3.040590	1.520295	a/d 294.289 ***
b	Nematicides	2	0.454870	0.227435	b/d 44.025 ***
c	Doses	4	0.046840	0.011710	
d	Residual (1)	36	0.185958	0.005166	
e	Totals	44	3.728258		

(1) All interactions have been added up to make residual, because none of them were significantly different from H.N.D.

Table 59. Analysis of variance of final infestation data.

Fumigation of H.rostochiensis 1960 with C.B.P., E.D.B. and Nemagon using three host plants: Potato, Tomato and Eggplant.

Log (cysts/400g. of soil).

	Items	D.F.	S.S.	M.S.		F.	
a	Hosts	2	16.3294	8.1647	a/g	610.29	***
b	Nematicides	2	1.4171	0.7085	b/g	44.28	***
c	Doses	4	0.9762	0.2440	c/g	15.25	***
d	H.N.	4	0.6926	0.1731	d/g	10.81	***
e	N.D.	8	0.6903	0.0863	e/g	5.39	**
f	H.D.	8	0.1991	0.0249	f/g	1.55	n.s.
g	H.N.D.	16	0.2566	0.0160			
h	Treatments	44	20.5613				
i	Error	45	0.2034	0.0045			
	Total	89	20.7647				

Table 60. Fumigation of H.rostochiensis (1960) Pot Experiment No.18.

Hosts : potato, tomato and eggplant.

Mean log (c/400g.) : Interaction.

Host	C.B.P.	E.D.B.	Nemagon	∑ Hosts
Potato	3.3447	3.3196	2.8426	3.1689
				0.3196
Tomato	2.9894	2.8719	2.6866	2.8493
				0.7003
Eggplant	2.1401	2.1990	2.1079	2.1490
∑ Nematicides	2.8247	2.7968	2.5457	2.7224
		0.0279		

Standard error of mean :

0.0212	0.0122
0.0122	0.007

		C.B.P.	E.D.B.	Nemagon	∑ Doses
Dose µl	32	2.7938	2.9183	2.8145	2.8422
	45	2.8353	2.8988	2.7000	2.8114
	64	2.8655	2.7775	2.5888	2.7439
	89	2.8327	2.7297	2.4093	2.6572
	128	2.7963	2.6598	2.2158	2.5573
∑ Nematicides		2.8247	2.7968	2.5457	2.7224

Standard error of mean :

0.0274	0.0158
0.0122	0.007

Table 61. Fumigation of H.rostochiensis 1960. Pot Experiment No.18.

Fumigants : C.B.P., E.D.B. and Nemagon.

Hosts : Potato, Tomato and Eggplant.

Log (cyst/400g.)

Treatment totals, differences and critical differences.

Treatments (doses applied)	Totals	Differences
0.032 ml.	51.160	
		0.555 n.s.
0.045 ml.	50.605	
		1.214 *
0.064 ml.	49.391	
		1.561 *
0.089 ml.	47.380	
		1.798 *
0.128 ml.	46.032	

Critical difference = 0.804

Nematicides	Totals	Differences
C.B.P.	84.742	
		0.837 n.s.
E.D.B.	83.905	
		7.534 *
Nemagon	76.371	

Critical difference = 1.04

Hosts	Totals	Differences
Potato	95.069	
		9.590 *
Tomato	85.479	
		21.009 *
Eggplant	64.470	

Critical difference = 1.04

Table 62. Analysis of variance of final infestation data.

Fumigation of H.rostochiensis 1960, with C.B.P., E.D.B. and Nemagon using three host plants: Potato, Tomato and Eggplant.

Log (larvae/g. of soil).

	Items	D.F.	S.S.	M.S.	F.	
a	Hosts	2	20.366250	10.183125	a/g 649.558	***
b	Nematicides	2	2.321120	1.160560	b/g 73.611	***
c	Doses	4	0.804300	0.201075	c/g 12.826	***
d	H.N.	4	0.427730	0.106932	d/g 6.821	**
e	N.D.	8	0.695070	0.086883	e/g 5.542	**
f	H.D.	8	0.105390	0.013174	f/g <1	n.s.
g	H.N.D.	16	0.250832	0.015677		
h	Totals	44	24.970692			

Table 63. Percentage mortality of H.rostochiensis using 3 fumigants: C.B.P., E.D.B. and Nemagon. (Experiment No.18). Host: Potato.

Doses ml. per pot	Cyst/g. of soil			Larvae/g. of soil		
	C.B.P.	E.D.B.	Nemagon	C.B.P.	E.D.B.	Nemagon
0.032	12.51	-	40.26	15.48	9.29	52.90
0.045	18.30	2.07	51.62	17.25	13.55	68.64
0.064	12.88	22.77	72.14	13.70	20.29	82.08
0.089	30.88	41.66	86.38	32.43	38.17	92.26
0.128	26.90	50.22	94.98	22.77	46.72	97.63

N.B. For other doses see Appendix Tables 50A, 51A, 52A.

Table 64. Percentage mortality of H.rostochiensis using 3 fumigants: C.B.P., E.D.B. and Nemagon. (Experiment No.18). Host: Tomato.

Doses ml. per pot	Cyst/g. of soil			Larvae/g. of soil		
	C.B.P.	E.D.B.	Nemagon	C.B.P.	E.D.B.	Nemagon
0.032	2.05	0	14.54	1.77	0	22.38
0.045	0	0	37.59	0	0	37.82
0.064	0	17.78	60.99	0	28.36	51.35
0.089	0	17.92	65.60	0	29.07	54.09
0.128	0	38.98	83.68	0	33.68	66.19

Table 65. Percentage mortality of H.rostochiensis using 3 fumigants: C.B.P., E.D.B and Nemagon. (Experiment No.18). Host: Eggplant.

Doses ml. per pot	Cyst/g. of soil			Larvae/g. of soil		
	C.B.P.	E.D.B.	Nemagon	C.B.P.	E.D.B.	Nemagon
0.032	79.13	57.28	62.25	76.78	56.25	66.07
0.045	73.84	58.94	73.50	75.89	58.92	83.03
0.064	70.52	70.19	76.49	71.42	65.17	86.60
0.089	58.27	74.50	83.77	56.25	69.64	90.17
0.128	88.08	76.15	89.73	82.14	78.57	91.07

4. Y Samples.

The results obtained from a root diffusate assay of batches of 50 cysts fumigated at different doses with Nemagon are set out in Table 49 and are compared with the results of the final density assay (larvae/g. of soil) (Z sample). The percentage kill values of the Y and Z samples were transformed to angles and analysed.

The analysis of variance showed a clear difference between the methods and confirmed previous results on the delayed effect of this nematicide.

Analysis of Variance of Y and Z samples.

(Angular transformation).

Items	D.F.	S.S.	M.S.	F.
Samples	1	1195.85	1195.85	19.57 **
Error	8	488.91	61.11	
Total	9	1684.76		

Totals, differences and critical differences.

Samples	Totals	Differences
Z sample	241.44	97.81 *
Y sample	143.63	

Critical difference = 71.49

PART IV. MISCELLANEOUS EXPERIMENTS.

(a) Studies on nematicidal effects of Tagetes spp. on the final population of Heterodera rostochiensis Wollenweber.

A preliminary study on the hatching stimulus of Tagetes root diffusates on H.rostochiensis larvae (Omidvar 1961, appended), revealed that the leachings extracted from three species of Tagetes did not hatch larvae from cysts. Oostenbrink et al. (1957) have shown that the cultivation of Tagetes appears to suppress the population of some nematodes.

The reduction of population of Heterodera rostochiensis in the presence of Tagetes has also been claimed by some workers (Doubleday Association Report 1960; Uhlenbroek and Bijloo 1958). Therefore if the reduction of the population of H.rostochiensis in the presence of Tagetes is not due to a hatching stimulus, present in the roots, to force them to hatch and die from starvation, it could be due to a nematicidal effect of some kind.

One possible way to explore this effect was to expose the parasite in soil planted with Tagetes and some other plant (non-host) and then to examine the effect of the plants on the viability of the nematode. The other possibility was to grow a host plant in the soil infested with H.rostochiensis in the presence and absence of Tagetes plants and at the end of the growing season to compare the final eelworm populations. Accordingly two sets of experiments were carried out.



1 2 3



1 2 3

Fig.25. 1. Tagetes signata.

2. Tagetes minuta.

3. Cineraria maritima.

i. Observations of nematicidal effect of two Tagetes species on H.rostochiensis cysts. Experiment No.19.

Medium clay pots (19 cm. diameter) were each filled to 2 cm. from the rim with a mixture of 1 part loam and two parts sand, artificially infested with 1000 H.rostochiensis cysts (size $> 599\mu$).

Similar clay pots were also filled with a soil naturally and heavily infested with H.rostochiensis of the Solanum nigrum type.

Seeds of Tagetes minuta and Tagetes signata were sown, and when the seedlings were five weeks old they were transferred, one each, to the above mentioned pots (12 July 1960), replication being threefold. Three pots of each type were also planted with Cineraria maritima seedlings about 6 weeks old acting as a control. The latter plant is not a host and it is not known to have any effect on H.rostochiensis. Threefold replication plus six Cineraria as controls gave 18 pots in all.

The pots were left in the greenhouse and the plants allowed to continue their growth (Fig.25). Watering was carried out equally and when necessary. On November 22, at the end of the growing season (Fig.26), the haulms were lifted and the soil of each pot was washed by the Fenwick can apparatus. The cysts recovered from each pot were collected.

Two batches of 100 cysts extracted from each treatment were soaked 8 days in water followed by potato root diffusate. The hatching test was carried out for 64 days. At the end of each week the hatched larvae from each treatment were collected in 3" × 1" glass tubes and observations were made on any delayed action or other abnormality in the hatching



1 2 3

At later stage of growth.



1 2 3

Before lifting.

Fig.26.

1. T.signata.
2. T.minuta.
3. Cineraria maritima.

process.

Results.

The results are presented in Appendix Tables 66A and 67A. The data obtained from this experiment suggests that the cultivation of Tagetes signata and Tagetes minuta had a slight nematicidal effect on H.rostochiensis but there was no significant difference between the two species. However when these Tagetes were planted in naturally infested soil, Tagetes signata more strongly depressed the population of Heterodera larvae, and this effect was significantly different from that of Tagetes minuta (see Appendix Table 67A).

Table 68. Geometric means of larvae per cyst hatching in potato root diffusate. (Data obtained from Appendix Tables 66A and 67A).

	Artificially infested soil.	Naturally infested soil
Cineraria maritima	199	134
T.minuta	167	118
T.signata	163	85
Critical ratio	1.183	1.1103

ii. Observations on the effect of Tagetes minuta on the final density assay of H.rostochiensis in the soil planted with a host plant. (Experiment No.20).

Ten $4\frac{1}{2}$ inch clay pots were filled to 2cm. from the rim with a loam-sand mixture (3 : 1) which had previously been sterilised.

Each pot was planted with a 20 day old tomato seedling and left for 3 days to allow the plants to become established after which five of them were selected by randomization and in these, 20 seeds of Tagetes minuta were sown around each seedling. When the Tagetes seedlings had begun to grow both sets of pots were inoculated with a known number of H.rostochiensis larvae at weekly intervals as follows:

Batches of 100 cysts were soaked in water and exposed to the action of potato root diffusate. The emerging larvae at the end of each week were collected and estimated. Eelworm suspensions of known concentration were prepared in 100 c.c. measuring cylinders, and with a pipette two c.c. aliquots were injected at five equidistant points into the soil around the growing plants, using the tip of the pipette to make the hole.

When a total number of 32000 larvae had been introduced in this assay to each pot the plants were left and growth was allowed to proceed.

Six weeks after the last inoculation the haulms of tomatoes and Tagetes were lifted and the soil in each pot was washed in the Fenwick can. The total numbers of recovered cysts in each pot was counted.

Results.

The results of this experiment are presented in Appendix Table 63A.

An analysis of variance revealed that there was no significant difference between the number of newly formed cysts in the pots planted with tomato alone and those with Tagetes plants. This suggests that the growth of Tagetes minuta in soil infested with H.rostochiensis has no immediate effect on H.rostochiensis larvae. In comparison with the previous experiment, many of the eelworm larvae in this experiment were exposed to possible nematicidal action for only a very short time, before entering the host.

During the growth of the plants a slight reduction in the height of Tomato plants growing with Tagetes could be detected, which may have been due to competition for the nutrients in the soil. The small reduction of the cyst population in the pots with both plants, if real, would probably be a result of the somewhat poorer growth of the hosts due to competition.

(b) Determination of the hatching power of different concentrations of root diffusate extracted from Potato, Tomato and Eggplant.

i. Introduction.

Early German work with H.schachtii had shown that larvae were stimulated to hatch in the presence of a substance excreted from the roots of the host plant (Baunacks 1922). This striking feature of the



Fig.27. Collection of root diffusate.

bionomics of the genus Heterodera has been studied for many years. The activating agent has been referred to as "root diffusate". Similarly, Triffit (1930) showed that H.rostochiensis larvae were stimulated to hatch by diffusates extracted from the roots of potato and tomato plants which has been confirmed by later workers. She found that the substance was heat resistant, remaining in full activity even after being reduced to half its volume by boiling (Peters 1950; Franklin 1951). The rate of production of root diffusate varies with different species of host plants. Fassuliotis and Feldmesser (1954) have shown that leachings from eggplant roots showed a similar stimulation to potato. In the present section leachings were examined at different concentrations to determine the stimulatory effect of root diffusates extracted from different host plants. The methods of collecting root diffusate are briefly described and the results are discussed. The form of regression lines when the hatch from a series of dilutions of diffusates was plotted against dilution has been investigated.

ii. Root diffusate collection.

