POPULATION STUDIES ON SOIL AND PLANT NEMATODES IN RELATION TO THE APPLICATION OF SOIL STERILANTS.

by

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Abstract

Population studies on nematodes in untreated plots under monocultured potatoes showed seasonal fluctuation in numbers of plant parasitic nematodes and no distinct variation in the free living particulate feeders. Pratylenchus populations showed maxima in late summer, autumn and spring and minima in winter and summer. There were no distinct seasonal fluctuations in Paratylenchus spp. Crop rotation had a marked effect on Pratylenchus spp. and Paratylenchus spp. Vegetation had a pronounced influence on the depth distribution of plant parasitic nematodes, and variation in depth distribution associated with seasonal changes was clearly demonstrated. The effect of herbicide treatments on soil and plant nematodes was investigated.

The repeated applications of fumigants; Ethylene dibromide, Chloropicrin and Dazomet, did not affect the free living nematode populations, but the plant parasitic nematodes especially Pratylenchus spp. showed a very poor recovery. Paratylenchus populations rapidly increased following Ethylene dibromide treatments. Thionazin (inrow and broadcast applications) were ineffective in controlling soil nematodes. Pot experiments using very high doses of thionazin effectively controlled both soil and root inhabiting nematodes.

Studies on some aspects of the morphology and taxonomy of the genus Pratylenchus established the usefulness of the oesophageal overlap as a diagnostic feature for species differentiation. Rate of increase and subsequent reproduction of Pratylenchus spp. was demonstrated. Pratylenchus fallux completed its life cycle in 40-45 days. Seinhorst's mistifier was the most efficient method of extracting Pratylenchus spp. from plant roots.

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Population studies of free living and plant parasitic nematodes in relation to the application of soil sterilants.

General Introduction

The rapidly growing awareness of nematodes as plant pests has prompted the use of several regulatory measures. The oldest and perhaps most widely used method of field control of nematodes is crop rotation. This is primarily used when the nematode pest has a limited host range (e.g. Heterodera and Meloidogyne).

The production of plant varieties resistant to or tolerant of nematode attack is also used to combat nematode disease.

Chemical control of nematodes and other soil borne pests and diseases by the application of pesticides to the soil is an established practice in high value crops.

The present investigation includes observations on the effectiveness of crop rotation and chemical methods in controlling plant and soil nematodes. The work was carried out as a part of a study on the long term effects of pesticides on Heterodera rostochiensis on potato. Field trials consisted of the use of four pesticides; Chloropicrin, Dazomet, Ethylene dibromide and thionazin applied at three dosage rates each (0, low and high). Two crop regimes were investigated, monocultured potatoes and a three course rotation of potatoes, field beans and barley.

In most of the work involving nematicide treatments, the primary objective has been to determine the immediate effect of the nematicide. Most of these reports fail to indicate any subsequent sampling or other investigations to determine the influence that treatments have

had on the future nematode populations.

The long term effect of nematicides on cyst forming nematodes is relatively easily evaluated by examining cysts at the end of the growing season. With non cyst formers, the absence of nematodes from the soil soon after treatment does not necessarily mean that the nematodes have been killed. A complete knowledge of the life cycle under field conditions is therefore necessary to draw conclusions about the effectiveness of the treatment.

The primary objective of the present investigation was to study the influence of pesticide treatment on the population dynamics of free living and migratory plant parasitic nematodes.

A number of workers have reported population fluctuations of soil and plant nematodes and these changes are of relevance to the present work in that they deal with changes occurring without the additional effect of the presence of nematicides.

Micoletzky (1922) was the first to present evidence of seasonal fluctuations in nematode populations. He found the number of nematodes to be highest in autumn and the lowest in late winter, rising again in the spring. These changes were attributed to seasonal changes in temperature, moisture and plant growth.

Siedenschwarz (1923) concluded that nematode numbers in the soil were very low in winter, with a steady increase in February, up to a maximum in August. Burkhalter (1928) found that nematode numbers were low in May and increased to high numbers in July. This high level was maintained until October.

Nielsen (1949) however concluded that there was very little seasonal fluctuation in nematode numbers in the mineral soils that he investigated in Denmark.

Data on seasonal variations in numbers of migratory plant feeding nematodes are mainly of recent origin. Several workers have demonstrated seasonal fluctuations in populations of Pratylenchus spp. including P.zeae on tobacco and corn (Graham, 1951), Pratylenchus spp. on cultivated brambles (Goheen and Williams, 1955) and P.coffeae on strawberries (Riggs, Slack and Faulton, 1956).

Miller et al (1962) carried out an extensive study on population trends of adult Pratylenchus spp. in corn roots as well as those in the rhizosphere and non rhizosphere. They found two distinct peaks in the population of Pratylenchus in corn roots, in early July and early September. The population in the rhizosphere varied inversely with that in the roots whilst the non rhizosphere populations remained constant and low.

Edmunds et al (1967) found that populations of Pratylenchus spp. reached highest levels in corn roots in mid July and mid September, these increases were more pronounced for larval populations. The adult populations in the rhizosphere tended to drop early in the season while those in the roots increased. Towards the end of the season, however, when about 65% of the roots had rotted the populations in the rhizosphere had increased.

Wehunt (1957) related seasonal population trends of Pratylenchus spp. and Tylenchorhynchus spp. to growth of white clover, where maximum clover yields and highest nematode populations occurred from January to June. Fewer P. penetrans were found in winter in a fruit tree nursery in Germany (Decker, 1960) and around strawberry roots in Jersey (Di Edwardo, 1961). Lownsberry (1961) suggests that soil moisture fluctuations may be responsible for populations of Criconemoides xenoplax decreasing in some Californian orchards during summer. Winslow (1964) reported that soil populations of Tylenchida showed strong seasonal fluctuations with minima in May, June and July and maxima in late summer or autumn. Banage (1966) found seasonal fluctuations of nematodes in peaty soils but not in mineral soils. Wolff Shoemaker (1968) discusses the population fluctuations of several Tylenchida in relation to soil type in tropical climates where temperature changes are not pronounced.

The thesis reports, therefore, the following studies:

1. A study of the seasonal fluctuation of nematode numbers in relation to crop growth (monoculture and rotation).
2. (A) The influence of depth on the distribution of nematodes.
   (B) Population variations associated with herbidide applications.
3. Population dynamics in relation to pesticide application.
4. Of the non cyst forming plant parasitic nematodes, Pratylenchus spp. and Paratylenchus spp. occurred in the largest numbers. The difficulties encountered in the identification of Pratylenchus spp. led to a detailed study on the taxonomy and morphology of the group. This was followed by biological studies on Pratylenchus spp. under laboratory and green house conditions.

PART 1. INFLUENCE OF CULTIVATION AND THE APPLICATION OF PESTICIDES ON THE POPULATION DYNAMICS OF FREE LIVING AND PLANT PARASITIC NEMATODES.

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Experimental

Sites and design

One randomized block containing 24 plots was established on Church field, Silwood Park, in April, 1964. Each plot was made to measure 12yds. x 6yds. with three foot wide pathways separating the plots. In Spring, 1967, four plots were added to include thionazin broadcast treatments at low and high dosage rates.

For vertical distribution studies, a permanent pasture with apple trees was selected for comparison with the arable land.

Soil treatments

The plots were cultivated at regular intervals under two agronomic regimes; monocultured potatoes and a three course rotation consisting of potatoes, field beans and barley.

Pesticide treatments were given first, before a potato crop, i.e. fumigants applied in the autumn preceding a potato crop and thionazin in the following Spring immediately before planting. It follows that, since the monoculture plots were treated every year, the rotation plots were treated every third year.

Sampling

Field sampling was done from October, 1966 to June, 1968. One method of estimation was used throughout the investigation, the sampling tool being a steel auger, one inch in diameter.

Six four inch deep soil cones were taken at random from each plot, bulked together and mixed thoroughly, two 100 ml. subsamples were extracted using a modified Baermann funnel (Whitehead's Trays - Whitehead and Hemming, 1965). The resulting nematode suspension was concentrated to 50 ml. by decanting, and two 5 ml. samples were drawn into shallow counting trays and the nematodes examined and counted under a stereomicroscope. Replication was fourfold.

For vertical distribution studies, six 24 cm. deep soil cores were taken at random and each core was subdivided into six, four cm. length portions. The corresponding portions from each core were then bulked together and extracted as previously.

The nematode genera were identified using Goodey's (1963) classification.

For quantitative estimations, the nematode genera were grouped as in Table 1.

Table 1.

Free living (Particulate feeders)	Rhabditida Aerolaimida Monohysterida Enoplida
Dorylaimida	Dorylaimoidea Mononchidea Diphtherophoridea
Tylenchus group	Tylenchus Psilenchus Ditylenchus Neotylenchidae

Easily identifiable Tylenchida:-

Paratylenchus
Pratylenchus
Aphelenchus
Aphelenchoides
Tylenchorhynchus
Helicotylenchus
Rotylenchus
Heterodera (larvae)

SECTION I

A Seasonal distribution of nematodes in untreated plots under monoculture

These estimates were made on an arable field planted with potatoes. The soil is a light sandy loam with weeds common to the soil type (see Appendix table 1). The population estimates were made over a period of 20 months (October 1966 - June 1968). The relative abundance of both larvae and adults was determined separately for most of the genera from August 1967 - June 1968.

Results and Conclusions

The list of nematode genera recovered (shown in Appendix Table 2) is by no means comprehensive. Of the 44 genera recovered, the Neotylenchidae, Rotylenchus (arable fields), Criconemoides and several particulate feeders; Acrobeles, Monohystera, Prismatolaimus and Tripyla were found in very low numbers. The number of Dorylaimida recovered was also relatively low.

The recovery of certain genera is dependent on the method of extraction. The efficiency of the Whitehead Tray method depends on the mobility of nematodes and thus recovery of the slow moving, sluggish nematodes, e.g. Criconemoides and some Dorylaimida is poor. The larger Dorylaimida tend to float on the surface of the soil extract and are lost during decantation. For the best results a combination of several extraction methods would be required. This was not within the scope of this investigation.

The genera found in the permanent pasture were the same, but speciation was different.

Seasonal distribution of nematodes in the monoculture plots

Fig. 1 shows the monthly recovery of nematodes from 100 ml. of soil. The results are a mean of 16 replicates. There is a closely marked seasonal fluctuation in the number of free living and plant parasitic nematodes. Although the pattern of this fluctuation was similar in both groups there was a greater overall increase in the free living nematodes.

The annual curves obtained during this investigation can only apply to the particular conditions of the experiment. After a comparatively quiescent period in winter, the nematode activity increases in the spring. The subsequent rise in numbers is mainly a result of larval emergence from eggs as the temperature increases. The fall in numbers in April is possibly caused by soil disturbances i.e. rotovation, which directly decreases the food supply by removing the plant cover, resulting in a poorer survival of the larvae. The increase in the plant parasitic nematodes in May (1967) and June (1968) is due to the hatch of Heterodera larvae. The summer reduction of the plant parasitic nematodes is a result of root invasion by endoparasitic nematodes and also by feeding of ectoparasitic nematodes. The late summer and autumn peak seems to be closely associated with harvesting, when the plant parasitic nematodes re-enter the soil, or are dislodged from the roots. The saprophytic forms increase in number following the increase in organic debris in the soil,

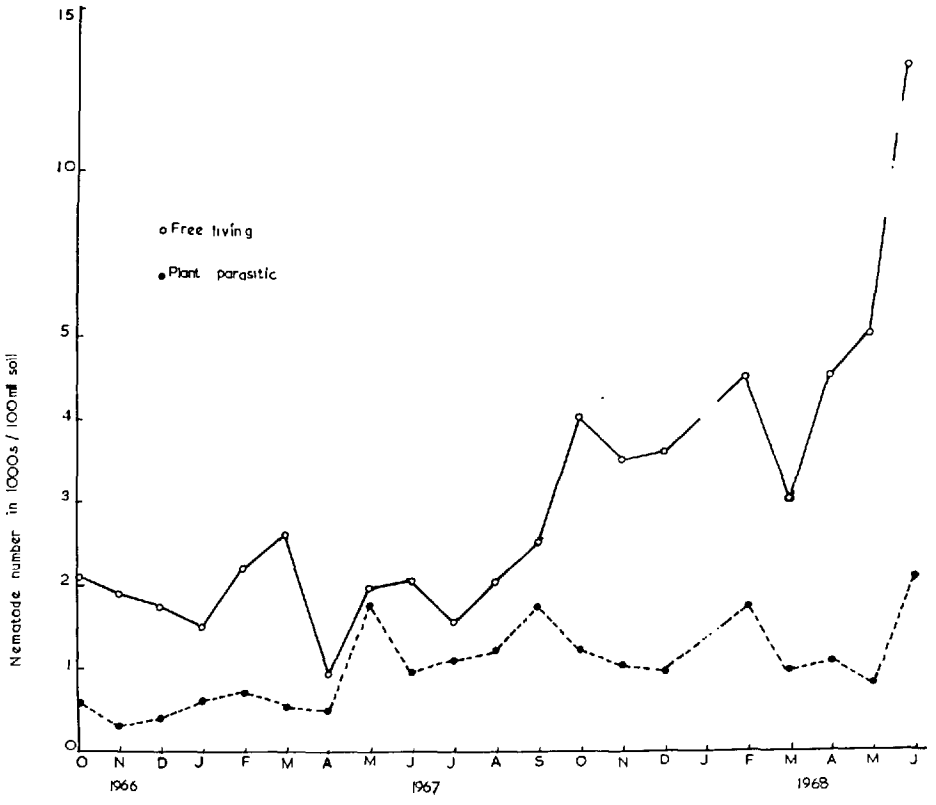

Fig1. Monthly recovery of soil nematodes in monoculture plots

The winter fall in numbers results from a poorer survival due to lowering of temperatures and lack of vegetation.

The seasonal variations of the plant parasitic nematodes were similar to those observed by Winslow (1964) for Tylenchida on light sandy soils, where the seasonal fluctuations showed a fall in numbers in May, June and July, and a rise in late summer or autumn.

Other investigators have reported different results. Micoletzky (1922) found that Eucephalobus elongatus increased steadily from October to April, while another common soil nematode Plectus granulosus decreased greatly over the same period. Seidenschwarz (1923) investigated the nematode fauna of an alpine meadow in the Austrian Tyrol, and found minima in winter with a steady increase from February to a peak in August, followed by a rapid decrease to very low numbers in November. Nielsen (1949) reported that seasonal variation in numbers of nematodes was very small in mineral soils in Denmark. Banage (1966) investigated some British upland and moor soils and reported a seasonal variation in the nematode numbers in peaty soils, (a rise in the nematode numbers in late summer and autumn, with a fall in winter) but no variations in the mineral soils.

The seasonal fluctuation in the population of separate nematode genera or groups.

Free living - Particulate feeders

The seasonal fluctuation in the population of free living particulate feeders is shown in Fig. 2 . There is no marked variation in the number of adults and larvae present, except in the spring. There is, however, an overall increase in numbers from October, 1967 until the end of the experimental period (June, 1968). The June (1968) rise in numbers follows the application of herbicides (in April and June), and is related to the higher levels of organic debris in the soil.

Free living - Fungal feeders

The fungal feeders Aphelenchus and Aphelenchoides did not show a wide seasonal fluctuation in population and are similar to the free living particulate feeders. Here again, the very high increase in June (1968) may be related to the higher organic debris in the soil following weeding and the application of a herbicide.

Dorylaimida

The recovery of Dorylaimida was somewhat irregular. Thus the results obtained (see Appendix table 5) may not be a true representation of the seasonal fluctuation in the population of this group.

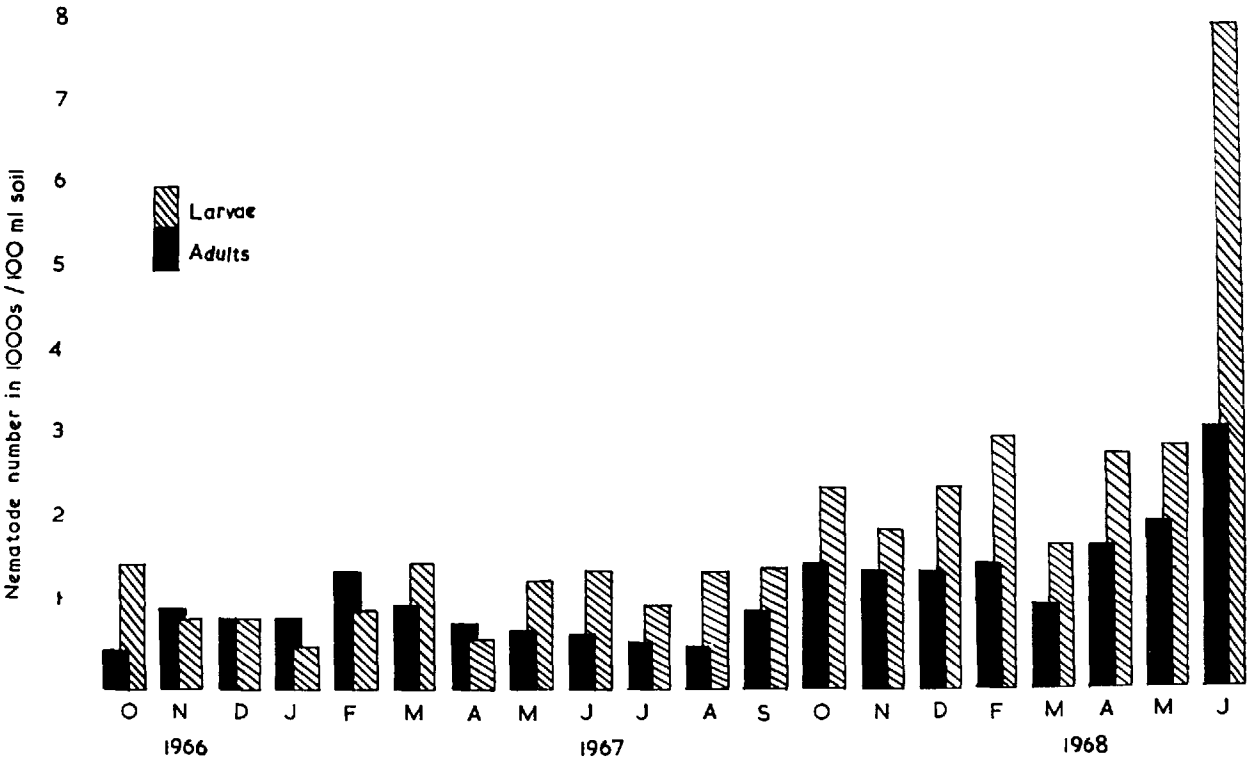

Fig. 2. Seasonal population fluctuations of free living particulate feeders in monoculture

Tylenchus group (Fig. 3)

There was no distinct seasonal variation in this group of nematodes. There was, however, an overall increase in numbers during the latter part of the investigation (August, 1967 - June, 1968), with very high numbers in June (the end of the experimental period).

Tylenchorhynchus spp. (Fig. 3)

The Tylenchorhynchus spp. remained very low during the first year of the investigation and did not show a seasonal fluctuation in numbers. The second year of investigation showed a distinct increase in numbers, exhibiting a seasonal variation. The February and June (1968) increases are largely due to a rise in the larval populations.

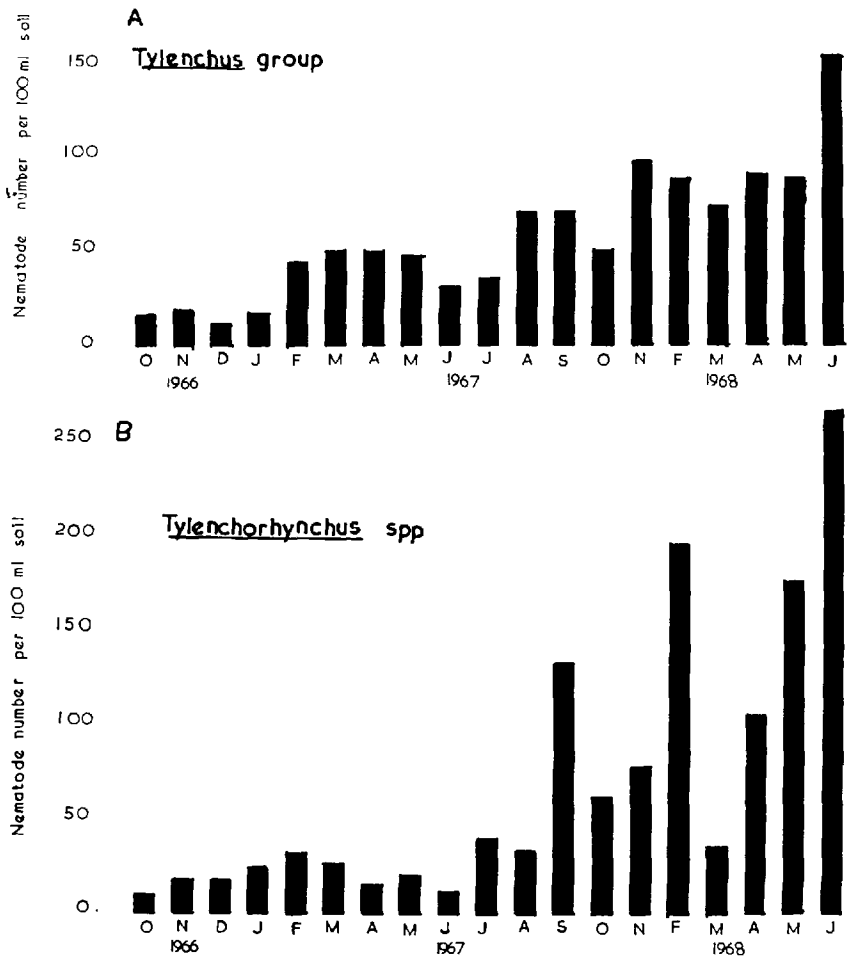

Fig.3. Seasonal population fluctuations of Tylenchus group & Tylenchorhynchus spp in monoculture plots

The seasonal fluctuation in the population of Pratylenchus spp.

Fig. 4 shows the population changes of Pratylenchus spp. under monocultured potatoes. The seasonal rhythm in numbers is similar for the two years investigated. The slight winter reduction results from a lowering of the larval population. The subsequent spring peak is associated with larval emergence, as a result of the temperature increase. The reduction in April is probably due to an inadequate food supply following soil rotovation. Rotovation removes the weeds which have served as a food supply, and the host crop, potatoes has not started to grow.

The low recovery in the summer months is clearly due to root invasion, since plant growth is at its maximum during this period. This is followed by a post harvest autumn increase in numbers, when lifting of the crops causes a liberation of nematodes into the soil. These results agree with those recorded by Goheen and Williams (1955), Wehunt (1957), Winslow (1964) and Di Edwardo (1961).

The unusual increase in June, 1968 may be due to the nematodes re-entering the soil following the application of herbicides and the subsequent death of the weed hosts.

Table 2 shows the larvae and adults observed over a period of eleven months. The post harvest results (October) show an increase in the proportion of adults. This remains the same until the spring when the populations showed a higher proportion of larvae. The proportion of larvae remained high through the summer months.

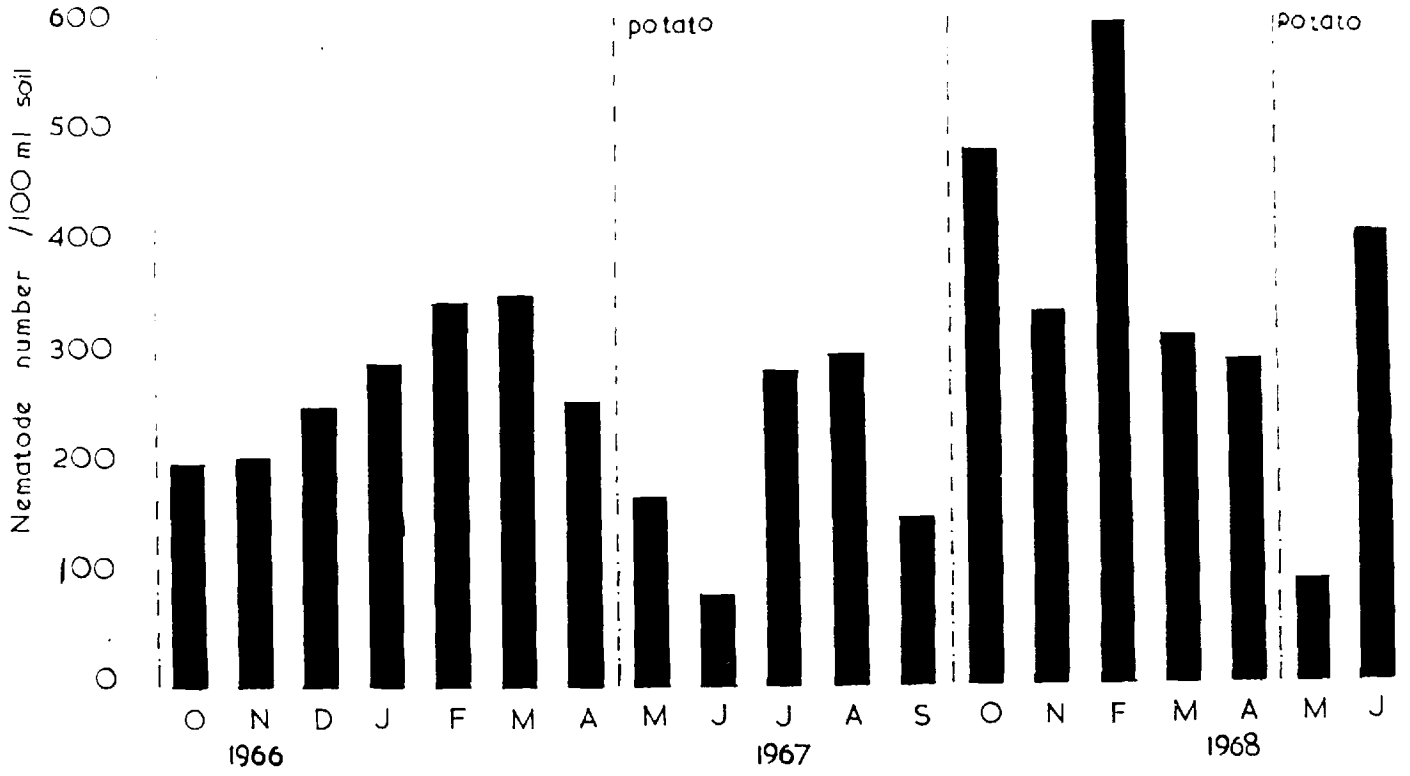

Fig.4. Seasonal fluctuation in population of Pratylenchus spp in monoculture

Table 2

Variation in the adult and larval populations of *Pratylenchus* spp. associated with monocultured potatoes

(Mean of 16 replicates)

Sampling time		Adults	Larvae
Year	Month		
1967	August	106	192
	September	56	68
	October	236	250
	November	153	151
1968	February	223	368
	March	43	265
	April	67	207
	May	60	63
	June	115	280

The seasonal fluctuation in the population of Paratylenchus

The distribution of Paratylenchus spp. was very variable (Fig. 5). The population level was very low initially, and remained somewhat low during the subsequent winter and spring. There was, however, a marked increase in numbers in the summer, reaching a peak at harvest time (September). The numbers remained high through the winter months reaching a very high peak in February (1968), showing the hatch of larvae. The decrease in numbers following rotovation is more marked with Paratylenchus than with Pratylenchus. The progressive increase in numbers shows that the continued growth of potato has a favourable influence on the increase of Paratylenchus populations. Table3 shows the number of adults and larvae recovered. There was a greater proportion of larvae recovered throughout the investigation. Most of the larvae recovered were either 4th stage larvae or pre-adults. These are known to resist dessication and low temperatures (Rhoades & Linford, 1961). This explains the prevelance of the 4th stage larvae in the soil, especially in winter.

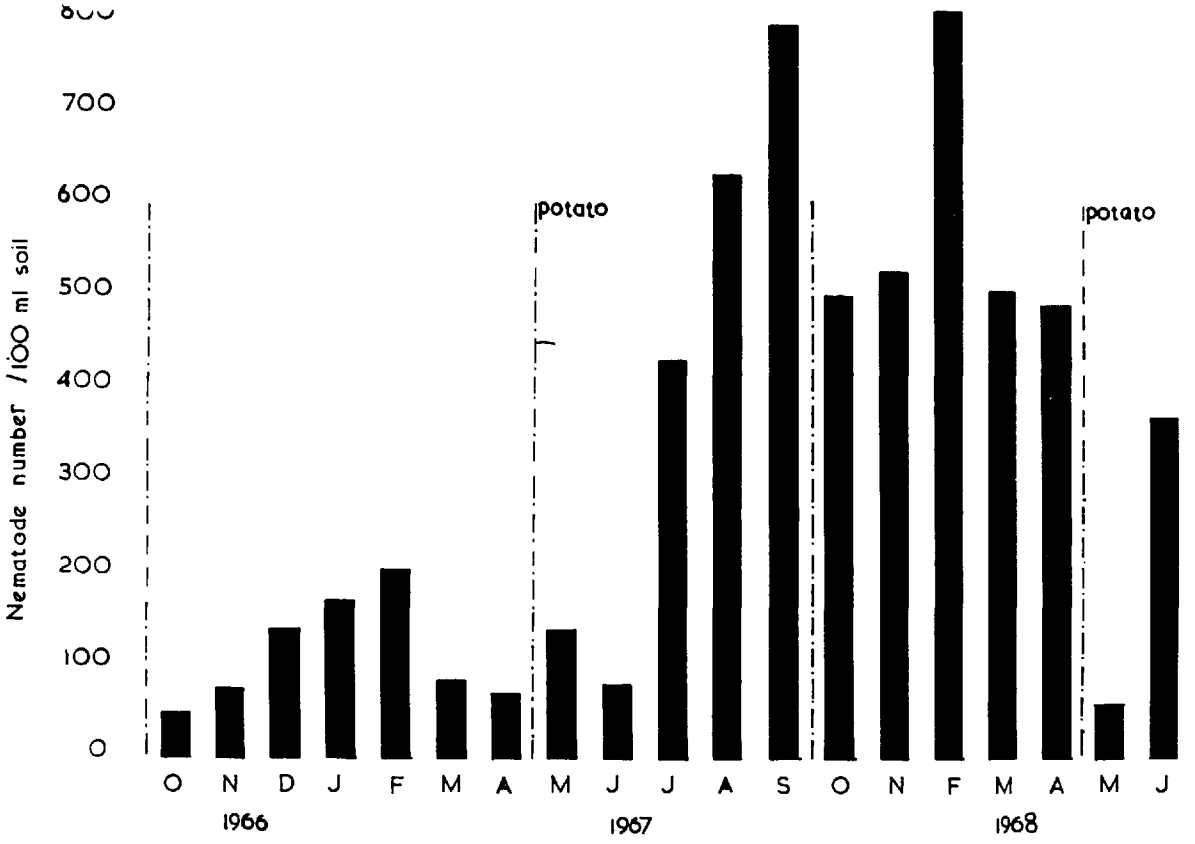

Fig.5. Seasonal population fluctuations of Paratylenchus spp in monoculture

Table 3

Variation in the adult and larval populations of *Paratylenchus* associated with monocultured potatoes

(Mean of 16 replicates)

Sampling time		Adults	Larvae
Year	Month		
1967	August	97	532
	September	177	617
	October	58	434
	November	37	467
1968	February	58	773
	March	83	417
	April	75	409
	May	13	40
	June	137	220

The low recovery of adults may be due to their sluggish nature which results in low rates of extraction through Whitehead trays, or the adults are not easily dislodged from the plant roots and are therefore present in lower numbers in the soil samples.

