AN ECOLOGICAL STUDY OF SOME NEMATODES ASSOCIATED WITH APPLE TREES IN A GRASSED ORCHARD

A THESIS

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INTRODUCTION

Plant nematology appears to have been studied in New Zealand for the past 68 years, during which period a number of publications have accumulated on this subject (Kirk, 1899; Reid & Cottier, 1935; Jacks, 1944; Cottier, 1956; Stanton, 1956; Atkinson, Brien, Chamberlain, Cottier, Jacks, Reid & Taylor, 1949; Blair & Morrison, 1949; Morrison, 1957; Clark, 1964). However until 1961 when Clark returned to New Zealand from the United Kingdom, there was no trained nematologist in this field and most of the earlier identifications were done either through the disease symptoms or by overseas authorities. Hence, there is no record of specific ecological studies for this country.

The first attempt to study the New Zealand soil nematode ecolology was made by Clark at the commencement of his Ph.D. studies, but because of the high endemism encountered, which presented an absorbing taxonomic problem, he ended up merely opening the gate to this field by describing a vast number of the hitherto undescribed species. This work is the second attempt made, and it aims, not only to venture into the yet untouched ecological realm, but also to investigate some of the possible relationships of nematodes and apple trees in this primarily agricultural country.

CHAPTER I

LITERATURE REVIEW

There are three aspects to this review. For convenience, these are set out as shown below:

1.1. The association of nematodes with fruit trees, with particular reference to apple trees.

(a) Introduction.

(b) Nematodes and fruit trees.

(c) General conclusions.

1.2. The ecological aspect.

(a) Introduction.

(b) The effect of the seasons on the population dynamics of nematodes.

(c) Cropping effect on population dynamics of nematodes.

(d) Influence of soil type on population dynamics of nematodes.

(e) Vertical zonation of nematodes.

(f) Effect of soil moisture content and rainfall on nematode populations.

(g) Effect of nutrients, fertilizers and pH. on nematode populations.

(h) The effect of temperature on nematode populations.

1.3. On plant parasitic nematodes associated with fruit trees in New Zealand.

1.1. The association of nematodes with fruit trees with particular reference to apple trees.

(a) Phytonematology is not a very recent discipline. It is about two and a quarter centuries old. It dates back to 1743 when Needham first discovered nematodes (*Anguina tritici*) as the causative agent of the wheat cockle. Very little progress was made between this date and the middle of the 19th Century, when the sugar beet nematode (*Heterodera schachtii*) first directed attention to the tremendous economic importance of plant parasitic nematodes. Although such nematodes as the root-knot ones were known to be destructive to many crops before 1900, it was not until 1925-1950 that phytonematology really began to receive intensive attention. The cyst formers, which constituted a major problem to beet, peas and other vegetable crops still dominated the scene. The importance of nematodes to fruit trees was realised only recently when Ark and Thomas (1936) found *Anguillulina pratensis* (= *Pratylenchus pratensis*) associated with poorly growing apple, pear, plum and grape trees in California. This has been followed up by a series of similar reports from most parts of the world: America (Hastings and Bosher, 1938; Thorne, 1948; Ducharme & Suit, 1953, 1954; Parker & Mai, 1956;

Bosher, 1959; Bosher & Newton, 1957; Tamburo & Adams, 1962; Lownsberry & Serr, 1963); Australia (Colbran, 1963; Goss, 1961; Fisher, 1961; Anderson, 1966); Germany (Decker, 1959; D'Herde & van den Brande, 1963); Netherlands (Oostenbrink, 1962); Canada (Bosher & Orchard, 1963; Mountain & Boyce, 1958); Italy (Marinari, 1959); South Africa (Koorts, 1961); England (Pitcher, Patrick & Mountain, 1960; Pitcher, Way & Savory, 1966), etc.

(b) In 1943, Binchet pointed out the importance of nematodes to fruit trees in his article "Eelworms or nematodes: parasites dangerous to fruit and market garden crops." Thorne, 1948, described Pratylenchus spp. as the factor responsible for "a baffling slow decline" of apple trees in several areas in the U.S.A. In many countries the replant problems set off a host of investigations which resulted into the publication of many of the papers available on this subject today. Colbran (1963), investigating the apple tree replacement problem in Australia, associated this with P. coffeae Zimmerman. Goss (1961) came to a similar conclusion when she found that the decline and dieback of apple trees and replant difficulties in Western Australia were caused mainly by three species of the same genus (Pratylenchus). Parker & Mai (1956) showed that P. penetrans was the major factor in the failure of replant in old orchards in Western New York. Koorts (1961) described nematodes as a serious pest of orchards and deciduous fruit trees, "but," he wrote, "the problem is particularly serious where a few trees or even whole orchards are planted to replace old trees." Wilson & Hedden, (1961) found that nematodes were the cause of stunted young trees in replanted cherry orchards in Ohio. Mountain & Boyce (1958) confirmed that nematodes form the main factor in the Ontario peach replant problem. D'Herde & van den Brande (1963) experimentally linked poor growth of young apple trees in old orchards with Pratylenchus penetrans.

Because these replant problems played an important role in directing attention to the seriousness of nematode problems of fruit trees, they were, for a while, regarded as serious pests of young plants only. The hope that older plants might be free of the 'all invading' nematode problem seems to have been disipated by more recent work. Tamburo & Adams' (1962) work on established apple orchards in West Virginia convincingly linked poor growth in old apple trees with nematodes. Such trees could be easily spotted as occasional stunted patches or individual poorly growing ones. A later work by Palmiter, Braun & Keplinger (1966) in the Hudson Valley agreed with this conclusion. Reduction in the number of plant parasitic nematodes, especially the predominant Paratylenchus by nematicides corresponded to the resulting increase in plant vigour.