Tomato and eggplant seedlings were transferred into 2" clay pots, with an un-sterilised soil mixture of three parts loam to one part sand. Small tubers of potato were also planted in 2" clay pots. Five pots of each plant were placed on the concave grooves of a sloping asbestos sheet and root leachings were collected by the method described by Fenwick (1949), which consists essentially of passing excess water through pots containing the host plant, (Fig.27). The plants were leached twice a week for a fortnight. Collected root diffusates were filtered and stored in a

refrigerator at about 5°C.

iii. The effect of heat on potato root diffusate (Experiment No.21).

Storage of root diffusate occupies valuable space in refrigerators. According to Triffit's report (1930), The volume of the collected root diffusate could be reduced to half by boiling but Hague (1958) stated that he was unable to confirm this result. Consequently it was desirable to test the claims of the above workers. A preliminary experiment was carried out as follows:

Forty ml. of potato and tomato root diffusates were heated until only 20 ml. remained. Batches of 100 cysts were soaked in tap water for a week followed by their exposure to the reduced root diffusate. No significant difference in the number of hatched larvae was detected from the controls in tap water. The experiment was repeated by heating to get lower concentrations of root diffusates. The use of a bath of boiling water instead of direct heat was also tried but in all cases the heated root diffusates remained inactive.

iv. Changes in the dilution curve resulting from the use of three samples of root diffusates extracted from different host plants (Experiment No.22).

The activity of the hatching process is dependent on the variety of plant and the degree of purity of the sample of root diffusate. Fenwick (1951a, 1952) has shown that if a sample of root diffusate is successively diluted in a geometric series and batches of cysts are exposed

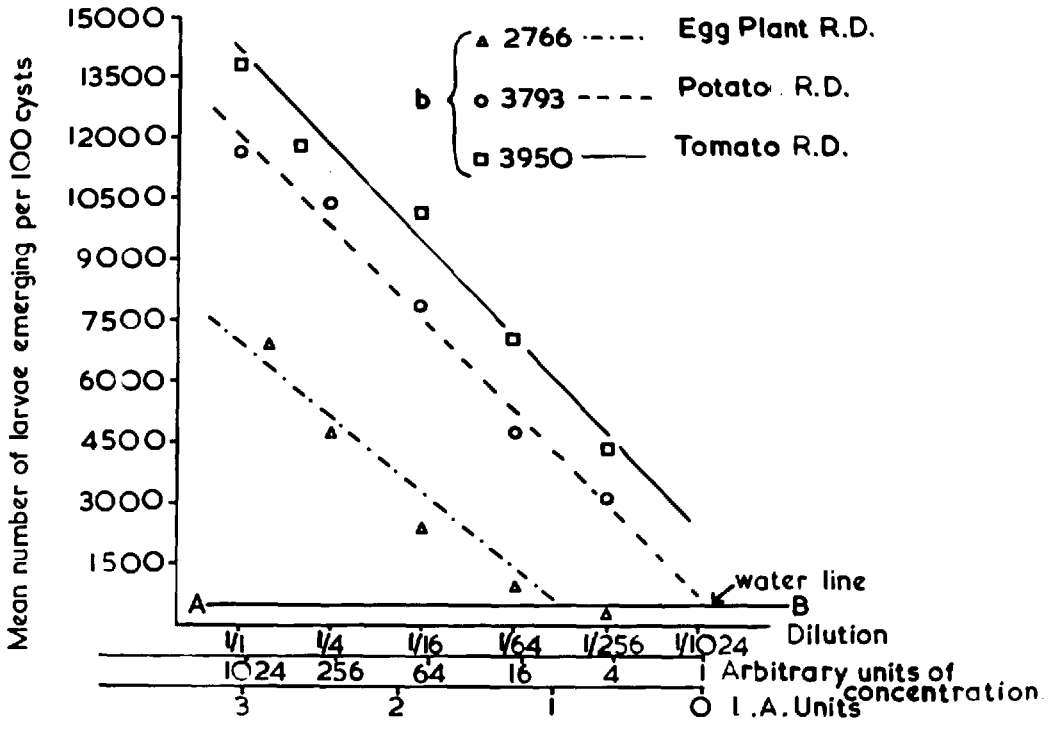


Fig.28 Effect of graded solutions of different diffusates on larval emergence and derivations of L.A. values

to the action of each dilution, a straight line will result from plotting the number of emerged larvae against log dilution of the root diffusate.

Experiments to investigate differences in the dilution curves of diffusates from tomato, potato and eggplant were carried out. Collected root diffusates were progressively diluted in the proportions of 1/1, 1/4, 1/16, 1/64, 1/256. Batches of 100 presoaked cysts were exposed to the action of each dilution and emerging larvae were collected in glass tubes at 8-day intervals. The larval emergence in tap water was also reported and was used as a control. Replication was threefold at each dilution. At the end of 64 days the total larval emergences were estimated The results are presented in Appendix Table 70A. I have already shown (Omidvar, 1961) that the number of emerged larvae expressed per g. of dry root from which the root diffusate has been collected is more clearly in agreement with the hypothesis put forward by Fenwick (1952), that root diffusates from different sources give parallel slopes. Therefore in this experiment the numbers of larvae emerging in root diffusates have been expressed as the number per g. of dry root (see Appendix Table 71A).

v. Results and discussion.

Computed regression lines showing the effect of dilution of each sample on larval emergence per g. of dried root are given in Fig.28. The hatch in tap water was also estimated and is represented on the graph by the horizontal line AB. An analysis of variance for each graph also has been calculated and is represented in Appendix Table 72A. The standard

error of each value of b (the regression coefficient) has been calculated. The results are set out in the table below.

Table 73. Comparison of regression coefficient of Fig.28.

	b	S.E. b	L.A. Value
Tomato	3950	274.64	3.49
Potato	3793	270.15	3.08
Eggplant	2766	306.20	2.10

In counts per g. of dried root the slopes of the three lines do not differ significantly, the only differences in the curves being in position. This is in agreement with Fenwick (1952). It may be suggested that in comparing the root diffusate extracted from different hosts the best curves will be obtained only when the number of liberated larvae in dilutions are calculated in terms of number per g. of dried roots in each plant, otherwise there is the possibility of a difference in slope.

From the data obtained the L.A. value (Log Activity) i.e. hatching power of each root diffusate, was calculated (Fenwick 1952) and is presented in Table 73.

The following conclusions from the results could be drawn and summarized as below.

1. The activities of root diffusate extracted from potato and tomato are similar and more powerful than that of eggplant.

2. The hatching power of different dilutions of various root diffusates on cysts are proportional to the logarithm of their concentrations and related to the weight of their dried root.

(c) Observation on the population build up of H.rostochiensis with different hosts. (Experiment No.23).

(The present chapter, which is the result of a study of the development of H.rostochiensis "Solanum nigrum type" in 8 different plants, was designed in association with Dr.N.G.M.Hague).

i. Introduction.

Populations of H.rostochiensis have been studied by many workers. Fassuliotis and Feldmesser (1954) found that eggplant could be attacked by the parasite, but it has been shown in this thesis, that although the eggplant was a host for the ordinary type of H.rostochiensis, the number of newly formed cysts in soil planted with eggplant was far less than those formed in soil planted with either potato or tomato.

It is also known that the ordinary type of H.rostochiensis which attacks the potato and tomato crop is not able to develop fully on Black nightshade, Solanum nigrum (Doncaster, 1953).

Nevertheless, a local gardener (Mr.J.Osbaldstone) has observed cysts on the roots of this weed and these have been found to be a specialized race of H.rostochiensis. In 1959 this race caused failure in a potato crop.

Having this source of a new race of cysts a further set of experiments was designed with the following purposes:

1. To determine the population build-up of H.rostochiensis in the presence of different hosts.

2. To investigate the degree of infestation of the eggplant with this new type of cyst and also the susceptibility of different varieties of Solanum nigrum.

3. The difference in the cyst content of similar sized cysts produced on the roots of different host plants.

ii. Experimental procedure.

Samples of the infested soil were washed and the number of eggs per g. of soil estimated in the Fenwick can, and the homogenizer technique (see Part II). One part of infested soil and 14 parts of a sterilised sand -loam mixture (1 : 3) were thoroughly mixed in order to obtain an initial density of 50 eggs per gramme of soil. The mixed soil was put into size 32 clay pots (holding about 3 Kg. of air-dried soil) and seedlings of the plants listed below were transferred to each pot. Replication was fivefold. The experiment was divided into two blocks: outside and in the greenhouse.

1. Potato "Arran Banner"
2. Solanum nigrum English variety.
3. Solanum nigrum German variety.
4. Solanum minuta.

} Outside.

5. Eggplant "Long purple" Persian Variety
6. Eggplant rounded "Dolmei" Persian variety
7. Tomato "Leader"
8. Tobacco

} Greenhouse.

The pots were left until the end of the season, and watered when necessary. When the plants died the haulms were removed and final eelworm densities were estimated in each pot (see technique).

iii. Results.

The data obtained are presented in Appendix Table 73A and Table 74.

The criterion new cysts per 200g. (Table 74) shows clear differences in the susceptibility of different host plants to H.rostochiensis. Tobacco was not infested and is excluded from further analyses.

Two different varieties of eggplant showed the same response to this strain of parasite: both were poor hosts.

In Column 3 of Table 75 and also Table 76 it can be seen that the number of larvae per cyst (the latter picked out by randomization) and even the number of larvae per cyst of the same graded size show remarkable differences as between host plants. So if we are dealing with the infectivity of the parasite in different hosts, the number of new cysts is satisfactory only as a representation of the larvae penetrating the plant root.

As a representation of the reproductive capacity of the parasite it is inadequate. This is because not only will the size of the cysts be different in each host, being largest in potato and smallest in eggplant, but also the number of eggs and larvae per cyst of similar size differs from host to host. The analysis of variance (Appendix Table 77A) shows that the number of cysts in potato, tomato and eggplant are significantly

different, whereas none of the other hosts differs significantly from eggplant.

Table 75. Population build-up of H.rostochiensis with different hosts. (Experiment No.23).

Plant	Mean No. of cysts per 200g. of soil	No. of new cysts per 200g. of soil	No. of larvae per cyst	No.of Larvae per g. of soil
Potato	1241	1193	274	1635
Tomato	401	353	170	301
Eggplant Dolmei	197	149	98	74
Eggplant Long Purple	148	100	8	41
S.nigrum English	124	76	18	69
S.nigrum Germany	93	45	15	34
S.minuta	104	56	122	34
Tobacco	38	-	-	-

Initial infestation: 48 cysts per 200g. of soil.

210 larva per cyst.

50 larvae per g. of soil.

Table 76. Total number of viable eggs and larvae per cyst of graded size (422 - 599μ) obtained from soil planted with different host plants. (Data from Experiment No.23).

Host plant	No. of larvae per cyst.
Potato	369
"	388
Tomato	177
"	152
Eggplant Dolmei	157
"	175
Eggplant Long purple	126
"	142

GENERAL DISCUSSION.

A series of experiments has been carried out which were concerned with the fumigation of Heterodera rostochiensis, and different methods of assay. In addition, the susceptibility to attack by potato root eelworm of several species of host plants was compared. The hatching power of root diffusates extracted from the various plants was also compared.

Fumigation experiments were mainly concerned with the efficacy of three halogenated hydrocarbon nematicides: Nemagon, ethylene dibromide and chlorobromopropene.

Experiments were conducted during 1958-1961 in order to obtain the median lethal dose of the fumigants under different conditions. Mortality was assessed by one of the methods of assay described which were separately examined to determine the degree of accuracy which could be expected with each one.

The L.D.50 and the slope of the regression line were calculated. The L.D.50 is known to be statistically the most sensitive part of the line relating dosage to mortality. Any other points away from a 50% kill towards either 0% or 100% will have increasing standard errors and consequently be less accurately determined.

The results of the work in this thesis will be discussed under four general headings:

1. Bio-assay methods for nematicides.
2. Fumigation of H.rostochiensis.
3. Host-parasite-fumigant relationships.

4. Miscellaneous observations.

1. Bioassay methods for nematicides.

There have always been difficulties in determining the precise differences between "hatchability" and full "viability" in the sense of capacity both to invade the host and to reproduce. H.rostochiensis has been reported to penetrate the roots of a number of different plants but in some of them the larvae failed to complete development and reach maturity (Fenwick and Franklin, reported by Doncaster 1953). From the results of my own experiments, many larvae which successfully penetrated the roots of eggplants were unable to complete their life cycle. Therefore in fumigation experiments it is difficult to assess the efficacy of the fumigants. Three different methods of assay are described, namely, the root diffusate assay, the larval invasion assay, and an assay based on the final eelworm population. The suitability of these three methods is difficult to assess; first, the method of the root diffusate stimulation in vitro is only an artificial and indirect measure of the effect of the root excretion on encysted larvae. In the two other methods the number of penetrated larvae or the number of newly formed cysts, which form the criteria will be far less than the number of emerged larvae in the root diffusate assay. This is due to many factors present in the soil which play an important part in preventing the total number of hatched larvae from entering the root system of the host plant. Thus the efficiency of these different methods varies.

In the root diffusate assay the number of larvae emerging in each treatment up to a given time will be one measure of the efficacy of a fumigant. But this measure can not be a satisfactory one. It has been shown in this thesis, which is a confirmation of the work of others (Fenwick 1957; McBeth 1957), that fumigation of cysts with different fumigants, in addition to reducing hatchability, also delays the time of larval emergence. Furthermore, a greater mortality can be expected some months after treatment than just after. In some species such as H.schachtii the larvae will hatch both in root diffusate and water, whereas with H.göttingiana no root diffusate hatch has been found. Consequently the root diffusate assay would be limited to those species in which hatching occurs in this medium. Determination of the time necessary in the hatching process in nematicidal experiments is another factor which has been considered. These factors indicate that the larval hatch is not an ideal method of assay for determining the efficacy of different fumigants and caution is needed in evaluating results based on root diffusate assays.