B The influence of crop rotation on free living and plant parasitic nematodes

The population fluctuation of soil nematodes is greatly influenced by crops. This influence has been convincingly demonstrated for several plant parasitic nematodes by Oostenbrink (1952, 1954, 1960 et al; 1956, 1957). Beet was found by these authors to suppress Pratylenchus penetrans, Tylenchorhynchus and Saprozoic nematodes, but leguminous crops favoured Pratylenchus. Ley increased Paratylenchus and Tylenchorhynchus populations and cereals were good hosts for Pratylenchus crenatus. Crop rotation has a limited influence in controlling populations of these migratory plant parasitic nematodes, since they are known to be polyphagous.

With cyst forming nematodes, the crop is of prime importance in the population growth, since these nematodes are highly specialized and have a limited host range. This influence has been manifested in the use of crop rotation for the control of Heterodera spp. and Meloidogyne spp. (Wilson 1962, 1963, 1964; s'Jacob 1960; Oostenbrink 1952; Ayala 1967 et al).

The present investigation was made with a view to studying the effect of crop rotation as a control measure in nematode infestations. The study was made over a period of 20 months on an arable field under two agronomic regimes; monocultured potatoes and a three-course rotation consisting of potatoes, field beans and barley.

Results

The influence of crop rotation on free living nematodes

Fig. 6 shows the seasonal fluctuation of free living nematode populations in monoculture and rotation plots. The seasonal rhythm in numbers was similar for the two regimes, resulting in an overall increase in the population at the end of the experimental period. Although the initial population levels were the same for both regimes, the subsequent increase in numbers in the rotation plots was higher than those of the monoculture plots.

The influence of crop rotation on plant parasitic nematodes

The results shown in Fig. 6 are somewhat reversed when compared with the observations made for the free living nematodes. The nematode population in the rotation plots remained the same at the end of the experimental period. Although the initial monoculture populations were lower than those of the rotation plots, the results show a steady increase in numbers reaching a level about 9 times the initial level. This increase is due to a rise in numbers, especially of *Paratylenchus* and *Heterodera* larvae.

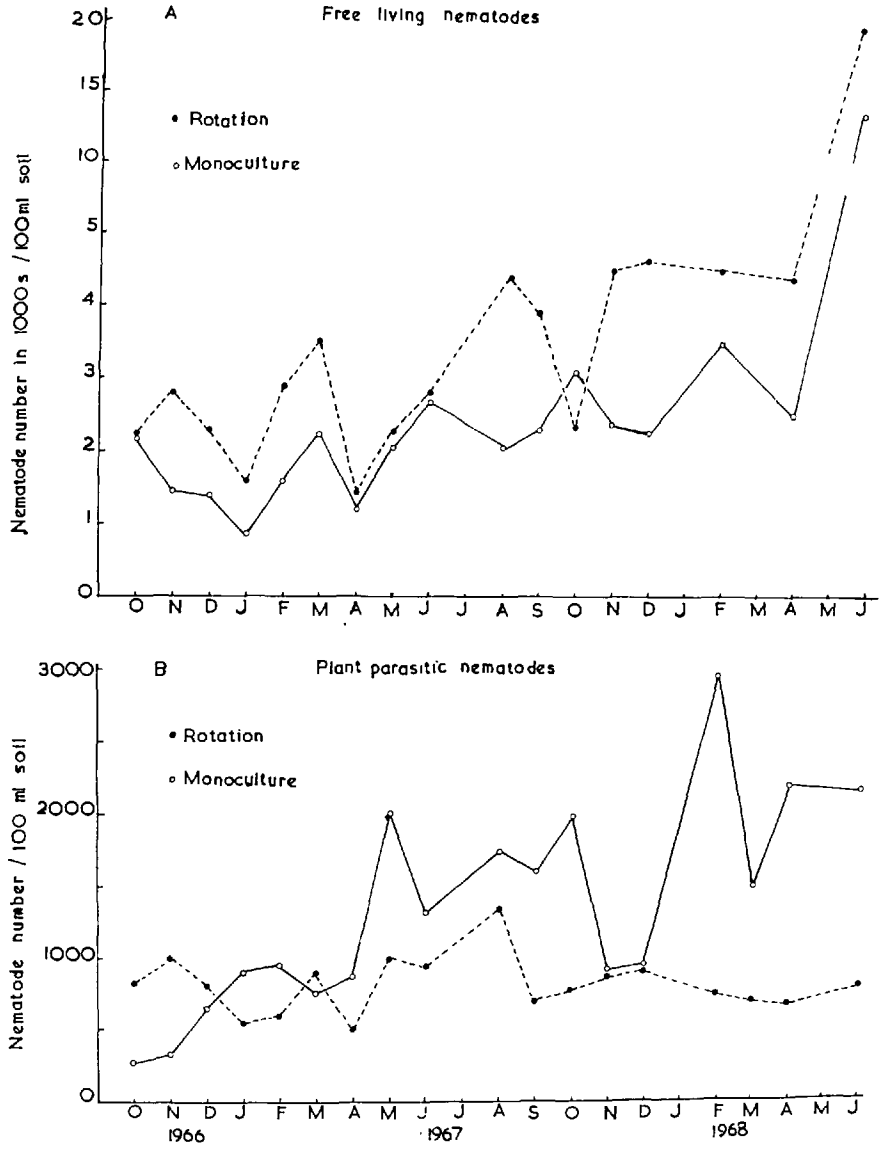

Fig.6. Seasonal population fluctuations of free living and plant parasitic nematodes in rotation and monoculture plots

Detailed analysis of the influence of crop rotation on some Tylenchida

The distribution of Pratylenchus spp.

Fig. 7 shows the seasonal variation in the population of Pratylenchus in the monoculture and rotation plots. The October, 1966 results in the monoculture plots show the numbers of nematodes following field beans in the rotation, and potato in the monoculture. The winter reduction in the population is more marked in the rotation plots. Although the nematode population was similar for both regimes during the Summer months, the post harvest results show a greater increase in numbers in the monoculture plots. The subsequent increase in numbers in the Spring was much higher in the monoculture than in the rotation plots.

The distribution of Paratylenchus spp.

Fig. 8 shows the seasonal variation in the Paratylenchus populations associated with monoculture and crop rotation. Although the population levels remained somewhat similar in both regimes during the Winter and Spring (1966), a wide fluctuation in numbers was observed during the Summer months. This was most marked in the rotation plots. The monoculture plots showed a regular build up of the Paratylenchus populations and the Spring (1968) results reflect a marked increase of 4th stage larvae in the soil.

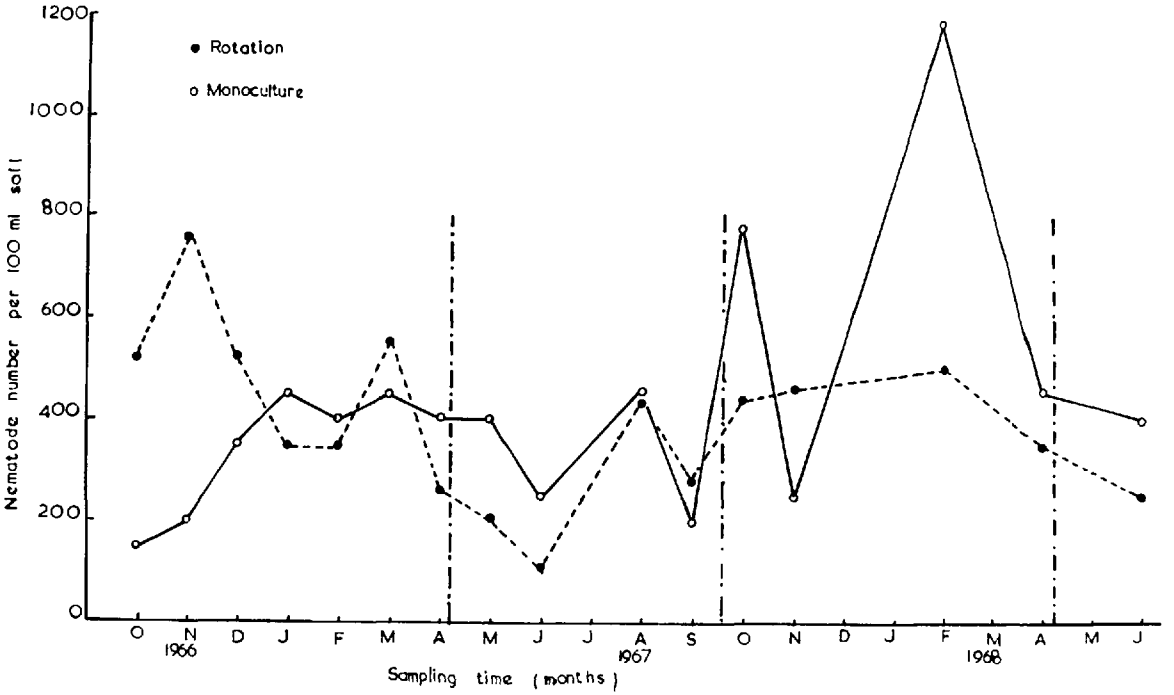

Fig .7. Seasonal variation in Pratylenchus populations in rotation and monoculture plots

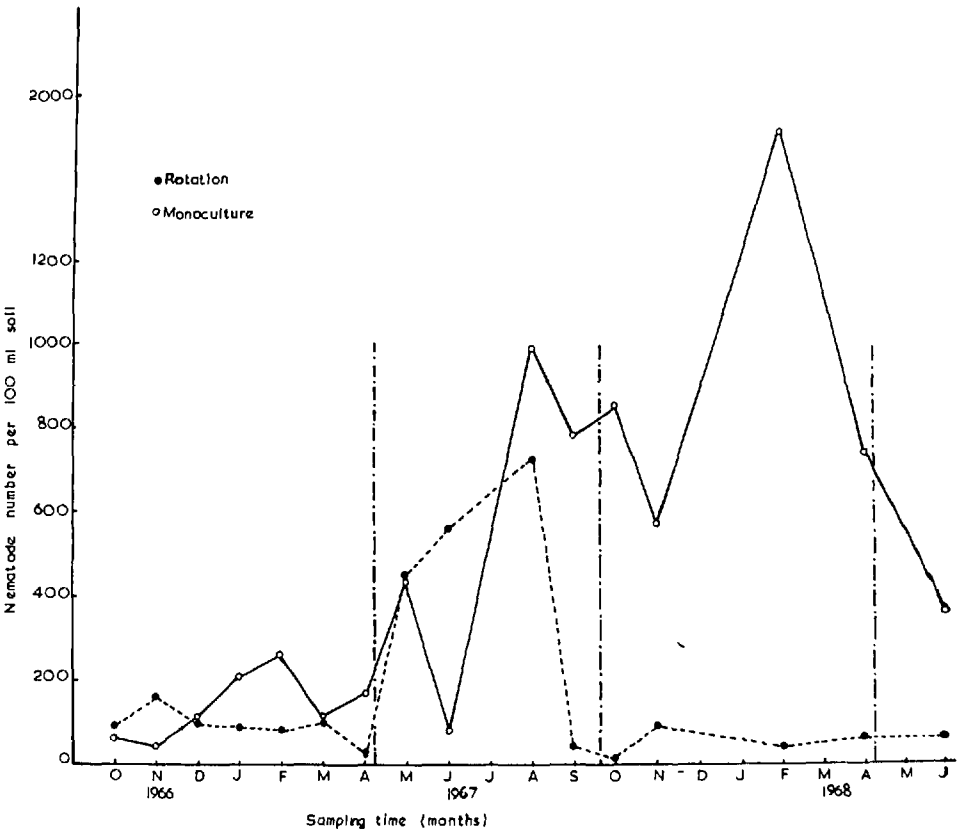

Fig.8. Seasonal variation in the populations of Paratylenchus spp in rotation and monoculture plots

The distribution of Aphelenchus and Aphelenchoides

The population level of the fungal feeders was higher in the rotation plots, especially the numbers following the barley crop. (See Appendix table 10.7).

The numbers of Heterodera larvae observed in the monoculture plots were 15 - 16 times greater than those seen in the rotation plots (see Appendix table 11).

Discussion and Conclusions

The increase in the population of particulate feeders in the rotation plots may be due to an increase in the organic content of the soil. This is also true for the populations of the fungal feeders Aphelenchus and Aphelenchoides. Although detailed studies were not carried out on the plant debris in the soil, observations showed that a great deal of organic matter remained in the soil consequent to the barley harvest. The June (1968) increase of particulate and fungal feeders might be attributed to a herbicide application and the subsequent increase in plant debris.

The high initial population of Pratylenchus spp. in the rotation plots indicates the influence of field beans as a host crop. Although the Pratylenchus spp. populations were expected to increase in the rotation plots following barley, the post harvest results did not show an increase in the population. These results therefore disagree with those of Oostenbrink (1954, 1956, 1960) and Winslow (1964). The continuous potato cultivations showed a progressive increase in the Pratylenchus populations. The Pratylenchus population was a mixed one with P. fallux, P. penetrans, P. crenatus and P. neglectus present. A further detailed study on the nematode species associated with the host crop might help to explain the changes in the population seen here.

Paratylenchus populations were markedly controlled by crop rotation. It is, however, difficult to explain the increase in numbers associated with barley in the rotation plots since barley has not been cited as a favourable host plant for Paratylenchus spp.

The crop rotation effect in reducing the Heterodera populations (Heterodera larvae) is clearly demonstrated here.

C. The vertical distribution of nematodes

The samples described so far were confined to the top 4 inches of the soil. It was thought of interest to investigate the distribution pattern of soil nematodes in relation to depth. The results obtained were compared with similar observations in orchard pasture.

i. The influence of vegetation on the vertical distribution of soil nematodes.

ii. Seasonal variation in the vertical distribution of soil nematodes.

1. The influence of vegetation on the vertical distribution of soil nematodes

The sampling was done in December, 1966. The two sites selected contained the same soil type. The arable field (rotation) had a very sparse vegetative cover in comparison to the orchard which had a dense grass cover.

Fig. 9 shows the vertical distribution of nematodes in pasture and arable land. The distribution of nematodes is closely associated with the vegetation cover. This is clearly seen for the permanent pasture where both free living and plant parasitic nematodes are more abundant in the upper layers; whereas in arable land the plant parasitic nematodes are confined to the lower layers.

This is also seen for the individual genera Pratylenchus and Paratylenchus, Fig.10. When the overall numbers are low, as for Pratylenchus in pasture, and Paratylenchus in arable land, there is no variation in the depth distribution. The distribution of Paratylenchus in the permanent pasture is closely associated with the feeder roots of the apple trees. The distribution of Pratylenchus in the arable soil may be influenced both by lack of vegetation and low temperatures.

Other nematodes Tylenchorhynchus, Tylenchus and spiral nematodes were found in large numbers in the upper 8 cms. in the permanent pasture, but at lower depths in the arable soils (See Appendix table 13).

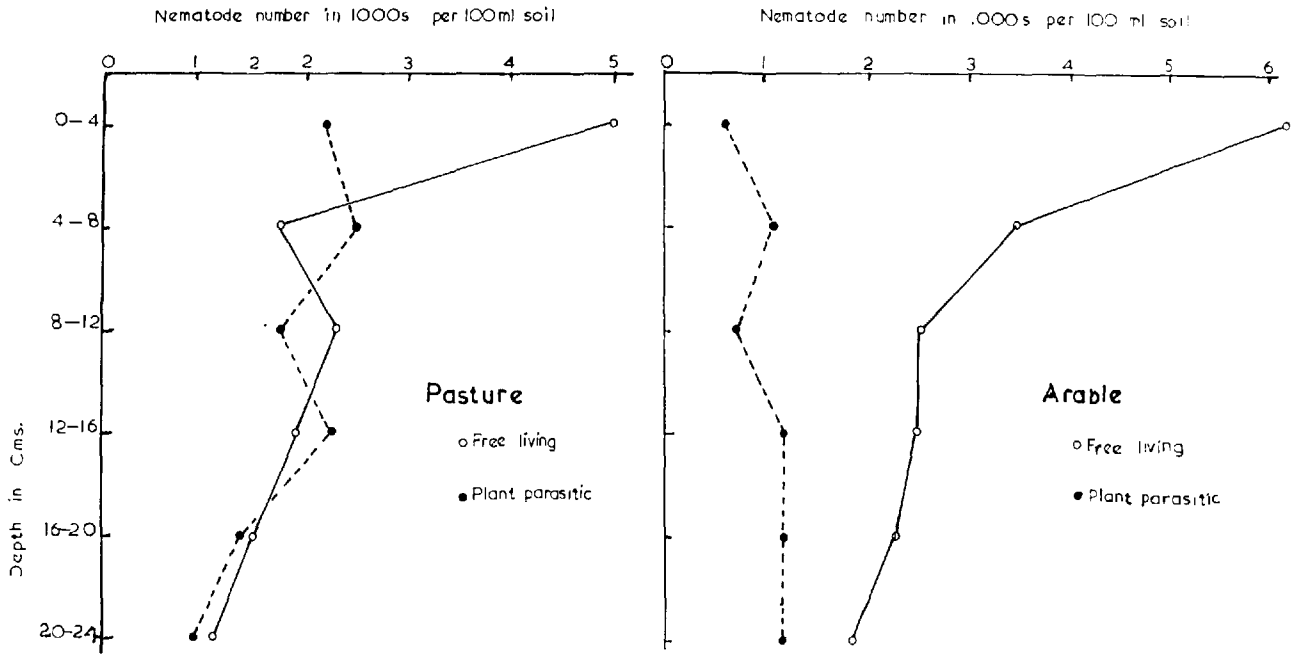

Fig.9. Vertical distribution of nematodes in permanent pasture and arable land

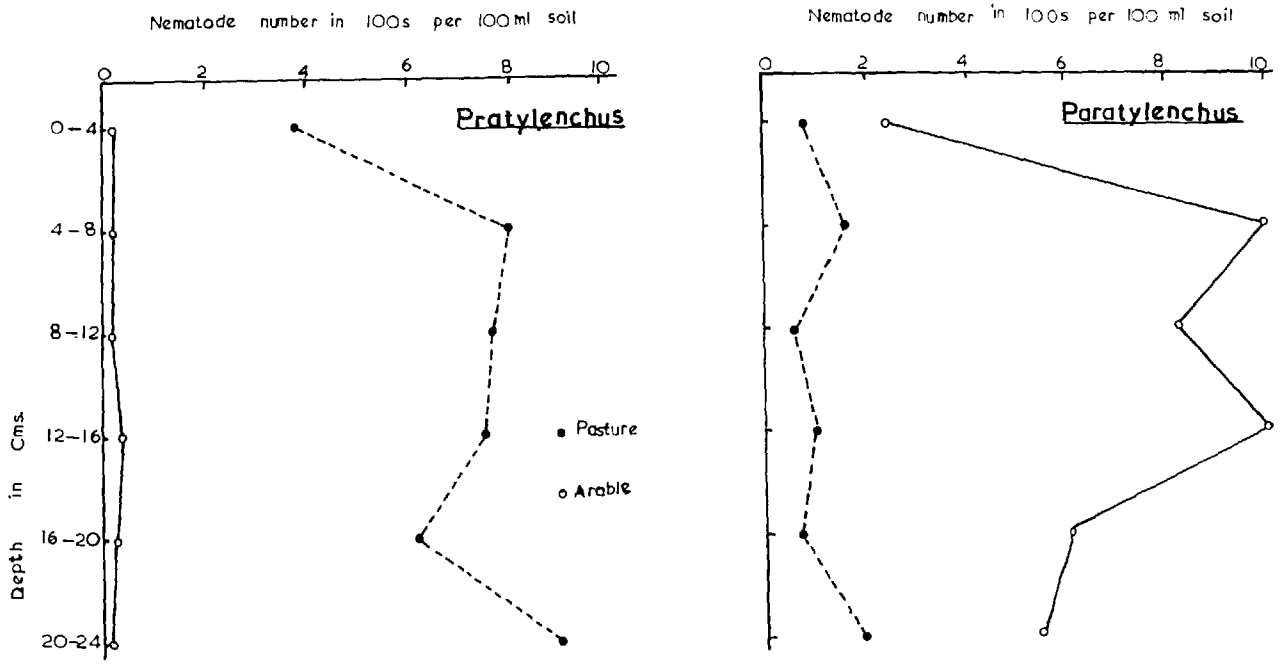

Fig.10. Vertical distribution of Pratylenchus and Paratylenchus in Pasture and arable land

11. Seasonal variation in the vertical distribution of soil nematodes

Figs. 11 & 12 show the influence of seasonal variation on the vertical distribution of nematodes on arable land from January - August, 1967. The effect of the decrease in soil temperature is seen in the variation in the depth distribution of both soil and plant nematodes in January and February. The increased activity especially in the upper layers with rise in temperature is shown by the increased number of free living nematodes in March, and continuing into August. The plant nematodes show only a slight increase in numbers.

Fig. 12 shows the depth distribution of Pratylenchus and Paratylenchus which indicates a slight increase in activity associated with seasonal variations ~~in the depth distribution.~~

The variations seen in April are largely due to the effect of rotovation. The plant parasites are still found in the deeper layers in the Summer months closely associated with plant roots, and the reduction in numbers especially of Pratylenchus, is due to root invasion.

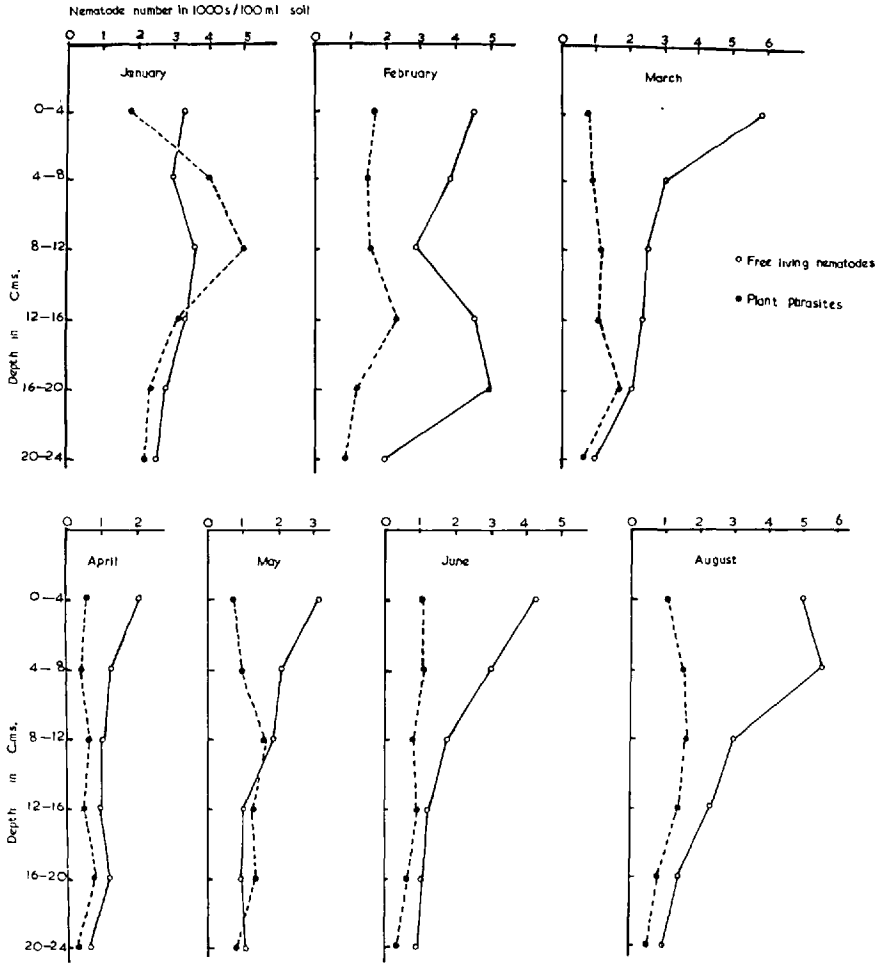

Fig.1 Seasonal variation in depth distribution of free living and plant parasitic nematodes in rotation plots

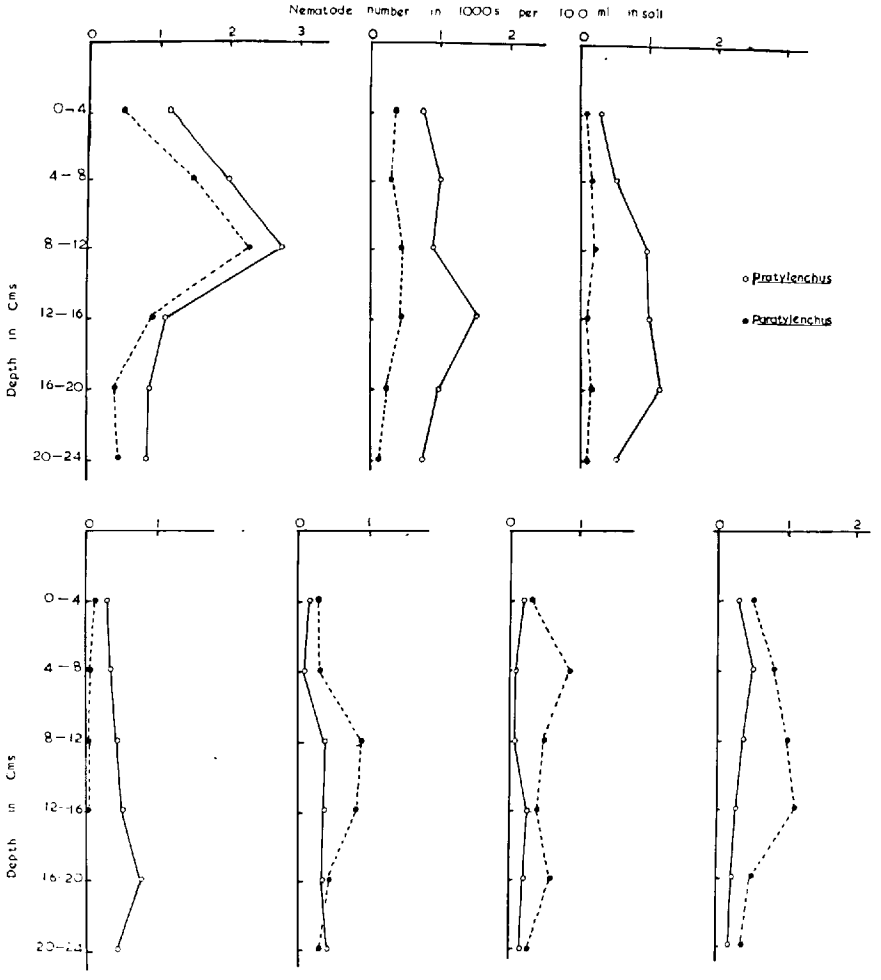

Fig.12 Seasonal variation in depth distribution of Pratylenchus and Paratylenchus in rotation plots

D. The influence of Herbicides on soil and plant nematodes

Experimental

Weeds common to the soil type (see Appendix table 1) were prevelant throughout the growing season, and caused poor growth of the cultivated plants. The borders between plots were treated with a herbicide before cultivation, and several times during cultivation, and the plots were weeded by hoe. In 1968, most of the plots were treated with a herbicide, before planting in April and after planting (between rows containing potato) in June.

The herbicide used was 1,1 - dimethyl - 4,4' - bipyridilium caction (active ingredient) commercially known as Paraquat (Gramoxane W)which was applied as a 5% solution (25 cc. of Gramoxane to one gallon of water).

Samples were taken as before, one month after the application of the herbicide. Although the top growth of the weeds were adversely affected by the herbicide, the roots still remained intact.

25 gram samples of weed roots from the treated and untreated plots were taken to estimate the Pratylenchus population within roots.

The results shown in Table 4 are the means of four replicates.

The population of Pratylenchus spp. was unaffected, both in the roots and in the soil. Paratylenchus spp., Helicotylenchus spp., and the Dorylaimida were adversely affected, the decrease in the larval population between treatments was highly significant ($P=0.001$).

With Tylenchorhynchus, Aphelenchus and Aphelenchoides, Tylenchus group and the particulate feeders (Rhabditida etc.) there was a significant increase in the larval populations (P=0·001%).

Table 4 Effect of herbi ides on soil nematodes (Paraquat).

A

		Control (Mean of 4)	Treatment (Mean of 4)	t test
Pratylenchus spp	a	118	85	n.s.
	1	68	58	n.s.
	T	186	143	n.s.
Paratylenchus spp.	a	70	15	P = 0.001%
	1	77	17	P = 0.001%
	T	177	32	
Tylenchorhynchus	a	32	35	n.s.
	1	27	67	P = 0.01%
	T	59	102	
Helicotylenchus sp.	a	15	10	n.s.
	1	95	43	P = 0.001%
	T	110	53	
Tylenchus sp.	a	35	43	n.s.
	1	40	135	P = 0.001%
	T	75	178	

Table 4 contd.

		Control (Mean of 4)	Treatment (mean of 4)	t-test
aphelenchus & aphelenchoides	a	77	100	n.s.
	1	80	295	P =0.001%
	T	157	395	
Dorylaimida	a	40	13	P = 0.01%
	1	40	10	P = 0.001%
	T	80	23	
Particulate feeder (Rhabditids etc)	a	1145	1208	n.s.
	1	1345	1832	P = 0.001%
	T	2590	3040	

B Effect of herbicides on Pratylenchus within root tissue.

		Control	Treatment	t test
Pratylenchus	a	230	240	n.s.
	1	370	275	n.s.

The increase in the organic debris due to the application of the herbicide may subsequently result in an increased activity of soil bacteria and fungi, thus providing adequate food for the particulate feeders and fungal feeders.

It may seem that the increase in the Tylenchus and Tylenchorhynchus is also due to this fact.

Although Pratylenchus populations were unaffected by herbicide treatment, other plant parasitic nematodes were reduced in number. It is not possible to explain this effect with data presented here.

The evidence obtained during the course of this experiment helps to explain the rise in the field populations of particulate feeders Aphelenchus and Aphelenchoides, Tylenchus and Tylenchorhynchus during June, 1968.

Section 1. General Discussion

The work described in Section 1 deals mainly with the influence of cultivation on the population dynamics of soil nematodes. The effects of depth distribution and of herbicide application are also examined in relation to population changes.