It seems, therefore, that vigour of both old and young apple trees can be seriously impaired by nematodes. That younger trees, especially in replanted orchards are most seriously affected is not unexpected since the growth of infective nematodes would have been encouraged by the previous cropping. The initial high population of fruit-tree parasitising nematodes would naturally drastically reduce the vigour of young fruit trees, whose roots had been greatly damaged at transplanting and whose new tender roots provide a better food supply for such nematodes.

Although the genus Pratylenchus has been much emphasised in most of the literature cited above, this does not indicate that all other genera are of less importance. In fact, a few have been shown to be more significant than this genus. Marinari (1959) reviewed work done in Italy in orchards and nurseries. Nematodes recorded as potentially dangerous to fruit trees include Pratylenchus, Tylenchulus, Rotylenchus, Xiphinema, Criconemoides and Trichodorus. The genus Meloidogyne was reported as constituting a most important problem to fruit trees especially in warm temperate climates. This agrees with the conclusions of Lownsberry and Thomason (1959) in their review entitled "Progress in Nematology Related to Horticulture" where they wrote "The root-knot nematodes, Metoidogyne ....are probably the most important nematode parasites of fruit and nut crops in the southern half of the United States .... P. penetrans appears more common in the northern half of the United States ....". With reference to apple trees, however, it must be mentioned that in places where Meloidogyne are known to constitute an outstanding problem, apple trees are seldom included in the list of affected hosts (Marinari, 1959; Lownsberry & Thomason, 1959).

Besides these, many other genera have been associated with apple and fruit trees. From a survey of ten different orchards in New York, Mai, Dolliver, Kirkpatrick and Parker (1957) reported the following potentially pathogenic nematode genera: *Pratylenchus*, *Xiphinema*, *Tylenchus* and *Paratylenchus*. Anderson (1966) examined fruit tree nurseries and orchards in New South Wales and found the following genera associated with apple trees: *Helicotylenchus*, *Criconemoides*, *Pratylenchus*, *Trichodorus* and *Xiphinema*. In bearing orchards only the following three genera were reported: *Helicotylenchus*, *Pratylenchus* and *Xiphinema*. Other similar reports include that of Oteifa and Tarjan (1965) where fourteen species of potentially pathogenic nematodes were encountered in a survey of established orchards in U.A.R. Palmiter et al (1966) associated the poor growth of old apple trees with *Paratylenchus curvitatus*.

(c) From available evidence, it is clear that nematodes can constitute a major phytosanitary problem of apple trees. This is particularly true of the much emphasised genus *Pratylenchus*. There are indications that other potentially pathogenic ones pose a serious threat, e.g. *Paratylenchus* (Palmiter et al, 1966). Some statistical evidence showing the seriousness of the nematode danger to apple trees include results of Hoestra & Oostenbrink (1962). Reduction of shoot growth by more than 50% was to be associated with heavy infestation of *P. penetrans*. Tamburo & Adams (1962) found that while normal apple trees with soil nematode population of 83 per 50 ml of soil had an average terminal growth of 9.4 inches, trees in soil with average nematode population of 190 per 50 ml of soil had an average terminal growth of 2 inches only. Palmiter & Braun (1962) found that terminal growth in apple trees on soil treated with nemagon EC-2 to control Pratylenchus and Paratylenchus was 68% greater than that in trees on untreated soils. While the former produced an average of 6.6 boxes of fruits, the latter only produced 2 boxes.

It is worth noticing that the latitudinal effect and soil type (ecology) play an important role in the question of what particular species or genus constitutes the most important factor in the resulting low vigour observed in infested fruit plants. Marinari (1959) and Lownsberry & Thomason (1959) agreed that the genus Meloidogyne is most important in warm temperate climates. Pratylenchus seems to be the outstanding factor in the temperate regions. Palmiter et al, (1966) thought that the predominance of Paratylenchus in the Hudson Valley instead of Pratylenchus was due to the heavier soil type.

However, so far only the genus Pratylenchus has received sufficient attention to merit an undoubted status of a 'very important apple tree pathogen.' The many other potentially pathogenic nematodes which have been associated with this plant need further work to clarify the question of their probable importance to the apple tree economy.

1.2. The ecological aspect.

(a) Ecological investigations of migratory soil nematodes started only recently. Much of the earlier work was on the cyst forming nematodes which are highly specialised root parasites and are therefore mainly influenced by their host plants. The fact that the migratory soil nematodes are affected to a greater extent by a host of other factors, including soil type and crop, illustrates an important difference between the ecology of these and the cyst-formers (Heterodera spp.).

(b) Work done so far on seasonal population variations of nematodes can be reviewed in three parts: (1) Population changes of nematodes within plant roots; (2) In soils around a specific plant, and (3) both within the roots and in soils around the roots.

In South Carolina, Graham (1951) found an increase in Pratylenchus sp. population on tobacco plants in spring and summer which reached a peak in August and then decreased sharply. Riggs et al (1956) working on P. coffeae in Arkansas, found that root population increased from 1,000 per gram of root in winter to a maximum of 1,400,000 per gram of root in spring. There was a decrease in summer and autumn. In the roots of cultivated brambles in North Carolina, Goheen & Williams (1950) recorded maximum population of P. vulnus in early June (spring) and an appreciable decrease in summer and minor fluctuations in autumn and winter. In Ontario, Mountain & Boyce (1958) suggested that P. penetrans population in the roots of the young peach plants was highest in spring, but gradual decline accompanied a gradual increase in temperature, resulting in a late summer minimum.

While most workers reported a maximum root population in spring, soil population results are less uniform. For example, a winter minimum was reported for P. penetrans in Germany (Decker, 1960) and New Jersey (Edwardo, 1961), but a winter maximum was found by Jensen (1950) for P. vulnus. Fisher (1961) found the minimum population of Paratylenchus spp. in summer. This agrees with Winslow's (1964) findings for the total nematode populations and the Tylenchida. Both results are contrary to those of Norton (1963) for Xiphinema americanum and Yuen's (1966) for Helicotylenchus vulgaris where spring and summer maxima were reported. These discrepancies tend to emphasise the importance of different soil conditions and crop on population behaviour of soil nematodes.