In the larval invasion assay the number of larvae penetrating the roots is used as a means of assaying the nematicidal effect of a fumigant. Gemmel (1939) employed this method to determine the degree of infestation of the soil with potato root eelworm. The counts can be expressed as the number of nematodes in the whole root system, or as the number of nematodes per unit length of root (Franklin 1938; Miles 1930), or finally as the number per gramme of root, (Chitwood and Feldmesser 1948; Taylor and Loegering 1953; Williams and Winslow 1955; Doncaster 1957).

This method of assaying the efficacy of a fumigant involves many problems. The delayed action of the fumigant and consequently the early invasion in the controls and delayed invasion in the other treatments is one of the difficulties. Another problem is the differences in the ability of penetrated larvae to complete their development; this is influenced by two factors: the nature of the host plant and the delayed effect of some fumigants. It is difficult to know just what stage of the nematode's life history the fumigant affects, but this certainly varies with different fumigants.

Peters and Fenwick (1949) found that for testing nematicidal effect the seedling invasion assay was no better than the hypochlorite technique. A disadvantage of the root invasion assay was that it involves great expenditure of time and the procedure is laborious. Another problem is to determine what stage of plant growth is most suitable for examining the roots.

The question therefore arises - which method of assay should be adapted for measuring the efficacy of a nematicide against H.rostochiensis and other species of this genus. Providing the required time for a complete hatch at different treatments has elapsed, say at least 8 to 9 weeks, the root diffusate assay could be a rough guide for a preliminary estimation of the efficacy of treatments against H.rostochiensis. But unfortunately this assay even when all precautions are taken cannot be applicable to all nematicides. Nemagon is a good example of this. It has been shown that in some treatments with this fumigant the larvae hatch

and even penetrate the host plant roots but most of these hatched and penetrated larvae are not able to complete their life cycle and will die before making a new generation. (See Experiments No.10 and 14, pages 52 and 65).

The time factor in assays is most important and a short and precise method of assay is urgently needed. I have demonstrated (Experiment No.4., page 27) in this thesis that larvae fumigated with Nemagon are less active than non-treated controls. The activity of eelworms was measured by observing their downward movement in a column of sand which was just in contact with root diffusate (see Part II, eelworm movement).

Little experimental evidence is as yet available to show precisely how much the above method is applicable to other nematicides. However it seems to me that a combination of the hatching process with the above method might be a better way of measuring the efficacy of fumigants and might perhaps throw some light on the problem of finding a short method of assay.

The final eelworm population assay takes a long time, requiring the whole of a growing season before the efficacy of a fumigant can be assessed. This appears to be a disadvantage of the method, but on the other hand it has the advantage that overall effects of the fumigant can be taken into account and there is less danger of getting false results due to delayed effects.

I have shown in this thesis that in the root diffusate assay and root invasion assay the results were misleading due to the delaying

effect of the fumigants on the time at which the larvae hatched, but in the final eelworm population where larvae per gramme of soil was the criterion errors due to the delaying effect of the fumigant disappeared. Consequently in any proposed short method for measuring nematicidal effects attention should be given to the possibility of immediate delaying effects and later delayed effects of the nematicide.

Chitwood and Feldmesser (1948) proposed another method of assay called "washed root technique" in which potato plants were dug when the nematodes had become adults but before the females turned colour. The roots were shaken gently to remove excess soil and after immersing them in 10% formalin they were shaken vigorously in a pan with water to dislodge females. The water was then passed through a series of sieves to separate the white females from the debris after which the volume of the liquid was measured. Aliquot samples were withdrawn and the number of females estimated. The old cysts, being darker were not counted. By weighing the root system the number of new cysts per unit of root weight was calculated. In the "washed root technique" the number of larvae entering the root late in the season was not taken into account. Therefore the authors proposed a combination of this technique with root invasion assay and although laborious the new method seems to be more accurate than root diffusate or root invasion assay alone.

However, although the new method may be satisfactory for estimating field populations of nematode it has a number of disadvantages when used as the basis for a bioassay technique.

1. Larvae unable to complete their development as a result of the delaying effect of the fumigant will be included in the count.
2. No account is taken of the effect of fumigation on the size, and consequently on the egg- and larval- contents of the cysts.

2. Fumigation of H.rostochiensis.

The fumigation experiments fall into two types:

(a) Experiments under laboratory conditions namely in vitro, which will now be discussed more fully. The results obtained in this series of experiments showed that environmental conditions such as relative humidity and temperature have a great influence on the effect of the fumigant on the potato root eelworm. Three features of the results were interesting; first, at higher temperatures an increase in percentage mortality occured. Secondly, 100% R.H. resulted in the highest kill, which indicates that a higher relative humidity prior to and during fumigation leads to the fumigant penetrating more effectively to the parasite. Thirdly in the case of C.B.P. and E.D.B. a lower larval emergence was obtained, when the root diffusate assay was carried out six months after fumigation (see Table 40), thereby confirming Fenwick's investigation (1957). However the storage of cysts fumigated with Nemagon did not affect the hatchability of the larvae very much.

(b) Pot experiments. The results of pot experiments in the first year were very poor, but were a good guide for pot experiments in 1960. From experiments in 1959 it could be concluded that the eelworm population appears

to undergo a rapid increase in number towards the end of its development, which is in agreement with other workers (Peters 1953b; Clements 1959). The number of cysts per g. of soil from potato pots which were lifted early due to virus attack was significantly lower than the number recovered from those which remained until the end of the season (See Table 42 and 43). In 1960 a similar situation occured again because of a virus attack. An entire replication of potato plants was lifted (16 weeks after planting). The number of cysts/g. of soil in this series did not differ significantly from the number found in the soil of the pots which were removed at the end of the season. The reason was probably that the cysts in both series were allowed sufficient time (16 and 18 weeks respectively) to continue their development.

Implications of the results of C.B.P., E.D.B. and Nemagon in vitro with special reference to soil fumigation.

Chlorobromopropane.

Van den Brande et al. (1956) reported that C.B.P. was effective against H.rostochiensis cysts which might adhere to Gloxinia and Begonia tubers. The tubers and cysts were dipped in 0.025% (250 ppm) emulsions for 20, 40 or 60 minutes. Similar experiments were carried out in the Zoology Department at Silwood (1958), using C.B.P., Nemagon and E.D.B. by dipping cysts in emulsions. C.B.P. gave an effective kill at 0.025% assayed by both the root diffusate and final density assays (Hague, private communication).

It has been shown above in Part III (experiments in vitro) that a considerable measure of kill was obtained when cysts were exposed to C.B.P. for only 4 and even 2 minutes (see Appendix Tables 28A, 39A and 30A). It was clear from the above results that chlorobromopropane is an extremely effective nematicide. But the results of pot experiments revealed that the use of this fumigant for controlling H.rostochiensis on a field scale is not advisable. The reason for this contrast is not easy to find but it seems to me, as Thorne (1951) has mentioned; "the efficacy of a soil fumigant depends on its ability to penetrate the soil mass in sufficient quantity to build up lethal concentrations in the soil atmosphere". It may be that C.B.P. did not penetrate into soil particles either because it escaped too quickly, or lost its property due to decomposition in the soil. From the results obtained, the use of C.B.P. can be recommended as a promising contact nematicide for quarantine fumigation, but its effect in soil fumigation was not satisfactory. Further research on the behaviour of C.B.P. in pot experiments would be of very great interest.

Ethylene dibromide.

The physico-chemical processes involved in fumigation with ethylene dibromide have been studied by Call (1955) who has summarized the reason for its only moderate success as a soil fumigant in Great Britain with its cold and wet soils. These are (1) the rapid escape of the fumigant from the soil surface, (2) the low soil temperatures delaying volatilisation and dispersion of vapour, and (3) rain, which washes the fumigant out of the surface layers before fumigation is complete. He

also found that when E.D.B. is applied as a fumigant to soil, between 97 and 99% of the E.D.B. is present in the sorbed phase, the remainder being available in the vapour phase to exert a fumigant action against the parasite. Clements (1959) showed that the L.D.50 for H.göttingiana was between 0.00809 ml. E.D.B. and 0.0728 ml. E.D.B. for a pot holding 7½ Kg. of soil, while Peters (1952) suggested that 0.13 ml. of pure E.D.B. would give a 50% kill of H.rostochiensis in pots holding 10 Kg. of soil.

The E.D.B. used in the experiments described here was purified by fractional distillation under reduced pressure followed by fractional crystallisation. In in vitro experiments E.D.B. showed a slightly better effect than Nemagon whereas in soil fumigation a 50% kill was achieved by using 0.135 ml. E.D.B. in a pot holding 7½ Kg. of soil.

Nemagon.

The stability and retention of the nematicide in the soil for an optimum time is of primary importance because the response of an organism has been found to be proportional to the C × T. Product. The degree of sorption of a specific nematicide on a particular soil type, and the losses of the nematicide by ventilation are factors which influence the time of contact of a lethal dose of nematicide with the parasite. In the soil fumigation experiments I have noticed that in pots fumigated with Nemagon the smell of the fumigant could be detected at most dosages even 6 weeks after fumigation, which indicates the retention of the fumigant in the soil. However no trace of phytotoxicity due to this fumigant was observed on the growing host plants even at the highest rates. This stability and

slow action of the fumigant due to a very low vapour pressure could be one explanation of the efficacy of this nematicide.

An estimation of the L.D.50 to H.rostochiensis in terms of ml. of Nemagon, E.D.B. and C.B.P. has been presented in Table 78 below.

In in vitro experiments by the root diffusate and root invasion assays Nemagon gave a poor result and could be considered of no use as a contact nematicide. A delaying action in the hatching process was observed at all doses but at lower doses this concealed the real lethal effects of the nematicide.

Table 78. Estimation of the L.D.50 to Heterodera rostochiensis in terms of ml. of different fumigants per $7\frac{1}{2}$ Kg. of soil.

(Pot experiments).

Host	Fumigant	Year			
		1959-60		1960-61	
		Criterion		Criterion	
		cyst/g. of soil	larvae/g. of soil	cyst/g. of soil	larvae/g. of soil
Potato	C.B.P.	-	-	0.562	2.290
"	E.D.B.	-	-	0.135	0.135
"	Nemagon	0.039	-	0.043	0.029
Tomato	Nemagon	-	-	0.081	0.060

3. Host-parasite-fumigant relationships.

Potato.

The data obtained in the fumigation experiments when potato was the host, resulted in reductions in the final population which corresponded to a graded series of doses applied.

In the final density assay one could count the number of newly formed cysts and use it as a criterion, or count the number of larvae and eggs per g. of soil (which is a product of the number of cysts per g. of soil and number of eggs and larvae per cyst). The criterion of the number of cysts per g. of soil eliminates the estimate of "eggs per cyst" and thus requires less labour and seems to be preferable. But as it has been shown above the final density assay is clearly dependent on three factors, the host plant, the effect of the fumigant on the number of cysts, and the effect of the fumigant on the size and contents of the cysts. Table 79 shows the direct effect of Nemagon on the number of eggs and larvae per cyst which was significantly different at different treatments (see also Table 37). The data of Appendix Table 57A and Table 80 are other reasons for considering the number of eggs per g. of soil as the criterion of choice. In Fig.22 which consists of three charts, showing the differences between cyst count and larval count, the criterion of new cysts per g. of soil shows clear treatment differences which with some exceptions are in close agreement with the criterion l/g. of soil.

However I feel that assays on the final eelworm population should be expressed in terms of larvae per g. of soil which will be the only

ultimate means of estimating the efficacy of a nematicide, and which reflect the significant treatment differences of both cysts/g. of soil and larvae per cyst, these latter differences constitute a newly discovered phenomenon.

Table 79. Data extracted from Experiment No.18. Larvae per graded size (422 to 235μ) cysts in soil fumigated with Nemagon and planted with potato. (All new cysts).

Treatment	Larvae/cyst	Log. (larvae/cyst)
Control	233	2.367
"	222	2.346
0.016 ml.	176	2.246
"	182	2.260
0.0224 ml.	167	2.223
"	186	2.270
0.032 ml.	135	2.130
"	163	2.214
0.045 ml.	131	2.117
"	144	2.158
0.064 ml.	126	2.100
"	132	2.121
0.089 ml.	115	2.060
"	94	1.973

The graded cyst size in initial infestation was 599 to 422μ and 359 eggs and larvae per cyst.

Table 80. Data extracted from Experiment No.18. Larvae per cyst of different sizes produced in soil planted with different hosts. (Untreated controls).

Potato		Tomato		Eggplant	
No. of larvae/cyst		No. of larvae/cyst		No. of larvae/cyst	
Graded size 422-235μ	All sizes	Graded size 422-235μ	All sizes	Graded size 422-235μ	All sizes
211	337	127	143	143	123
233	389	115	142	131	139
233	353	131	140	135	131

The size of the cysts in the original infestation was 599 - 422μ

The number of eggs and larvae per cyst was 359.

Table 81. Analysis of variance of no. of larvae/graded size (325-422μ) cysts in soil fumigated with Nemagon and planted with Potato (Log. larvae/cyst). Data of Table 79. Experiment No.18.

	Items	D.F.	S.S.	M.S.	F.
a	Treatments	6	0.148369	0.02472816	a/b 17.66 ***
b	Error	7	0.009799	0.00139985	
c	Total	13	0.158168		

Continued on next page.

Table 81 continued.

Treatments	Totals	Differences
Control	4.713	
		0.207
0.016 ml.	4.506	
		0.013
0.0224 ml.	4.493	
		0.149 *
0.032 ml.	4.344	
		* 0.069
0.045 ml.	4.275	
		0.054
0.064 ml.	4.221	
		0.188
0.089 ml.	4.033	

Critical differences = 0.177

Tomato.