The free living particulate feeders and fungal feeders, showed very little seasonal fluctuation in numbers; although rotovation prior to cultivation in the spring resulted in reduction in numbers, this may have been due to soil disturbances and subsequent dessication. The influence of organic matter on the free living nematodes was seen clearly in June 1968, when as a consequence of herbicide applications, there was an increase in the plant debris in the soil and a corresponding increase in these populations.

Free living nematode populations were not greatly affected by crop rotation.

The distribution of migratory parasites e.g. Pratylenchus has been examined by several workers. The influence of the interaction of soil type, moisture, temperature and air permeability on Pratylenchus spp. has been emphasised by Jones & Mai (1964), Di Edwardo (1968), Miller and Boothroyd (1964), Kable and Mai (1968) and Koen (1967).

The reasons for the population fluctuations of Paratylenchus spp. associated with crop rotation is not clearly understood. Pratylenchus populations showed distinct variations associated with monoculture and rotation but the results did not agree with reports of other workers (Winslow, 1964; Oostenbrink, 1954, 1956).

There was a tendency for Pratylenchus numbers to increase in the monoculture plots. Detailed studies on the specific host parasite relationships of this genus (which generally has a very wide host range) might explain these observations.

Potato cyst eelworm populations, estimated mainly as larvae, were greatly influenced by crop rotation. This lays emphasis on the effectiveness of crop rotation in controlling nematodes with a limited host range.

Herbicide applications had an adverse effect on Paratylenchus populations as well as on the Dorylaimida. It is not clear why this should be so. Particulate feeders and fungal feeders increased in number as expected. The increase in Tylenchus and Tylenchorhynchus spp. cannot be explained until more information on the feeding habits of these genera is available.

The influence of vegetation on the depth distributions is clearly seen from the results obtained in Section 1.C.(1). There have been several reports of the influence of roots on the depth distribution of plant nematodes. (Baines, Van Gundy and Sher, 1959; Suit et al, 1953; Chitwood and Feldmesser, 1948; Harrison and Winslow, 1961.) Yuens' (1966) studies on the spiral nematodes and Flegg (1968) on Xiphinema and Longidorus spp. showed that depth distribution was not entirely related to root distribution.

It has also been reported that each nematode species maintained itself at a specific depth; which for some species may differ with different habitats (Yuen, 1966; de Maeseneer, 1963; Hoff and Mai, 1964).

Seasonal variations in depth distribution were clearly marked for both free living and plant parasitic nematodes. The results suggest that this variability might have been largely due to nematode survival rather than a vertical migration. Yuen (1966) showed that depth distribution of spiral nematodes did not reflect a downward migration. Evidence in support of vertical migration associated with seasonal changes has been shown by Lewis and Mai (1960) and Koen (1966, 1967).

The change in Pratylenchus spp. populations seen here may be largely associated with root invasion in the summer months.

Section 2.A.

Field studies on the action of soil applied pesticides on the free living and plant parasitic nematodes.

The use of chemicals in the control of plant parasitic nematodes is generally considered to be a modern technique. Soil fumigation was practiced as early as 1884 in France where carbon disulphide was used against the aphid Phylloxera vastatrix on grape vines. It is seldom used today because of its high cost, and difficulty of application. Tear gas or chloropicrin left over from the First World War was used for the control of "Heterodera marioni" by Newton, Hastings and Bosher (1937).

The materials most widely used today are halogenated hydrocarbons. Methyl bromide has been extensively used in the control of insect pests of stored products and it was first used against root knot nematodes by Taylor and Macbeth in 1940. D-D, a mixture of Dichloropropane and Dichloropropene (Carter, 1943) and Ethylene dibromide (E.D.B.) (Christie, 1945) were the first chemicals to give both economic and practical control of nematodes under field conditions. They are still used widely because of their ease of application and relative cheapness. Nemagon (1-2 dibromo - 3 Chloropropane) introduced in 1955, is not only effective against nematodes but also has a low phytotoxicity and persists longer in the soil.

A group of compounds allied to fumigants are some dithiocarbamates, introduced in 1955. These have the property of breaking down in moist soil into methyl isothiocyanate which has a fumigant action.

Some of the common ones are: Trapex (methyl isothiocyanate), Vapam (sodium methyl dithiocarbamate), Mylone or Dazomet (tetrahydro - dimethyl thiadiazine - thione).

Methyl isothiocyanate is claimed to have fungicidal, herbicidal and bacteriocidal properties. Most of the fumigants with the exception of nemagon are more or less phytotoxic and have to be applied to the soil well in advance, and removed from the soil before planting.

The use of compounds with chemotherapeutic properties, i.e. taken up by the growing plant to give control of the nematodes within the plant tissues, is of recent origin. The best known is probably Parathion which was used effectively against Aphelenchoides spp. in chrysanthemum as a foliar spray. (Dimock and Ford 1950) and Tarjan (1950) used it against Pratylenchus spp. in boxwood. Another organophosphorus compound with systemic properties is thionazin, which has been found to give effective control of Ditylenchus dipsaci in narcissus, as a dip and drench.

The repeated use of pesticides in field plots might be expected to have changing effects on the pests and other organisms as well as on the crops themselves; also the physico-chemical characteristics of the soil might become changed.

The present investigation was primarily planned to study the effect of continued applications of pesticides on Heterodera rostochiensis. Simultaneous studies were made on the effects on other soil nematodes (free living and plant parasitic), the effects on natural enemies and other soil fauna.

Three fumigants, Chloropicrin, Ethylene dibromide and Dazomet and a systemic compound thionazin were applied at three dosage rates.

The population studies on free living and migratory plant parasitic nematodes were made only after the second application of the pesticides.

Materials and Methods

Soil treatments

Three fumigants and one systemic compound were applied at two dosage levels.

Chloropicrin $CCl_3 NO_2$

Commonly known as trichloronitromethane or nitrochloroform. Known to be effective against fungi and is highly phytotoxic.

Ethylene dibromide $CH_2 Br. CH_2 Br.$

Also known as 1,2, dibromoethane or E.D.B., is highly phytotoxic. Commercially known as Dowfume W-85, containing 85% E.D.B. is marketed as a liquid.

Dazomet $C_5 H_{10} N_2 S_2$

Tetrahydro - 3 dimethyl - 2H - 1,3 5, thiadiazine 2 thione (Mylone)., breaks down in moist soil to release methylisothiocyanate, which has a high fumigant action; usually formulated as an 85% dust, it is highly phytotoxic and suppresses the growth of many weeds.

Thionazin $C_8 H_{12} N_2 O_3 P.S.$

O - 2 pyrazinyl phosphorothioate or known in the United States as O - O - diethyl O - 2 pyrazinyl phosphorothioate. Usually known under the trade name Nemafos or Zinophos, is formulated as 5% or 10% granules or 46% E.C.

Table 5 Dosage rates of pesticides applied

	Rate	lb/acre	per plot	per point
Chloropicrin	low	200	830 ml.	1•3 ml.
	high	800	3320 ml.	5•12 ml.
Ethylene dibromide	low	200	625 ml.	1•0 ml.
	high	800	2500 ml.	4•0 ml.
Dazomet	low	100	685•5 g.	1•5 g.
	high	400	2734 g.	3•8 g.
Thionazin in row	low	2	270 g.	
	high	8	1080 g.	
Broadcast	low	10	1350 g.	
	high	40	5400 g.	

Method of Treatment

Ethylene dibromide and chloropicrin were applied using the Shell fumigant injector, Model H 1. Each plot was divided cross-wise and lengthwise into one foot square sections to form a checker board pattern, and injections were applied at the intersections to a depth of nine inches in the soil. After each injection the soil was pressed down over the hole produced by the injection nozzle, to prevent a loss of fumigant from the soil.

Thionazin was applied as 5% granules in row treatments, using a combined dipper hopper (Call - private communication), in which the dipper held the correct volume of granules required for the given length of row. The potatoes were next planted by hand, i.e. six rows per plot and 24 potatoes per row.

Plots receiving broadcast applications of thionazin were marked into one yard squares and the correct amount of material sifted as evenly as possible, using a sugar sifter.

Dazomet was applied broadcast in a similar manner to the thionazin granules. After application the plots were immediately rotovated to a depth of nine inches.

Application and sampling times

The fumigants were applied in the Autumn after the crops had been harvested. The thionazin inrow was applied in the Spring, and potatoes planted soon after. Broadcast applications were made after planting potatoes.

All fumigant treatments were sampled one month after application (Autumn, 1966) and the monthly sampling continued for twelve months. After Autumn, 1967, the first sampling was done after one month and the subsequent samples were taken every two months until June, 1968.

After thionazin treatments, however, samples were taken every two weeks up to eight weeks, and thereafter at monthly intervals.

Results

The effect of chloropicrin, Dazomet and Ethylene dibromide, on populations of soil nematodes in the monoculture plots.

Effect on free living nematodes.

The effect of repeated applications of fumigants on free living nematodes; Fig. 13 shows that there is a marked reduction in the nematode populations immediately after treatment (November, 1966). The nematode numbers remained low during the winter months, but in the Spring there was a progressive increase in nematode numbers; in all treatments the population reached the initial population levels at the end of twelve months.

The results after treatment in Autumn, 1967, showed a similar effect over the eight months sampled. At the high dose, dazomet gave a more effective control of the nematode populations. The rise in the population level in June, 1968 is probably associated with the herbicide application (see Part 1, Section 1(c))

The effect on plant parasitic nematodes

The three fumigants reduced the number of plant parasitic nematodes greatly (Fig. 14). Before treatment the population levels of plant parasitic nematodes were very low, due to the previous application of the same chemicals, i.e. about 50% of the controls in chloropicrin and dazomet plots, and almost zero in the high dose ethylene dibromide plots.

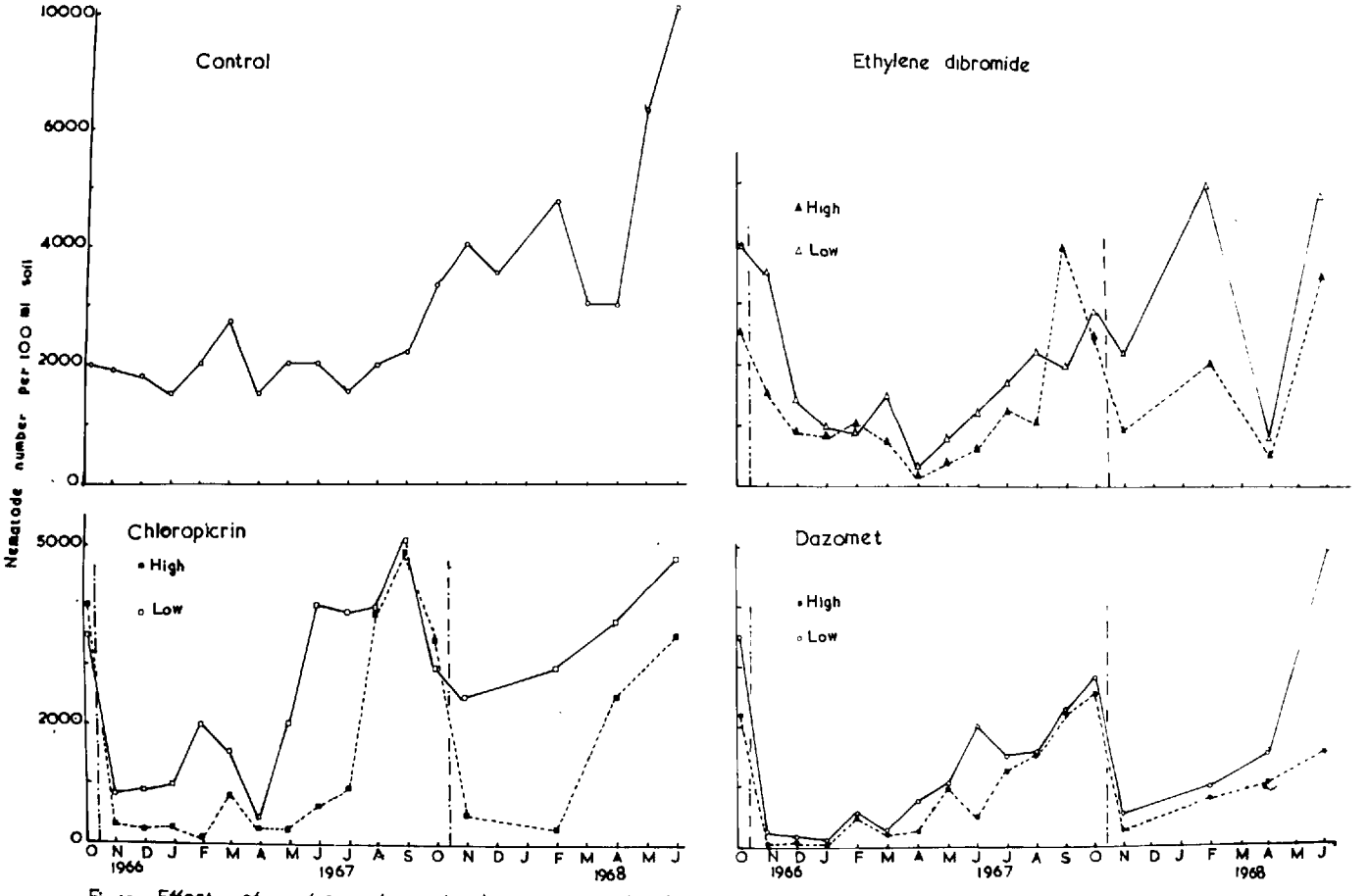

Fig 13. Effect of fumigants on freeliving nematodes in monoculture plots

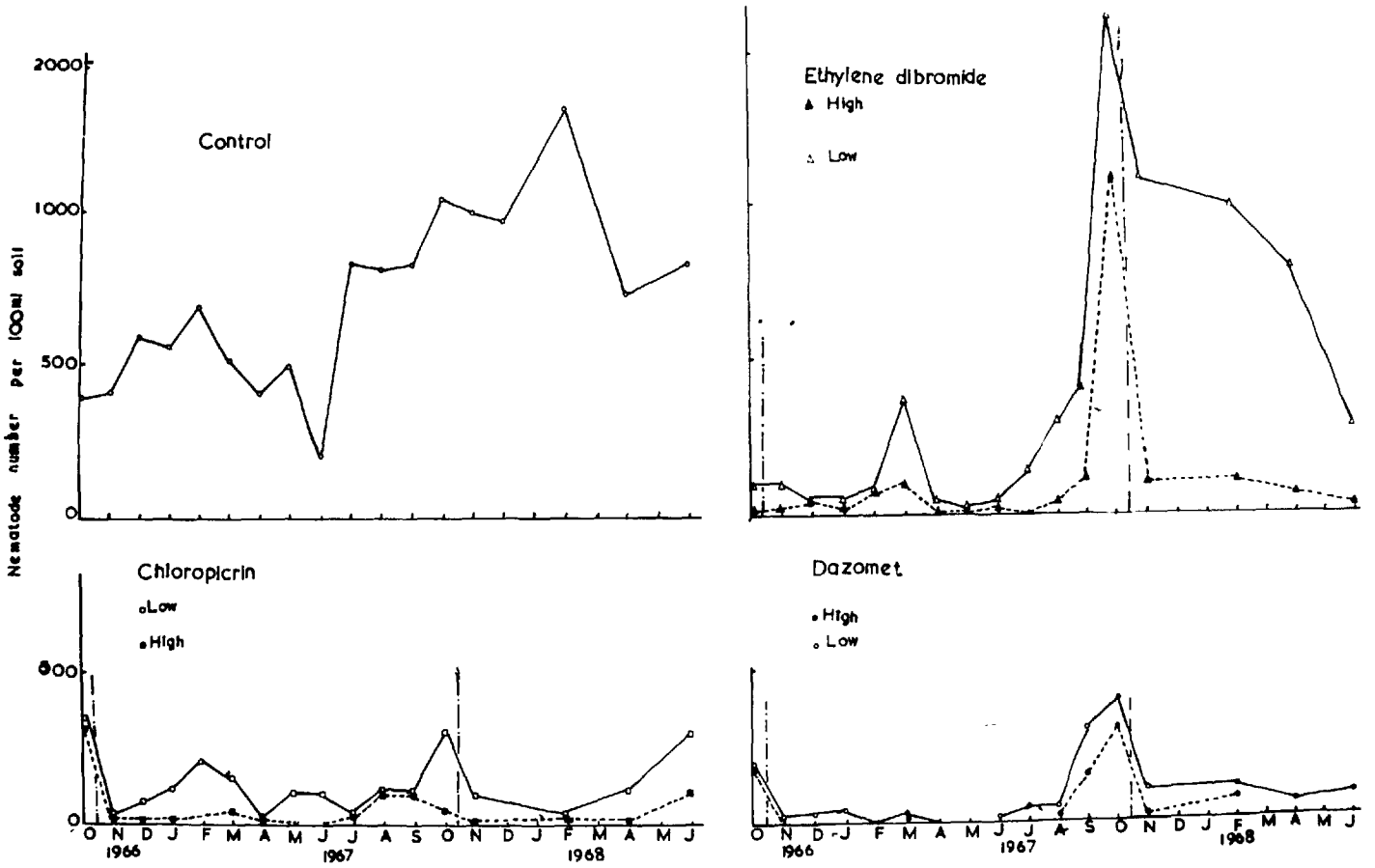

Fig. 14. Effect of fumigants on plant parasitic nematodes in monoculture plots

The immediate effect of the fumigant application was to reduce the nematode numbers. The effect of ethylene dibromide was not very marked. The overall effect was an immediate reduction of the population followed by a slow increase, except in the low ethylene dibromide plots where plant parasitic nematodes reached population levels higher than in the controls. In the chloropicrin and dazomet plots, the population levels after twelve months were the same as before treatment.

Effect of fumigants on individual genera

Effect on Pratylenchus

The comparative effect of the three fumigants on Pratylenchus populations is shown in Fig. 15 . All three fumigants, chloropicrin, dazomet and ethylene dibromide effectively reduced the number of Pratylenchus. The dazomet treatments markedly affected the recovery of Pratylenchus; but chloropicrin and ethylene dibromide plots showed a wide fluctuation in the Pratylenchus populations following the pesticide applications.

Effect on Paratylenchus

Table 6 shows the results of the repeated application of fumigants on Paratylenchus populations in monoculture plots. Prior to treatment (October, 1966) very low nematode numbers were found in the chloropicrin and dazomet plots. Although the subsequent fumigant treatments resulted in a further reduction in the populations, the ethylene dibromide (low dose) application was less effective in controlling the nematode numbers. The results in October, 1967, indicated that there had been a rapid build-up of the Paratylenchus populations. The ethylene dibromide plots showed a population increase of 20 - 30 times greater than the October, 1966 populations. Numbers in the dazomet plots indicated an increase of 30 - 80 times greater than numbers observed in October, 1966. The chloropicrin applications were more effective in controlling Paratylenchus populations.

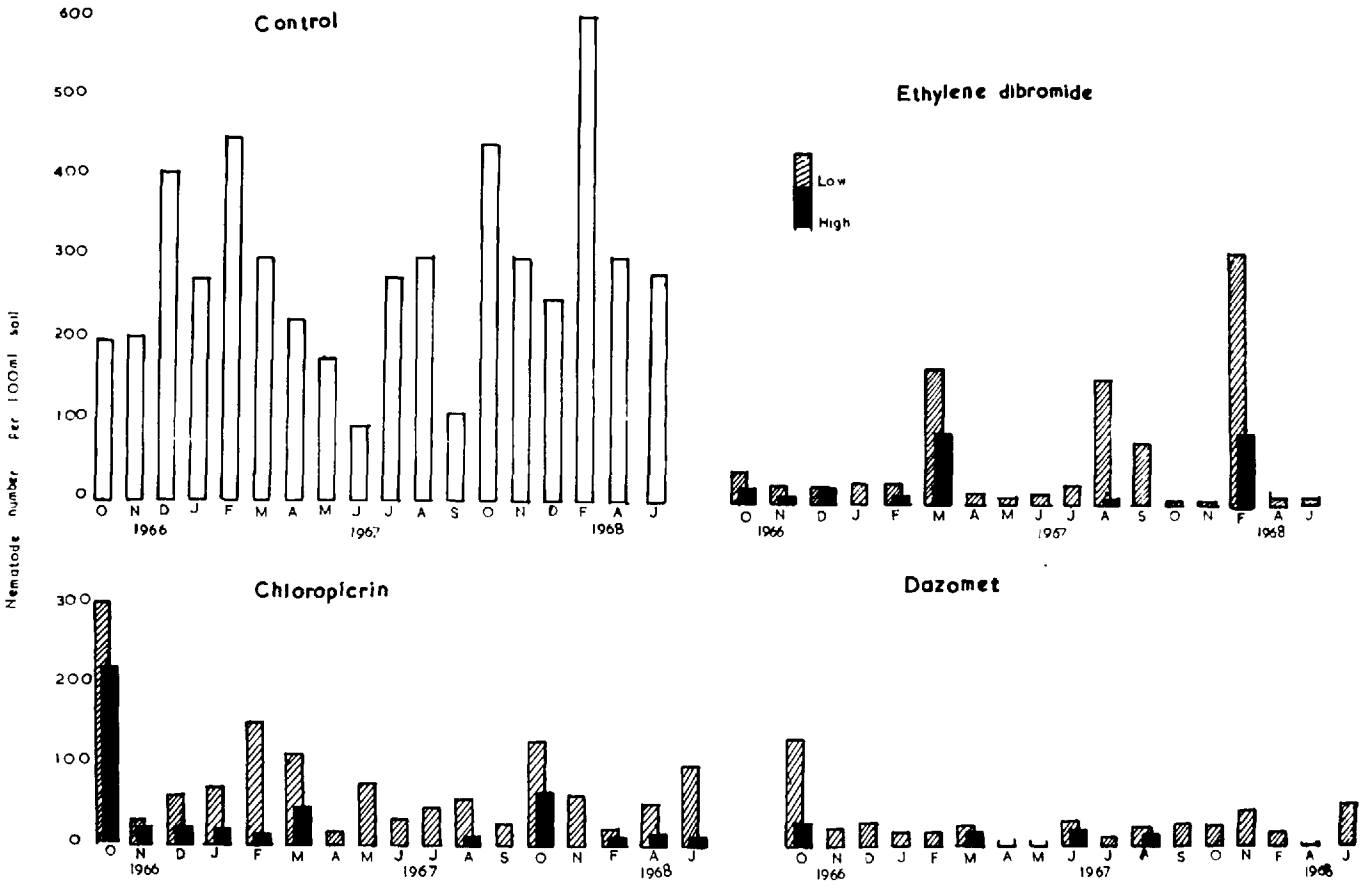

Fig. 16

Effect of repeated applications of fumigants on Pratylenchus populations.

Effect on other genera

The number of Tylenchus, Tylenchorhynchus, Helicotylenchus and Dorylaimida were very low throughout the experimental period.

The fungal feeders, Aphelenchus and Aphelenchoides were recovered in very low numbers throughout the experimental period, but a slight increase in the population was observed at the end of the sampling in June, 1968.

Table 6 Effect of fumigants on Paratylenchus in monoculture (mean of 4 replicates)

Sampling time	Control	E.D.B.		Chloropicrin		Dazomet	
		Low	High	Low	High	Low	High
Oct 66	51	60	50	30	10	10	10
Nov	74	65	-	20	-	-	-
Dec	141	35	30	20	-	-	-
Jan 67	171	28	20	10	-	-	-
Feb	198	72	10	20	-	-	-
Mar	77	172	23	-	-	10	5
Apr	76	28	-	-	-	-	-
May	142	8	-	13	-	8	-
June	78	15	13	13	-	10	-
July	427	107	-	18	-	25	20
Aug	344	90	15	15	13	42	10
Sept	427	148	138	128	13	272	164
Oct	492	2342	1203	115	13	852	312
Nov	505	1222	98	20	-	15	10
Feb 68	831	210	28	20	-	85	28
Apr	312	790	10	20	-	18	13
June	270	519	88	45	-	10	-

A comparison of the effect of fumigants on soil nematodes in fields under rotation and monoculture.

The monoculture plots received three annual fumigant applications in the Autumns of 1965, 1966 and 1967, but the rotation plots were only fumigated once in Autumn, 1967.

Effect on free living nematodes

The immediate post-treatment effects of fumigants on free living nematodes are similar in both monoculture and rotation plots (Fig. 16). Ethylene dibromide and chloropicrin applications resulted in higher populations at the end of the experimental period (June, 1968). Of the three fumigants used, dazomet was more effective in controlling free living nematodes.

Effect on plant parasitic nematodes

The immediate effect of the fumigant applications on plant parasitic nematodes was the same in both monoculture and rotation plots (Fig. 17). October, 1967, results for the monoculture plots i.e. chloropicrin and dazomet treated plots, showed populations lower than those of the rotation plots, but not in the plots treated with ethylene dibromide. The Pratylenchus populations were effectively controlled by all these fumigants in both regimes. The chloropicrin (low dose) plots showed a higher recovery of Pratylenchus.

The Paratylenchus populations were considerably reduced by the fumigants, dazomet and chloropicrin being more effective than ethylene dibromide.

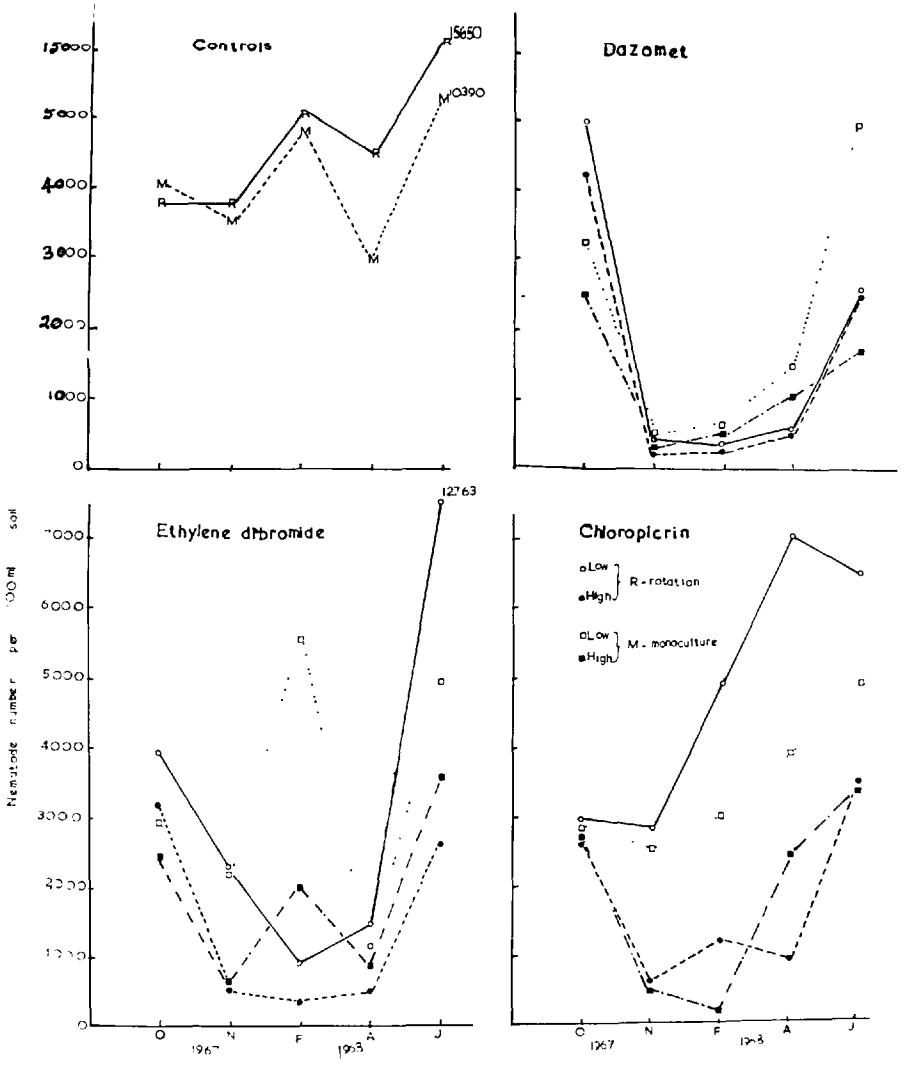

Fig. 16 Short term effect of fumigants on free living nematodes in rotation and monoculture plots

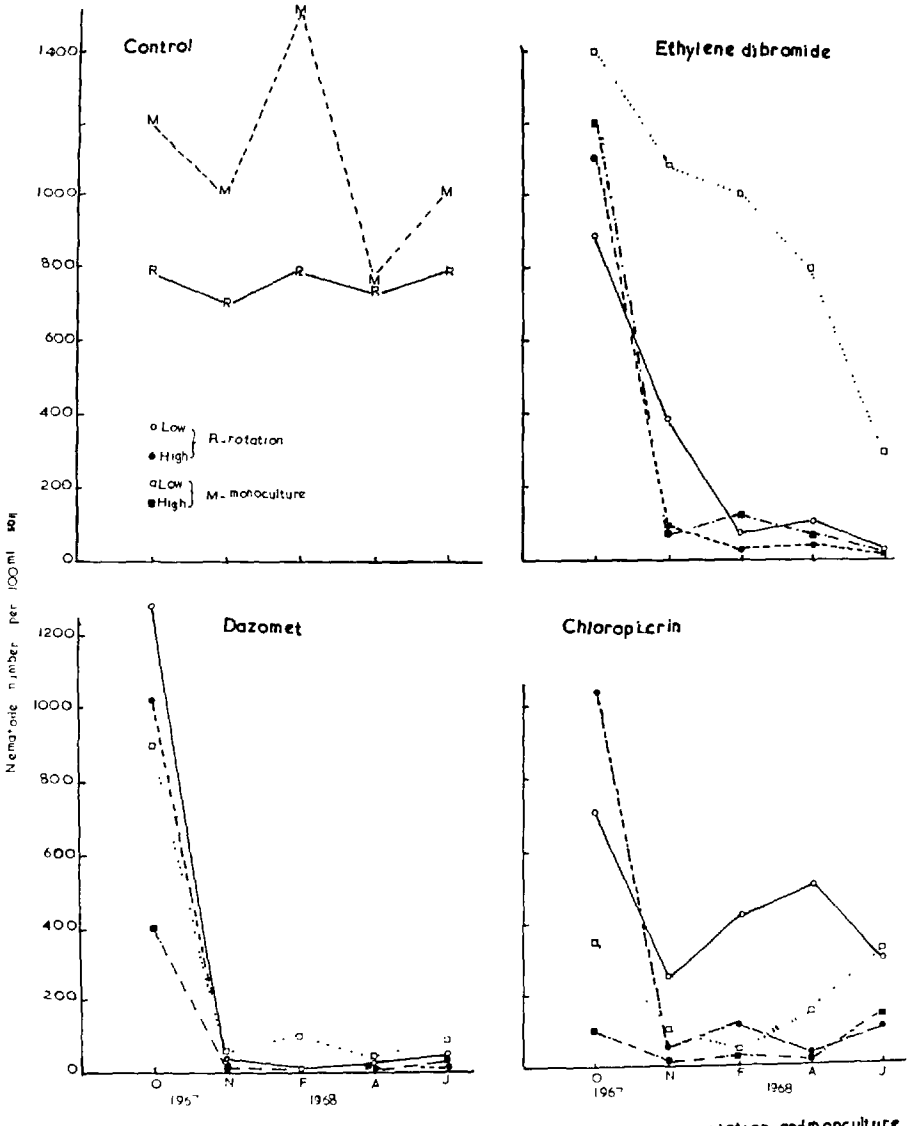

Fig.17 Effect of fumigants on plant parasitic nematodes in rotation and monoculture Plots

Effect of Thionazin on soil nematodes in monoculture plots

In row application

Fig. 18 shows the results of the repeated application of thionazin on free living and plant parasitic nematodes. Although there was a slight reduction in numbers immediately following the pesticide application, the long term effect has been an overall increase in free living nematodes. This increase occurred gradually after the April, 1967 treatment, but the increase following the April, 1968 application was very rapid.