(c) However, most workers seem to agree on one point - that the period of maximum population corresponds to the period of maximum plant growth (Dieter, 1959; Wehunt, 1957; Yuen, 1966). It has also been shown that different plant hosts can have a considerable influence on soil nematode population levels (Oostenbrink, 1961; Kleyburgh & Oostenbrink, 1959; Winslow, 1955; Endo, 1959; Henderson & Katznelson, 1960). Graham (1951) working on Pratylenchus sp. found a population peak in tobacco plants in early August, but in corn roots, the peak was in early September, while in cotton and crab grass, populations continue to increase until October. These differences in the root population would respectively result into different soil population levels around each plant. In 1956, Seinhorst pointed out the existence of a passive winter phase of Ditylenchus dipsaci in soils with annual crops, but this is not true in soils with perennial crops where root material persists all the year round. Winslow (1964) stated, "Within each soil type, the crop affected the prevalence of these nematodes ....". Even in a monocultural plantation, populations of plant parasitic nematodes are known to fluctuate with the root distribution patterns of the plant hosts. Fisher's work (1961) on apple trees in Adelaide, provided substantial evidence in support of this. He found that differences in root weights accounted for about 50% of the extreme variations usually found between samples from individual trees.

Edwardo's work (1961) covered population changes both in the roots as well as the surrounding soil. Slight differences in the seasonal behaviour of root and soil populations of P. penetrans in strawberry plantations in New York was demonstrated. While soil population reached a peak in June, root population was maximum in July.

Ecology however, involves more than already indicated. Other aspects of nematode ecology include their spatial distribution in the soil and the influence of soil type, soil moisture, rainfall and pH values.

(d) The great influence of soil type on the distribution of migratory nematodes has been observed by a number of workers, especially for Pratylenchus spp. in Holland (Oostenbrink, 1961;

Seinhorst, 1957; England (Wallace & Winslow, 1964; Winslow, 1964) and in North America (Parker & Mai, 1956; Sher & Bell, 1965; Mountain & Boyce, 1958; Endo, 1959) etc. Pratylenchus' obvious preference of light textured, well drained soils rather than heavy ones, has been emphasised. Parker & Mai (1956) reported an apparent absence of P. penetrans in heavy soils, and Koorts (1961), writing on nematodes in orchards and deciduous fruit trees in South Africa stated, "Fortunately, orchards on clay soils are almost invariably free of eelworms".

(e) Changes in soil nematode populations with depths have been recorded (Godfrey, 1924; Chitwood & Feldmesser, 1948; Harrison & Winslow, 1961; Decker, 1959). Overgaad Nielsen (1949) showed that such vertical zonations differ in cultivated and uncultivated soils. Wallace (1963) concluded from his literature survey that vertical distribution of plant parasitic nematodes is chiefly related to root distribution.

(f) Many investigators have also associated soil moisture content and rainfall with increase in nematode populations (Frandsen, 1951; Nolte, 1957; Seinhorst, 1950; Wallace, 1962; Norton, 1959; Wallace & Greet, 1964).

(g) Among other factors, the effect of nutrients and fertilisers on nematode populations (Oteifa,1952 and 1953; Ross,1959; Bird, 1960; Shafiee & Jenkins, 1962; Kirkpatrick et al, 1961, 1964) and the effect of pH have both received some attention. Relatively very little field work has so far been published on the latter aspect. The contradictory nature of the few results obtained give no scope for generalisations. E.g., the results of Peters (1926), Bird (1959), Mai & Harrison (1959) and Lownsberry (1961) agreed on a negative correlation between nematode populations and pH, but on the contrary, results of Simon (1955) and Duggan (1964) both suggest a strong correlation between the two factors. All reports given above, except that of Lownsberry, concerned only the cyst forming nematodes.

(h) Most of the published work on temperature effects on nematode populations also concerned the cyst forming nematodes. To a lesser extent, the genera Ditylenchus and Meloidogyne have also been investigated. Besides general deductions such as those of Winslow (1960) and Mountain & Boyce (1958), only very few workers, e.g., Yuen (1966) seem to have seriously considered the effect of soil temperature on nematode population changes. Mountain & Boyce attributed the significant reduction in P. penetrans population in summer to the limiting high temperature. Winslow stated that D. dipsaci occurs chiefly in cooler countries. Whether these observations result from a direct effect of the soil temperature on the nematodes or indirectly through the host plants' reaction to same is a question yet to be answered through further work. The difficulty involved in trying to distinguish between the effects of temperature and moisture in the field is probably responsible for the limited interest shown in this aspect.

1.3. On the whole, nematode ecology is receiving more attention lately than in the past, but because there are so many aspects to ecology, so much still remains to be done. Because so many geographical factors influence the nematode population dynamics, a complete ecological picture is only possible when information is available from most geographically different parts of the world. Although there are publications from various parts of the world, especially from Europe and America, there is relatively very little work of this nature done in this country so far. All New Zealand publications on plant parasitic nematodes were reviewed by Clark (1964). Several nematodes have been recorded on a number of plants (Clark, 1964). The only record of nematodes on fruit trees seems to be that of Newhook (Clark 1964) where *Tylenchulus semipenetrans* was reported on sweet oranges in Auckland. There are no published records of nematodes associated with apple trees in New Zealand.