Almost at all different rates of C.B.P. and in some lower treatments with E.D.B. and Nemagon (Appendix Table 55A) where tomato plants were used the number of newly formed cysts per g. of soil was higher than those of the controls, whereas in potato it was lower than in the corresponding controls.

It has been shown (Peters and Fenwick 1955; Hague and Hesling 1958) that the final density in terms of "eggs per gramme of soil" increased to a maximum and then declined when potatoes were exposed to a geometric series of initial infestations. These observations indicated that low initial densities could give rise to final ones greater than those produced by high initial densities. Consequently in nematicidal experiments



Fig. 29 Eggplants – English Long Purple

the initial infestation will be a very important factor and should be such that the final eelworm population in all treatments will be not greater than in the controls. Hague and Hesling (1958) have shown that an initial density of 50-60 eggs per g. of soil will give a satisfactory result in pot experiments.

It is also important that no adverse effect on the growth of the plant or the weight of the yield should occur, otherwise the final eelworm population in the damaged plant will be reduced. Ambiguous results obtained in some treatments in the tomato experiments together with the noted reduction in size of tomato seedlings (Figs.15, 16 and 17) in the root invasion assay led me to measure the height of tomato plants after 60 days growth and the weight of the fruits after ripening (Appendix Table 82A). Analysis of variance in both (height and weight) (Appendix Tables 83A, and 84A) revealed a significant difference between control and other treatments. This result combined with previous observations suggest that an initial infestation of 50-60 eggs per g. of soil for tomato is too high. This needs further investigation.

In eggplant seedlings the damage was not as considerable as in tomatoes (Fig.14), but an initial infestation of about 100 eggs per g. of soil seems to be high enough to affect the natural growth of the plant and to cause a considerable reduction in fruit (Fig.29).

In pot experiments in 1959 the height of the tomato plants and the weight of the tomatoes in each treatment were not significantly different from the controls, although the initial infestation was the same or

even a little higher than in 1960.

The reason that these differences were observed only in the second year was probably that the cysts used in the 1959 experiments were very old. Since the invasion of roots by larvae from old cysts does not take place quite as rapidly as from new cysts (Franklin 1938b) the plants were able to become well established before they were attacked and therefore no obvious damage occured.

In all the experiments in this thesis when potato and tomato were used as host plants, the total number of newly formed cysts and also the size of the cysts in the soil planted with potato were significantly greater than those in the soil planted with tomato. These differences are unlikely to be due only to the different susceptibilities of the host plants, but are partly due to the different conditions necessary for the growth of the plant. In England tomatoes are usually grown in greenhouses with a higher temperature than outdoors where potatoes are grown. This difference in temperature and other environmental conditions during the growing season affect the eelworm population, the parasite being much more successful under temperate conditions.

Eggplant as host.

A series of experiments was carried out which was concerned with the stimulatory power of eggplant root diffusate, the degree of susceptibility of the plant and its ability to act as a host to H.rostochiensis.

The first conclusion was that leachings from eggplant roots, although able to stimulate the larvae to hatch, had lower L.A.value than



1 2 3

Fig.30. Three varieties of eggplants.

1. Long purple.
2. Dolmei.
3. Beauty.

potato and tomato leachings; the L.A. value being 2.10.

The results of the root invasion assay and final density assay showed that some of the larvae which penetrated the eggplant roots could not complete their life cycle. Similar observations have been made by other workers on Solanum nigrum (Fenwick and Franklin quoted by Doncaster 1953).

In the population build-up experiment (page 124) where two varieties of eggplants (Persian long purple and Dolmei) were used as hosts the final eelworm population revealed that they were both very poor hosts and the number of newly formed cysts was only about twice the initial infestation (see Experiment No.23).

In pot fumigation experiments the variety of eggplant was "English long purple" and the data obtained showed that this variety was also a poor host. Appendix Table 57A showed that the final eelworm population appears to have increased about twofold when the number of new cysts were used as the criterion, but, taking the number of l/g. of soil into account, a reduction in the population was revealed, due to a decrease in (l/cyst).

After many different experiments and using three varieties of eggplants (see Fig.30) and two strains of H.rostochiensis, I consider that this plant is not comparable with potato and tomato as a susceptible host to H.rostochiensis.

4. Miscellaneous Observations.

(a) Three different types of experiments were carried out to investigate the nematicidal effects of Tagetes plants. It was concluded that in the cultivation of Tagetes spp. in soil infested with H.rostochiensis, there would be a slight reduction in population (11-16% with Tagetes minuta and 18-36% with Tagetes signata).

The great increase in the eelworm population at even a very low initial infestation suggests that no nematicidal effect is worth considering unless the percentage mortality is very high and not less than 96% (Peters 1959).

Therefore it would be very doubtful, whether the cultivation of Tagetes plants in infested soil would be advantageous.

A combination of the results obtained from experiments on Tagetes plants in this thesis, in addition to tests of their root diffusates (Omidvar 1961 appended), suggests that a long period of time is required for a slight nematicidal effect and during this long period the Tagetes are removing soil nutrients. Thus their use is probably uneconomic.

(b) In the experiment on population build up (page 124), where different Solanum spp. were tested as hosts, it was found that the particular strain of H.rostochiensis could complete its life cycle on these weeds. If crop rotation is considered as a method of control, attention should be paid to the eradication of these weeds, otherwise the parasite could be maintained at a low level permanently.

Further research is also desirable to find out whether this strain of the parasite can overcome the natural resistance of Solanum spp. by continuous attack over several years.

SUMMARY.

This work has been concerned with the fumigation of Heterodera rostochiensis under different conditions.

Cysts of potato root eelworm were fumigated with chlorobromopropene, ethylene dibromide and Nemagon under laboratory conditions and in pots of soil under natural conditions.

Attempts have been made to assay any nematicidal effects of the fumigants. Different methods of bioassay were used to estimate the mortality of the parasite under different conditions, with a view to ascertaining which of them is the most reliable in nematicidal experiments. Host plants were grown in pots of soil to which treated cysts were added. The roots of growing plants in infested soil were examined at different stages of growth to measure the efficacy of fumigation by the root invasion assay. The height of the host plants, the number of potato tubers and the total weight of tomato fruits, were measured to investigate the effect of initial infestation when used for different host plants.

Comparisons were made between the susceptibilities of many different host plants. Samples of root diffusates extracted from three host plants and three Tagetes species were compared. Dilutions of the root diffusates were also examined and the L.A.value (Log-Activity) of each was calculated.

The effect of temperature and relative humidity during fumigation in in vitro experiments was studied.

In pot experiments the final eelworm population after growing a host crop was used to measure the efficacy of the fumigants. The estimation of mortality was expressed as a percentage based on a comparison of the final population level of each treatment with that of the untreated control.

Cysts of potato root eelworms were exposed to geometric series of doses of the fumigants and the results were analysed. Regression lines relating the kill transformed to angles and the log dose were computed for each fumigant and the median lethal dose was calculated.

With some fumigants, the final density assay will yield different results according as cysts or larvae are estimated per gramme of soil.

Experiments were also carried out to study the population changes of H.rostochiensis when using different host plants. It was found that some strains of potato root eelworm could build up a new population using Solanum nigrum as a host. It was also found that the eggplant was a poor host which caused a decrease in eelworm population as measured by larvae per g. of soil when used in fumigation experiments, although under ordinary conditions it supported a slight increase in population.

The activity of fumigated larvae was measured by their vertical movements and it was found that they are less active than unfumigated larvae.

The nematicidal effect of two species of Tagetes has been studied. It was concluded that the cultivation of Tagetes in soil infested with H.rostochiensis will cause a slight reduction in population due to a weak nematicidal secretion. The results suggested that a long period of time is required to obtain this slight nematicidal effect.

REFERENCES.

Bijloo, J.D. (1954). "A new method for estimating the cyst contents of the potato root eelworm Heterodera rostochiensis Wollenweber." J.Helminth. 28 (3/4), 123-126.

Bishop, D. (1953). "Hatching the contents of cysts of Heterodera rostochiensis with alternating temperature conditions." Nature. 172, 1108.

Bishop, D. (1955). "The emergence of larvae of Heterodera rostochiensis under conditions of constant and alternating temperature." Ann.appl.Biol. 43 (4), 525-533.

Brande, Van den. J., Kips, R.H. and D'Herde, J. (1954). "Invloed van de vochtigheid bij de Scheikundige bestrijding van het aardappel-cystenaaltje Heterodera rostochiensis Woll." Meded.LandbHoogesch.Gent., 19, 353-372.

Brande, Van den. J., Kips, R.H. and D'Herde, J. (1955). "Doding van Heterodera rostochiensis - cysten op Begonia - en Gloxinia - Knollen." Meded.LandbHoogesch.Gent. 20 271-278.

Call, F. (1955). "The distribution of ethylene dibromide in soils." Ph.D. Thesis. University of London.

Call, F. (1957). "Microsampling method of determining gases and vapours, particularly halogenated hydrocarbons, in air." J.appl.Chem., 7, 210-215.

Call, F. & Hague, N.G. (1957). "Control of plant nematodes." Rep.Progr.appl.Chem. 42, 605-612.

Chitwood, B.G. (1951). "The golden nematode of potatoes." U.S. Dept.Agric. Circ. 875, 48 pp.

Chitwood, B.G. and Buhrer, E.M. (1945). "Summary of soil fumigant tests made against the golden nematode of potatoes Heterodera rostochiensis Wollenweber, 1942-1944." Proc.helminth.Soc.Wash. 12 (2), 39-41.

Chitwood, B.G. and Feldmesser, J. (1948). "Golden nematode population studies." Proc.helminth.Soc.Wash. 15 (2), 43-55.

Christie, J.R. (1950). "Soil fumigation for control of nematodes and other soil-inhabiting organisms." Plant.Dis.Reptr. Suppl.170 (1947 revised) 22 pp.

Christie,J.R. (1959). "Plant nematodes. Their bionomics and control." Science 130 (3367), 94-95.

Clements, D.W.G. (1959). "Some problems in the bio-assay of substances used in the chemical control of plant parasitic nematodes." Ph.D. Thesis. University of London.

Doncaster, C.C. (1953). "A study of host-parasite relationships. The potato root eelworm (Heterodera rostochiensis) in Black nightshade (Solanum nigrum) and Tomato." J.Helminth. 27 (1/2), 1-8.

Doncaster, C.C. (1957). "Growth, invasion and root diffusate production in tomato and black nightshade inoculated with potato root eelworm." Nematologica 2 (1), 7-15.

Ellenby, C. (1951). "Mustard oils and control of the potato root eelworm Heterodera rostochiensis Wollenweber: further field and laboratory experiments." Ann.appl.Biol. 38 (4), 859-875.

Fassuliotis, G. and Feldmesser, J. (1954). "Infection of eggplant, Solanum melongena by the golden nematode of potatoes Heterodera rostochiensis." Plant Dis.Reptr. 38 (11), 791-793.

Feldmesser, J., Fassuliotis, G. and Spruyt, F.J. (1951). "Investigations on the control of the golden nematode of potatoes." Plant.Dis.Reptr. 35, 515-518.

Fenwick, D.W. (1940). "Methods for the recovery and counting of cysts of Heterodera schachtii from soil." J.Helminth. 18 (4), 155-172.

Fenwick, D.W. (1949). "Investigations on the emergence of larvae from cysts of the potato root eelworm Heterodera rostochiensis. 1. Technique and variability." J.Helminth. 23 (3/4), 157-170.

Fenwick, D.W. (1950). "Investigations on the emergence of larvae from cysts of the potato root eelworm Heterodera rostochiensis. 2. The form of the hatching curve." J.Helminth. 24 (1/2), 75-86.

Fenwick, D.W. (1951). "The effect of temperature on the development of the potato root eelworm Heterodera rostochiensis." Ann.appl.Biol. 38 (3), 615-617.

Fenwick, D.W. (1951). "Investigations on the emergence of larvae from the cysts of the potato root eelworm Heterodera rostochiensis. 4. Physical conditions and their influences on larval emergence in the laboratory." J.Helminth. 25 (1/2), 37-48.

Fenwick, D.W. (1952). "The bioassay of potato root diffusate." Ann.appl.Biol. 39 (4), 457-467.

Fenwick, D.W. (1952). "The estimation of the cyst contents of the potato root eelworm Heterodera rostochiensis." J.Helminth. 26 (2/3), 55-68.

Fenwick, D.W. (1956). "The breakdown of potato root diffusate in soil." Nematologica 1 (4), 290-302.

Fenwick, D.W. (1957). "Preliminary studies on the effect of ethylene dibromide fumigation on the hatchability of Heterodera rostochiensis (Woll.)." Nematologica 2 (3), 242-249.

Fenwick, D.W. and Reid, E. (1953). "Population studies on the potato root eelworm, Heterodera rostochiensis Woll." J.Helminth. 27 (3/4), 119-128.

Fenwick, D.W. and Widdowson, E. (1958). "The conduct of hatching tests on cysts of the potato root eelworm Heterodera rostochiensis (Woll.)." J.Helminth 32 (3), 125-134.

Franklin, M.T. (1938). "Experiments with cysts of the potato eelworm (Heterodera schachtii) of different ages." J.Helminth. 16 (2), 67-76.

Franklin, M.T. (1951). "The cyst-forming species of Heterodera." Commonwealth Agricultural Bureaux. 147 pp.

Goodey, J.B. (1957). "Laboratory methods for work with plant and soil nematodes." Tech.Bull.Min.Agric.Fish. & Fd. No.2 (3rd.edit.)

Grainger, J. (1956). "Progress in soil mixing for nematode control." Nematologica, 1 (1), 31-46.

Hague, N.G. (1958). "Emergence of encysted potato root eelworms responding to controlled and variable stimuli." Ph.D.Thesis. Univ. London.