Plant parasitic nematodes were reduced slightly immediately following application, but again there was a rapid recovery in all treatments reaching the pre-treatment populations at the end of nine months. The population levels following April, 1968 applications were lower than the controls at the end of eight months.

Broadcast applications

Population fluctuations associated with broadcast applications of thionazin are shown in Fig. 19. Free living nematode numbers were reduced immediately after the pesticide applications (low numbers were recovered up to eight weeks in low dose plots, and up to twelve weeks in the high dose plots). This was followed by a rapid increase in the free living nematodes, reaching a level higher than that of the control at the end of thirty two weeks.

Pre-treatment sampling in the thionazin broadcast plots, showed very low numbers of plant parasitic nematodes. The immediate post-treatment results did not indicate a marked variation in the population levels.

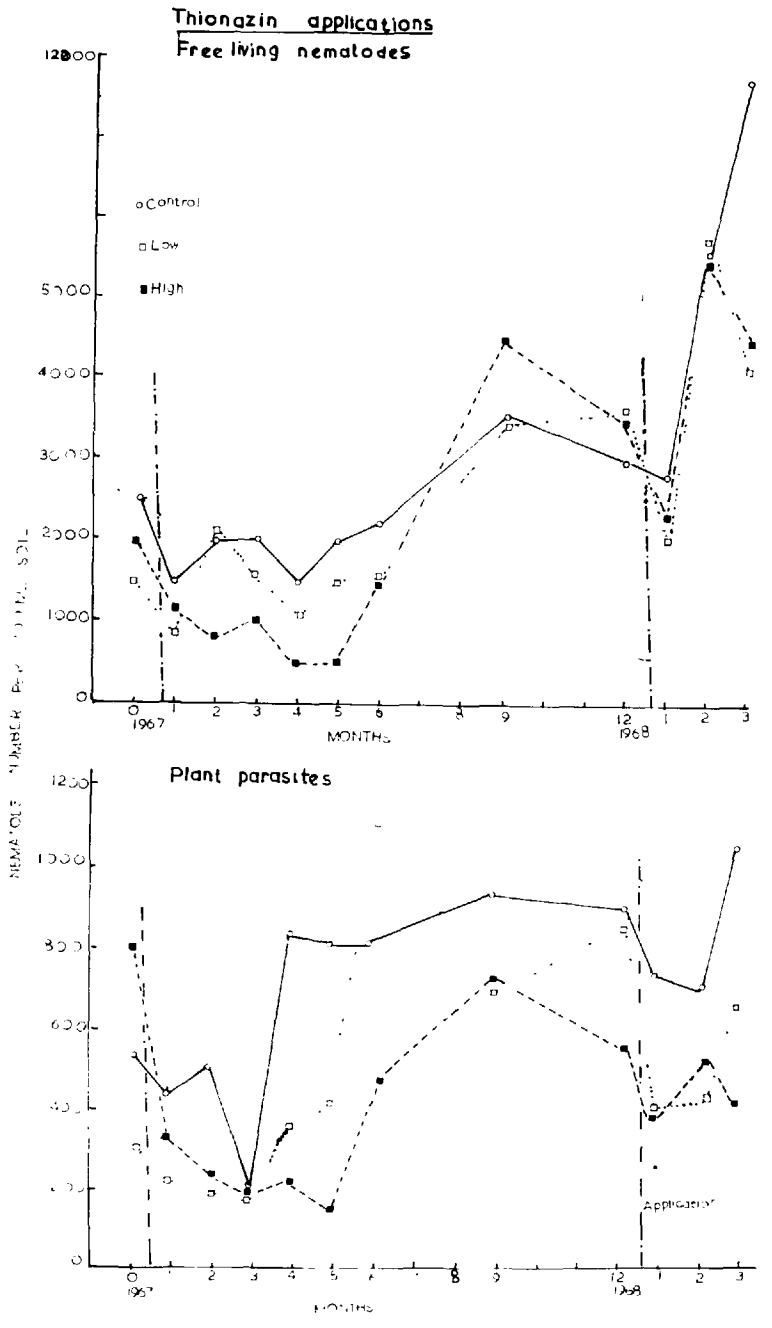

Fig.18 Effect of repeated (in row) applications on soil nematodes

A rapid increase was observed by the sixteenth week (post treatment), where the nematode numbers were very much higher than those in the untreated plots.

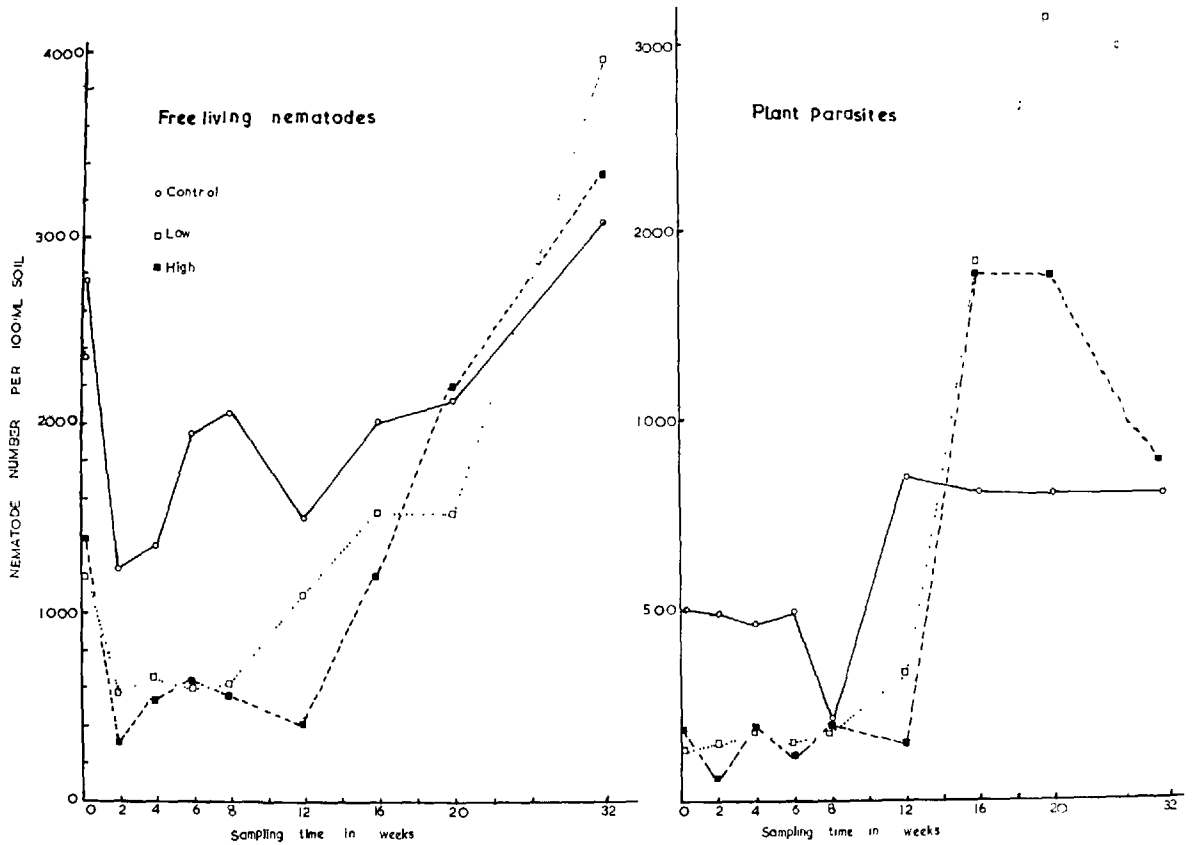

Fig.19. Effect of Thionazin (broadcast application) on soil nematodes

The effect of Thionazin applications on some Tylenchida in monoculture plots

Effect on Pratylenchus

Table 7 shows the results of population changes of Pratylenchus associated with broadcast and inrow applications of thionazin.

Pratylenchus numbers were slightly reduced immediately following thionazin inrow treatment, but the post-treatment results over a nine month period showed a progressive increase in the nematode numbers.

The pre-treatment populations were very low in the broadcast plots and the immediate post-treatment results did not show much variation in the nematode numbers. The post-treatment results (at the end of nine months) indicated a population increase of 5 - 7 times greater than the pre-treatment population.

Table 7. Effect of Inrow and Broadcast application of Thionazin on Pratylenchus spp. (monoculture plots)

Sampling time months after application	Control	In row application		Broadcast application	
		Low	High	Low	High
0	306	238	448	40	60
1	228	115	143	65	52
2	176	48	60	55	15
3	95	55	25	30	18
4	274	180	120	58	35
5	295	85	55	162	148
6	110	90	130	170	165
9	255	240	397	300	200
12	318	80	243		

Effect on Paratylenchus spp.

Results of the effect of inrow and broadcast application of thionazin on Paratylenchus populations is shown in Table 8. Paratylenchus numbers were slightly reduced soon after thionazin inrow treatments; this was followed by a rapid increase, especially in low dose plots.

The pre-treatment sampling in the broadcast plots showed very low populations of Paratylenchus. The immediate post-treatment results showed an increase in numbers in low dose plots and reduced numbers in high dose plots. Paratylenchus populations recovered after five months (post-treatment) were 300 times greater than pre-treatment populations, and 6 - 7 times greater than those of the controls.

Table 8. Effect of inrow and broadcast applications of Thionazin on Paratylenchus species. (Monoculture plots)

Sampling time months after application	Control	Inrow application		Broadcast application	
		Low	High	Low	High
0	77	42	140	10	20
1	76	20	43	53	8
2	142	17	25	30	5
3	78	13	8	18	18
4	427	38	23	153	62
5	344	28	18	1355	1355
6	427	995	253	3052	1330
9	515	205	80	2460	510
12	500	113	93		

The comparative effects of Thionazin (inrow) applications on soil nematodes in rotation and monoculture plots

The monoculture plots received three annual applications of thionazin, April 1966, 1967 and 1968, but the rotation plots were only treated once in April, 1968.

The results (Figs. 20 & 21) show the short term effect of this compound on soil nematodes.

The population changes of free living nematodes, associated with thionazin application, is the same for both monoculture and rotation plots.

Plant parasitic nematode populations prior to thionazin application, were higher in monoculture plots than in rotation plots, but the immediate response to pesticide application was the same for both regimes. High dose applications appeared to be more effective in controlling plant parasitic nematodes.

Of the plant parasitic nematodes, Pratylenchus populations were reduced by thionazin treatments, but there was no effect on the Paratylenchus populations.

Both monoculture and rotation plots showed an increase in Tylenchorhynchus populations following thionazin application. Aphelenchus and Aphelenchoides populations, however, were adversely affected by thionazin treatments.

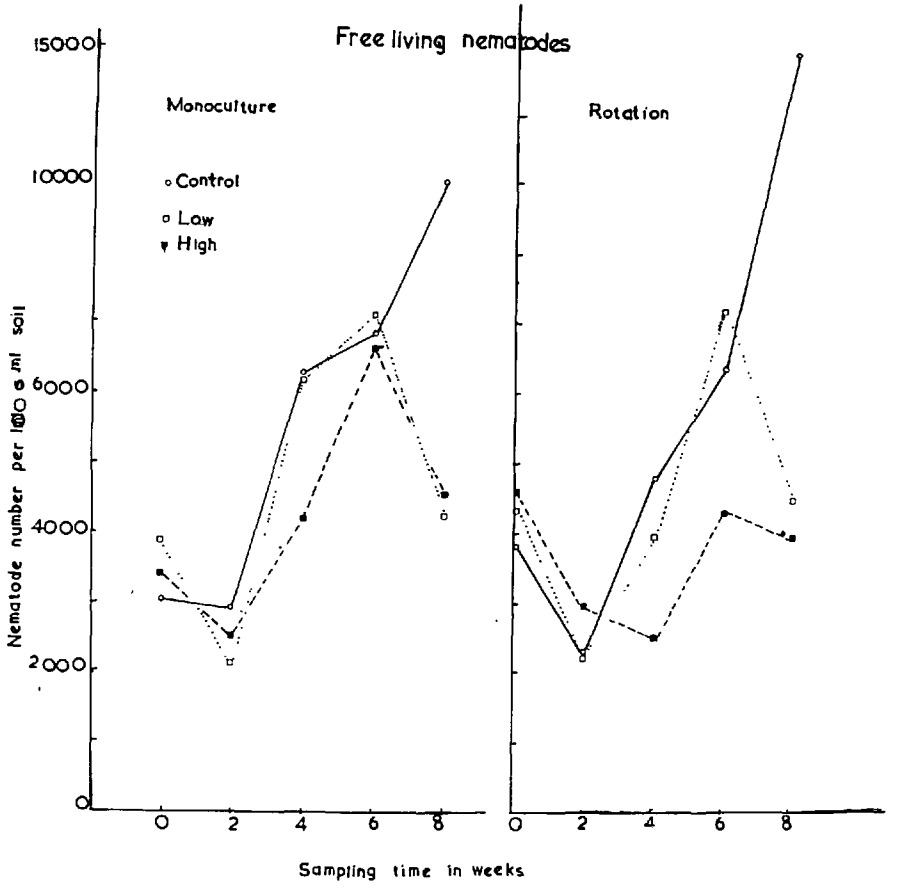

Fig. 20 Effect of thionazin (inrow) applications on free living nematodes in rotation and monoculture plots.

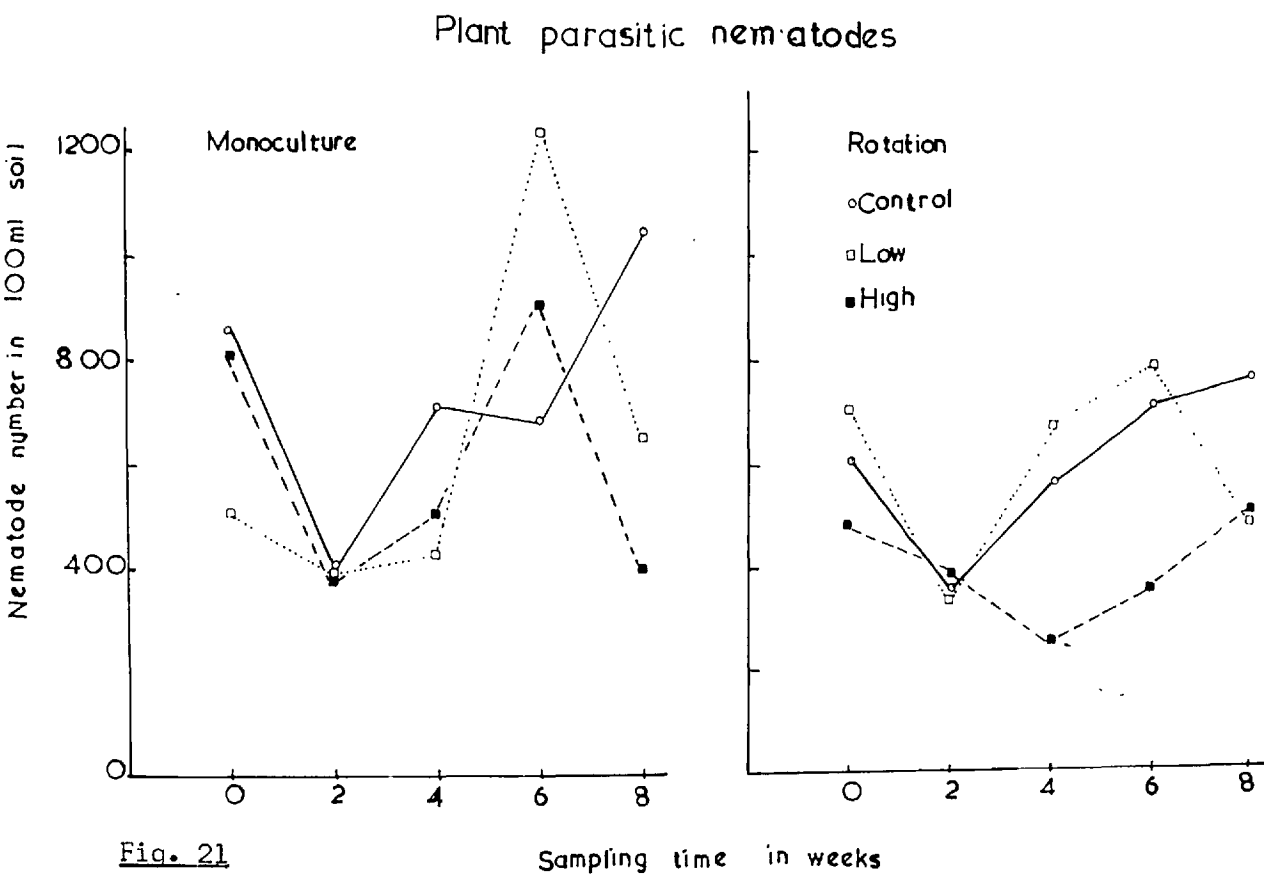

Fig. 21

Effect of thionazin (inrow) applications on plant parasitic nematodes in rotation and monoculture plots.

Effect of pesticide treatments on crop growth

In the thionazin treatments slight phytotoxic effects were seen in the early stages of crop growth, especially following the high dose application. Stunting of plants and poor growth were most evident in chloropicrin and ethylene dibromide plots (high dose applications). These also showed effective herbicidal properties.

Dazomet treatments resulted in an improved growth of the potato plants and very low weed growth.

The control plots showed an extremely poor growth of the potato haulms early in the season and continued to do so later, mainly due to very high numbers of potato cyst nematodes in these plots.

Table 9 shows the potato yields for three years in the monoculture plots. These results indicate the effect of chemical treatments in increasing potato yields.

The low yields obtained in 1967 may be due to ineffective weed control. Excessive weed growth early in the season may have prevented a proper growth of the potato haulms.

Table 9 Potato yields expressed as plot yields and tons per acre in the monoculture plots.

Treatments		1966		1967		1968	
		Kg/plot	tons/acre	Kg/plot	tons/acre	Kg/plot	tons/acre
Controls		30.5	6.1	2.4	.44	22.9	4.55
Thionazin	low	32.0	6.4	8.4	1.66	13.15	2.61
	high	47.5	9.4	9.1	1.81	17.12	3.40
Ethylene dibromide	low	65.0	12.9	20.6	4.10	32.71	6.49
	high	75.0	14.9	0.9	0.17	2.72	0.54
Chloropicrin	low	58.5	11.6	8.6	1.70	28.12	5.58
	high	68.5	13.6	38.1	7.5	59.65	11.84
Dazomet	low	71.0	14.1	38.3	7.6	31.06	6.17
	high	92.0	18.3	61.2	12.1	57.15	11.34

Section 2. Discussion

The present investigation was mainly concerned with the influence of pesticides on population dynamics of free living and plant parasitic nematodes. The results show that of the pesticides used, the fumigants were more effective in controlling soil nematodes than the systemic compound thionazin.

The effectiveness of a soil nematicide depends on several factors i.e. the biological and chemical nature of the soil environment. In addition a fumigant depends for its efficiency on the ability to penetrate the soil mass. Most halogenated compounds, e.g. Ethylene dibromide and Chloropicrin have a high vapour pressure and volatalize readily. Dazomet, which is not itself a fumigant, depends on its chemical decomposition to a volatile fumigant (Methyl isothiocyanate) in contact with moisture. Thionazin hydrolyses into the sodium salt of 2 Pyrazinol (Kiegemaji and Terriere, 1963), which does not persist in the soil. Getzin (1964) shows that one of the degredation products of thionazin in ths soil is CO_2. The loss of this pesticide from the soil has been shown to be largely due to leaching rather than volatalization (Critchley, 1968).

The present investigation formed part of a long term project on the effect of repeated applications of soil pesticides on Heterodera rostochiensis.

With non cyst forming nematodes, a great deal of confusion exists in estimating the direct effect of nematicides. The apparent absence of nematodes immediately after the nematicide application does not necessarily mean an effective control of the nematode.

As shown in Section 2. A, there was a distinct reduction in the free living nematode populations immediately following the fumigant application, but observations over a period of twelve months showed a regular build up of the nematodes to population levels higher than those of the controls. Thionazin treatments were ineffective. The control of fungal feeders by thionazin might have been due to a systemic effect since fungi are known to take up thionazin (Oliff, 1966).

With migratory plant parasitic nematodes, the continued applications of fumigants resulted in very low populations. Thionazin applications resulted in an initial decrease followed by an increase in the nematode populations. This was most marked in the thionazin broadcast applications. In this case, the increase appeared to be independent of chemical treatment, for these plots were cultivated for the first time when thionazin was applied in April, 1967. The presence of the crop may therefore have merely served as a boosting crop for the nematodes present in the soil.

The fumigants appeared to have a selective action on some Tylenchida present. Chloropicrin was more effective in controlling Paratylenchus than Pratylenchus, and least effective in controlling free living nematodes. Dazomet treatments were more effective in controlling Pratylenchus populations. After Ethylene dibromide treatments, a very rapid increase of Paratylenchus populations occurred.

The selective action of fumigants has been shown by van den Brande et al, 1955, in control of Heterodera rostochiensis where the fumigants in ascending order of efficiency were, Chloropicrin, D-D., and E.D.B.

Dieter (1954) showed that D-D was better than E.D.B. for the control of cyst nematodes, but that E.D.B. was more effective in the control of potato root and sting nematodes. Nusbaum (1955) reported that D-D was more effective against the meadow nematode than against the stunt nematode and the reverse was true for E.D.B. Feldmesser et al (1951) and Hague (1959) have reported a selective action of fumigants on the potato cyst nematode.

The build up of Saprophagous nematode populations in all treatments is perhaps due to the reduction of natural enemies. Also the increase in organic debris in the soil (putrefying plant and animal remains) provides an increased food supply for reproduction of these nematodes. The plant parasitic nematodes, especially Pratylenchus were mainly found in the adult stage at the time of fumigation. It is thus possible that not only are the potential egg layers killed by the fumigants, but the absence of a host crop during the subsequent winter period prevents the reproduction of the surviving nematodes.

In contrast Paratylenchus populations exist mainly as fourth stage larvae and pre-adults which are known to be resistant to dessication and might even be resistant to fumigation.

None of the pesticides gave good control of potato cyst nematodes in the present investigation (Pain & Hague, personal communication). This suggests that the egg stage of the nematode present at the time of pesticide application is not reduced sufficiently by the nematicide to prevent subsequent build up of the populations to initial levels.

There have been many reports of an increase of cyst forming nematodes as a result of nematicidal treatments (Peters and Fenwick, 1949; Goffart 1961; Guile, 1964; Epps et al, 1964; Murant and Taylor, 1965). Dieter (1954) explains that the nematode population increase was due to less competition from other organisms for the existing food supply. Goffart (1961) also points out that the nematicidal treatment resulted in a better growth of the host plant and therefore a better food supply for the nematodes. Perry (1953) ascribes the nematode increase to the reduction of its natural enemies.

Thorne (1951) suggests that some nematodes (especially the fast moving, free living ones) are able to detect the approach of a gas, and thus migrate away from it. He also suggested that saprophagous nematodes appear to be accustomed to gases from decaying matter on which they feed and may therefore be more resistant to fumigation.

Crop responses alone do not indicate a nematode kill because other pathogenus are also killed. Further, the control of Paratylenchus and Pratylenchus populations cannot be directly linked with the potato yields since the potato cyst nematode was certainly at a population level likely to cause damage, in the fields under investigation.

Two of the fumigants, Dazomet and Chloropicrin, were also effective in controlling fungi and weeds, but the latter was phytotoxic. The nutrient status of the soil also affects crop growth. The poor plant growth on Ethylene dibromide treated plots is perhaps due to a nutrient effect. Koile (1961), Munnecke and Ferguson (1960) have shown that fumigants cause a retardation of the nutrification of soil nitrogen.

This causes an increase in the total mineralization which in turn affects the availability of other nutrients to the plant.

Section 2. B.

Laboratory and greenhouse tests on the effect of thionazin on plant and soil nematodes.

Chemicals with systemic properties have become widely used in recent years for the control of nematodes. The best known systemic compound is probably parathion, which combines low phytotoxicity with high toxicity to nematodes. The most successful results of systemic treatments have followed applications to soil or roots.

Of the systemic compounds in use, thionazin has been used successfully in soil applications for the control of root knot nematodes (Peacock, 1960, 1963; Sasser, 1952; Dias, Nelto & Falanger, 1962; Motsinger and Morgon, 1960; Motsinger, 1961). Bare root dips have been used to control the nematodes in situ (Miller and Perry, 1965).

Potato cyst nematodeshas been effectively controlled by soil applications of thionazin (Guile, 1964; Lewis, 1965; Ouden and Kaai, 1963) and stem and bulb eelworms by thionazin dips (Hague and Purnell, 1964; Oliff, 1966). Soil applications of thionazin have also been used against Radopholus similis (Suit and Felman, 1961; Collins and Felman, 1960), and the citrus nematode (Cohn and Minz, 1965).

In the foregoing field experiments thionazin applications were ineffective in controlling both migratory plant nematodes and free living nematodes. The purpose of the experiments described here was to obtain information on the effect of thionazin applications on plant and soil nematodes under laboratory and greenhouse conditions.

Experiment 1.

Effect of Thionazin (used as a chemical dip) on Pratylenchus populations invading barley roots

Materials and Methods

Barley seeds were germinated on filter paper and were transferred after three days to five inch plastic pots, containing naturally infected field soil. (Five plants per pot.)

The plants were lifted after 20 days, and the roots washed free of soil, first in tap water and then in distilled water. Commercial thionazin (3•5% granules) was ground to a fine powder and solutions prepared in distilled water. The treatment consisted of four concentrations of the chemical; 0, 25, 100 and 400 p.p.m. The plant roots were dipped for 24 hours in 200 ml. of the solutions. The roots were subsequently washed several times. The weighed roots were cut into approximately 1 cm. pieces, placed in 25 ml. of water and macerated in an MSE atomiser for 15 secs. The resulting suspensions were poured onto trays lined with facial tissue and the nematodes extracted for seven days. The water was changed every 24 hours.

The remaining root debris was stained with acid fuschin in lactophenol and cleared overnight in plain lactophenol. Replication was fourfold.

Results

The nematodes remaining within the root tissue were assumed to have been killed by the chemical. Root examination, however, showed that a small proportion of the nematodes still remained even within the untreated roots.

Thus a corrected percentage kill was obtained using the following formula:-

$$\text{Percentage corrected } (P') = \frac{(p1 - C)}{(1 - C)} \times 100$$

$$\text{Corrected percentage kill} = \frac{\%\ \text{kill} - \text{control}\%}{100 - \text{control}\%} \times 100$$

Fig. 22 shows the effect of thionazin on Pratylenchus populations expressed as a percentage kill assuming a 0% kill for the untreated populations.

The percentage nematode counts appear in Appendix table 27 and the analysis of variance of the transformed data is shown in Appendix table 28.

The effect of different concentrations on the nematodes present within the plant tissue was significant at the 0.05% level. The nematode number extracted was not significant.

It was observed that, of the nematodes remaining within the root tissue, a large number were adults.T Tests of significance carried out showed that, for all treatments the difference between the number of larvae extracted and the number of larvae remaining was significant, but the difference between the number of adults extracted and those remaining was significant only in the high dose treatment (400 p.p.m.) See table 10.

E= Extracted
R= Remaining

Treatment	Adults (mean) E	Adults (mean) R	Larvae (mean) E	Larvae (mean) R	t values Adults	t values Larvae	Significance Adults	Significance Larvae
25 p.p.m.	169	124	262	62	1.8	2.6	n.s	*
100 p.p.m.	150	156	282	68	0.24	4.9	n.s	**
400 p.p.m.	148	221	210	132	2.36	7.5	*	***

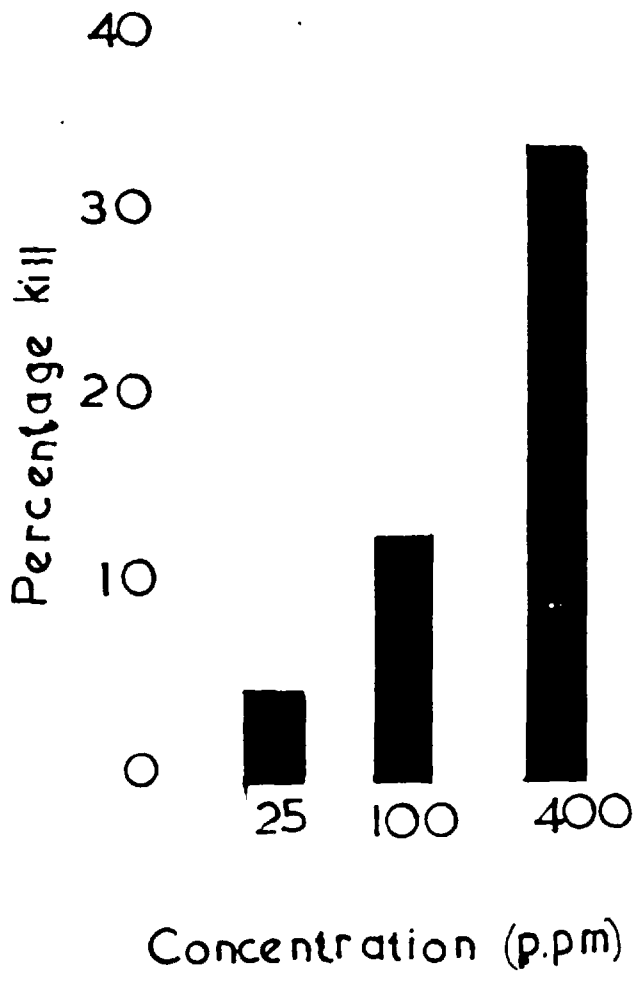

Fig. 22 Effect of thionazin (used as a chemical dip) on Pratylenchus spp. infecting barley roots.