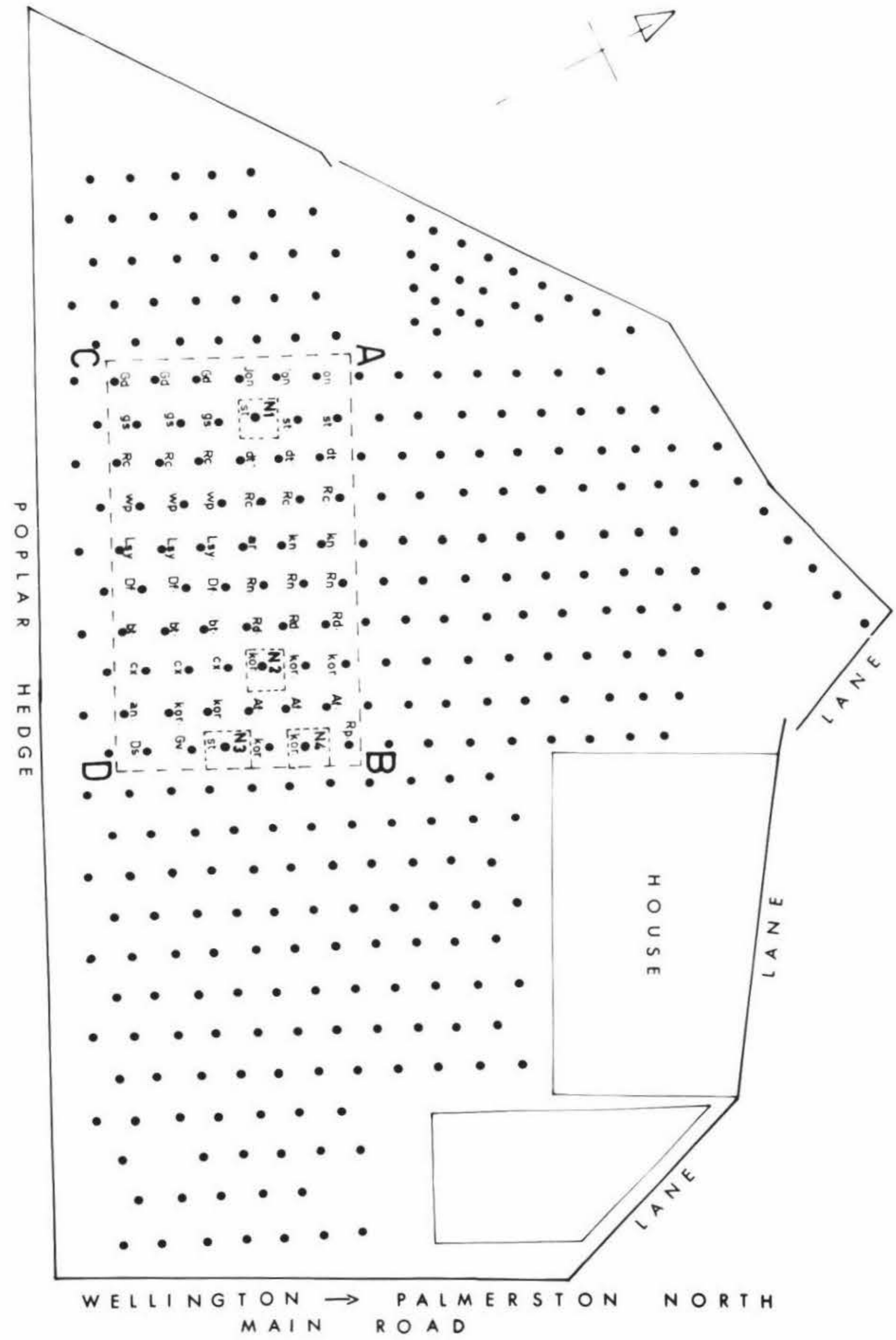

Fig. 1. Map of Batchelar Orchard, Massey University. ABCD. Experimental plot showing the varieties of the apple trees. st. = Sturmer; kor. = Kidd's Orange Red.

CHAPTER II

SITE OF WORK

2.1. Topography.

The Batchelar orchard where this work was done (illustrated in fig. 1) is an experimental orchard belonging to the Department of Horticulture, Massey University. Situated on a low river terrace, the orchard occupies the western side of the main University campus, about 13 metres south-west of the Batchelar Homestead. The campus proper is on higher ground and is separated from the orchard by State Highway 57, which connects Wellington to Palmerston North.

It is located between two rivers, at a distance of about 198 metres north of the Tiritea River and about 396 metres south of the Manawatu River.

The soil is relatively young aluvium soil, the plot being directly situated on the old course of the Manawatu River. The slight depressions present in some parts of the orchard mark the course of a more recent river. These depressions, aggravated by the several truck tracks running through the orchard, encourage the water accumulations observed during the rains. Along these tracks, soil is more compact and mat formation seems to be more pronounced.

2.2. History.

The orchard was planted about 24 years ago. Over the years, many replantings which gradually turned the original monocultural apple orchard to a polycultural one have taken place. At the commencement of this work, the orchard contained fruiting pear, plum, peach, apricot, nectarine, sweet cherry and quince trees, besides the apple trees.

Constant weeding was done for the first six years, but for the past eighteen years the orchard has been put into pasture. Grasses form the main cover crop and these include rye grass (Loluim spp.), Yorkshire fog (Holcus lanatus) and Browntop (Agrostis tenuis). Besides these, clover (Trifolium repens) and other weeds such as Ranunculus repens, Solanum nigrum, Poa sp., Plantago major, Cerastium glomeratum and Bromus catharticus are fairly common.

Spraying with insecticides and fungicides has also been a common practice. Spraying is done annually between September and February - i.e., in spring and summer. Details of the chemicals used for the whole period are not available. Some of those used in the recent years are as follows : Superior oil; Lindane 50%; Bordeaux; Thiram 80%; Kerathane 25%; Colloidal sulphur; DDT 50%; DDD 50%; Ferbam 80%; Lead arsenate; Captan; Triton B - 1956; Chlorocide. These chemicals are used at various dilutions and some are applied several times during each spray period. Some, however, have one application only, e.g., Bordeaux.