Hague, N.G.M. (1959). "Control of plant parasitic nematodes. 2. Some aspects of the fumigation of the potato root eelworm, Heterodera rostochiensis (Woll.) with methyl bromide." Nematologica 4, 115-121.

Hague, N.G.M. (1960). "Control of plant parasitic nematodes. 3. The final eelworm density in soil as a means of assessing the efficacy of a fumigant." Nematologica Suppl.II, 13-21.

Hague, N.G.M. and Hesling, J.J. (1958). "Population studies on cyst-forming nematodes of the genus Heterodera." Proc.Linn.Soc.Lond. 169 Session. 1956-57. Pts. 1 & 2. pp.86-92.

Harrison, M.B. (1957). "A new approach to the evaluation of nematicides for the control of the golden nematode." Phytopathology. 47 (9), 523.

Harrison, M.B. and Mai, W.F. (1956). "Fumigation of encysted golden nematode under controlled environmental conditions." Abstract: Phytopathology 46 (1), 14.

Henry Doubleday Research Association. (1960). "Interim report on the control of potato eelworm."

Hesling J.J. (1952). "An improved method of separating eelworm cysts from debris." J.Helminth. 26 (2/3), 69-70.

Hesling, J.J. (1959). "The emergence of larvae of Heterodera rostochiensis (Woll.) from single cysts." Nematologica 4, 126-131.

Johnson, L.R. and Townsend, W.N. (1949). "The inhibition of hatching of potato root eelworm (Heterodera rostochiensis Woll.) in partially sterilized soil." Ann.appl.Biol. 36 (4), 504-512.

Jones, F.G.W. (1955). "Quantitative methods in Nematology." Ann.appl.Biol. 42 372-381.

Jones, F.G.W. (1955). "A microplot technique for the study of soil populations of cyst-forming root eelworms of the genus Heterodera." (in Kevan, Soil Zool., 390-402).

Jones, F.G.W. (1956). "Soil populations of beet eelworm (Heterodera schachtii Schmidt) in relation to cropping. 2. Microplot and field plot results." Ann.appl.Biol. 44 (1), 25-26.

Jones, F.G.W. (1957). "Soil populations of beet eelworms (Heterodera schachtii) in relation to cropping. 3. Further experiments with microplots and with pots." Nematologica 2 (4) 257-272.

Jones, F.G.W. (1960). "Plant parasitic nematodes." Advancement of Science, 174-180.

Jones, F.G.W. and Winslow, R.D. (1953). "Hatching responses in root eelworms (Heterodera spp.)." Nature 171 478.

Lear, B., Mai, W.F., Feldmesser, J. and Spruyt, F. (1952). "Soil fumigation experiments on Long Island, New York, to control golden nematode of potatoes." Phytopathology 42 (4) 193-196.

Lownsbery, B.F. and Peters, B.G. (1955). "The relation of the tobacco cyst nematode to tobacco growth." Phytopathology, 45 (3), 163-167.

Lubatti, O.F. and Blackith, R.E. (1956). "Fumigation of agricultural products. 13. Trials of onion seed treated with methyl bromide, and an improved method for its analysis." J.Sci.Fd.Agric. 7 (2), 149-159.

Lubatti, O.F. and Harrison,M.(1944). "Determination of fumigants. xv. Determination of trichloroacetonintrile and methyl bromide." J.Soc.Chem.Ind. LXIII, 140-144.

Mabbot, T.W. (1956). "Potato root eelworm. A report on an experiment to free seed potatoes from adhering soil and cysts." Scot.Agric. 36 (2), 73-74.

McBeth, C.W. (1954). "Some practical aspects of soil fumigation." Plant Dis.Reptr. Suppl.227, 95-97.

McBeth, C.W. (1957). "Chemical control - principles and practices." (A manual of plant nematology for experimental station workers in the Northeastern region). Cornell University.

McBeth, C.W. and Bergeson, G.B. (1953). "Methods of assaying nematicides." Phytopathology 43 (5), 264-267.

McBeth, C.W. and Gilpatrick, J.D. (1956). "The effect of placement depth on the activity of Nemagon." Phytopathology 46 (10) 529-531.

McBeth, C.W., Taylor, A.L. and Smith, A.L. (1941). "Note on staining nematodes in root tissues." Proc.helminth.Soc.Wash. 8 (1), 26.

McClellan, W.D., Christie, J.R. and Horn, N.L. (1949). "Efficacy of soil fumigants as affected by soil temperature and moisture." Phytopathology 39 (4), 272-283.

Mai, W.F. and Lautz, W.H. (1953). "Relative resistance of free and encysted larvae of the golden nematode Heterodera rostochiensis Wollenweber to D-D mixture and hot water." Proc.helminth.Soc.Wash. 20 (1), 1-7.

Martin, H. (1957). "Guide to the chemicals used in crop protection." Science Service Lab., Univ. of West.Ontario, London, Ontario.

Miles, H.W. (1930). "Field studies on Heterodera schachtii Schmidt, in relation to the pathological conditions known as potato sickness." J.Helminth. 8 (2), 103-122.

Morgan, D.O. and Peters, B.G. (1929). "The potato root eelworm in Lincolnshire." J.Helminth. 7 (2), 63-80.

Omidvar, A. (1961). "On the effects of root diffusates from Tagetes spp. on Heterodera rostochiensis (Woll.)." Nematologica 2 (6), 123-129.

Oostenbrink, M. (1950). "Het aardappelaaltje (Heterodera rostochiensis Wollenweber) een gevaarlijke parasiet voor eenzidige aardappelcultur." Versl-PlZiekt.Dienst.Wageningen 115, 1-230.

Oostenbrink, M. (1954). "Een overzicht van de nematologie als onderdeel van de Plantenziektendunde." Landbouwvoorlichting 11 (5) 215-226.

Oostenbrink, M., Kuiper, K. and S'Jacob , J.J. (1957). "Tagetes als feindpflanzen von Pratylenchus-Arten." Nematologica 2, 424-433.

Peters, B.G. (1951). "The term 'eelworm-free soil' in plant quarantine regulations." Nature, Lond. 167, 368.

Peters, B.G. (1950). "Review of work on potato root eelworm." Rep.Rothamst.Exp.Sta., 147-156.

Peters, B.G. (1952). "Pot tests of nematicides against potato root eelworm. I. Pilot test and methods." Ann.appl.Biol. 39 (4), 447-456.

Peters, B.G. (1953). "The golden nematode in Britain." Amer.Pot.J. 30 (9), 226-230.

Peters, B.G. (1953). "Changes in potato root eelworm population with time and depth." J.Helminth. 27 (3/4), 113-118.

Peters, B.G. (1953). "Vertical migration of potato root eelworm." J.Helminth. 27 (3/4), 107-112.

Peters, B.G. (1954). "Control of plant nematodes." Rep.Progr.appl.Chem. 39, 721-726.

Peters, B.G. (1955). "The combined use of nematicidal soil fumigants and solubilized chemicals." J.Helminth. 29 (1/2), 81-86.

Peters, B.G. and Fenwick, D.W. (1949). "Field trials with D-D mixture against potato root eelworm." Ann.appl.Biol. 36 (3), 364-382.

Shepherd, A.M. (1959). "Increasing the rate of larval emergence from cysts in hatching test with beet eelworm, Heterodera schachtii Schmidt." Nematologica 4, 161-164.

Shepherd, A.M. and Wallace, H.R. (1959). "A comparison of the rates of emergence and invasion of beet eelworm Heterodera schachtii Schmidt and pea root eelworm, Heterodera gottingiana Leibscher." Nematologica, 4 227-235.

Solomon, M.E. (1951). "Control of humidity with Potassium-hydroxide, KoH, Sulfuric acid, H_2SO_4, or other solutions." Bull.ent.Res., 42, 3.

Simon, M. (1949). "La lutte contre le nématode de la betterave par la désinfection du sol."
Pub.Inst.belge.Amelior.Better. 2 1-15.

Thorne, G. (1939). "Some factors governing the success of chemical treatment of soil for nematode control."
Proc.helminth.Soc.Wash., 6 (2), 60-62.

Thorne, G. (1951). "Diffusion of soil fumigants."
Proc.helminth.Soc.Wash. 18 (1), 18-24.

Uhlenbroek, J.H. and Bijloo, J.D. (1958). "Investigations on nematicides. 1. Isolation and structure of nematicidal principle occuring in Tagetes roots." Rec.Trav.chim.Pays-Bas. 77 (11); 1004-1009.

Uhlenbroek, J.H. and Bijloo, J.D. (1959). "Investigations on nematicides. 2. Structure of a second nematicidal principle isolated from Tagetes roots." Rec.Trav.chim.Pays-Bas. 78 (5), 382-390.

Wallace, H.R. (1958). "Movement of eelworms. I. The influence of pore size and moisture content of the soil on the migration of larvae of the beet eelworm, Heterodera schachtii Schmidt."
Ann.appl.Biol. 46 (1) 74-85.

Wallace, H.R. (1958). "Movement of eelworms. II. A comparative study of the movement in soil of Heterodera schachtii Schmidt and of Ditylenchus dipsaci (Kuhn) Filipjev." Ann.appl.Biol. 46 (1) 86-94.

Wallace, H.R. (1958). "Observations on the emergence from cysts and the orientation of larvae of three species of the genus Heterodera in the presence of host plant roots." Nematologica 3 236-243.

Wallace, H.R. (1958). "Movement of eelworms. III. The relationship between eelworm length, activity and mobility." Ann.appl.Biol. 46 (4), 662-668.

Wallace, H.R. (1959). "Movement of eelworms. IV. The influence of water percolation." Ann.appl.Biol. 47 (1), 131-139.

Wallace, H.R. (1959). "The movement of eelworms in water films." Ann.appl.Biol. 47 (2), 366-370.

Wallace, H.R. (1960). "Movement of eelworms. VI. The influences of soil type, moisture gradients and host plant roots on the migration of the potato root eelworm Heterdera rostochiensis Woll." Ann.appl.Biol. 48 (1), 107-120.

Williams, T.D. and Winslow, R.D. (1955). "A synopsis of some laboratory techniques used in the quantitative recovery of cyst-forming and other nematodes from soil." (In Kevan: Soil Zool. 375-384.)

Winslow, R.D. (1954). "The hatching responses of some root eelworms of the genus Heterodera." Ann.appl.Biol. 43 (4), 19-36.

Winslow, R.D. (1955). "Experiments on the control of the potato root eelworm by trap cropping with black nightshade." Plant Path. 4 (4), 139-140.

Winslow, R.D. (1955). "A new method for the production and recovery of cysts of root eelworms (Heterodera spp.) for use in bioassay." J.Helminth. 29 (1/2), 49-54.

Winslow, R.D. (1957). "Studies on hatching stimulation in the beet nematode, Heterodera schachtii Schmidt." Canad.J.Bot. 35, 619-634.

Appendix Table 7A.

χ^2 test for the migration of the first, second and third week emerged larvae in column of sand.

Time of emergence	Total migrated larvae	Non-migrated larvae	Grand Total
	Observed:		
1st week	2521	1779	4300
2nd week	3182	1518	4700
3rd week	1318	3182	4500
	7021	6479	13500
	Expected:		
1st week	2236	2064	4300
2nd week	2444	2256	4700
3rd week	2340	2160	4500

$$\chi^2 = \Sigma\left[\frac{(O-E)^2}{E}\right] = \frac{(285)^2}{2236} + \frac{(-285)^2}{2064} + \frac{(738)^2}{2444} + \frac{(-738)^2}{2256} + \frac{(1022)^2}{2340} + \frac{(-1022)^2}{2160} =$$

$$\frac{81225}{2236} \quad \frac{81225}{2064} \quad \frac{544644}{2444} \quad \frac{544644}{2256} \quad \frac{1044484}{2340} \quad \frac{1044484}{2160} =$$

$$36.33 + 39.35 + 222.85 + 241.42 + 446.36 + 483.56 =$$

$$\Sigma\left[\frac{(O-E)^2}{E}\right] = 1469.87^{***}$$

Appendix Table 13A.

(χ^2 test.)

Penetration of tomato roots by larvae emerging in the 1st, 2nd and 3rd weeks.

(Experiment No.6).

Type of larvae	No. of roots	Observed			Expected		
		Penetrated	Non-penetrated	Total	Penetrated	Non-penetrated	Total
First Week	10	2780	3620	6400	2840	3560	6400
Second Week	10	2912	2958	5870	2605	3265	5870
Third Week	10	2416	3584	6000	2663	3337	6000

$$\chi^2 = \sum \frac{(O - E)^2}{E} = 108.51 \ ***$$

Appendix Table 14A.

(χ^2 test.)

Penetration of tomato roots by larvae emerging in the 1st, 2nd and 3rd weeks.

(Experiment No.7).

Type of larvae	No. of roots	Observed			Expected		
		Penetrated	Non-penetrated	Total	Penetrated	Non-penetrated	Total
1st week	10	2554	8696	11250	2669	8581	11250
2nd week	10	3279	6071	9350	2215	7135	9350
3rd week	10	1241	8019	9260	2194	7066	9260

$$\chi^2 = \sum \frac{(O - E)^2}{E} = 1218.751 \ ***$$

Appendix Table 19A.

The effect of relative humidity during fumigation of H.rostochiensis with Ethylene dibromide and Nemagon at saturation concentrations and different periods of exposure. (Root diffusate assay).