Experiment 2

Pot experiments on the effect of Thionazin on soil nematodes

Since the field experiments on thionazin were ineffective, the following experiment was carried out to obtain more information on the effect of the chemical on free living and migratory plant feeding nematodes in pot cultures.

Materials and Methods

Granules of commercial thionazin were ground into a fine powder and mixed thoroughly with soil by coning and quartering. Naturally infected field soil was used. The treatments consisted of four concentrations of the chemical, 0, 100, 300, 900 p.p.m.

Barley seeds germinated for 3 days were planted in five inch plastic pots (1 kg. of soil per pot) containing the treated soil. Each pot contained five seedlings, and the replication was sixfold.

The experiment was initially designed to determine the effect of thionazin on the rate of invasion and the subsequent reproduction of Pratylenchus, and other soil nematodes, on barley. The estimates were to be made at 10 day intervals over a period of 60 days. Since the 10th day observations showed very poor root invasion, even in the untreated pots, the subsequent ten day observations were discontinued. Thus all estimates were made after 60 days.

The roots were washed free of soil, cut into 1 cm. pieces, and extracted over a Seinhorst Mistifier for 5 days. The soil was washed over a series of sieves onto a 400 mesh sieve, and the sediment extracted over Whitehead Trays.

Results

Effect of Thionazin applications on Pratylenchus spp.

Fig. 23 shows the effect of the different concentrations of thionazin on the Pratylenchus populations in the roots as well as in the soil. Thionazin used at the above concentrations was very effective in controlling these nematodes. The treatments 100, 300 and 900 p.p.m. of the chemical exhibited 68%, 75% and 89% kills respectively.

Effect of Thionazin applications on other soil nematodes

Table 11 shows the effect of thionazin on other soil nematodes in pot culture. There is a marked reduction in numbers of all nematode groups, especially of Helicotylenchus, Aphelenchus and Aphelenchoides populations.

Table 11 Effect of Thionazin on plant and soil nematodes

(mean of 6 replicates)

Nematode Groups	Thionazin concentration p.p.m.			
	0	100	300	900
Paratylenchus	558	87	48	26
Helicotylenchus	47	4	1	1
Tylenchorhynchus	1871	131	45	18
Tylenchus spp.	161	53	13	8
Aphelenchus and Aphelenchoides	2912	132	24	8
Particulate feeders	10270	5940	4246	1970

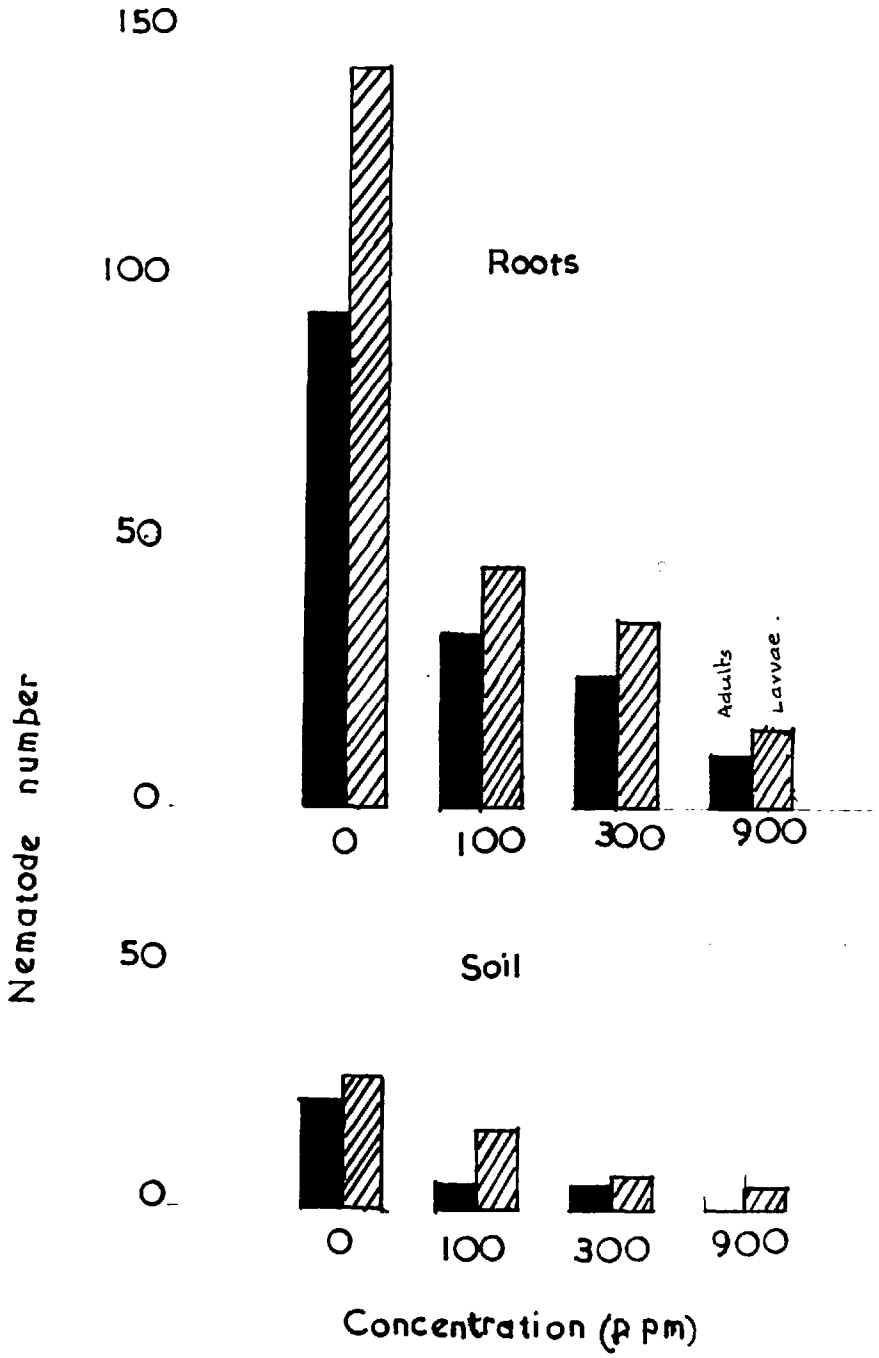

Fig. 23 Effect of thionazin on Pratylenchus spp. in pot cultures.

Experiment 3.

The purpose of this experiment was to obtain more information on the effect of the time and method of application of thionazin on Pratylenchus populations.

Materials and Methods

Poa annua plants were collected from a field infected with Pratylenchus spp. Several one gram samples were initially selected at random and were examined for nematode infection. An equal number of plants of approximately equal weight were used in each replicate. Finely ground commercial thionazin was used at four dosage rates, i.e. 0, 25, 50 and 100 p.p.m. The chemical was applied to sterilized soil and thoroughly mixed by coning and quartering.

The Poa annua was replanted in 5 inch plastic pots (5 plants per pot). The replication was sixfold.

Results

Table 12 shows the results of the change in the population of Pratylenchus observed at the end of 40 days. The results are expressed as the number of adults larvae and eggs present per gram of root, and the rate of increase shown is a ratio of the initial population.

Although the observations appear to indicate a difference in some of the treatments when compared with the controls, an analysis of variance of the data (Appendix table #31) showed no significance.

Table 12 Effect of Thionazin (in the soil) on Pratylenchus sp. within roots of Poa annua

Initial Population	Adults	Larvae	Eggs
Per gram of root	26	22	45

Change in population after 40 days. (mean of 6 replicates)

Treatment p.p.m.	Number per gm. of root			Rate of increase		
	Adults	Larvae	Eggs	Adults	Larvae	Eggs
0	205	125	96	7•8	5•7	2
25	140	96	78	5•4	4•4	1•7
50	119	73	54	4•6	3•3	1•2
100	112	61	38	4•3	2•8	0•8

Discussion and conclusions

The results of Experiment 1 show that thionazin is effective as a systemic nematicide. Further the Pratylenchus adults were more susceptible to the chemical than were the larvae. More marked differences might have been obtained had the dipping time been prolonged.

The high kills obtained in the pot tests (Experiment 2.) are of interest but give little specific information on the chemical. Peters (1962) points out that higher kills are to be expected in pot experiments than are found under field conditions. Furthermore, the concentrations of the chemical used here (100, 300 and 900 p.p.m.) are very high compared with concentrations in the field. The highest field applications of 40 lbs/acre resulted in a concentration of 20 p.p.m. in the top six inches of soil.

The high kills of free living particulate feeders may be due either to a contact effect or to active ingestion of the chemical. 'In vitro' studies on Turbatrix aceti have shown that thionazin has a nematicidal effect when ingested by the nematode (Oliff, 1955). Critchley (1968) showed that high doses of thionazin have a fumigant effect on Carabidae. Another possibility may be that the high concentrations of the chemical may bring about a reduction in soil organisms which serve as a source of food for the particulate feeders.

Studies on thionazin uptake by fungi (Oliff, 1965) show that small amounts of the compound are taken up by fungi. Therefore the high kills of the fungal feeders Aphelenchus and Aphelenchoides may be partly due to a systemic effect.

Other possibilities are the fumigant action of the chemical when present in high concentrations and perhaps a retarding effect of the chemical on growth of fungi, thereby reducing the food available to the nematodes.

The migratory endoparasite, Pratylenchus, may have been affected both by contact and systemic action of thionazin. Den Ouden and Kaai (1963) suggest that potato cyst nematode larvae are probably killed by the chemical just before or while penetrating the plant. This may well apply to Pratylenchus.

The reduction in the ectoparasitic nematodes, e.g. Paratylenchus, Tylenchorhynchus, Helicotylenchus and Tylenchus may be again due to a fumigant or a systemic action.

Another factor that should be taken into consideration is the persistence of the chemical in the soil. Field studies on thionazin show that the chemical persists over a very short period in the soil. Since the concentrations used in the pots were very much greater than those in the field, it is to be expected that it may persist in larger amounts in the pots, the absence of other environmental factors such as temperature and moisture present in the field may result in a longer period of persistance in the soil.

The third experiment was intended to yield further information on the systemic effect of thionazin on Pratylenchus in *situ*. Although the bare root dips gave effective control of the nematodes, the results obtained here were not statistically significant. This may be due to lower concentrations of thionazin (25, 50, 100 p.p.m.) as well as the different method of application.

Page.

PART 2. STUDIES ON SOME ASPECTS OF THE MORPHOLOGY, TAXONOMY AND BIOLOGY OF THE GENUS PRATYLENCHUS... 95

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PART 2

Some aspects of the morphology, taxonomy and biology of the genus Pratylenchus

Pratylenchus spp. are difficult to identify because they are very similar.

There is a great deal of variability in character within species. The present morphological and taxonomic study reviews some of these diagnostic characters and attempts to define more precise diagnostic features for species differenciation.

Biological studies on Pratylenchus species were carried out subsequently under laboratory and greenhouse conditions, in an attempt to study the influence of the plant on the population dynamics of the nematode.

Section 1. Morphological and taxonomic studies on the genus Pratylenchus.

Section 2. Biological studies on Pratylenchus spp.

Section 1.

Morphological and taxonomic studies on the Genus Pratylenchus - Tylenchida

INTRODUCTION

Since the present study was based on the effect of soil sterilants on migratory plant parasitic nematodes of which the genus Pratylenchus was most common, a special study has been made on the morphology and taxonomy of this genus.

The nematode genus Pratylenchus is of great phytopathological and economic importance. Commonly known as the lesion nematode, it is endoparasitic especially on the subterranean parts of plants, e.g. roots; tubers; rhizomes etc., and very rarely on the aerial parts. Pratylenchus are usually found to occur in the cortex of roots, brown necrotic lesions or pustules on the roots being the main evidence of their presence: these symptoms however are not seen in every infestation.

Considerable confusion still exists in the literature on the morphology and taxonomy of this genus. About thirty species have been described, many of which have been synonymized with the type species Pratylenchus pratensis (de Man, 1880). Thorne (1939) was the first to suggest that Pratylenchus pratensis contained a complex group of closely related species.

Pratylenchus was first described by various workers under the generic name Tylenchus. Goodey (1932) assigned the name Anguillulina in accordance with the revision made by Baylis and Daubney (1926). Filipjev, (1934) classified and defined it further, establishing the name Pratylenchus. In their revision of the genus Pratylenchus, Sher & Allen (1953), the synonyms resulting from the transfer is recorded in detail. Although the confusion in the nomenclature was eliminated by this work, the variations within the group make it taxonomically difficult, and thus it requires intensive study. The wide variation within the species was first discussed by Taylor and Jenkins.(1957). Both morphological characters and measurements were used in their study showing the variations within: P. zeae, P. penetrans, P. hexincisus and P. subpenetrans. Variations in the values (according to de Man's formula) were investigated, and the value of 'V', (the position of the vulva as a percentage of the total length) was found to be the most consistent feature and hence was regarded as a reliable diagnostic character. The most variable feature was found to be the shape of the tail tip.

Loof (1960) made a further detailed study, confirming the identity of de Man's type species Tylenchus pratensis along with several other species from de Man's collection. Variations in the tail shapes among progeny of a single female and variations seen in the lateral fields, among Pratylenchus spp. common to the Netherlands, have been investigated. Seinhorst (1968) discusses the use of other characters, i.e. number of annules on tail, the presence or absence of annules on the tail tip, shape and position of the spermatheca as distinguishing characters for different

species of Pratylenchus.

Materials and Methods

The Pratylenchus spp. were obtained from the soil as well as plant roots taken from Church field (Silwood Park).

The specimens from the soil were extracted, using the Whitehead's tray method. Those from the root tissue were extracted through a mistifier. Fresh specimens examined were relaxed over gentle heat and mounted in water. The permanent mounts were made by fixing the nematodes either in T.A.F., or F.A. 4:1 solutions and processing by the glycerol ethanol method (Seinhorst, 1959).

All measurements made were based on the de Man formula:-

L = Total length of body in mm.

$$a = \frac{\text{Total length of body}}{\text{width of body}}$$

$$b = \frac{\text{Total length of body}}{\text{anterior end to end of oesophagus}}$$

$$c = \frac{\text{Total length of body}}{\text{length of tail}}$$

$$V = \frac{\text{Distance from anterior end to Vulva \%}}{\text{Total length}}$$

$$T = \frac{\text{Length of testis \%}}{\text{Total length}}$$

The extent of the oesophageal overlap, (i.e. the distance from the centre of the median bulb to the end of the oesophageal overlap), and the number of tail annules were also determined. All measurements and illustrations were made by means of a Wild Microscope drawing apparatus. The identifications were mainly based on females, since males were rare or unknown.

The chief diagnostic features used were based on Sher & Allen (1953) and Seinhorst (1968).

1. Annules in the lip region - this was a consistent feature easily recognizable when there were two annules but indistinct when there were more.
2. Shape of tail - Although used as a diagnostic character, it was not very reliable since a wide variation in tail shape was evident within a single species.
3. Number of lines in the lateral field - The reliability of this character seems doubtful since the number of lines varied in the different regions of the same individual.
4. Cephalic framework - was used by Sher & Allen (1953) as a diagnostic feature for Pratylenchus thornei. Further examination of other species showed a considerable overlap of this character.
5. Shape of stylet knobs - Largely depends on the state of the specimens examined; viz, freshly killed specimens show the clear outline of the basal knobs, while it is somewhat obscure in permanent mounts.
6. The position of the vulva - Although regarded as being a reliable character, there was a considerable overlap among the species investigated.
7. Size - There was a great deal of variability in size influenced by external conditions. Example: Specimens extracted from plant tissue were usually larger than those from the soil. Fixed specimens were smaller than the freshly killed ones. Variations in size appeared to be influenced by the soil type.

8. Length of the posterior uterine branch - Although very little importance was attached to this character, Seinhorst (1968) has shown that a long post-uterine sac with a rudimentary ovary appears to be characteristic of certain species.

9. Number of tail annules - used as a diagnostic character, although there is considerable variation within species (Seinhorst, 1968).

10. The presence or absence of males - used as a diagnostic feature, this bearing a direct relationship to the spermatheca. In species where males were known to exist a spermatheca was present, and filled with sperms; in species without males an empty spermatheca was most common, or absent altogether.

11. Oesophageal gland overlap - The extent of the oesophageal gland overlap has not been regarded as a salient feature. The present work has shown this feature to be consistent within the species studied, and hence indicates its possible use as a diagnostic feature.

Descriptions of the four species of Pratylenchus found in the experimental site are given below, with a list of the plants in which Pratylenchus fallux was found.

Pratylenchus fallux (Seinhorst, 1968)

Measurements

Female. Length 0·501 mm. ± 0·047 mm. (·397 - ·581 mm.)

a = 25·2 ± 3·8 (20·1 - 32·5) b = 6·6 ± ·8 (5·6 - 8·7)

c = 14·3 ± 2·1 (11·2 - 19·1) V = 80% ± 2·0 (75 - 83·4%)

Stylet length 18 μ (16 - 20 μ).

Shown in Fig. 24.

Body slightly curved ventrally when killed by gentle heat. Three annules in the lip region, rather low and flat and almost obscure, prominent annules about 2 μ wide. The lateral field originates as a narrow groove in the region of the stylet guiding apparatus. It broadens posteriorly occupying one third of the body width in the vulval region and narrows in the tail region. It usually consists of four lines, a fifth oblique line between the inner two is common in the region of the vulva. The inner lines extend up to the phasmids, placed half way on the tail 10-13 annules from the tail tip. The tail tip ranges from almost smooth to distinctly crenate. The cephalic framework is heavily selerotized with its lateral margins extending posteriorly about 2 annules behind the lip region. A long stylet with round, flattened or forwardly directed basal knobs is present.

The dorsal oesophageal gland duct opens into the lumen of the intestine about 2 - 4 μ behind the base of the stylet. The nerve ring encircles the narrow isthmus very near to the base of the median bulb. The oesophageal gland overlaps the intestine ventrally, the extension averaging 42 microns. The excretory pore opens slightly posterior to the junction of the oesophagus and intestine. It is usually preceded by a clear space, the hemizonid, extending about two body annules.

The intestinal cells are filled with refractive granules, terminating in an oblique rectum opening by a very faint anus.

The single outstretched ovary extends anteriorly, usually not reaching the oesophageal region. It contains a single row of oocytes except for the region of multiplication consisting of a double row.

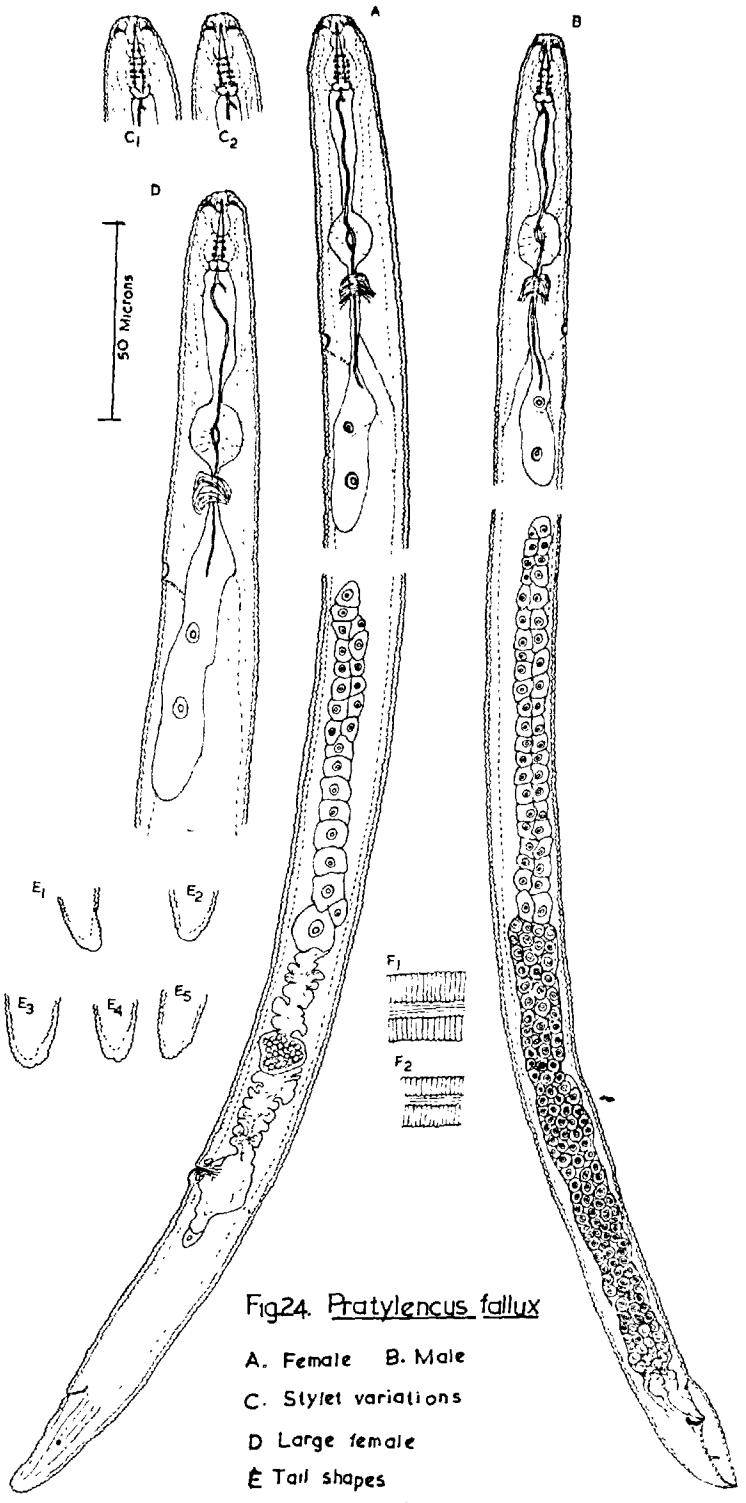


Fig24. Pratylencus fallux

A. Female B. Male

C. Stylet variations

D Large female

E Tail shapes

F Lateral fields

The oviduct is tubular and often forming invaginations. The spermatheca is very prominent, circular to square shaped filled with circular bodies about 2μ across containing small granules. The distance between the positions of the vulva and spermatheca is 37-71% of that between vulva and anus. The uterus is cellular and extends into a transverse vagina which opens to the exterior through a conspicuous vulva, the lips of which are sometimes protruded. The post-uterine sac is undifferenciated but sometimes may contain a single cell which represents the rudimentary ovary:-

Male:

Measurements

Length: •462 mm. (•397 - •5M mm) a = 26•7 (25 - 29•8)

b = 6•0 (5•2 - 7•3) c = 16•0 (14 - 22)

T = 44•6% (33•6 - 50%) stylet = 16•6 (16 - 18μ)

Spicule = 16μ

Shown in Fig. 24.

The males were not very common, the ratio of males to females being 1:8. The males were similar to the females, but were slightly more slender, and shorter. The oesophageal gland overlap is shorter in the male than in the female. The testis contained a multiple row of spermatocytes, followed by a vas deferens which is longer than the testis. The bursal edge is distinctly crenate.

Variations within Pratylenchus fallux.

Although recent studies indicate that P. fallux is a very common species in England and the Netherlands, it has not been described before Seinhorst (1968), presumably because it had been confused with P. crenatus P. penetrans and P. pratensis.

A detailed study was made of the variations existing within the species, during the course of this work. There was a considerable variation in size (length and width) within the population. The adults extracted from roots were larger than those extracted from the the soil. One population in Barley contained exceptionally large individuals; females ranging from •550 - •600 mm. and males •490 - •520 mm.

Shape of tail and tail annules.

The tail shape varies from smooth to distinctly crenate, conical, circular and almost straight tail tip. (Fig. 24). The tail annules are usually uniform and very distinct, but larger forms contained faint annulations, persumably due to the expansion of the body. The number of annules in the tail region ranges from 17-24.

Lateral field.

Generally consisted of four lines clearly visible in small specimens and barely visible in the larger forms. The outer lines were usually crenate, but appeared straight in the larger forms. A fifth line was often observed in the Vulval region, appearing either as a clear oblique line or as a series of short oblique lines. The lateral lines posterior to the phasmids were aerolated in some specimens.

Oesophageal overlap.

This was measured as the distance from the centre of the median oesophageal bulb to the end of the oesophageal overlap. This was fairly consistent within individuals of similar size. The length varied from 71-75μ in the larger females and 68-71μ in the smaller females. In the males the oesophageal length varied from 53-59μ in the smaller forms, and 63-70μ in the larger forms.

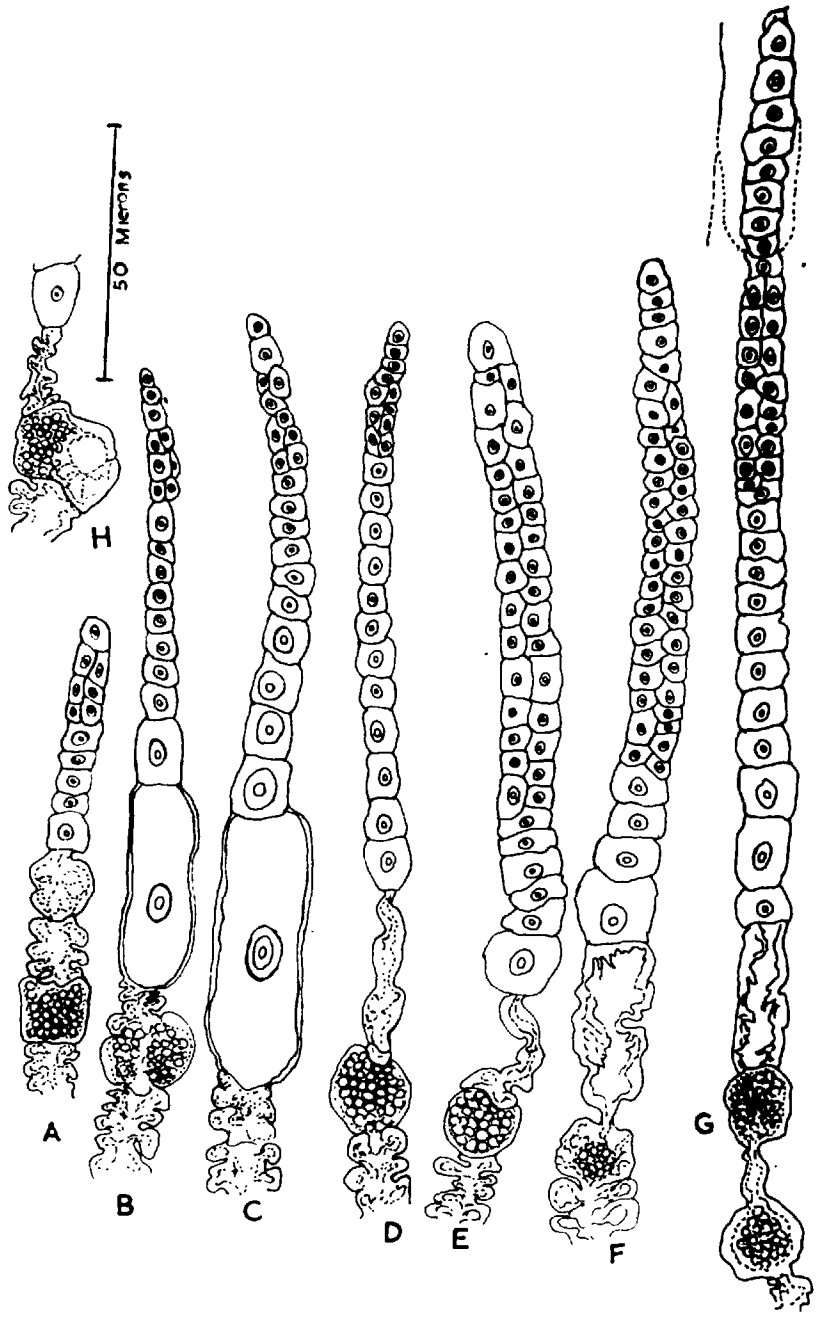


Fig. 25 Variations observed in the ovaries and spermathecae of Pratylenchus fallux.

A - F and H - normal females

G - large females.

Ovary

In general the ovary contains a single row of cells, except for a double row of 5-6 cells at the anterior end, and does not reach the oesophageal region. However, the large specimens had long ovaries extending beyond the overlap of the oesophagus. In these forms the number of cells in the region of multiplication varied from 8-17 (Fig. 25).

Spermatheca

Circular to square in shape, filled with sperms about 2 μ in diameter. Females with fully developed eggs contained empty spermathecae resembling those of P. crenatus. A large specimen contained a bilobed spermatheca, presumably a part of the oviduct acting as a reservoir. Other variations observed are shown in Fig. 25.

Pratylenchus crenatus. (Loof, 1960)

Measurements

Female: Length: •487 mm. (•384 - •589 mm.) a = 26•12 (23 - 28•7)

b = 6•09 (5•4 - 7•1) c = 15•8 (11•6 - 19)

V = 79•7% (76 - 82%)

Stylet = 17•5 μ (15 - 20 μ)

Somewhat slender species lying coiled ventrally when killed by gentle heating. Lip region contains 3 annules, which are not very distinct. The number of lines in the lateral field varies from 4 - 6. The outer lines are distinctly crenate. An oblique fifth line or two more lines lying very close to the inner lines have been observed. The tail is conical in shape and distinctly crenate. The tail tip may be circular, spathulate, forked or somewhat straight. The number of tail annules vary from 20 - 25. Fig .26.