In choosing the experimental plants, trees of relatively constant and uniform history were chosen in a portion undisturbed by replantings. Because such undisturbed areas are few and in

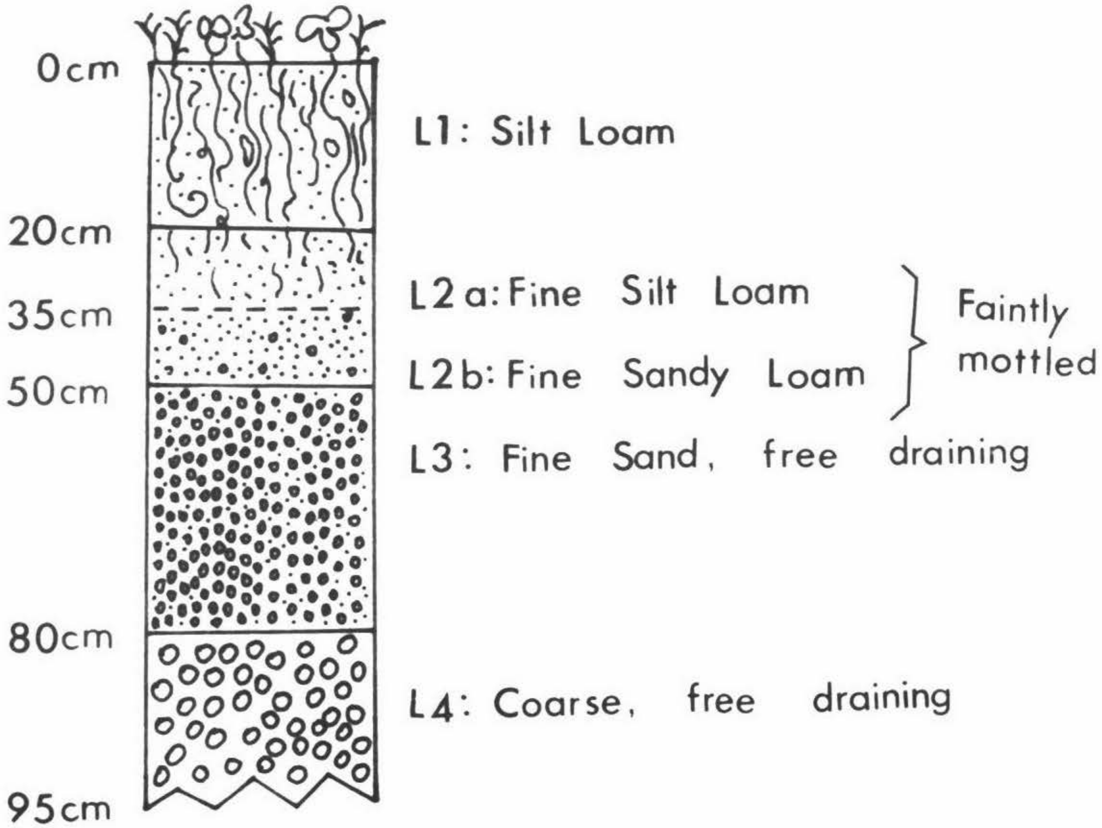

Fig. 2. The Soil Profile. (After Pollock, J., Soil Dept., Massey University, Palmerston North).

trying to avoid the 'edge effect' from the poplar hedge, the eligible part was limited to a rather small area (cf Fig.1, ABCD). Within this small area, it was impossible to choose four apple trees of the same variety 'at random' due to the planting system used. Therefore, four plants belonging to two different varieties - viz., Malus pumila (Mill) var. Sturmer and Malus pumila (Mill) var. Kidd's Orange Red were chosen 'at random' (viz., as far apart as possible).

2.3. Soil Type

The soil belongs to the "Karapoti silt loam" series in the New Zealand soil map. It is here described in two parts: (A) The soil profile description, shown diagramatically in Fig. 2 and fully described in Table 1 for 0 - 40 cm top soil, and (B) Soil texture description shown in Table 2.

In taking this soil profile, the river depressions were avoided. Although serious water logging is not expected, occasional pots of water were encountered at depths below 10 cm during sampling, especially in winter. This is probably due to the slight imperfection in drainage mentioned in the profile description.

TABLE 1

Detail Description of 0-40 cm Top Soil

(Compiled from the records of the Soil Dept., Massey University)

	L1: 0-20 cm	L2(a): 20-40 cm
Colour	10 Yr. 4/2, dark greyish brown	2.5 Y 414 - 514, olive brown to light olive brown, faintly mottled; 2.5 Y 7/0, light grey and 10 Yr 5/8, yellowish brown.
Texture	Silt-loam with no stones or nodules	Silt-loam, rather lighter than in layer above, no stones or nodules.
Structure	Well developed, medium crumb structure, becoming nutty with depth.	Very weakly developed structure, does not fit any established category, digs out into weak clods of medium size.
Consistency	Firm, friable, the friability being conferred by the good structure.	Friable when dug out, very slightly compact in situ.
Internal drainage	Fairly free.	Slightly imperfect.
Moisture	Moist.	Moist.
Organic Matter	Good supply of the normal continuously decaying plant tissues under grass, with mat. *	Nil.
Roots	Fairly numerous grass roots.	Some, but much less than in layer above.
Fauna	Earthworms and beetle grubs.	Fairly numerous.
Outstanding feature.	Typical silt loam, well structured.	Slight imperfection in drainage.

* Although no mat was reported in the original description, a mat of about 1 - 1½ cm was observed in many parts of the orchard

TABLE 2

Soil texture at various depths (28.10.60).

(Compiled from the records of the Soil Dept. Massey University).