Fumigant	Period of exposure	Total larval emergence per 100 cysts (Control Value: 7295)					
		60% R.H.	Log	30% R.H.	Log	100% R.H.	Log
EDB	4 hours	4470	3.650	1478	3.170	0	-
"	"	4013	3.603	3224	3.508	0	-
"	8 hours	4242	3.628	2968	3.472	0	-
"	"	4678	3.670	2281	3.358	0	-
"	16 hours	4962	3.696	1859	3.269	0	-
"	"	4189	3.622	1239	3.093	0	-
"	32 hours	4431	3.647	1684	3.226	0	-
"	"	4064	3.609	1922	3.284	0	-
Nemagon	4 hours	5552	3.744	4061	3.609	2367	3.374
"	"	4038	3.606	3507	3.545	1746	3.242
"	8 hours	4359	3.639	3810	3.581	1645	3.216
"	"	4665	3.669	2643	3.422	2155	3.333
"	16 hours	4409	3.644	3587	3.555	1425	3.154
"	"	4366	3.640	3087	3.490	1097	3.040
"	32 hours	3507	3.545	3542	3.549	1163	3.066
"	"	3523	3.547	2834	3.452	1167	3.067

Appendix Table 28A.

The effect of fumigating H.rostochiensis cysts with Nemagon, E.D.B. and C.B.P. in glass chambers at 20°C. and 100% R.H.

(Root diffusate assay).

Fumigant	Period of Exposure	Emergence per 100 cysts	Mean	% Mortality	Angular Transformation
Nemagon	64 minut s	26111	23427		
"	"	20743		16.712	24.12
"	128 minutes	19524	20123		
"	"	20723		28.46	32.27
"	178 minutes	18784	17539		
"	"	16294		37.65	37.88
"	256 minutes	4011	5027		
"	"	6043		82.13	64.97
"	512 minutes	7	7		
"	"	7		99.9	88.19
C.B.P.	2 minutes	13050	12032		
"	"	11013		57.22	49.14
"	4 minutes	1331	1250		
"	"	1168		95.56	77.89

Continued on next page.

Appendix Table 28A Cont.

Fumigant	Period of Exposure	Emergence per 100 cysts	Mean	% Mortality	Angular Transformation
C.B.P.	8 minutes	792	611		
"	"	430		97.81	81.47
Ethylene dibromide	32 minutes	13377	13999		
"	"	14622		50.23	45.11
"	45 minutes	1003	1494		
"	"	1984		94.69	76.69
Control	-	28804	28128	-	
"	-	29728			
"	-	24227			
"	-	29753			

Appendix Table 29A.

The effect of fumigating H.rostochiensis cysts with Nemagon, E.D.B. and C.B.P. in glass chambers at 20°C. and 100% R.H. (Root invasion assay).

Fumigant	Period of Exposure	Host Plant	Mean wt.of roots. g.	Mean Nematodes per pot	Mean Nematodes per g.of root	% Mortality Tomato	% Mortality Eggplant
Nemagon	64 mins.	Tomato	0.87	2940	3603	-	8.2
"	"	Eggplant	0.465	749	1668		
"	128 mins.	Tomato	1.005	2282	2270	16.69	27.13
"	"	Eggplant	0.41	556	1324		
"	178 mins.	Tomato	1.565	3017	1931	29.14	45.51
"	"	Eggplant	0.82	701	990		
"	256 mins.	Tomato	1.115	674	606	77.76	47.33
"	"	Eggplant	0.39	369	957		
"	512 mins.	Tomato	1.81	-	-	100	100
"	"	Eggplant	0.6	-	-		
C.B.P.	2 mins.	Tomato	1.255	1441	1250	54.13	57.95
"	"	Eggplant	0.535	412	764		
"	4 mins.	Tomato	1.390	36	26	99.04	90.97
"	"	Eggplant	0.620	80	164		
"	8 mins.	Tomato	1.675	-	-	100	100
"	"	Eggplant	0.815	-	-		
Ethylene dibromide	32 mins.	Tomato	1.370	217	160	94.13	84.86
"	"	Eggplant	0.567	150	275		
"	45 mins.	Tomato	1.755	-	-	-	100
"	"	Eggplant	0.620	-	-		
Control	-	Tomato	0.400	1090	2725	0	0
"	-	Eggplant	0.307	558	1817	0	0

Appendix Table 30A.

The effect of fumigating H.rostochiensis cysts with Nemagon, E.D.B. and C.B.P. in glass chambers at 20°C. and 100% R.H. (Final density assay).

Host: Potato. (Initial infestation 50 cyst/pot),

Fumigant	Period of Exposure	New cyst per pot	Cyst/200g. of soil	No. of larvae /100 cysts	L/g of soil
Nemagon	64 minutes	430	338	18850	320
"	"	245		19250	
"	128 minutes	399	335	13800	247
"	"	271		15770	
"	178 minutes	332	266	9950	158
"	"	201		13900	
"	256 minutes	132	173	10175	101
"	"	213		9750	
"	512 minutes	38	44	-	-
"	"	50		-	
C.B.P.	2 minutes	166	175	18900	159
"	"	184		17500	
"	4 minutes	51	74	17400	62
"	"	98		16120	
"	8 minutes	31	48	-	-
"	"	64		-	
Ethyene dibromide	32 minutes	44	54	21200	-
"	"	65		20740	
"	45 minutes	40	49	-	-
"	"	57			
Control	-	725	818	22850	901
"	-	910		21250	

Appendix Table 32A.

Data from Tables 28A, 29A, 30A. (See also Fig.11).

Analysis of variance for regression lines.

1. Root diffusate assay.

	Items	D.F.	S.S.	M.S.		F.	
a	Variance attributable to regression	1	2529.2254	2529.2254	a/b	26.81	*
b	Residual	3	283.0523	94.3508			
c	Total	4	2812.2777				

2. Root invasion assay.

	Items	D.F.	S.S.	M.S.		F.	
a	Variance attributable to regression	1	4736.1186	4736.1186	a/b	105.83	**
b	Residual	3	134.2513	44.7504			
c	Total	4	4870.3699				

3. Final density assay (cyst/g. of soil).

	Items	D.F.	S.S.	M.S.		F.	
a	Variance attributable to regression	1	809.25278	809.25278	a/b	13.52	*
b	Residual	3	179.58034	59.860			
c	Total	4	988.8331				

4. Final density assay (L/g. of soil).

	Items	D.F.	S.S.	M.S.		F.	
a	Variance attributable to regression	1	740.7271	740.7271	a/b	37.51	**
b	Residual	3	59.2452	19.7484			
c	Total	4	799.9723				

Appendix Table 33A.

Analysis of variance (for three regression lines).

(1. Root diffusate assay. 2. Root invasion assay. 3. Final density assay (L/g.) Nemagon as fumigant in "in vitro".

Data: From Tables 38A, 29A, 30A. (Experiment No.14). See also Fig.11.

	Items	D.F.	S.S.	M.S.	F.	
a	Common regression	1	7149.9405	7149.9405	a/d 135.24	***
b	Between regression (slopes)	2	857.5910	428.7955	b/d 8.11	**
c	Between intercepts (positions)	2	1753.6250	876.9125	c/d 16.59	***
d	Residual	9	475.8115	52.868		
e	Total	14	10237.1680			

Appendix Table 34A.

Analysis of variance (for two regression lines).

(1. Final density assay c/g. of soil. 2. Final density assay L/g. of soil).

Data from Experiment No.14.

	Items	D.F.	S.S.	M.S.	F.	
a	Regression line	1	1549.277	1549.277	a/d 38.931	*
b	Between regression (slopes)	1	0.758	0.758	b/d < 1	n.s.
c	Between intercepts (positions)	1	34.065	34.065	c/d < 1	n.s.
d	Residual	6	238.770	39.795		
e	Total	9	1822.870			

Appendix Table 35A.

Regression of angular transformation on log dose.

Final eelworm population (cyst/g. of soil).

Experiment No.14 in vitro.

Dose	1.8	2.10	2.40	2.70	
a	45.1	47.6	70.9	90.0	
b	59.7	57.5	62.6	90.0	
Σ	104.8	105.1	133.5	180	
$\bar{m}$	52.4	52.6	66.8	90	A = 65.4

	Total y	$y\xi_1$	$y\xi_2$	$y\xi_3$	
x_1	104.8	-314.4	+ 104.8	-104.8	$B = \frac{245}{40} = 6.35$
x_2	105.1	-105.1	- 105.1	+ 315.3	
x_3	133.5	+133.5	- 133.5	-400.5	$C = \frac{46.2}{8} = 5.78$
x_4	180.0	+540.0	+ 180.0	+180.0	
Σ	523.4	+254.0	+ 46.2	- 10.0	
$(\Sigma)^2$		64516.0	2134.44	100.0	
$\Sigma n\xi_1^2$		40	8	40	
$(\Sigma)^2/\Sigma n\xi_1^2$		1612.9	266.80	2.5	

$$\hat{y} = A + B\xi_1^1 + C\ \xi_2^1$$

$$\hat{y} = 65.4 + 6.35\ \xi_1^1 + 5.78\ \xi_2^1$$

Appendix Table 36A .

(1) ξ_1^1	(2) $6.35\ \xi_1^1$	(3) $\xi_1^1\ 2/4-1.25\ \xi_2^1$	(4) $5.78\ \xi_2^1$	(5) (2)+(4)	(6) Y = (5)+65.4
-3	-19.050	+ 1	5.78	-13.27	52.13
-2.5	-15.875	+ 0.31	1.79	-14.08	51.32
-2	-12.700	- 0.25	-1.45	-14.15	51.25
-1.5	- 9.525	- 0.69	-3.99	-13.52	51.88
-1.0	-6.350	-1.00	-5.78	-12.13	53.27
-0.5	- 3.175	-1.19	-6.88	-10.06	55.34
0	0	-1.25	-7.23	- 7.23	58.17
0.5	3.175	-1.19	-6.88	-3.70	61.70
1.0	6.350	-1.00	-5.78	+ 0.57	65.97
1.5	9.525	-0.69	-3.99	5.54	70.94
2.0	12.700	-0.25	-1.45	11.25	76.65
2.5	15.875	+0.31	1.79	17.67	83.07
3.0	19.050	+1.0	5.78	24.83	90.23

$$\xi_2^1 = \xi_2 = \xi_1^2 - 1.25 = \xi_1^1\ 2/4 - 1.25$$

Appendix Table 39A (2)

Total emergence from cysts treated with Nemagon and stored for 90 days before hatching test. (Relative humidity during fumigation 100%).

Time of exposure (Saturation concentration) Hours	Larval emergence (100 cysts)	
	(after 90 days)	(After 32 days)
4	2145	2367
4	2023	1746
8	1611	1645
8	2121	2155
16	1412	1425
16	1210	1097
32	940	1163
32	811	1167
Control	5411	6513
	6914	8077

Appendix Table 41A.

Fumigation of H.rostochiensis (in 1959) with Nemagon.

Summary of the final eelworm population. (Experiment No.17)

Initial infestation:

Cyst/1000g.	Larvae/cyst	Larvae/g. of soil.
180	335	60.3

The final population of cysts. 2 Blocks (A & B). Wt.of sample 500g.

Treatment	Block	No.of cysts per 1000g.of soil			Total No. of cysts/ 1000 g.	% Kill
		> 599μ	599-422μ	< 422μ		
Control	A	467	1060	434	1961	-
"	B	340	1180	252	1772	-
0.001 ml.	A	401	1155	518	2074	-
"	B	346	959	450	1755	-
0.004 ml.	A	513	1243	583	2339	-
"	B	327	1324	399	2050	-
0.016 ml.	A	303	1139	307	1749	11.90
"	B	62	473	180	715	
0.064 ml.	A	48	558	162	768	66.98
"	B	33	360	185	578	
0.128 ml.	A	18	229	18	265	95.23
"	B	12	172	8	192	
0.256 ml.	A	7	188	10	205	98.60
"	B	18	158	12	188	
0.512 ml.	A	5	197	4	206	98.54
"	B	-	178	-	178	

Appendix Table 45A.

Fumigation of H.rostochiensis (Experiment No.17) with ethylene dibromide.

Summary of the final eelworm population.

Initial infestation:

Cysts/1000g.	Larvae/cyst	Larvae/g. of soil
180	335	60.3

The final population of cysts. Wt. of sample 500g.

Treatment	No.of cysts per 1000g. of soil			Total No. of cysts/1000 g.	% Kill
	>599μ	599-422μ	<422μ		
Control	271	1171	451	1893	-
0.016 ml.	326	1271	516	2113	-
0.032 ml.	324	1397	330	2052	-
0.064 ml.	125	555	274	954	54.82
0.128 ml.	104	536	44	684	70.58
0.256 ml.	51	373	51	475	82.78

Appendix Table 50A.

Fumigation of H.rostochiensis 1960 in vivo. Summary of initial and final infestation data.

Initial infestation :	Cyst/400g.	Larvae/cyst	Larvae/g. of soil.
	64	359	57

Host plant : Potato.

Fumigant : C.B.P.

Final Infestation.

Treatment	c/400g.	L/cyst	L/g.	% Kill (c/400g.)	% Kill (L/g.)
0.004 ml.	9923	347	2535	-	6.14
0.008 ml.	2704	392	2653	2.58	1.78
0.016 ml.	1781	374	1665	36.64	38.36
0.032 ml.	2435	375	2283	12.51	15.48
0.045 ml.	2278	393	2235	18.30	17.25
0.064 ml.	2425	385	2331	12.88	13.70
0.089 ml.	1937	375	1825	30.88	32.43
0.128 ml.	2045	408	2086	26.90	22.77
Control + Acetone	2774	389	2701	-	-

Appendix Table 51A.

Fumigation of H.rostochiensis 1960 in vivo.

Summary of initial and final infestation data.

Initial infestation :	Cyst/400g.	Larvae/cyst	Larvae/g. of soil.
	64	359	57

Host plant : Potato

Fumigant : E.D.B.

Final Infestation.