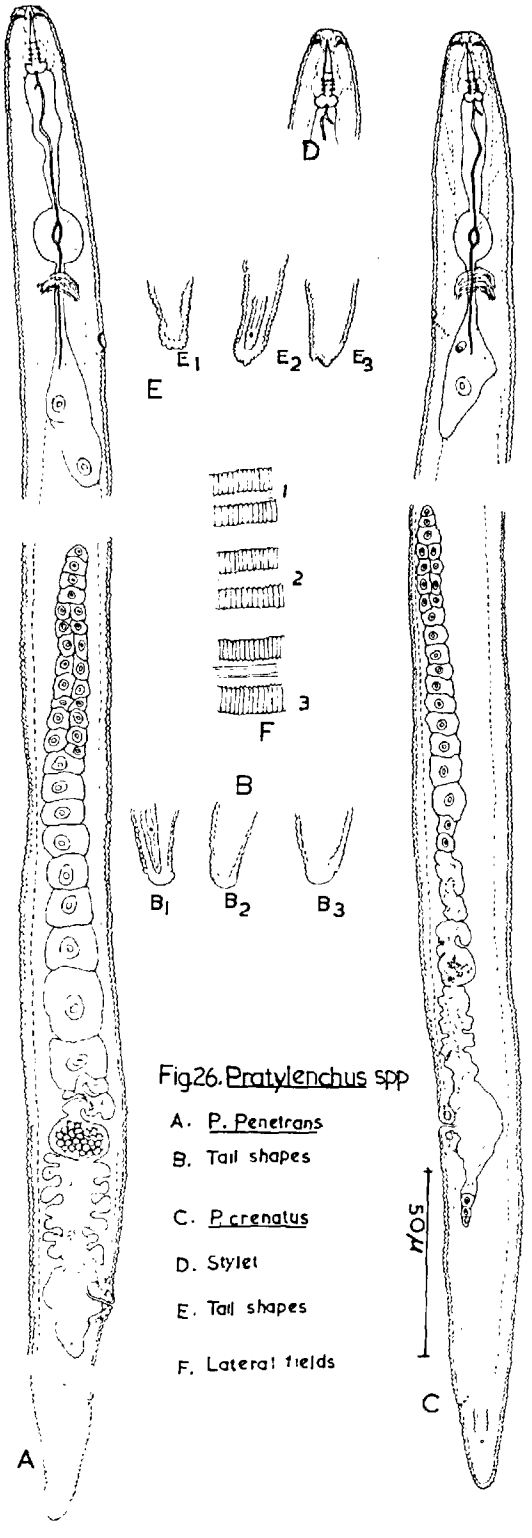


Fig.26. Pratylenchus spp

A. P. Penetrans

B. Tail shapes

C. P crenatus

D. Stylet

E. Tail shapes

F. Lateral fields

The overlap of the oesophageal gland is distinctly shorter than in P. fallux, ranging from 32 - 37μ. The distance from the centre of the median bulb to the end of the oesophageal overlap was 52 - 65μ. The ovary is straighter and much shorter than in P. fallux, never reaching the oesophageal region, even in the larger specimens. A few specimens were found with a double reflexed ovary (Fig. 27). The region of multiplication did not exceed 4 - 5 cells. The tubular oviduct usually ended in a body of varying size and shape which is an empty spermatheca (Spermagonium, Dickinson 1962). This resembled the empty spermatheca of P. fallux (Fig. 25). The distance between the vulva and the spermatheca is 50 - 59·6% of that between vulva and anus. The post-uterine sac is usually elongated and contains a rudimentary ovary of 3 - 4 cells.

Pratylenchus penetrans (Cobb, 1917; Chitwood & Oteifa, 1952).

Measurements

Female: Length: ·540 (·490 - ·570 mm.) a = 23 (21 - 26·3)

b = 6·5 (5·7 - 7·9) c = 18· (16 - 21)

V = 80% (79 - 82·6%) Stylet = 18μ (16 - 20 μ)

Larger than P. fallux, usually lying straight when killed by gentle heat. The 3 annules in the lip region are indistinct. Body annulations are somewhat faint. Lateral field has four incisions extending to the tip of the tail and encircling the phasmid, the outer lines are faintly crenate. Tail tip is conical to broadly rounded and smooth. Tail annules ·18 - 22 in number, and not prominent. The median oesophageal bulb is broad and almost spherical. The oesophageal gland overlap ranges from 32 - 38μ. The distance from the centre of the median bulb to the end of the oesophageal overlap is 52-62 μ.

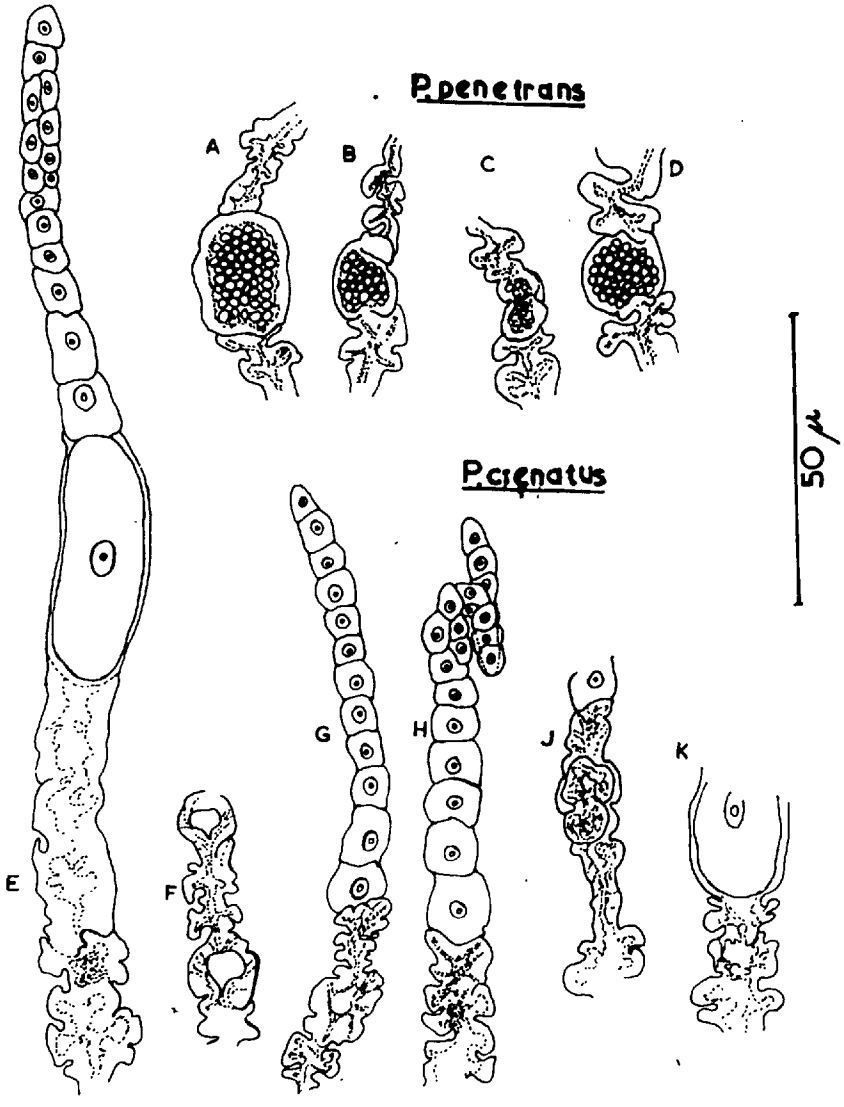


Fig. 27 Variations in the spermathecae and ovaries of Pratylenchus spp.

A - D P. penetrans

E - K P. crenatus

Single, outstretched ovary not reaching the oesophagus, region of multiplication extending up to 6 - 8 cells. The spermatheca is usually circular, and is filled with granular sperms. The shape of the spermatheca my be very variable (Fig.27). The empty spermatheca resembles that of P. crenatus. The distance between the vulva and the spermatheca is 43% (32 - 53%) of that between the vulva and the anus (Fig.26).

Pratylenchus neglectus. (Rensch, 1924;) Chitwood & Oteifa, 1952.

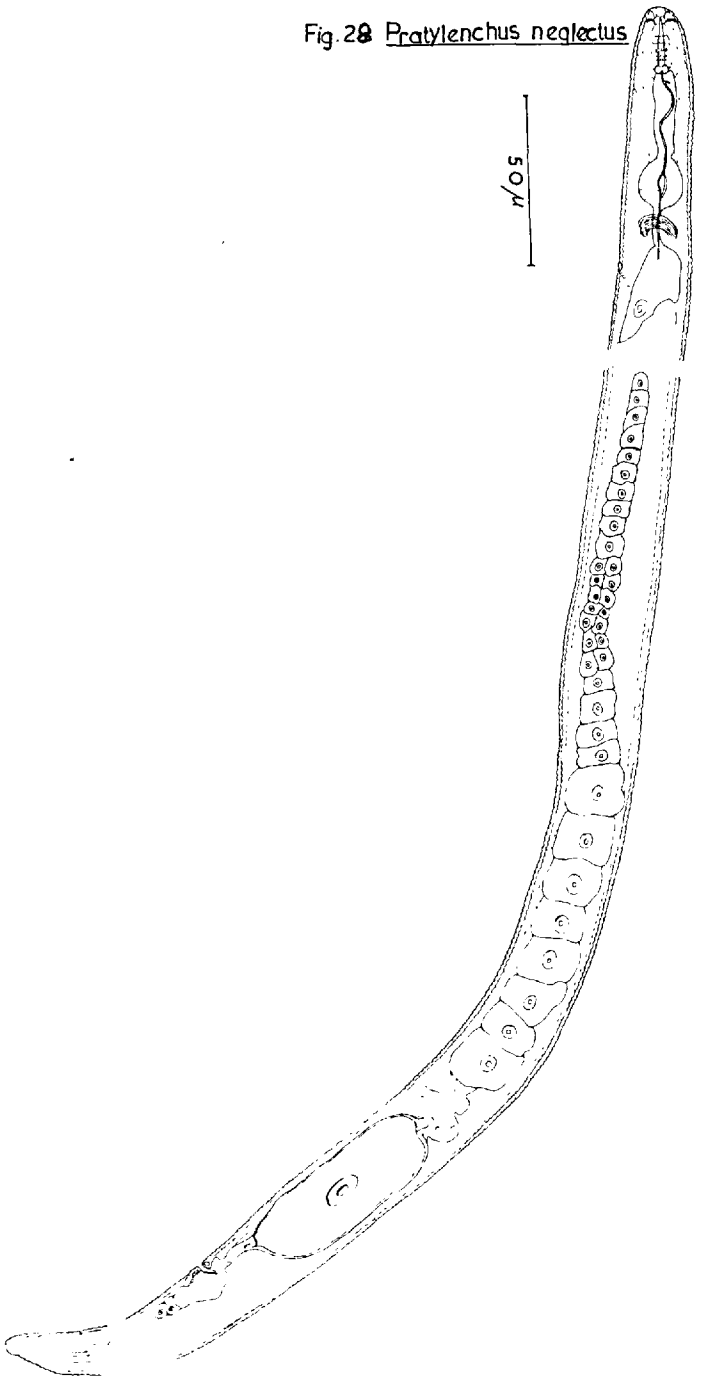
Measurements

Female: L = •456 mm. (320 - 528 mm.) a = 18•2 b = 6•4 c = 20•1

V = 89% Stylet = 17 μ.

Short but stout species, lying almost straight when killed by gentle heat. Lip region with 2 prominent annules, the anterior margin is convex. Body annulation is very faint especially in females with fully developed ova in the uterus. The lateral field contains four indistinct lines. Tail tip is broadly rounded and smooth with 17 - 20 annules. The median oesophageal bulb is broad and the overlap of the oesophageal gland is about 24 microns. The distance from the median bulb to the end of the oesophageal overlap is 47 - 53 μ. It has a long single outstretched ovary (Fig. 28), and spermatheca were not observed (specimens examined contained only fully developed ova). A post-uterine sac is slightly elongate and has a rudimentary ovary of one to two cells.

Fig. 28 Pratylenchus neglectus



Relationships

Of the four species of Pratylenchus present in the population studied, P. neglectus can be easily distinguished from the other three by the presence of two distinct annules in the lip region. P. crenatus, P. penetrans and P. fallux are not easily distinguished from each other because of the variations found within the species. In a recent study (Seinhorst, 1968), which describes P. fallux, the main diagnostic feature was the annulation around the tail tip. Of the diagnostic features considered in the description of the species, the shape of the tail, the tail tip annulation and the number of tail annules appeared to be very variable. The tail tip may appear smooth as in P. penetrans, or distinctly crenate or even forked as in P. crenatus. The number of tail annules ranges from 16 - 26 in P. fallux; 15 - 27 in P. penetrans; 20 - 24 in P. crenatus (Seinhorst, 1968). These differences make it extremely difficult to distinguish P. fallux from P. penetrans and P. crenatus.

A long post vulvular uterine branch, an empty spermatheca and the absence of males appeared to be conspecific for P. crenatus. Here again, P. fallux females often appeared with empty spermatheca similar to those of P. crenatus.

During the present study, the extent of the oesophageal overlap, (i.e. the distance from the origin of the oesophageal gland to the end of the oesophageal gland, and the distance from the centre of the median oesophageal bulb to the end of the oesophageal gland) was measured. The latter measurement was more consistent because the origin of the oesophageal gland was often indistinct in fixed specimens, the former measurement was therefore not very reliable.

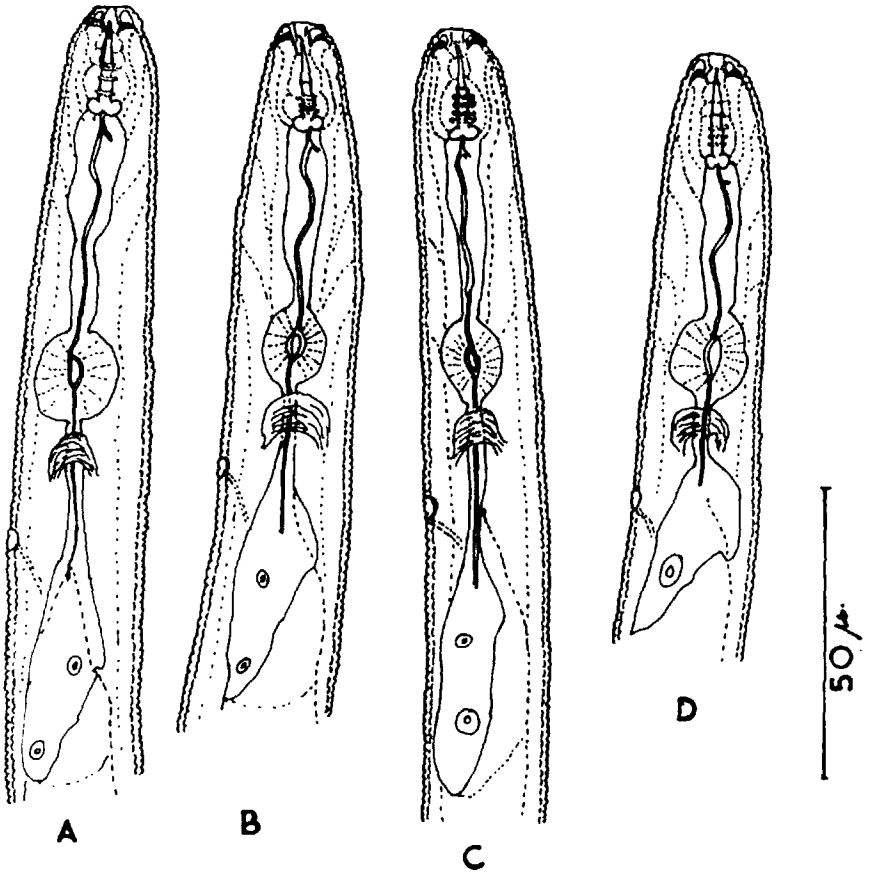


Fig. 29 Oesophageal overlap of four Pratylenchus spp.

A - P. penetrans B - P. crenatus

C - P. fallux D - P. neglectus

The shape of the oesophageal gland varied to a large extent (varying with fixation), and hence could not be considered as a diagnostic character. The extent of the oesophageal overlap was consistent for a particular species (Fig. 29).

P. neglectus showed a mean of 50·8 microns; P. fallux had a mean of 70 microns while P. crenatus and P. penetrans had a mean of 60 microns. Fig. 30 shows the distribution of this value expressed as a percentage of the total length of the worm. The distinct difference in the value for P. fallux **vs** that for P. crenatus and P. penetrans is very clear and shows very little overlap in the values.

Statistical analysis of the data showed the difference between the means to be highly significant (P = 0·001). Thus the measurement of the oesophageal overlap is a reliable character when distinguishing P. fallux from P. penetrans, and P. crenatus. The presence of a rudimentary ovary with 2 - 3 cells clearly distinguished P. crenatus from P. penetrans and P. fallux (Seinhorst, 1968).

The present study has shown that the extent of the oesophageal overlap should prove to be a reliable diagnostic character for distinguishing Pratylenchus fallux.

Distribution

The four Pratylenchus spp. were obtained from an arable field, with sandy loam soil and cultivated at regular intervals.

P. fallux, hitherto unknown, was found to be very common in several sites examined. These included peat soils of Lancashire. The unnamed species of Pitcher, Way & Savoury, 1960, and Corbett and Webb, 1967 may also belong to this species (Seinhorst, 1968).

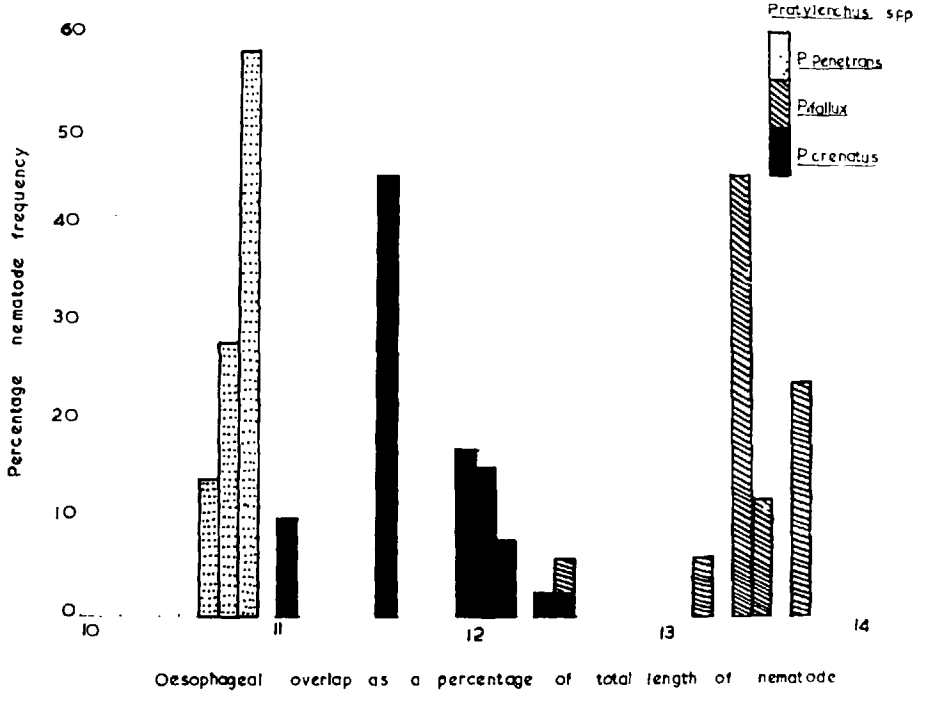


Fig 30 Oesophageal overlap of Pratylenchus spp expressed as a percentage of total length

Although the number of the separate species was not recorded, observations suggest a greater abundance of some species. The species in their order of abundance were: P. crenatus, P. fallux, P. penetrans and P. neolectus, the last named being found in very low numbers.

The distribution of P. fallux in plant roots was determined by staining roots with acid fuschin in lactophenol. The nematodes were found invading the roots of both cultivated crops (i.e. Field beans, barley and potato), and weeds common to the soil type (Appendix table 1).

Section 2.

The previous field experiments dealt with the population fluctuation of Pratylenchus spp. only in the soil. The present investigation deals with the population changes occuring in the soil as well as within the plant tissue.

Experiment 1. The rate of invasion and the subsequent reproduction of of Pratylenchus spp. (P. fallux, P. crenatus, P. penetrans) on Barley and chickweed.

Experimental.

Barley was selected, since it was known to be a good host for Pratylenchus spp. Chickweed was selected since it was the most prevelant weed in the plots and supported a large number of Pratylenchus.

Naturally infected field soil was sieved and mixed thoroughly by coning and quartering. Two 100 ml. subsamples were extracted to determine the initial population level and the remainder placed in plastic pots (350 ml. soil). Barely and chickweed seeds that had been germinated for 3 - 4 days were planted (two Barley seedlings per pot; 10 chickweed seedlings per pot); replication was fourfold. The observations were made over a period of 60-70 days at 10 day intervals. The first estimation for chickweed (10th day) was not possible because the plants had not grown sufficiently and the nematode number was therefore estimated on the 20th day.

The roots were washed free of soil and stained in 0·05% acid fuschin in lactophenol, cleared for one day in lactophenol. These were crushed between two glass plates and examined under the stereomicroscope.

The soil nematodes were extracted using Whitehead trays. As the root system enlarged the staining method by itself was tedious. Therefore the roots were macerated on an M.S.E. atomiser and the nematodes extracted for 3 days over nylon sieves. The distilled water in the trays was changed daily. The root tissue was subsequently stained and examined for nematodes remaining within.

Changes in the population level of Pratylenchus spp. associated with Barley and chickweed.

Fig.31 shows the population changes in the total number of Pratylenchus spp. (soil and roots) associated with barley and chickweed. The population in Barley gradually reached a peak followed by a sudden decrease, but in chickweed the population remained at a constant level. The decrease in barley was possibly related to the physical condition of the roots. The peripheral roots were often tangled and dried up, and might have prevented a further increase in the nematodes.

The rate of invasion and the subsequent increase in the population of Pratylenchus.

Fig. 32 shows rate of invasion, and the increase in the population level of Pratylenchus species on barley and chickweed. Although the total nematode level over the first 10 days remained the same as the initial in barley, the population in chickweed showed a reduction of 16% of the initial population. After 10 days growth the barley roots were sufficiently large for root invasion by Pratylenchus, whereas the chickweed roots were too small. The increase in number of nematodes in the roots seems to be directly related to the invasion and the loss from the soil.

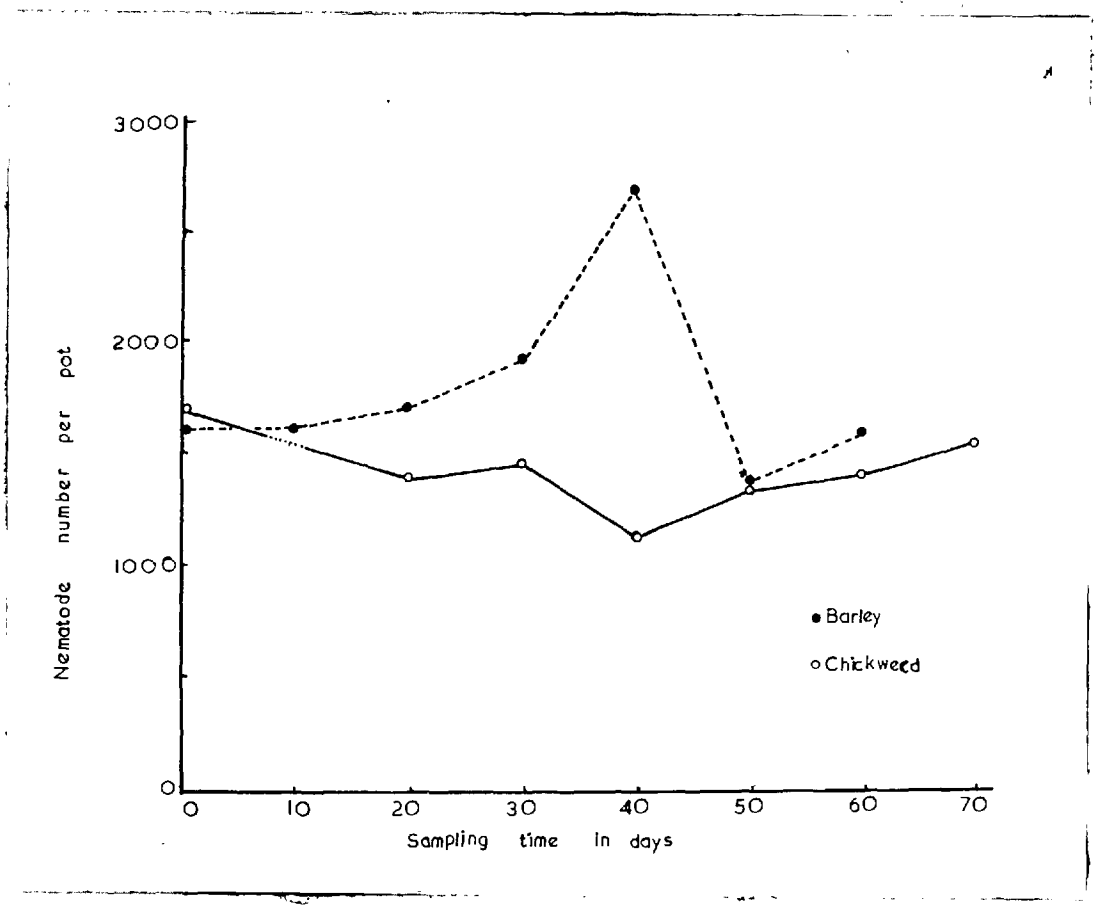


Fig. 31 Total number of Pratylenchus spp. associated with barley and chickweed.

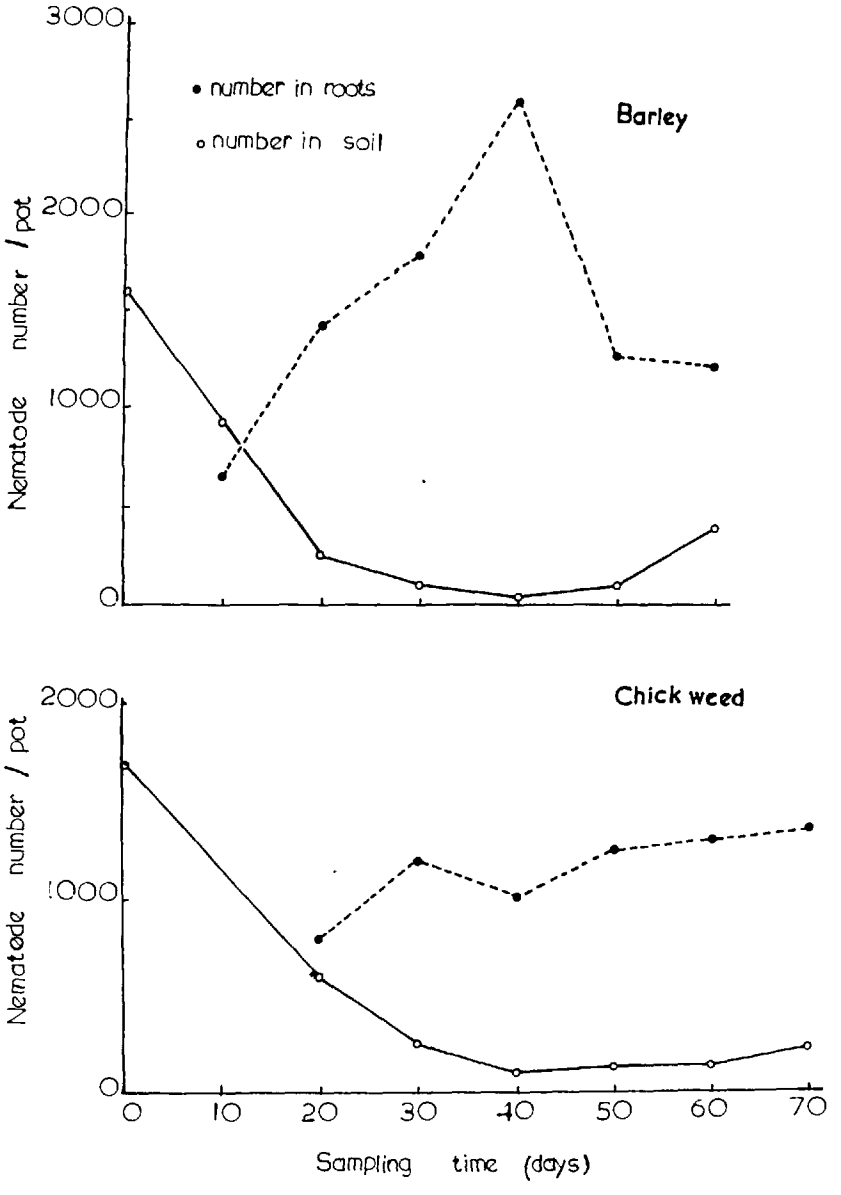


Fig.32 Rate of invasion and subsequent reproduction of Pratylenchus spp in barley & chickweed

In barley the nematode population increased until an equilibrium level was reached, further increase has been prevented probably by the physical condition of the roots in pot culture, as explained earlier. The slight increase in numbers in the soil after 50 days indicates that the nematodes are starting to migrate from the roots.

The results show that chickweed is a poor host, although it is able to support some of the nematodes. Therefore only a small number migrate back into the soil.

Fig. 33 showing the population fluctuation of Pratylenchus (larvae and adults) in barley and chickweed, helps to emphasise further that the latter is a poor host. The increase in adults by the 20th day in barley shows the presence of new adults, but in chickweed the adult population remained at the same level.

Relationship between increase in root weight with change in population of Pratylenchus

Fig. 34 shows the change in the root weight of barley and chickweed with change in time and the variation in the population of Pratylenchus. The rate of increase in the population of nematodes is directly related to the increased root weight in barley up to the 40th day. This effect, however, is reversed soon after. The nematode population changes in chickweed, however, is not closely related to a change in root weight.

Discussion and Conclusions.

The field studies on Pratylenchus spp. were confined to the population fluctuations of the nematodes in soil. The results seen here illustrate the population changes of Pratylenchus spp. directly associated with plant roots.