Sampling Depths	0-15 cm	22-30 cm	38-48 cm	63-73 cm	90-95 cm
Coarse sand	0.8	1.3	9.3	13.6	55.6
Fine Sand	45.2	48	59	76.7	39.1
Silt	34	30.3	17.1	5.7	1.8
Clay	20	20.4	14.6	4.0	3.5
Sum	100	100	100	100	100
Organic matter	3.55	0.78	0.44	0.41	-

All figures expressed as percentage of dry weight.

Organic matter recorded as percentage weight lost in ignition.

IV. Climatology.

In this work, only the climatic changes that are relevant to the immediate environment of the nematodes are taken into consideration, e.g., rain fall, soil temperature and ground frosts. These are shown in Fig. 3 and Table 3. The soil temperatures are shown to a depth of 20 cm. The monthly averages for a period of thirty years (1936-1965) are used for plotting the graphs.

The mean annual rainfall is 98.35 cm. The average monthly rainfall ranges between a rather narrow limit of a minimum of 7 cm

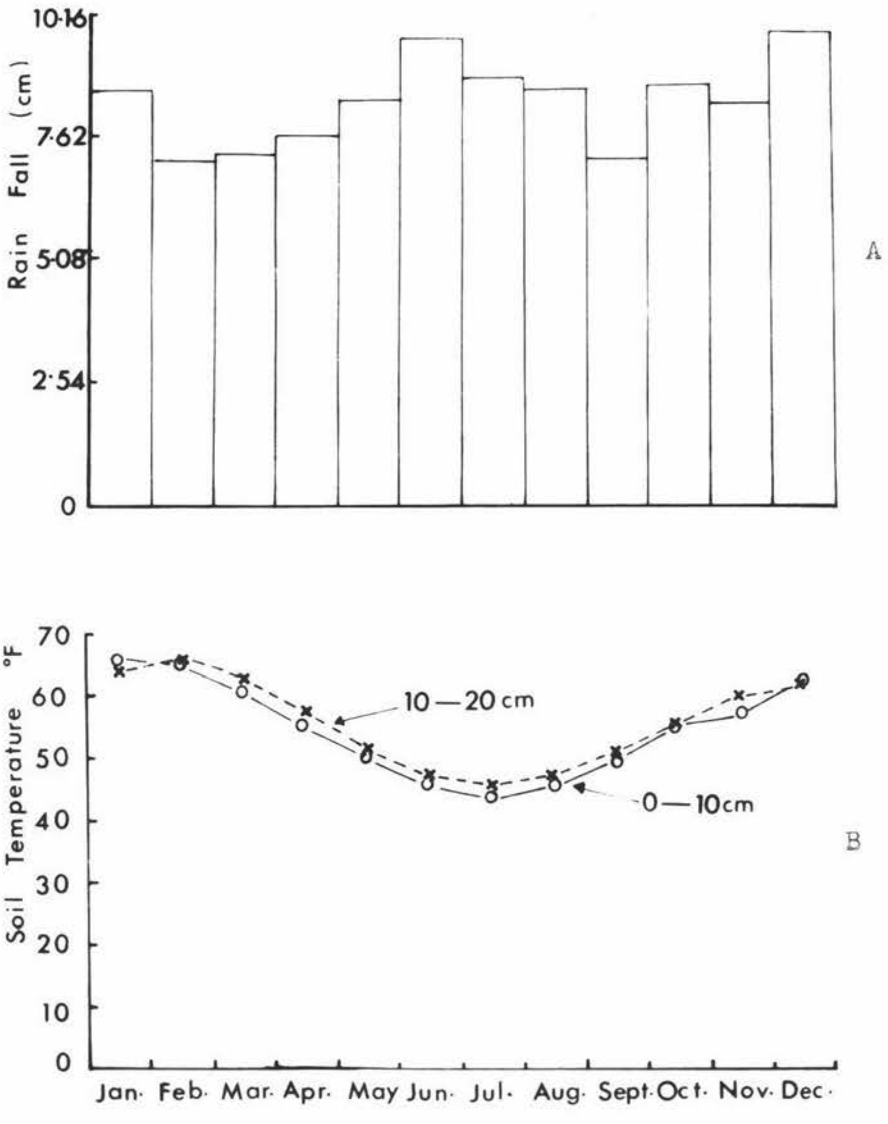

Fig. 3. Rainfall and soil temperature monthly variations, showing the means for 1936 - 1965. A. Rainfall; B. Soil temperature.

recorded for February, to a maximum of 9.8 cm for December (Fig. 3, A). Rainfall is more or less evenly distributed throughout the year, but there is a short spell of lower rainfall between February and May and another in September, December (summer) seems to be the wettest month of the year, whilst February seems to be the driest.

Fig. 3, B shows the monthly variations of the soil temperature. Unlike the rainfall, the soil temperature monthly fluctuations is well defined. The general trend at the different soil levels seems to be a high temperature of about 60-70° F. in January to March, followed by a gradual decline from March to a minimum of about 44-50° F. in July and a gradual increase from August to December when temperature ranges between 60 and 65° F. in the latter month. While the top soil tends to remain colder than the bottom part most of the year, the fact that the top soil is more affected by the external environment is also apparent since the 10 cm curve is higher than the 20 cm one between December and January (summer) while remaining colder for the rest of the year.

The ground frosts record for 1966 to January, 1967, is given in Table 3 below. In collecting this data, ground frost was recorded whenever the minimum grass temperature was at or below 30.3° F. Except for a single day in April, ground frosts were confined to the winter and spring months.

TABLE 3

Ground Frosts Record for 1966 to January, 1967
(Compiled from the Meteorological Records, D.S.I.R., Palmerston North).

	Months.	No. of Days.
1966	January	0
	February	0
	March	0
	April	1
	May	0
	June	15
	July	18
	August	15
	September	10
	October	3
	November	2
	December	0
1967	January	0

The highest record was for July when ground frosts were recorded for 18 days with the grass minimum temperature of 19.8^{o} F. on the 15th day of the month. The figures gradually declined through September to November to zero value in December which remained more or less constant throughout summer and autumn.