Treatment	c/400g.	L/cyst	L/g.	% Kill (c/g.)	% Kill (L/g.)
0.032 ml.	2921	335	2450	-	9.29
0.045 ml.	2718	343	2335	2.07	13.55
0.064 ml.	2157	399	2153	22.77	20.29
0.089 ml.	1645	406	1670	41.66	38.17
0.128 ml.	1413	407	1439	50.22	46.72
0.178 ml.	1148	384	1103	60.00	59.16
0.256 ml.	734	382	701	75.28	74.04
Control + Acetone	2774	389	2701	-	-

Appendix Table 52A.

Fumigation of H.rostochiensis 1960 in vivo.

Summary of initial and final infestation data.

Initial infestation :	Cyst/400g.	Larvae/cyst	Larvae/g. of soil.
	64	359	57

Host plant : Potato

Fumigant : Nemagon

Final Infestation.

Treatment	c/400g. (mean of 2)	L/cyst	L/g.	% Kill (c/400g.)	% Kill (L/g.)
0.016 ml.	2673	297	1988	3.73	26.39
0.0224 ml.	2330	305	1776	16.38	34.25
0.032 ml.	1683	287	1209	40.26	52.90
0.045 ml.	1375	246	847	51.62	68.64
0.064 ml.	819	236	484	72.14	82.08
0.089 ml.	433	193	209	86.38	92.26
0.128 ml.	200	128	64	94.98	97.63
Control + Acetone	2774	389	2701	-	-

Appendix Table 55A.

Fumigation of H.rostochiensis (1960) in vivo.

Summary of initial and final infestation data.

Initial infestation :	Cyst/400g.	Larvae/cyst	Larvae/g. of soil
	64	359	57

Host : Tomato.

Fumigant	Dose per pot ml.	Mean No. of cysts/400g.	L/cyst	L/g.	% Kill (c/400g.)	% Kill (L/g.of soil)	Ø L/g. of soil
C.B.P.	0.032	780	142	277	2.05	1.77	-
"	0.045	1011	136	345	0	0	-
"	0.064	1097	121	330	0	0	-
"	0.089	856	142	304	0	0	-
"	0.128	1197	126	377	0	0	-
E.D.B.	0.032	1007	156	392	0	0	-
"	0.045	1005	134	336	0	0	-
"	0.064	665	122	202	17.78	28.36	-
"	0.089	664	121	200	17.92	29.07	-
"	0.128	510	147	187	38.98	33.68	-
Nemagon	0.032	928	104	241	0	14.54	22.38
"	0.045	640	110	176	21.20	37.59	37.82
"	0.064	527	84	110	36.66	60.99	51.35
"	0.089	360	108	97	59.51	65.60	54.09
"	0.128	243	76	46	75.51	83.68	66.19
Control	with acetone	795	142	282	-	-	

Appendix Table 57A.

Fumigation of H.rostochiensis 1960 in vivo.

Summary of initial and final infestation data.

Initial Infestation :	Cyst/400g.	Larvae/cyst	Larvae/g. of soil
	64	359	57

Host : Eggplant.

Fumigant	Dose per pot ml.	Mean No. of cysts/400g.	L/cyst	L/g.	% Kill (c/400g.)	% Kill (L/g. of soil)
C.B.P.	0.032	127	81	26	79.13	76.78
"	0.045	143	78	27	73.84	75.89
"	0.064	153	85	32	70.52	71.42
"	0.089	190	104	49	58.27	56.25
"	0.128	100	79	20	88.08	82.14
E.D.B.	0.032	193	102	49	57.28	56.25
"	0.045	188	98	46	58.94	58.92
"	0.064	154	102	39	70.19	65.17
"	0.089	141	97	34	74.50	69.64
"	0.128	136	71	24	76.15	78.57
Nemagon	0.032	178	84	38	62.25	66.07
"	0.045	144	54	19	73.50	83.03
"	0.064	135	45	15	76.49	86.60
"	0.089	113	41	11	83.77	90.17
"	0.128	95	44	10	89.73	91.07
Control	with acetone	366	123	112	-	

Appendix Table 66A.

Nematicidal effect of Tagetes plants on H.rostochiensis.

(Artificially infested soil).

Replication	Total number of emerged larvae per 100 cysts in Potato R.D.		
	Tagetes signata	Tagetes minuta	Cineraria maritima
1	14300	13650	20300
2	18550	19100	17700
3	15450	13850	23300
4	17650	18900	17400
5	18750	17500	20350
6	13700	18400	21700
Mean and % Kill	16400 (18.51%)	16900 (16%)	20125

Analysis of variance

Log (larvae/cyst).

	Items	D.F.	S.S.	M.S.	F.
a	Plant sp.	2	0.028900	0.014450	a/b 4.132 *
b	Residual	15	0.052462	0.003497	
c	Total	17	0.081362		

Treatments (Plant spp.)	Totals	Differences
Cineraria	13.808	
		0.469 *
Tagetes minuta	13.339	
		0.074 n.s.
Tagetes signata	13.265	

Critical difference = 0.436

Appendix Table 67A.

Nematicidal effect of Tagetes plants on H.rostochiensis.

(Naturally infested soil).

Replication	Total number of emerging larvae per 100 cysts in Potato R.D.		
	Tagetes signata	Tagetes minuta	Cineraria maritima
1	9600	12200	12700
	8610	12550	14300
2	8715	10100	12700
	7607	12300	13600
3	7814	11150	14000
	8600	13200	13200
Mean and % Kill	8491 (36.6%)	11917 (11.2%)	13417

Analysis of variance

Log. larvae/cyst.

	Items	D.F.	S.S.	M.S.	F.
a	Plant sp.	2	0.12635	0.06317	a/b 526 ***
b	Residual	15	0.01785	0.0012	
c	Total	17	0.14420		

Treatments (Plant spp.)	Totals	Differences
Cineraria	12.764	
		0.321 *
Tagetes minuta	12.443	
		0.878 *
Tagetes signata	11.565	

Critical difference = 0.255

Appendix Table 69A.

The number of newly formed cysts obtained from the soil of the pots planted with tomato seedlings and inoculated with 32092 H.rostochiensis larvae per pot.

Replication	Total number of newly formed cysts			
	Pots planted with tomato and Tagetes minuta		Pots planted with tomato without Tagetes minuta	
		Log.		Log.
1	769	2.886	723	2.859
2	602	2.780	1139	3.057
3	829	2.919	729	2.863
4	488	2.688	692	2.840
5	753	2.877	720	2.857
Total	3441		4003	
Mean	688		800	

Analysis of variance (data above).

Log (no. of cysts per pot).

	Items	D.F.	S.S.	M.S.	F.
a	Tagetes	1	0.0106224	0.0106224	a/b 1.23 n.s.
b	Residual	8	0.0689680	0.0689680	
c	Total	9	0.0795904		

Appendix Table 70A.

Total larval emergence from batches of 100 cysts exposed to the root diffusate of three host plants and water. Time of exposure 64 days.

Concentration	Host plant.		
	Potato	Tomato	Eggplant
1	28830	30913	9406
1/4	25896	26394	6623
1/16	19490	23147	3226
1/64	11610	15813	1276
1/256	7641	9662	544
Water	539	495	404

Appendix Table 71A.

The number of larvae hatching in root diffusate extracted from three host plant roots. Time of exposure 64 days. Number of larvae per g. of dry root.

Concentration	Host plant		
	Potato	Tomato	Eggplant
1	11578	13800	6767
1/4	10400	11783	4765
1/16	7827	10333	2321
1/64	4662	7059	918
1/256	3068	4313	391
Root weights (g. oven dried)	2.49	2.24	1.39

Appendix Table 72A.

Analysis of variance for data of Fig.28.

A. Tomato.

	Sources	D.F.	S.S.	M.S.	F.
a	Variance attributable to regression	1	56164260	56164260	a/b 206.833 ***
b	Residual	4	1086179	271544	
c	Total	5	57250439		

B. Potato.

	Sources	D.F.	S.S.	M.S.	F.
a	Variance attributable to regression	1	51792656	51792656	a/b 197.12 ***
b	Residual	4	1050980	262745	
c	Total	5	52843636		

C. Eggplant.

	Sources	D.F.	S.S.	M.S.	F.
a	Variance attributable to regression	1	27552680	27552680	a/b 81.62 ***
b	Residual	4	1350231	337557	
c	Total	5	28902911		

Appendix Table 73A.

Results of the final density assay (population build-up) of H.rostochiensis (Solanum nigrum type) with different hosts.

Replication	Number of cysts per 200 grammes of soil							
	Potato	Tomato	Eggplant v.Dolmei	Eggplant v.Long purple	Solanum nigrum Rothamsted	Solanum minuta	Solanum nigrum Germany	Tobacco
1	1215	328	246	116	119	92	132	37
2	1328	436	204	173	128	110	65	41
3	1310	402	197	149	112	107	98	41
4	1157	427	189	147	133	105	101	38
5	1198	410	148	156	126	104	71	35
Mean	1241	401	197	148	124	104	93	35

Appendix Table 74A

Number of eggs and larvae per 100 cysts obtained from infested soil with H.rostochiensis (Solanum nigrum type) and planted with different hosts.

Initial infestation.

Replicates	Potato	Tomato	Eggplant v.Dolmei	Eggplant v.Long purple	S.nigrum Rothamsted	S.minuta	S.nigrum Germany
1	25900	16600	10050	10075	20500	15300	19200
2	28925	17550	9675	6150	15850	9200	10850

Initial infestation 48 cysts per 200g. of soil

210 larvae per cyst

50 larvae/g. of soil.

Appendix Table 77A.

Analysis of variance the number of cysts with different host plants (Experiment No.23).

	Items	D.F.	S.S.	M.S.	F.
a	Host plants	6	5183146	863857	a/b 617.26 ***
b	Error	28	39186	1399.5	
c	Total	34	5222332		

Total number of cysts in descending order of magnitude, differences and critical difference.

Host plant	Totals	Differences
Potato	6208	
		4205 *
Tomato	2003	
		1019 *
Eggplant Dolmei	984	
		243 n.s.
Eggplant Long purple	741	
		123 n.s.
S.nigrum English	618	
		100 n.s.
S.minuta	518	
		51 n.s.
S.nigrum German	467	

Critical differences = 638

Appendix Table 82A.

The effect of initial infestation (57 eggs and larvae per g. of soil) of the potato root eelworm on the resultant weights of tomato fruits and heights of haulms. (Pot experiments 1960).

Doses and Fumigant	Total weight of tomato fruits g.			Maximum height of haulms after 60 days. Cm.			
	E.D.B.	Nemagon	C.B.P.	E.D.B.	Nemagon	C.B.P.	
0.032 ml.	322	757	682	105	136	142	
"	520	651	715	129	136	129	
0.045 ml.	716	829	738	129	158	121	
"	795	866	663	157	125	131	
0.064 ml.	845	783	866	137	125	126	
"	684	595	781	144	149	144	
0.089 ml.	801	682	686	143	133	150	
"	856	761	707	146	153	145	
0.128 ml.	638	873	825	149	130	152	
"	734	752	726	138	142	148	
Control		531			121		
"		552			127		

Appendix Table 83A.

Analysis of variance of the weight of tomato fruits in pot experiments (1960) using three fumigants at different rates. Log. (weight of the tomatoes). Data of Table 82A.

	Items	D.F.	S.S.	M.S.	F.
a	Nematicides	2	0.0121	0.0060	a/c < 1 n.s.
b	Doses	5	0.1331	0.0266	b/c 2.955 *
c	Interaction	10	0.0864	0.0086	
d	Treatment	17	0.2316	0.1362	
e	Residual	18	0.0163	0.0090	
f	Total	35	0.2479		

Treatment totals, differences and critical differences.

Doses applied:

Treatment	Totals	Differences
0.045 ml.	17.301	
0.128 ml.	17.265	
0.064 ml.	17.262	
0.089 ml.	17.236	
0.032 ml.	16.605	0.696 *
Control	16.401	0.900 *

Critical difference = 0.691

Appendix Table 84A.

Analysis of variance of the height of tomato plants after 60 days of growth. Pot experiments 1960. Log. (height in cm.). Data of Table 82A.

	Items	D.F.	S.S.	M.S.	F.
a	Nematicides	2	0.0001	0.00005	a/e < 1 n.s.
b	Doses	5	0.0200	0.0040	b/e 3.33 *
c	Interaction	10	0.01178	0.0011	
d	Treatment	17	0.03188		
e	Residual	18	0.02309	0.001283	
f	Total	35	0.05497		

Treatment totals, differences and critical difference.

Doses applied to the soil:

Treatment		Totals	Differences	
0.089 ml.	c	12.965		
			0.034	
0.128 ml.		12.931		
			0.108	
0.064 ml.		12.823		
			0.020	
0.045 ml.		12.803		
			0.140	
0.032 ml.		12.663		0.302 *
			0.102	
Control		12.561		0.404 *

Critical difference = 0.252

Nematologica 6 (1961): 123-129. Leiden, E. J. Brill

ON THE EFFECTS OF ROOT DIFFUSATES FROM *TAGETES* SPP. ON *HETERODERA ROSTOCHIENSIS* WOLL.

BY

A. M. OMIDVAR [1])

Imperial College Field Station, Silwood Park, Sunninghill, Ascot, Berks, England

The hatching stimulus or nematicidal effect of *Tagetes* root diffusates on potato-root eelworm was investigated.

Root diffusates of potato, tomato and *Solanum melongena* were also used. Plain water was used as a control. Neither hatching power nor nematicidal effects were detected from three species of *Tagetes* root diffusates.

Analysis of covariance suggests that the biological differences between active root diffusates from different sources are most clearly revealed when counts of emerged larvae are expressed in terms of the weight of oven-dried roots. *S. melongena* root diffusate showed a weaker stimulatory power than those of potato and tomato.