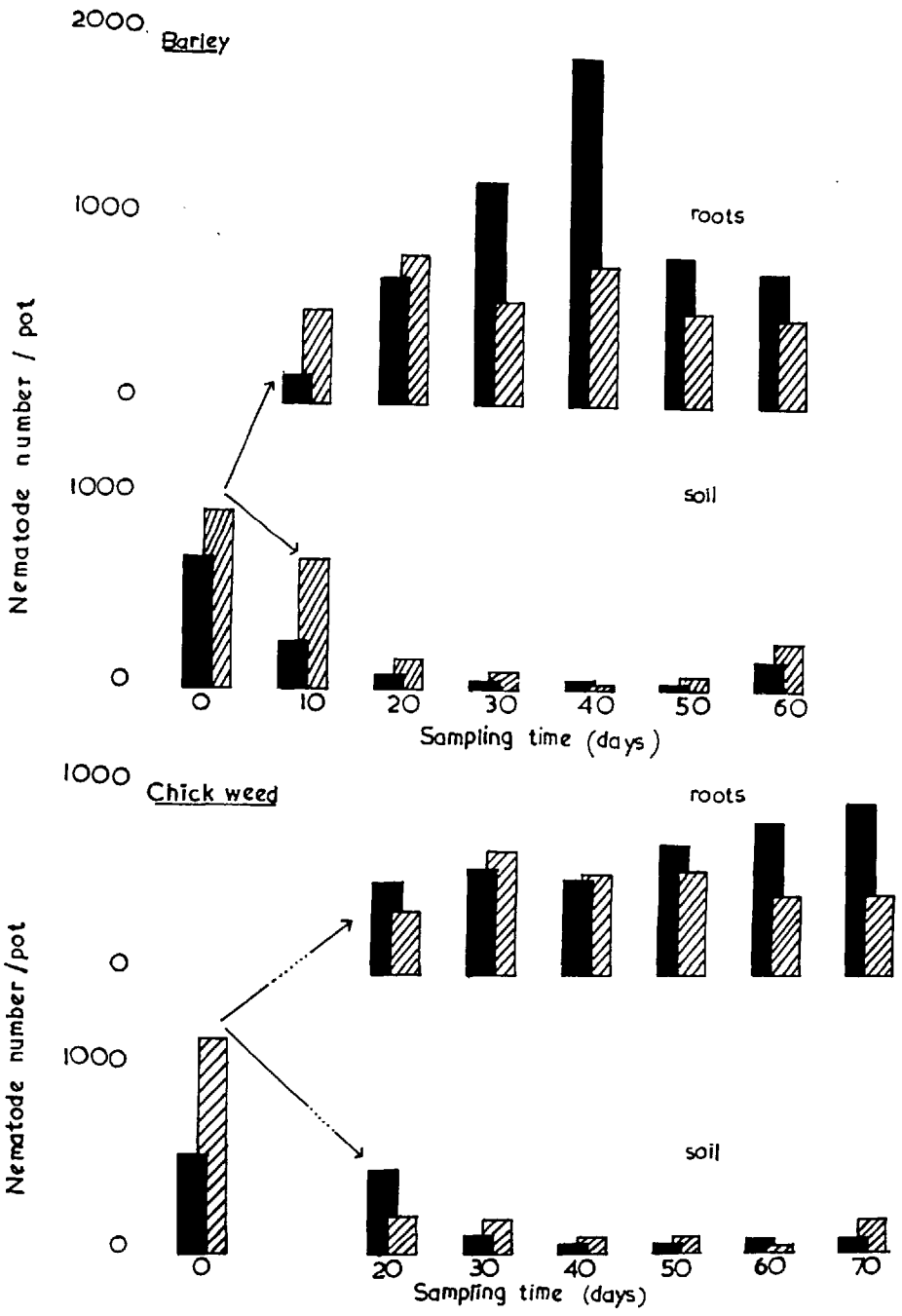


Fig.33. Population changes in Pratylenchus spp associated with barley & chick weed

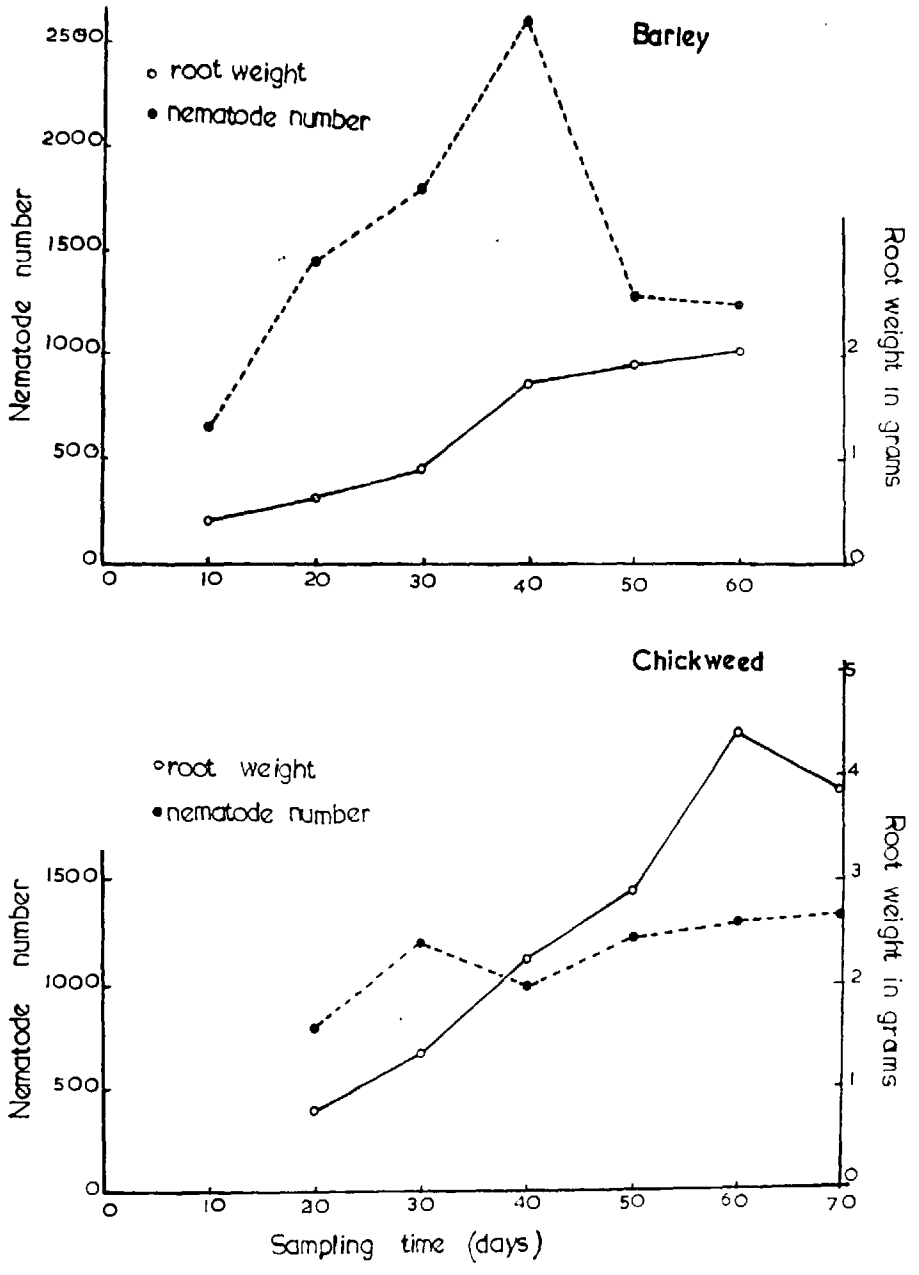


Fig.34. Change in root weight associated with changes in Pratylenchus populations

As seen for barley the Pratylenchus populations reach a peak followed by a rapid decline. The subsequent decrease in the nematode population within the roots is largely related to the migratory habit of the nematode. Further, the root system (especially the peripheral roots) in the pot cultures were dessicated by the 50th day, and this might have inhibited any further increase in the nematode population. These results lay emphasis on the fact that when evaluating the population fluctuations of Pratylenchus, the estimates should include the numbers present in the plants as well as in the soil.

Seinhorst (1968) has shown that most plant nematodes are stimulated by the host plant, and a large proportion are therefore unable to survive in the absence of a host plant. This might explain the high mortality rate encountered at the beginning of the experiment, especially with chickweed.

There were no external manifestations of the effect of Pratylenchus spp. on either barley or chickweed. Pratylenchus spp. are known to cause brown necrotic lesions often associated with secondary organisms such as bacteria and fungi on a large number of crops. Mountain (1959) showed that Pratylenchus penetrans produces emulsin which hydrolyses amygdalin present in the peach roots; one of the biproducts of hydrogen cyanide is responsible for killing the surrounding root tissue and causing a discolouration. Wood (1967) shows that certain plants contain less glucocides which liberate HCN when attacked by pathogens and therefore show no discolouration. Certain substances present in plants either reduce or prevent the production of a particular enzyme when attacked by plant parasites, and subsequently prevent the formation of toxic substances. e.g. the secretion of cellulose which causes a

breakdown of cell walls is reduced when glucose is present. It is therefore possible that the lack of disease symptoms in barley and chickweed are associated with the above factors.

Mountain (1968) showed that the severity of lesion formation by Pratylenchus depends on the concentration and location of glycocides in the plant tissue and the concentrations of the hydrolytic enzymes released by the nematodes.

There are variations in pathogenicity caused by Pratylenchus penetrans on tobacco (Olthof, 1968). This again shows that there is much variability in the external symptoms caused by Pratylenchus invasion. Therefore, the problem as to whether these symptoms are species specific, race specific or entirely dependent on the physical and biochemical condition of the plant still remains unsolved.

Experiment 2.

The life cycle and the reproductive potential of Pratylenchus fallux (Seinhorst, 1968) using a single female.

Experimental:

Barley seeds were surface sterilized using a 0·002% mercuric chloride solution, followed by several washings in sterilized water and germinated until the roots were about an inch long. These were then placed in petri dishes containing 10% water agar (5 seedlings per dish).

Pratylenchus fallux females were washed several times in sterilized water, hand picked and placed on the root tip of the seedlings (one per plant). The petri dishes were left in the dark for 48 hours.

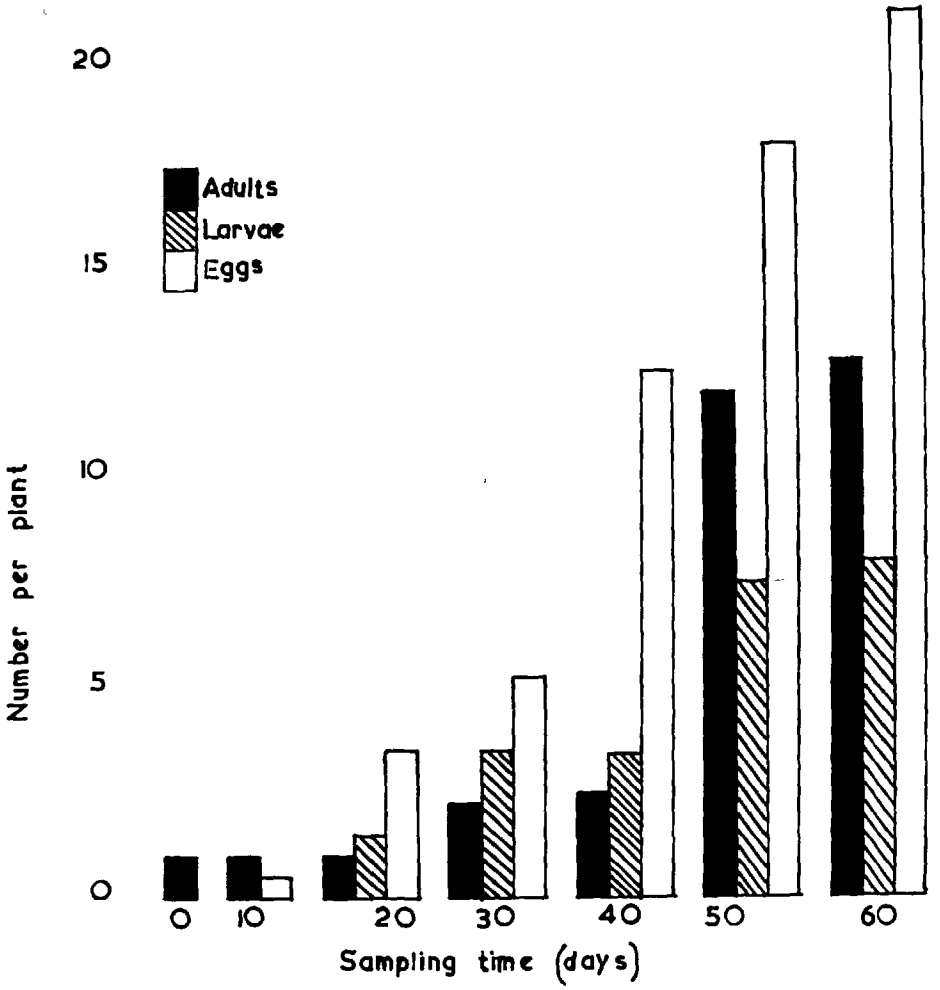


Fig.35. Reproductive rate of a single female of

Pratylenchus fallux on barley.

The dishes were examined for any worms that had not invaded the roots and each seedling was subsequently planted in 2½ inch plastic pots containing steam sterilized soil; replication was tenfold.

The roots were examined by staining with acid fuschin in lactophenol, the estimates were made every 10 days over a period of 60 days. The reproductive rate of a single female of Pratylenchus fallux is shown in Fig. 35, at the end of 10 days only two females had laid eggs. The number of eggs laid by the 20th day varied from 1-6 per female, and 6 plants contained larvae. New adults were present on the 30th day. The eggs produced varied from 16-1 per female. By the 40th day the nematodes were distributed in several roots. The number of eggs varied from 1-33 per female. The 50th day results showed a larger number of new adults and some of these had laid eggs. The number of eggs per plant varied from 2-53. On the 60th day the number of adults per plant varied from 5-20, and eggs 12-37. Several males were present.

Discussion and Conclusions.

The biology and development of Pratylenchus species have been investigated by several workers. Zimmerman (1898) was the first to describe the development from eggs to adult of P. coffeae in coffee plants.

Hastings (1939) showed that the life cycle of Pratylenchus pratensis on oats was completed within 54-65 days; the development from larva to adult was 25-31 days and maturation to second generation was completed in 29-34 days. The largest number of eggs laid per female was 16.

Gadd and Loos (1941 a) observed that the life cycle of Anguillulina pratensis (synonym for Pratylenchus loosi) on tea was completed in 45-48 days; the eggs hatched in 15-17 days, while the larval period was completed in 15-16 days. They also found that egg laying depended on the age of the female and the presence of the male.

Jensen (1950) describes and illustrates four moults for Pratylenchus vulnus.

Targan (1950) indicated a short life cycle for Pratylenchus sp. on boxwood, where the eggs hatched in 5-6 days at 78°F. Graham (1951) has shown that Pratylenchus sp. on corn completed its life cycle from egg to adult in 30-35 days.

The present study shows that the life cycle of Pratylenchus fallux (from egg to maturity) is completed in about 40-45 days.

The low rate of egg production at the beginning of the experiment may have been due to either the females being immature and unfertilized or some may have completed their egg laying period. A larger number of eggs were found whenever males were found in the vicinity of the females. The exact number of eggs laid by a female cannot be determined accurately because of its migratory habit. The same applies to the accuracy of the adult and larval counts after the 30th day.

Experiment 3.

Evaluation of the methods of extraction of Pratylenchus spp. in roots

Experimental:

Most methods of evaluating the number of Pratylenchus spp. present in roots deal only with the number of nematodes extracted from the plant tissue. During the course of the present investigation, roots were examined and found to contain a large number of nematodes after the usual extraction process. It was therefore evident that an estimate of only the number of nematodes extracted was not a true representation of the total population in the root tissue.

Four extraction methods were compared; namely, the mechanical maceration technique (Taylor & Loegering 1953, Fallus 1943); Mistifier (Seinhorst, 1950); a root incubation technique (Mckeen & Mountain, 1960); and a combination of mechanical maceration and constant agitation using a wrist action shaker. Equal weights of infected material (Trifolium pratense grown in infected soil) were used with a fourfold replication.

Mechanical Maceration technique

Root tissue was washed free of soil and cut into approximately 1 cm. pieces, placed in 100 ml. flasks containing 50 ml. water. These were macerated in an MSE atomiser for 30 secs. (10 secs at $\frac{1}{2}$ speed, 10 secs at full speed and 10 secs at $\frac{1}{2}$ speed). As the roots were not sufficiently macerated this was repeated for a further 30 seconds. The root suspension was placed over nylon sieves lined with facial tissue and the nematodes extracted for five days. The water in the trays was changed daily.

Mistifier method

The roots were washed free of soil, cut into 1 cm. pieces and extracted under the mistifier for five days.

Incubation method

Plants washed free of soil were placed in 400 ml. conical flasks containing 200 ml. of distilled water. The roots were sprayed with a solution of 550 p.p.m. of dihydrostreptomycin . sulphate. Air was bubbled continuously through the solution. The roots were incubated for five days.

Maceration and constant agitation using a wrist action shaker

The roots washed free of soil were cut into 1 cm. pieces and blended as before on an MSE atomiser. The mixed roots were placed in 400 ml. conical flasks containing 200 ml. of distilled water. These were agitated at half speed for 3 days on a wrist action shaker. The extract was collected and the remaining root tissue placed on nylon sieves and the nematodes extracted through facial tissue for a further two days. Two 1 gram samples of the remaining root tissue (in all four methods) were stained in acid fuschin to estimate the nematodes remaining within the root tissue.

The number of nematodes extracted by the four different methods is shown in Fig. 36. The number of nematodes extracted expressed as a percentage of the total (i.e. number extracted and number remaining) was considerably low (See Table 13).

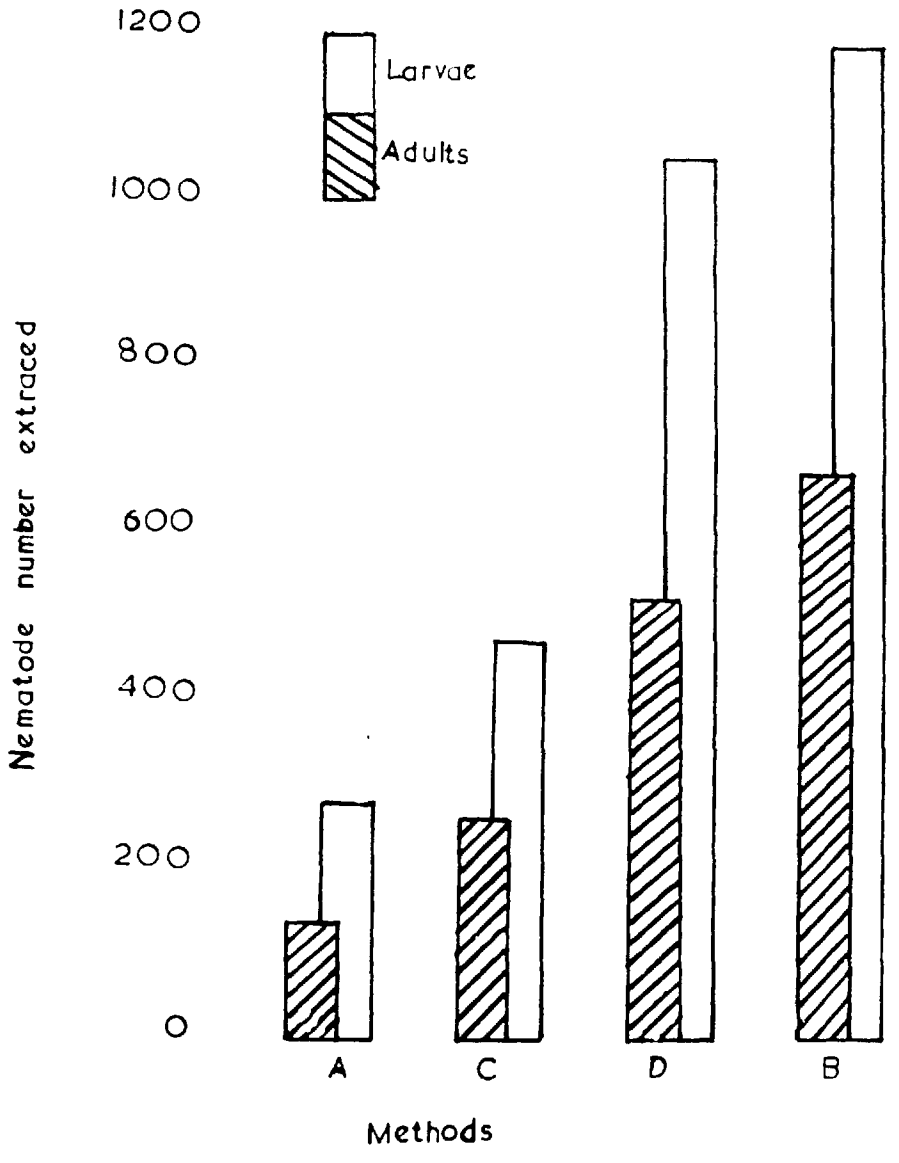


Fig. 36 Nematode number extracted from the roots-

A - Maceration method

B - Mistifier method

C - Root incubation

D - Maceration and wrist-action shaker

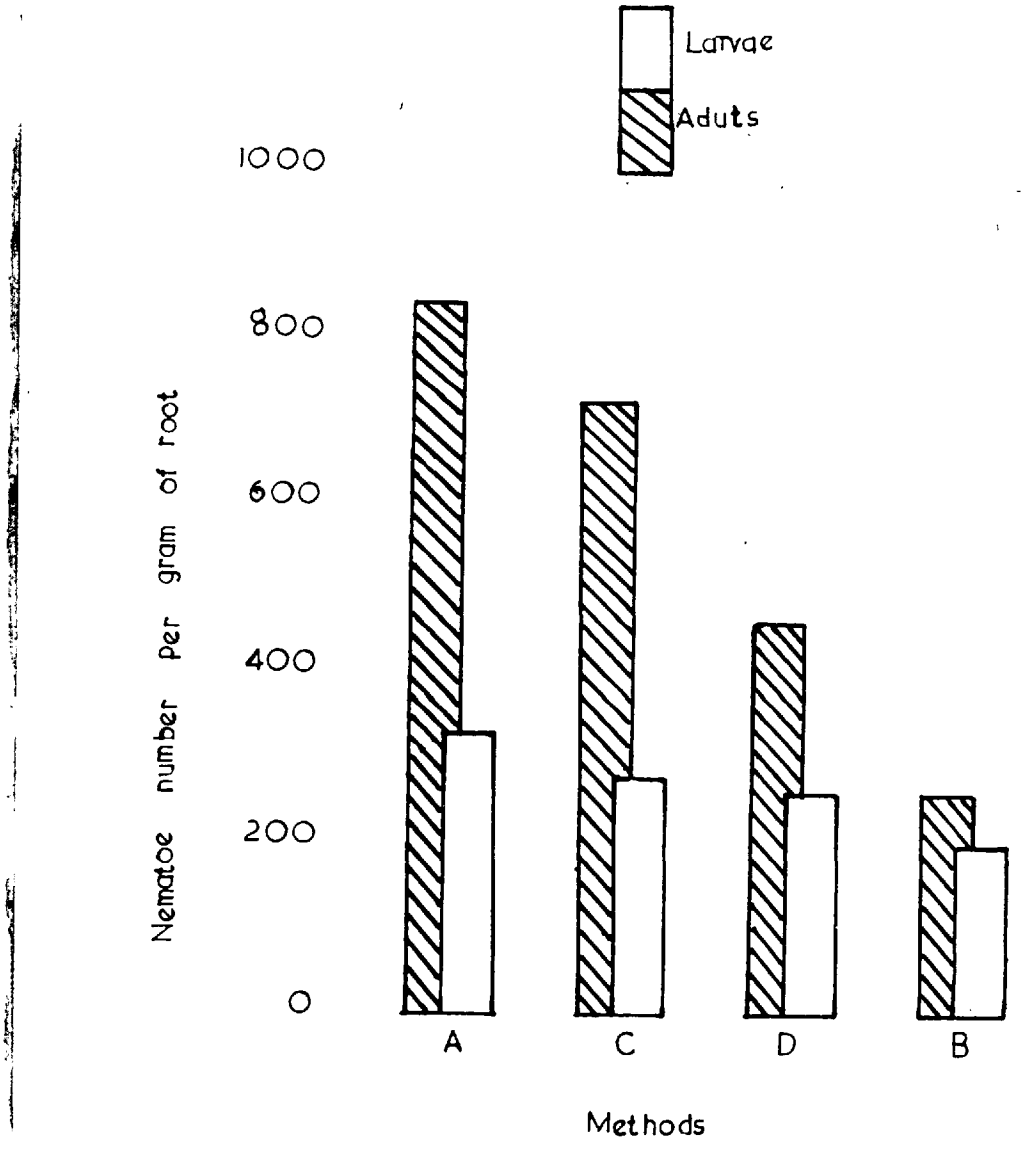


Fig. 37 Nematode number remaining per gram of root (using four extraction methods).

Method:

A - Maceration method

B - Mistifier method

C - Incubation method

D - Maceration and wrist-action shaker

In the mechanical maceration method, the roots were not fully macerated, and the resulting nematode suspension was dirty and the nematodes were not very active (probably due to a lack of aeration).

Table 13 Number of nematodes extracted and remaining expressed as a percentage of the total present.

	% Extracted	% Remaining
Maceration method	2	98
Incubation method	5	95
Maceration & wrist-action	11	89
Mistifier	19	81

Although a solution of streptomycin sulphate was used in the incubation method to prevent bacterial action, the resulting nematode suspension did not seem to be free of these bacteria, and the nematodes were not very active. The wrist-action shaker following the mechanical maceration was used to enable the nematodes in the partially macerated tissue to escape. The disdavantage of this method was again the resulting dirty suspension and less active nematodes. In contrast to these three methods, the nematode suspension from the mistifier was both clean and clear, and contained very active nematodes.

Table 14 shows the percentage of nematodes extracted (numbers expressed as a percentage of the total extracted), and those remaining within one gram of root tissue (expressed as a percentage of the total remaining per gram of root).

An analysis of variance showed that the variation between methods was highly significant (P = 0•1). The variation in the number of males, females, and larvae extracted by the four methods was significant (P=0•1). The variation between methods for nematodes remaining was significant (P=0•1, The adults were significant at P=0•1, and larvae at P=0•1).

Table 14. A

Nematode number extracted - expressed as a percentage of the total extracted.

	♀	♂	larvae
Maceration method	10	6	9
Incubation method	17	14	17
Maceration & wrist-action	33	32	35
Mistifier	40	48	39

B Nematode number remaining per gram of root expressed as a percentage of the total remaining.

	Adults	Larvae
Maceration method	36	30
Incubation method	31	25
Maceration & wrist-action	22	26
Mistifier	11	19

Discussion and Conclusions

Seinhorst's mistifier has been the most widely used method for extracting nematodes from plant tissue. In recent years, however, the mechanical maceration method has been used widely, especially for work with Heterodera, Meloidogyne and Pratylenchus in extracting from root tissue. Stemerding (1964) has shown that the mechanical maceration method gave better results that the mistifier. Longer than 5 secs blending however reduced the number of nematodes. Therefore it is possible that the longer macerating time used during the foregoing experiment may have affected the number of nematodes extracted. The success of this method also depends on the physical condition of the roots; for soft roots are easily macerated in a short time whereas hardier fibrous roots of Trifolium and barley are not sufficiently macerated. Although the incubation method has been used successfully by Young (1954) and Mckeen & Mountain (1960) for the extraction of Pratylenchus it did not prove to be efficient in the present experiment. Although the wrist action agitation following maceration was an improvement on the mechanical maceration method, it was not as efficient as the mistifier method. When nematodes are extracted from root tissue for purposes of reinfection, the physical condition of the nematode will influence the subsequent rate of invasion. The nematodes extracted through the mistifier are the most active and able to survive a longer period than those extracted by the other methods. A further advantage of this method is that the extraction process can be continued over a long period.

Two other maceration methods were used during the course of the investigation.

a. Chemical maceration - i.e. separation of plant tissue cells using a mixture of 10% nitric acid and 10% chromic acid followed by potassium hydroxide.

b. Enzyme maceration. This has been used successfully for root galls of the root knot nematode by Dropkin, Smith and Myers (1960). A solution of pectinase i.e. 0·8 g. of enzyme in 100 ml. of acetate buffer at pH 4 - 2 was used. The main disadvantage of both methods was that the nematodes were killed and could not therefore be easily separated from the plant debris.

Summary

The work described in this thesis is concerned with the population dynamics of free living and plant parasitic nematodes particularly as related to the application of soil sterilants. In addition, the effects of depth and of herbicide applications on nematode populations have been examined.

Surveys of soil nematodes were carried out over a two year period in untreated soil, and the nematodes recovered identified to generic level, and in some cases to the specific level.

Seasonal fluctuations were not marked for particulate and fungal feeding nematodes; increases in numbers of these groups were always associated with larval emergence. Some of the Tylenchida showed distinct seasonal changes in number. Pratylenchus populations had minima in winter and early summer and maxima in late summer, autumn and early spring. Adults were found in large numbers during the late summer and autumn, and in early spring the population consisted largely of freshly emerged larvae.

Paratylenchus populations were very low in the first year of observation, but numbers were very high in the second year; an increase in numbers mainly 4th stage larvae and pre-adults followed crop lifting. There was little seasonal variation in numbers of Tylenchus spp. After low levels in the first year, higher numbers of Tylenchorhynchus spp. were found in the second year and this population showed distinct seasonal variation.

Free living particulate feeders did not show much variation in numbers associated with crop rotation; a pronounced variation was seen with Pratylenchus spp. and Paratylenchus spp., while the number of Heterodera rostochiensis larvae was considerably reduced in the rotation plots.

The vertical distribution studies emphasised the influence of vegetation on the depth distribution of plant parasitic nematodes (Pratylenchus spp. and Paratylenchus spp). Variations in the depth distribution of both free living and plant parasitic nematodes associated with seasonal changes was clearly seen.

Herbicide treatments reduced the number of Paratylenchus spp., Helicotylenchus spp. and Dorylaimida, and increased populations of Tylenchus, Tylenchorhynchus, fungal feeders and particulate feeders. Pratylenchus spp. were unaffected.

The repeated application of fumigants, Ethylene dibromide, Chloropicrin and Dazomet resulted in an immediate reduction in numbers of free living nematodes (particulate feeders), followed by a rapid increase over a period of 6-7 months; the Tylenchida and Dorylaimida populations remained low. Pratylenchus populations failed to recover during the course of 2 years.

There was a rapid recovery of Paratylenchus populations, especially in the Ethylene dibromide treated plots.

Fumigant effects on soil nematodes in the rotation and monoculture plots were similar.

Thionazin treatments both inrow and broadcast applications, were ineffective in controlling soil nematodes. There was a distinct increase in plant and free living nematodes following treatments.

There was no variation in populations of nematodes associated with thionazin application in rotation and monoculture plots.

Thionazin used at high doses (100, 300, 900 p.p.m.) in pot experiments gave control of root infecting and soil nematodes.

Bare root dips in thionazin (400 p.p.m.) were effective in controlling Pratylenchus spp. Lower doses of thionazin (25, 50, 100, p.p.m.) in pot cultures were ineffective in the control of Pratylenchus spp. infecting Poa annua.

Treatments were effective in increasing potato yields (especially Dazomet).

The second part of the thesis reports studies on some aspects of the taxonomy, morphology and biology of the genus Pratylenchus. Four Pratylenchus spp. were studied in detail (P.fallux, P.penetrans, P.crenatus, P.neglectus). The extent of the oesophageal overlap as a useful diagnostic feature for species differentiation is emphasised.

Studies on the rate of invasion and subsequent reproduction of Pratylenchus spp. on barley and chickweed, clearly indicated barley as being a good host. The results help to emphasise the importance of both root and soil estimations when studying the population dynamics of Pratylenchus spp.

Studies on Pratylenchus fallux showed that its life cycle from egg to new adult was completed in 40-45 days under greenhouse conditions.

An evaluation of extraction methods emphasises the usefulness of Seinhorst's mistifier in obtaining large numbers and clean suspensions of Pratylenchus spp. from root tissue.

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ADDENDUM

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Table 1 Weeds present in the fields under investigation.

Anagallis arvensis
Capsella bursapastoris
Chaenopodium album
Crepis capillaris
Polygo um convolvulus
P. persicaria
Raphanus raphanistrum
Rumex acetosa
Rumex sp.
Senecio vulgaris
Solanum nigrum
Spergula sp.
Stellaria · edia
Trifolium repens
T. pratense
Viola arvensis

Table 2 List of genera recovered from Church Field (Silwood Park).