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

All material used was collected from the orchard discussed and illustrated in Chapter II. Samples were collected at intervals of 78 - 83 days each and they fall into four groups:

(i) Autumn samples collected on the 7th of May, 1966.
(ii) Winter samples collected on the 29th of July, 1966.
(iii) Spring samples collected on the 15th October, 1966.
(iv) Summer samples collected on the 7th of January, 1967.

3.2 Methods

These are described under two headings: A and B. In A, all methods used for soil collection, extraction and the general handling of the nematodes are described. In B, methods used for pH and soil moisture determinations are given.

A. This is described under the following headings:

1. Sampling technique
2. Storage
3. Extraction
4. Killing and fixing
5. Counting

6. Mounting
7. Examination
8. Drawing and measurements

1. Sampling Technique

The four experimental plants were tagged with metal labels, N 1, N 2, N 3, and N 4 for plants numbers one to four respectively. Samples were taken radially in four directions around each plant. In each direction, samples were collected at distances of 20 cm (hereafter referred to as OM), 1 metre and 3 metres away from the plant base. (cf Chapter IV). Attempts were made to sample right up at the base of the plants but this was not possible, using the soil borer. At each horizontal distance, three vertical samples were taken at depths of 0-10 cm, 10-20 cm and 20-30 cm. All samples from identical vertical and horizontal locations for each plant were pooled together in a labelled polythene bag to form a composite sample. (cf Chapter IV). For each plant, therefore, a total of nine sample bags were collected.

Labels were written directly on the polythene bags to avoid contamination of material by metal labels.

Stainless steel soil borer with a diameter of 2½ centimeters was used as this is easily kept clean, thus reducing cross contamination of material. The borer was thrust into the soil to a depth of about thirty centimeters. It was rotated and bent slightly to break the soil column and was then pulled out.

Because the rim is slightly narrower than the body diameter, the soil column often slid out when the borer was inverted at an angle on a polythene bag. Two probes were taken from each sampling point.

The usually intact core was then put against a ruler and cut with a knife into 0-10 cm, 10-20 cm and 20-30 cm portions, starting from the top soil.

Labels were designed to give information about the particular plant, horizontal distance and soil depth. Thus N1, 1M 0-10 indicates that the sample was taken from plant number one, at a distance of 1 metre away from the tree base and at a depth of 0-10 cm.

Occasionally, spade and hand trowel had to be used for sampling. In such cases, care was taken to ensure samples of equal weights as components of the composite sample.

2. Storage

Since all samples could not be extracted at once, each polythene bag was tied up and all were kept at $4^{\circ} \pm 1^{\circ}$ C., while awaiting extraction. This method has been shown by Overgaard Nielsen, (1949) and Oostenbrink (1960) to be quite reliable. W.C. Clark (1961) re-examined and re-affirmed its reliability.

3. Extraction

Each composite sample was emptied into a clean, dry, fairly large plastic bowl and was thoroughly mixed together. The

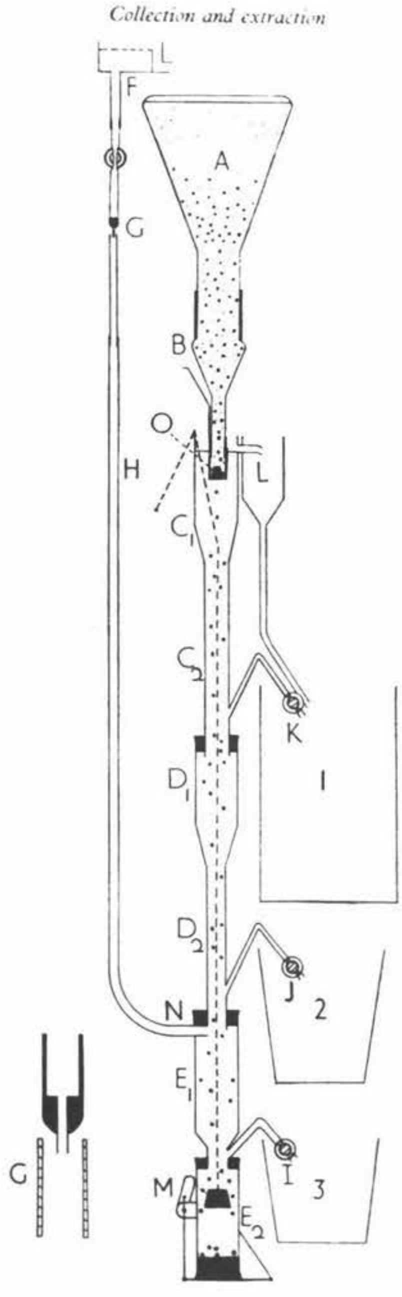

Fig. 4. Seinhorst's elutriator for the quantitative extraction of soil nematodes. (After Goodey, J.G., 1963).

quartering method was tried to eliminate bias, but because the samples were too muddy, it was soon abandoned. Besides, the samples were rather small and thorough mixing was easily achieved using the hand for about four minutes.

Seinhorst's elutriation method for the quantitative extraction of nematodes from the soil (Seinhorst, 1956, 1962[b], described in Goodey, 1963) was used throughout this work.

250 grams of soil was weighed into a 500 ml plastic beaker. The soil was then just covered with water and dispersed with a vibromixer immersed into the suspension so that the fans were just above the base of the container. The mixing was continued for about ten minutes to achieve fairly good dispersal. Any undispersed lumps were broken with the hand.

The resulting suspension was poured into an hemispherical domestic sieve of 2mm mesh, resting on a stoppered, water-filled funnel which led into the conical one litre flask (A*). The plastic beaker was properly rinsed into the sieve and the funnel plug was next pulled out, thus allowing the suspension to run into the flask. Any stones or trash left on the sieve was discarded.