Observations by Steiner (1941) and Oostenbrink, Kuiper & 's Jacob (1957) have shown that the cultivation of *Tagetes* appears to suppress the population of some nematodes. It has also been claimed that a population of *Heterodera rostochiensis* is reduced by as much as 80% in the presence of *Tagetes minuta* (Henry Doubleday Research Association 1960 [2])). Although it has been shown that the juice obtained from plants of *Tagetes nana* was ineffective in vitro against cysts of the potato-root eelworm, a preparation extracted from this plant had a high nematicidal activity against several nematodes, including *Heterodera rostochiensis* larvae (Uhlenbroek & Bijloo 1958).

The reduction of the population of *Heterodera rostochiensis* in the presence of *Tagetes* may be due either to a hatching stimulus present in the roots of the plant, inducing the larvae to hatch and die out, or to a nematicidal effect. The present paper records an investigation into cysts exposed to graded dilutions of root diffusates extracted from three species of *Tagetes* and three known hosts: potato (*Solanum tuberosum* L.), tomato (*Lycopersicon esculentum* L.) and egg-plant (*Solanum melongena* L.).

MATERIALS AND METHODS

In all experiments, cysts were set up in batches of one hundred in solid watch glasses and presoaked for a week in an incubator at 25°C. The water was then removed and replaced by the appropriate sample of root diffusate. To minimize errors due to the variability of the cysts, duplicated batches of 100 pot-raised cysts (cysts of known age) and of similar size (599-422 μ) were used in each treatment.

---

[1]) At present on study leave trom the University of Agriculture, Tehran, Iran.

[2]) Interim report on the control of potato eelworm.

Root diffusate collection

Seeds of three species of *Tagetes* (*T. minuta, T. florida, T. signata*) and of eggplant (*Solanum melongena,* long purple) and tomato (Leader) were planted in seed boxes and then transferred into 5 cm diam. clay pots, with a non-sterilized soil mixture of three parts loam to one part sand. Small tubers of potato (Arran Banner) were also planted in 5 cm diam. clay pots. Five pots of each plant were placed on the concave grooves of a sloping asbestos sheet and root leachings were collected by the Fenwick method (1949), i.e. after saturating the soil with tap water, 40 ml of excess water were added, and collected in a jar at the end of the asbestos sheet.

Leaching was carried out twice a week for a fortnight. The entire collection of root diffusate from all the leachings was stored in bottles, and kept in a refrigerator at 5°C.

Because of the suggestion that the most active root diffusate is produced in the early stages of growth (Fenwick 1941), the plants chosen for root diffusate were about 6-10 weeks old, and leachings were collected only 4 times at 3 day intervals.

Weights of haulms and roots

To relate the efficacy of the leachings of each plant to the weight of the haulms and the weight of the roots as well as the age of the plants, after taking the fourth root diffusate the haulms of the plants were removed and the roots were carefully washed and weighed. Then both were placed in an oven at 105°C and dried to constant weight. The results are shown in Table I.

TABLE I

Plant weights per 5 plants (gm)

Plant	Age when lifted	Fresh		Oven dried	
		Roots	Haulms	Roots	Haulms
Eggplant	16 weeks	17.500	81.100	1.390	8.200
Potato	6 weeks	26.120	65.100	2.490	7.185
Tomato	10 weeks	32.500	109.000	2.240	9.750
T. minuta	9 weeks	27.550	24.610	2.220	4.275
T. signata	8 weeks	10.320	23.700	0.870	2.720

Thus, the numbers of larvae hatching in root diffusate in a given time have been expressed in three ways: (a) the actual number counted, (b) the number per g of fresh root and (c) the number per g of dry root.

Setting up: sub-culturing

Collected root diffusates were set up in a graded geometric series of dilutions, the dilutions being 1/1, 1/10 and 1/100.

Duplicate batches of 100 cysts were exposed to the action of each dilution.

The larval emergence for each dilution was estimated every 8 days, up to a period of 48 days at which time emerging larvae in all dilutions were not greater than in tap water. Hatching tests were carried out at 25°C constant temperature. Tap water was used as a control. The results of the tests are expressed as total numbers of liberated larvae per 200 cysts and are shown in Table II.

TABLE II

The number of emerged larvae per 200 cysts from six root diffusates and water

Plant	Number of emerged larvae from 200 cysts		
	Concentration 1/1	Concentration 1/10	Concentration 1/100
Tagetes minuta	829	954	1041
Tagetes signata	735	1075	873
Tagetes florida	1159	777	958
Water	1079	991	808
Eggplant	20526	10506	3113
Potato	54475	36119	19517
Tomato	60038	43743	24343

RESULTS

Hatching power of Tagetes *plants*

An analysis of variance of the *Tagetes* data showed that there was no significant difference between the diffusates of the different species and plain water; but there was a possibility that owing to nematicidal effect, some larvae of the cysts exposed to the root diffusate of *Tagetes* plants were killed, and survivors which hatched happened to be equal to the hatch in water.

To deal with this possibility the batches of 100 cysts remaining after exposure to the leachings of *Tagetes minuta* and water were placed in potato root diffusate. The emerging larvae in each treatment were counted at 8 day intervals. The results are shown in Table III: there was no evidence of delayed hatching and no trace of nematicidal effect.

TABLE III

The number of emerged larvae from batches of 200 cysts already exposed to Tagetes minuta *root diffusate and water for 48 days and then immersed in ordinary root diffusate*

Root Diffusate	Concentrations	Total hatch in ordinary R.D.
Tagetes minuta	1/1	43589
	1/10	43334
	1/100	45141
Tap water	...	43754

Hatching power of the different hosts

Table IV shows the analysis of hatching data in response to the three active diffusates, using a logarithmic transformation. Similar analyses have also been

TABLE IV

Analysis of variance for data of Table II. Tomato, Potato, Eggplant (uncorrected). Log (emerged larvae)

Sources	Degrees of freedom	Sum of Squares	Mean Squares	Variance Ratio
a Concentrations	2	0.950	0.475	
				a/c 12.8*
b Varieties	2	1.518	0.759	
				b/c 20.5**
c Interaction	4	0.148	0.037	
				c/d 10.5*
d Error	9	0.032	0.0035	
e Total	17	2.648		

* = Significant at the 5% level of probability
** = Significant at the 1% level of probability

made for the same counts expressed per gramme of fresh roots and per gramme of dry roots. From these three analyses (the antilog of the treatment means being geometric means) the data of Table V have been computed. These data are presented graphically in Fig. 1 (1-3), which follows the technique of Fenwick (1952) with the difference that the dilution ratio was 10 instead of 4. The values of *b* shown are the decrease in larvae emerging from 100 cysts for each 10-fold increase in dilution.

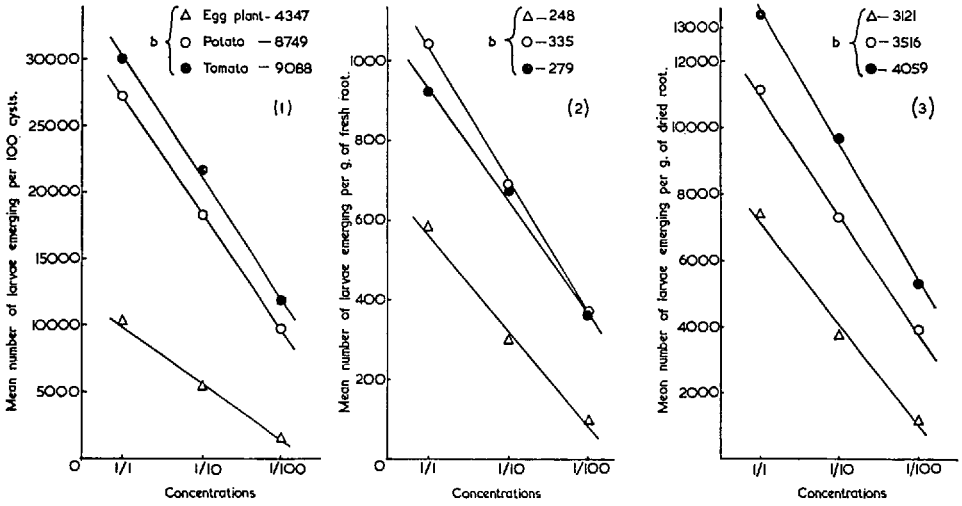


Fig. 1. Emergence of larvae from batches of 100 cysts, (1) uncorrected data, (2) per g of fresh root, (3) per g of dried root.

TABLE V

Number of emerged larvae (Geometric means)

	Plant	Uncorrected data		Data per g fresh root		Data per g oven dried root	
		Geometric Means	Ratio of Means	Geometric Means	Ratio of Means	Geometric Means	Ratio of Means
I	Tomato	19770		609		8831	
			I/II				
			1.175$^{n.s.}$		0.946$^{n.s.}$		1.306*
II	Potato	16827		644		6761	
			II/III				
			3.855*		2.586*		2.152*
III	Eggplant	4395		249		3141	
Concentration							
IV	1/1	20777		826		10257	
			IV/V				
			1.596*		1.601*		1.595*
V	1/10	17706		516		6427	
			V/VI				
			2.254*		2.243*		2.254*
VI	1/100	5636		230		2851	
		Critical ratio 1.194		Critical ratio 1.192		Critical ratio 1.194	

$^{n.s.}$ = not significant
* = significant at the 5 % level of probability

DISCUSSION

The three sets of data: (I) uncorrected hatched larvae for each root diffusate (Table IV and V), (II) the number of hatched larvae per gramme of fresh root and (III) per gramme of oven dried root, were analysed, results being shown in Table V. In uncorrected counts and counts per g of fresh root, there was no significant difference between the means for tomato and potato root diffusates but there was a significant difference between those of potato and eggplant. In counts per g of dried root the differences between tomato, potato and eggplant were all significant. The slope of each line was calculated and compared in three ways (Fig. 1).

The slopes of the three lines for dried root counts do not differ significantly, whereas for the uncorrected counts there is a strong suggestion of a shallower slope for eggplant diffusate. Thus, the dried root counts are more clearly in agreement with the hypothesis put forward by Fenwick (1952), namely, that root dif-

fusates from different sources give parallel slopes. It is also interesting that sensibly straight lines, as found by Fenwick, are given by all the present data.

Thus, in comparing diffusates from different sources there would seem to be a case for expressing counts of emerged larvae in terms of the weight of oven dried roots.

Fassuliotis & Feldmesser (1954) reported that the rates of hatching in root diffusates of eggplant and potato did not differ significantly. But after many different experiments some of which have been described in this paper, I am unable to confirm this result. Possibly it was due to using different strains of *Heterodera rostochiensis* cysts. Other experiments with another strain of cysts have revealed that in three kinds of assays (hatching test, penetration of larvae into the root and final cysts density assay) the eggplant was a host inferior to tomato and potato, and this is confirmed by the present experiment.

Uhlenbroek & Bijloo (1958) reported that some highly nematicidal polythienyls have been isolated from the roots of *Tagetes* plants and one of the compounds was shown to be α-terthienyl. After long extraction and purification processes only 0.2 g of pure α-terthienyl was obtained from 24 kg of *Tagetes* roots. This suggests that the amount of nematicide present in *Tagetes* roots is so small that in short-term pot experiments it is negligible. There is also other evidence (Oostenbrink, Kuiper & s'Jacob 1957) that cultivation of *Tagetes* for a short period either in spring or autumn had no effect on reduction of the nematodes present in the soil, whereas a period of 3-4 months cultivation was effective. Thus it may be that the very small production of nematicide requires a long period of time in order to reveal itself.

The author thanks Professor B. G. Peters for his advice and encouragement. Thanks are also due to Dr. N. Hague for many suggestions.

RÉSUMÉ

Effets des secrétions radiculaires de Tagetes *spp. sur* Heterodera rostochiensis *Woll.*

Differentes expériences ont été entreprises afin d'étudier l'action stimulante ou l'effet nématocide de la secrétion radiculaire du genre *Tagetes* sur les nématodes dorés de la pomme de terre.

Les secrétions radiculaires de trois hôtes ont été utilisées. L'eau pure a été utilisée comme témoin. Les secrétions radiculaires de trois espèces de *Tagetes* ne possèdent ni le pouvoir de faire éclore les larves ni des effets nématocides. L'analyse de covariance semble montrer que les différences biologiques entre les secrétions actives obtenues de différentes sources sont clairement démontrées quand le nombre de larves écloses est exprimé en fonction du poids des racines séchées à 105° C. Des secrétions radiculaires d'aubergine ont montré une plus faible stimulation que celles de la pomme de terre et de la tomate.

REFERENCES

FASSULIOTIS, G. B. & FELDMESSER, J. (1954). Infection of eggplant, *Solanum melongena,* by the Golden nematode of potatoes *Heterodera rostochiensis. Plant Dis. Reptr* **38**, 791-793.

FENWICK, D. W. (1949). Investigations on the emergence of larvae from the cysts of the potato root eelworm *Heterodera rostochiensis* (Woll.) I. *J. Helm.* **23**, 157-170.

—— (1952). The bio-assay of potato root diffusate. *Ann. appl. Biol.* **39**, 457-467.

FENWICK, D. W. & WIDDOWSON, E. (1958). The conduct of hatching tests on cysts of the potato root eelworm *Heterodera rostochiensis* (Woll.) *J. Helm.* **32**, 125-134.

OOSTENBRINK, M., KUIPER, K. & s'JACOB, J. J. (1957). *Tagetes* als Feindpflanzen von *Pratylenchus* Arten. *Nematologica* **2**, 424-433.

UHLENBROEK, J. H. & BIJLOO, J. D. (1958). Investigations on nematicides. I. Isolation and structure of a nematicidal principle occurring in Tagetes roots. *Rec. Trav. chim. Pays-Bas.* **77**, 1004-1009.

—— & —— (1959). Investigations on nematicides. II. Structure of a second nematicidal principle isolated from Tagetes roots. *Rec. Trav. chim. Pays-Bas.* **78**, 382-390.

Received for publication: December 23, 1960