Tylenchida
- Fam: Tylenchidae
  - Genus: *Tylenchus*
  - *Psilenchus*
  - *Tylenchorhynchus* 1.
  - *Tylenchorhynchus* 2.
  - *Tylenchorhynchus* 3.
  - *Ditylenchus*
- Fam: Heteroderidae
  - Genus: *Heterodera rostochiensis*
  - *Heterodera trifolii*
  - *Meloidogyne hapla*
- Fam: Hoplolaimidae
  - Genus: *Helicotylenchus*
  - *Rotylenchus*
  - *Pratylenchus fallux*
  - *P. crenatus*
  - *P. neglectus*
  - *P. penetrans*
- Fam: Criconematidae
  - Genus: *Criconemoides*
  - *Paratylenchus projectus*
  - *P. peraticus*
- Fam: Neotylenchidae
  - Genus: *Deladenus*
  - *Boleodorus*
  - *Paurodontus stictylus*
- Fam: Aphelenchidae
  - Genus: *Aphelenchus*
- Fam: Aphelenchoididae
  - Genus: *Aphelenchoides*

Rhabditida
- Fam: Diplogasteridae
  - Genus: *Diplogaster*
- Fam: Rhabditidae
  - Genus: *Rhabditis pelodera*
- Fam: Panagrolaimidae
  - Genus: *Panagrolaimus*
- Fam: Cephalobidae
  - Genus: *Eephalobus*
  - *Eucephalobus*
  - *Acrobeloides*
  - *Acrobeles*

Aerolaimida
- Fam: Plectidae
  - Genus: *Plectus*
  - *Wilsonema*

Monohysterida
- Fam: Monohysteridae
  - Genus: *Monohystera*
  - *Prismatolaimus*

Enophida
- Fam: Tripylidae
  - Genus: *Tripyla*

Dorylaimida
- Fam: Dorylaimidae
  - Genus: *Dorylaimus*
  - *Endorylaimus*
  - *Longidorus*
  - *Xiphinema*
  - *Monochus*
  - *Diphtherophora*
  - *Trichodorus*

Table 3 Monthly recovery of nematodes from 100 ml. of soil in monoculture (mean of 16 replicates.

Sampling time		Free living	Plant parasitic	Total	% free living	% plant parasitic
1966	October	2093	591	2684	77	23
	November	1957	352	2309	84	16
	December	1860	422	2282	82	18
1967	January	1500	539	2039	65	35
	February	2216	661	2768	80	20
	March	2665	546	3198	83	17
	April	1446	522	1995	74	26
	May	1961	1757	3718	53	47
	June	2095	921	3013	70	30
	July	1636	1015	2659	62	38
	August	2023	1170	3193	64	36
	September	2592	1739	4331	60	40
	October	4041	1194	5235	76	24
	November	3515	1028	4525	78	22
	December	3634	963	4597	80	20
1968	February	4685	1765	6450	72	28
	March	3046	936	3982	77	23
	April	4625	1077	5702	82	18
	May	5102	883	5985	85	14
	June	13644	2112	15875	86	14

Table 4 Seasonal fluctuation of Particulate feeders (Rhabditids etc.) in monoculture /100 ml. soil, (mean of 16 replicates).

Sampling time		Adults	Larvae
1966	October	482	1556
	November	963	909
	December	880	878
1967	January	871	516
	February	1305	802
	March	1501	1037
	April	773	595
	May	729	1152
	June	670	1367
	July	566	1012
	August	565	1357
	September	986	1402
	October	1555	2278
	November	1389	1848
	December	1137	2310
1968	February	1425	3003
	March	1017	1765
	April	1730	2705
	May	2027	2850
	June	3100	8012

Table 5 Seasonal fluctuation of aphelenchus and aphelenchoides, Tylenchorhynchus, Tylenchus group. Helicotylenchus, Dorylaimida in monoculture plots (mean of 16 replicates).

Time		Aphelenchus & Aphelenchoides	Tylenchor-hynchus	Tylenchus	Dorylaimida	Helico-tylenchus
Year	Month					
	Oct.	45	8	15	81	17
1966	Nov.	85	18	18	36	1
	Dec.	101	17	10	28	2
	Jan.	111	25	16	37	1
	Feb.	103	34	42	31	18
	Mar.	127	29	48	32	17
	Apr.	77	14	48	21	28
1967	May	80	19	46	16	21
	June	58	12	30	15	9
	July	58	40	35	25	40
	Aug.	101	28	72	32	24
	Sept.	204	135	70	31	27
	Oct.	208	56	53	38	42
	Nov.	278	69	95	22	11
	Dec.	187	37	82	37	18
	Feb.	257	138	87	36	37
	Mar.	264	65	70	50	10
1968	Apr.	190	102	91	52	22
	May	225	178	85	87	65
	June	2532	270	152	100	20

Table 6 Seasonal fluctuation in the population of Pratylenchus and Paratylenchus in monoculture (mean of 16 replicates).

Sampling time year	month	Pratylenchus spp.	Paratylenchus spp.
1966	Oct.	202	51
	Nov.	205	74
	Dec.	252	142
1967	Jan.	285	171
	Feb.	338	198
	Mar.	343	77
	Apr.	228	73
	May	175	141
	June	95	80
	July	274	427
	Aug.	298	629
	Sept.	124	794
	Oct.	486	492
	Nov.	304	504
	Dec.	255	515
1968	Feb.	591	831
	Mar.	308	500
	Apr.	274	484
	May	93	53
	June	395	357

Table 7 Free living nematodes /100 ml. soil in rotation and monoculture.

Sampling time		Monoculture					Rotation				
		Repli-cate 1	Repli-cate 11	Repli-cate 111	Repli-cate 1V	Mean	Repli-cate 1	Repli-cate 11	Repli-cate 111	Repli-cate 1V	Mean
1966	Oct.	1980	2210	1870	2110	2042	2220	1970	2130	2210	2132
	Nov.	1510	1490	1500	1480	1495	2670	2630	2600	2590	2622
	Dec.	1280	1380	1320	1360	1335	2250	2270	2200	2280	2250
1967	Jan.	1280	1350	1080	1100	1203	1670	1520	1680	1720	1697
	Feb.	1580	1630	1470	1590	1568	2890	2710	2780	2870	2812
	Mar.	1870	2140	2040	2100	2038	3300	3300	3000	3410	3252
	Apr.	1070	1040	1280	1250	1160	1360	1366	1370	1400	1374
	May	1990	1970	2220	2030	2053	2143	2183	2200	2190	2184
	June	2930	2840	2690	2570	2758	2783	3226	3210	2931	3037
	Aug.	1830	1920	1850	2200	1950	4043	4406	4310	4100	4215
	Sept.	2100	2440	2030	2070	2145	3660	4110	4090	4050	3977
	Oct.	2600	3000	3040	3020	2940	3000	2930	2450	2480	2765
	Nov.	2700	2410	1820	2260	2298	4540	4540	4630	4590	4575
1968	Feb.	3040	3240	3560	3380	3280	4500	3840	3850	4380	4143
	Apr.	2300	2850	2000	2100	2313	3820	4510	3580	4090	4000
	June	9180	10420	12440	12410	11113	18470	16820	16920	17520	17433

Table 8 Plant parasitic nematodes /100 ml. soil in rotation and monoculture.

Sampling time		Monoculture					Rotation				
		Repli-cate 1	Repli-cate 11	Repli-cate 111	Repli-cate 1V	Mean	Repli-cate 1	Repli-cate 11	Repli-cate 111	Repli-cate 1V	Mean
1966	Oct.	420	480	500	460	465	1150	810	860	990	952
	Nov.	390	420	440	380	407	1230	1060	1080	1160	1132
	Dec.	750	870	860	820	825	770	880	860	800	827
1967	Jan.	990	1130	1080	1040	1060	760	420	620	650	612
	Feb.	1210	1030	960	1040	1037	660	620	720	710	677
	Mar.	920	1070	820	970	920	1036	953	1010	1020	1005
	Apr.	870	950	960	970	938	610	547	600	593	587
	May	2310	1950	2160	1940	2065	1173	1200	1189	1190	1188
	June	1370	1380	1470	1290	1557	1043	804	1030	940	953
	Aug.	2090	2300	1770	1830	1998	1563	1430	1540	1480	1503
	Sept.	1740	1850	1750	1790	1805	1100	1450	1030	1060	1160
	Oct.	2240	2050	2360	2430	2245	1110	1090	1020	1200	1053
	Nov.	1080	1060	1010	1090	1035	1510	1560	1340	1550	1490
1968	Feb.	2870	3030	3800	3670	3368	1210	1080	1310	1280	1220
	Apr.	2080	2020	1270	1340	1677	970	1160	1220	1270	1155
	June	3390	3310	6300	6090	4762	2630	2970	2200	2220	2572

Table 9 Seasonal variation in the Pratylenchus and Paratylenchus population in monoculture and rotation (mean of 4 replicates).

Sampling time		Pratylenchus		Paratylenchus	
year	month	Mono-culture	Rotation	Mono-culture	Rotation
1966	Oct.	150	555	75	95
	Nov.	195	745	45	160
	Dec.	365	525	225	100
1967	Jan.	452	252	310	82
	Feb.	415	352	360	77
	Mar.	440	551	112	91
	Apr.	410	275	175	25
	May	407	231	440	438
	June	255	133	85	545
	Aug.	460	436	987	720
	Sept.	222	280	772	35
	Oct.	775	440	970	17
	Nov.	252	460	557	77
1968	Feb.	1250	487	1252	37
	Apr.	477	352	547	50
	June	395	228	358	68

Table 10 Seasonal variation in the population of Aphelenchus and aphelenchoides in monoculture and rotation (mean of 4 replicates).

Sampling time		Monoculture	Rotation
1966	Oct.	115	145
	Nov.	55	145
	Dec.	130	105
1967	Jan.	110	64
	Feb.	67	82
	Mar.	174	121
	Apr.	64	72
	May	67	196
	June	50	152
	Aug.	145	338
	Sept.	174	359
	Oct.	200	297
	Nov.	102	600
1968	Feb.	289	452
	Apr.	169	425
	June	2532	1743

Table 11 Seasonal variation in the population of Tylenchorhynchus, Tylenchus and Heterodera larvae in monoculture and rotation (mean of 4 replicates).

		Tylenchorhynchus		Tylenchus		Heterodera larvae	
		Mono-culture	Rotation	Mono-culture	Rotation	Mono-culture	Rotation
1966	Oct.		50		55		
	Nov.	40	5		10		
	Dec.	40	10		15		
1967	Jan.	60	12	57	15		
	Feb.	25	25	70	45		
	Mar.	57	66	80	93		
	Apr.	40	13	75	58	107	83
	May	22	120	80	81	970	41
	June	5	93	47	46	940	70
	Aug.	65	71	132	60	97	10
	Sept.	387	152	110	122	15	
	Oct.	80	85	65	75	22	90
	Nov.	47	110	55	110	17	52
1968	Feb.	282	77	102	72	22	10
	Apr.	162	135	117	77	73	17
	June	383	260	153	75	818	45

Table 12 Vertical distribution of nematodes in Pasture and Arable land. A - Distribution of free living and plant parasites
B - Distribution of Pratylenchus and Paratylenchus.

A

Depth in cm.	Pasture soil		Arable soil	
	Free Living	Plant Parasites	Free Living	Plant Parasites
1-4	5075	2215	6260	585
4-8	1880	2500	3440	1125
8-12	2310	1850	2560	700
12-16	1940	2190	2490	1115
16-20	1465	1330	2435	1130
20-24	1160	985	1975	1140

B

Depth in cm.	Pasture		Arable	
	Praty-lenchus	Paraty-lenchus	Praty-lenchus	Paraty-lenchus
1-4	20	250	390	65
4-8	10	1090	810	150
8-12	15	820	755	70
12-16	40	1005	750	90
16-20	35	615	630	85
20-24	20	530	895	180

Table 13 Vertical distribution of Tylenchorhgnchus, Tylenchus and spiral nematodes (Helicotylenchus and Rotylenchus) on arable and grassland soils. (Mean of 4 replicates).

	Arable / Grass-land	Depth in cms. 1-4	4-8	8-12	12-16	16-20	20-24
Tylenchorhynchus	A	60	50	75	65	40	10
	Gr.	2145	1040	770	930	520	370
Tylenchus	A	45	45	65	85	70	15
	Gr.	450	150	105	110	75	15
Spiral nematodes	A	-	-	10	20	10	-
	Gr.	165	150	100	70	55	35

Table 14 Seasonal variation in the vertical distribution of free living and plant parasitic nematodes.

Sampling time 1967		Depth in cms.					
		1-4	4-8	8-12	12-16	16-20	20-24
January	Free living	3250	3010	3655	2235	1820	1300
	Plant parasitic	1900	3965	5140	2205	1425	1210
February	Free living	4455	3845	2880	4575	5176	1995
	Plant parasitic	1720	1570	1415	2035	1265	680
March	Free living	5650	2935	1675	1300	950	845
	Plant parasitic	720	775	1130	1065	1530	575
April	Free living	1975	1250	1035	885	1100	520
	Plant parasitic	565	460	540	405	690	240
May	Free living	3300	2105	1730	800	815	695
	Plant parasitic	615	815	1485	1120	990	665
June	Free living	4215	2895	1865	1185	875	815
	Plant parasitic	1020	1035	705	600	405	385
August	Free living	4945	5425	2865	2450	1130	925
	Plant parasitic	1025	1435	1470	1425	730	595

Table15 Seasonal variation in the vertical distribution of Pratylenchus and Paratylenchus on arable land.

Sampling time		Depth in cm.					
		1-4	4-8	8-12	12-16	16-20	20-24
January	Pratylenchus	1185	1960	2685	1130	880	705
	Paratylenchus	510	1475	2295	965	445	435
February	Pratylenchus	730	960	830	1490	960	690
	Paratylenchus	350	335	270	280	140	90
March	Pratylenchus	280	515	855	910	1115	500
	Paratylenchus	35	95	145	50	105	45
April	Pratylenchus	280	270	275	305	545	185
	Paratylenchus	30	20	25	20	-	-
May	Pratylenchus	80	27	345	320	290	350
	Paratylenchus	165	210	910	770	340	215
June	Pratylenchus	220	75	75	205	160	170
	Paratylenchus	330	800	505	270	295	185
August	Pratylenchus	350	430	380	245	185	175
	Paratylenchus	485	710	965	990	510	410

Table 16

Effect of fumigants on free living nematodes (in monoculture).

Sampling time		Control	Treatments					
			Chloropicrin		Dazomet		Ethylene-dibromide	
year	month		low	high	low	high	low	high
1966	Oct.	2051	3555	4045	2220	3650	3800	545
	Nov.	1920	795	280	365	100	3005	1020
	Dec.	1836	790	225	140	70	1370	885
1967	Jan.	1505	935	210	50	45	960	867
	Feb.	1952	2073	80	615	572	880	1058
	Mar.	2673	1657	792	250	315	1530	777
	Apr.	1447	475	375	830	240	445	192
	May	1936	2050	620	1032	1062	778	388
	June	2090	4202	772	1974	565	1287	675
	July	1636	3737	914	1249	1377	1755	1388
	Aug.	2005	4002	4065	1628	1679	2240	1280
	Sept.	2192	5395	4952	2232	2294	1863	4084
	Oct.	4035	2857	2859	3356	2559	2966	2520
	Nov.	3517	2428	477	609	325	2296	935
1968	Feb.	4686	3105	315	974	797	5408	2108
	Apr.	2979	3861	2478	1121	1537	927	657
	June	10392	4960	3497	5006	1614	4960	3624

Table 17 Effect of fumigants on plant parasitic nematodes in monoculture plots.

Sampling time		Control	Treatments					
			Chloropicrin		Dazomet		Ethylene-dibromide	
year	month		low	high	low	high	low	high
1966	Oct.	391	370	335	200	75	100	20
	Nov.	397	30	40	35	-	105	75
	Dec.	579	65	30	35	-	55	55
1967	Jan.	549	93	15	18	13	50	40
	Feb.	678	212	-	-	-	115	119
	Mar.	524	155	65	40	25	380	110
	Apr.	441	15	13	-	-	47	-
	May	486	105	5	12	-	24	10
	June	226	101	-	35	35	33	25
	July	845	20	18	79	35	160	-
	Aug.	819	110	103	52	28	330	33
	Sept.	815	106	103	321	153	387	139
	Oct.	1186	346	73	891	431	2391	120
	Nov.	989	104	18	83	-	1224	97
1968	Feb.	1746	30	30	103	40	1032	136
	Apr.	738	156	10	28	18	810	40
	June	1058	290	142	80	-	290	15

Table 18 Effect of fumigants on Pratylenchus spp. in monoculture plots.

Sampling time		Control	Treatments					
			Chloropicrin		Dazomet		Ethylene-dibromide	
year	month		low	high	low	high	low	high
1966	Oct.	202	270	200	130	25	40	20
	Nov.	205	20	20	20	-	10	25
	Dec.	402	45	20	25	-	20	20
1967	Jan.	278	65	15	13	13	30	-
	Feb.	449	145	-	-	-	27	10
	Mar.	306	108	42	20	18	165	82
	Apr.	228	13	-	-	-	15	-
	May	176	75	-	5	-	10	-
	June	95	32	-	25	20	12	-
	July	274	40	-	5	-	22	-
	Aug.	295	55	10	15	10	155	13
	Sept.	110	18	-	23	-	68	-
	Oct.	485	125	65	23	-	-	-
	Nov.	304	60	-	38	-	-	-
1968	Feb.	591	20	10	13	12	310	80
	Apr.	311	52	5	-	-	-	-
	June	264	93	-	48	-	5	-

Table 19 Short term effect of fumigants on free living nematodes in monoculture and rotation plots.

Monoculture

Sampling time		Control	Treatments					
			Chloropicrin		Dazomet		Ethylene-dibromide	
year	month		low	high	low	high	low	high
1967	Oct.	4035	2857	2859	3356	2559	2966	2520
	Nov.	3517	2428	477	609	325	2296	935
1968	Feb.	4686	3105	315	974	797	5408	2108
	Apr.	2979	3861	2478	1121	1537	927	657
	June	10392	4960	3497	5006	1614	4960	3624

Rotation

Sampling time		Control	Treatments					
			Chloropicrin		Dazomet		Ethylene-dibromide	
year	month		low	high	low	high	low	high
1967	Oct.	3816	2942	2714	4956	4372	3912	3132
	Nov.	3812	2834	607	587	377	2310	695
1968	Feb.	5092	4095	1296	531	692	882	353
	Apr.	4677	7109	973	502	672	1412	528
	June	15683	6472	3402	2526	2664	12763	2542

Table 20 Short term effect of fumigants on plant-parasitic nematodes in monoculture and rotation plots.

Monoculture

Sampling time		Control	Treatments					
			Chloropicrin		Dazomet		Ethylene-dibromide	
year	month		low	high	low	high	low	high
1967	Oct.	1186	346	73	891	431	2391	1202
	Nov.	989	104	18	83	-	1224	97
1968	Feb.	1746	30	30	103	40	1032	136
	Apr.	738	156	10	28	18	810	40
	June	1058	290	142	80	-	290	15

Rotation

Sampling time		Control	Treatments					
			Chloropicrin		Dazomet		Ethylene-dibromide	
year	month		low	high	low	high	low	high
1967	Oct.	786	731	1059	1364	1072	912	1091
	Nov.	696	254	50	76	73	384	129
1968	Feb.	784	430	121	32	-	60	27
	Apr.	683	528	25	20	10	98	38
	June	754	268	50	40	25	43	30

Table 21 Short term effect of fumigants on Pratylenchus and Paratylenchus populations in rotation plots.

Pratylenchus

Sampling time year	month	Control	Treatments Chloropicrin low	Chloropicrin high	Dazomet low	Dazomet high	Ethylene-dibromide low	Ethylene-dibromide high
	Oct.	346	417	400	630	682	405	10
1967	Nov.	290	172	42	55	50	58	13
	Feb.	377	177	58	10	-	18	10
1968	Apr.	289	169	25	5	-	30	8
	June	213	62	60	17	-	-	-

Paratylenchus

Sampling time year	month	Control	Treatments Chloropicrin low	Chloropicrin high	Dazomet low	Dazomet high	Ethylene-dibromide low	Ethylene-dibromide high
	Oct.	122	37	297	220	90	88	110
1967	Nov.	99	13	7	10	-	50	50
	Feb.	77	30	5	13	-	43	18
1968	Apr.	64	28	-	5	-	30	18
	June	69	15	-	13	-	43	30

Table 22 Effect of Thionazin (in row) application on free-living and plant parasitic nematodes in monoculture plots.

Sampling time		Free-living nematodes			Plant parasitic nematodes		
		Thionazin			Thionazin		
Year	Month	Control	low	high	Control	low	high
1967	Mar.	2673	1443	2121	524	282	794
	Apr.	1447	805	1188	441	204	299
	May	1936	2033	905	486	190	235
	June	2090	1363	1040	226	165	187
	July	1636	1138	543	845	354	234
	Aug.	2065	1103	663	819	407	152
	Sept.	2192	1618	1535	815	1167	474
	Dec.	3636	3451	4453	939	647	689
1968	Mar.	3058	3828	3693	874	522	810
	Apr.	2979	2060	2448	406	388	377
	May	6765	7151	6525	738	433	507
	June	10392	4231	4543	1058	642	395

Table23 Effect of Thionazin (broadcast) application on free-living and plant parasitic nematodes in monoculture.

Sampling time in weeks	Free-living nematodes			Plant parasitic nematodes		
	Thionazin			Thionazin		
	Control	low	high	Control	low	high
Pre-treatment	2673	1230	1420	524	140	180
2	1305	722	360	480	150	60
4	1447	812	674	441	180	189
6	1936	742	769	486	173	103
8	2090	750	722	226	162	193
12	1636	1145	527	845	353	150
16	2005	1653	1242	819	1727	1605
20	2192	1663	2340	815	3487	1605
32	3130	3885	3355	800	3010	885

Table 24 Comparative effect of Thionazin (in row) applications on soil nematodes in monoculture and rotation plots.

A. Free living nematodes.

Sampling time (weeks)	Monoculture			Rotation		
	Thionazin			Thionazin		
	Control	low	high	Control	low	high
Pre-treatment	3058	3828	3693	3816	4310	4560
2	2979	2060	2448	2315	2330	2988
4	6633	6549	4218	4820	3990	2595
6	6765	7151	6525	6225	6575	4315
8	10392	4231	4543	15683	4482	3470

B. Plant parasitic nematodes.

Sampling time (weeks)	Monoculture			Rotation		
	Thionazin			Thionazin		
	Control	low	high	Control	low	high
Pre-treatment	874	522	810	599	682	440
2	406	388	377	355	322	384
4	738	433	507	573	683	259
6	682	1280	892	690	776	362
8	1058	642	395	754	448	485

Table 25

A - Effect on Pratylenchus spp.

Sampling time (weeks)	Monoculture			Rotation		
	Control	Thionazin low	high	Control	Thionazin low	high
Pre-treatment	318	80	243	370	460	225
2	311	108	98	130	83	88
4	83	40	103	289	158	18
6	130	133	175	205	283	48
8	264	120	110	213	60	62

B- Effect on Paratylenchus spp.

Sampling yime (weeks)	Monoculture			Rotation		
	Control	Thionazin low	high	Control	Thionazin low	high
Pre-treatment	500	93	113	85	80	50
2	312	43	43	78	58	23
4	465	23	-	30	5	25
6	165	43	-	48	18	27
8	230	38	43	69	72	-

Table 26 Experiment I - Effect of thionazin (used as a chemical dip) on Pratylenchus spp. infecting barley roots.

Treatment	0	25	100	400
Percentage	0	5.38	13.14	34.27

Table 27

Experiment I - Effect of thionazin used as a chemical dip on Pratylenchus spp. in barley.

Treatment p.p.m.	Replicate	Total recovered	Total remaining	% recovered	% remaining
0	i	357	100	78	22
	ii	423	121	78	22
	iii	159	456	74	26
	iv	586	175	77	23
	v	479	184	72	28
25	i	466	189	71	29
	ii	482	195	71	29
	iii	456	160	74	26
	iv	468	170	73	27
	v	475	215	69	31
100	i	371	200	65	35
	ii	439	208	68	32
	iii	548	277	66	34
	iv	439	259	63	37
	v	367	179	67	33
400	i	335	328	51	49
	ii	319	214	60	40
	iii	351	380	48	52
	iv	345	413	46	54
	v	359	433	45	55

Table 28 A

Experiment I - Analysis of variance of angular transformation values of percentage remaining within roots - Pratylenchus spp. treated with different concentrations of thionazin.

Source	d.f.	s.s.	M.S.	F.	P.
Treatments	3	26.5907	8.8635	2.4	*
Replicates	4	686.0860	171.5215	47.5	n.s.
Error	12	43.3576	3.6131		
Total	19	756.0343			

Table 28B

Experiment I - Analysis of variance of angular transformation values of percentage recovered - Pratylenchus spp. within roots treated with different concentrations of thionazin.

Source	d.f.	s.s.	M.S.	F.	P.
Treatments	3	27.25	9.06	2.5	n.s.
Replicates	4	726.77	181.69	50.0	n.s.
Error	12	43.59	3.63		
Total	19	797.61			

Table 29 Experiment II. Effect of Thionazin on Pratylenchus spp. in pot cultures.

Treatment p.p.m.	Replicates	Number extracted					
		Adult	Larvae	Total	Mean	Mean % survival	Mean % kill
0	i	59	120	179	245	100	0
	ii	74	140	214			
	iii	66	130	196			
	iv	167	208	375			
	v	121	141	162			
	vi	74	91	165			
100	i	18	30	48	78	32	68
	ii	11	21	32			
	iii	37	58	95			
	iv	56	84	140			
	v	40	47	87			
	vi	31	37	68			
300	i	42	51	93	62	25	75
	ii	33	48	81			
	iii	18	28	45			
	iv	15	32	47			
	v	27	25	52			
	vi	24	29	53			
900	i	12	13	24	26	11	89
	ii	12	29	41			
	iii	4	7	11			
	iv	4	12	16			
	vv	13	20	33			
	vi	12	18	30			

Table 30

Experiment III - Effect of thionazin on Pratylenchus spp. infecting Poa annua.

Treatment p.p.m.	Replicate	Total number per pot		
		Adults	Larvae	Eggs
0	i	1972	1043	976
	ii	1420	840	547
	iii	1592	1032	340
	iv	1992	1246	1079
	v	2198	1066	1295
	vi	731	701	416
25	i	1414	997	1087
	ii	1305	777	478
	iii	775	527	226
	iv	1449	865	579
	v	785	546	306
	vi	2210	1355	1575
50	i	889	546	275
	ii	1515	719	1154
	iii	1341	1036	216
	iv	948	741	1157
	v	1280	823	203
	vi	1610	708	1299
100	i	792	519	285
	ii	2513	979	276
	iii	1184	733	601
	iv	1166	744	209
	v	585	369	409
	vi	2646	1456	525

Table 31 Experiment III Analysis of variance of the data obtained on the effect of Thionazin on Pratylenchus spp. infecting Poa annua.

A. Analysis of variance of the effect on adults.

Source	d.f.	s.s.	m.s.	F	P
Treatment	3	1321719	440573	1.146	n.s.
Replicate	5	499586	139917	0.364	n.s.
Error	15	5761755	384170		
Total	23	7583060			

B. Analysis of variance of the effect on larvae.

Source	d.f.	s.s.	m.s.	F	P
Treatment	3	291324	97108	1.13	n.s.
Replicate	5	125691	25138	0.293	n.s.
Error	15	1284675	85645		
Total	23	1701708			

C. Analysis of variance of the effect on eggs.

Source	d.f.	s.s.	m.s.	F	P
Treatment	3	843188	281062	1.579	n.s.
Replicate	5	607187	121437	0.682	n.s.
Error	15	2669133	177942		
Total	23	4129508			

Table 32 Oesophageal overlap in Pratylenchus spp.

A Oesophageal overlap (Distance from the centre of the median oesophageal ult to end of oesophageal gland.

P.fallux		P.penetrans		P.crenatus		P.neglectus	
length nucrons	Freq. in nus.	length	7	length	7	length	7
60	2	52	1	52	1	48	1
66	1	58	1	56	1	49	5
68	13	59	1	58	6	53	2
69	3	60	1	59	3	58	1
70	2	62	3	60	7		
71	2			62	18		
75	8			63	3		
77	1			64	1		
78	1						
Total no.	33		7		40		9

B Oesophageal overlap as a percentage of total length of nematode.

P.fallux		P.penetrans		P.crenatus	
% overlap	% freq.	% O	% F	% O	% F
12.5	6	10.7	14	11.1	10
13.2	6	10.8	28	11.6	45
13.3	6	10.9	58	12.0	17
13.4	45			12.1	15
13.5	12			12.2	7
13.7	24			12.4	3
				12.5	3

Table 33 Pratylenchus spp. associated with barley and chickweed.

Time - days	Barley					Chickweed				
	Root		Soil			Root		Soil		
	Adult	Larv.	Adult	Larv.	Total	Adult	Larv.	Adult	Larv.	Total
0			682	910	1592			525	1148	1673
10	168	487	262	672	1589					
20	673	803	85	155	1716	474	319	436	182	1411
30	1210	606	50	57	1923	561	632	96	173	1461
40	1868	751	37	25	2681	496	507	57	68	1128
50	778	498	28	50	1354	668	559	57	70	1354
60	740	479	138	257	1615	774	417	58	52	1409
70						901	430	78	149	1558

Table 34 Root weight associated with changes in Pratylenchus populations.

Time	Barley		Chickweed	
	Root wt. (g.)	Nematode no.	Root wt.	Nematode no.
10	0.377	655		
20	0.555	1476	0.789	793
30	0.862	1816	1.349	1192
40	1.723	2619	2.254	1003
50	1.897	1276	2.868	1227
60	1.961	1220	4.404	1299
70			3.852	1331

Table 35 Mean number of adult larvae and eggs produced by a single female of Pratylenchus fallux.

Sampling time-days	Adults	Larvae	Eggs
10	1	0	0.4
20	1	1.5	3.7
30	2.2	3.5	5.3
40	2.5	4	12.5
50	11.7	7.5	18.2
60	12.3	8.6	21.7

Table 36 Evaluation of extraction methods. Numbers of Pratylenchus spp. extracted and remaining per gram of root.

Method	Replicate	No. Extracted		No. Remaining /g. root	
		A	L	A	L
Maceration method	i	167	328	910	388
	ii	152	294	777	290
	iii	135	262	819	352
	iv	121	238	840	330
	$\bar{m}$	143	280	840	330
Incubation method	i	162	260	622	216
	ii	202	356	679	300
	iii	145	252	759	23¼
	iv	529	1020	787	360
	$\bar{m}$	259	472	713	277
Wrist Action method	i	339	812	461	275
	ii	418	1117	492	223
	iii	424	986	409	270
	iv	811	1294	487	296
	$\bar{m}$	523	1052	462	266
Mistifier method	i	791	1254	368	275
	ii	761	1701	214	183
	iii	457	735	218	123
	iv	637	1012	257	240
	$\bar{m}$	661	1175	264	205