The flask was now filled up with water and the glass funnel (B) attached. The space in the glass funnel was left empty to allow proper mixing of the flask content without clogging the funnel stem.

Tube E 2 was fitted with a rubber bung with a bucket slipped under it. The whole column was then filled with water and the tap was left running to maintain an upward current through the

* All references refer to Fig. 4

apparatus.

The glass funnel (B) having been closed with the rubber bung (O), the flask content was thoroughly shaken and inverted onto the rest of the apparatus. Bung (O) was next removed by the aid of the wire guiding ring so that the contents of flask (A) can now sediment against the upward water current.

The apparatus was left running for seven minutes, after which it was reckoned (Seinhorst 1962) that most large soil particles must have passed into tube E 2. The bung was then closed. After another 20 minutes, flask (A) was removed and emptied into container 1 (Goodey 1963) and after another ten minutes, now a total of 37 minutes (Seinhorst's 45 minutes), the contents of tubes C1, C2 and D1, D2, were all emptied into container 1, while that from E1 was collected in container 2 (Seinhorst's container 3). Contents of tube E2 was run off by opening the rubber bung.

Sieving was done by pouring the contents of container 1 through a bank of five 50 μ sieves, held in a slanting position as this has been shown to minimise loss of nematodes through the sieves (Seinhorst 1962[b]).

For the first four preliminary samples extracted, the residues from these sieves were rinsed directly into a 8 cm wide 50 μ nylon sieve, supported by a plastic ring, sitting in a clean plastic petridish, suspended above its base by three short legs, the sieve having been covered with a double piece of "snowtex" tissue paper. Water was then gently added to the petridish until the base of the sieve was just awash. This was left for a minimum period of 24 hours during which time most or all nematodes move through the sieve into the petridish. But because extracts thus obtained were, despite the tissue paper, often too dirty for examination, residues from the five sieves were, in later samples, rinsed into a large bowl of clean water and this was re-sieved through the five sieves again before they were finally transferred to the nylon sieves and treated as described above. Extracts thus obtained are usually cleaner than the fore-described.

The content of container 2 was gently poured through an inclined, 75 μ sieve several times. The residues were rinsed into a large clean bowl of water after each sieving. The resulting suspension was again sieved, using the same sieve, two times, rinsing the residue after each sieving into a nylon sieve inlaid with "snowtex" tissue papers and sitting in a petridish, as described above. This repeated rinsing reduces, to a great extent, the large amount of mud which usually accompanies this sediment.

After 24 hours, the sieves were removed from the petridishes and the nematode suspensions in the petridishes were rinsed into large, labelled test tubes and left to settle.

The residues on the sieves were discarded, the sieves thoroughly washed and soaked for one or two hours in 3% hydrogen peroxide in water to prevent cross contamination.

4. Killing and fixing

A rough preliminary investigation shows that at least one hour is needed for complete sedimentation by gravity of most of the nematodes in suspension, so that loss through suction pull exerted in water reduction process by pipetting or using other simple suction mechanisms is reduced to a minimum. Therefore, each test tube was left for at least one hour and a half after which supernatant fluid was reduced to about 3 ml with a pipette.

This residue was rinsed into a previously labelled and clean 50 ml glass beaker. It was first examined for nematodes under the binocular stereoscopic microscope under a magnification of 30 diameters. Where nematodes were found, the beaker was gently heated on a small spirit lamp flame while checking on the condition of the nematodes from time to time. As soon as the nematodes were relaxed, heating was stopped. An equal volume of double strength fixative was added. TAF (Courtney, Polley and Miller, 1955) was used throughout. The nematode suspension, now in fixative, was run into a labelled 3 x 1 inch (7.62 x 1.54 cm) glass tube which was then corked and put away.

5. Counting

With large samples, counting every nematode leaves little or no time for anything else. Counting the nematodes in small aliquots of the suspension was, therefore, introduced as an unbiased, time-saving device. The original suspension in fixative was diluted to 50 ml, using normal strength TAF. For the first few samples, two aliquots each of 2.5 ml (5%) were measured for counting, using graduated bulb pipettes. Later, however, 5 ml pipette (10%) was substituted to reduce error. Each aliquot was taken after thorough stirring, before the nematodes could settle down again. Counting was done in 4.2 x 1 cm small glass dishes, each with its base divided into 49 roughly equal rectangles, specially made for this purpose. Counting was done with a binocular stereoscopic microscope at a magnification of 30 diameters.

6. Mounting

Temporary mounts were made in water or fixative as described by Goodey (1963). For permanent mounts, the Glycerol-ethanol method of Seinhorst (1959) described in Goodey (1963) was used. The nematode suspension in fixative was transferred into a small syracuse glass dish. The fixative was removed very carefully, using a small bulb pipette. About 0.5 ml of Glycerol-ethanol mixture 1 (cf. Goodey, 1963, pg. 27) was dropped into the dish which was then placed in a closed desiccator containing an excess volume of 95% ethanol and left for at least 12 hours at 35-40° C. In this way, much of the water was removed, leaving the nematodes in glycerol and ethanol mixture. The container was then filled with Glycerol-ethanol mixture 2 (cf Goodey ibid.) and left in a partly closed container at 40° C. for about 3 hours, when most of the ethanol should evaporate gradually, leaving the specimens in pure glycerine. The nematodes were then mounted as described by Goodey (1963). Transverse sections were also mounted as recommended by Goodey (1963) pg. 34-35.

7. Examination

Identification to the generic level was done mostly under the binocular stereoscopic microscope. For specific identity, the binocular compound microscope was used. Occasionally, the ortholux compound microscope was used to examine very fine structures. In the latter two cases, observations were under the oil immersion objectives, using the Kohler illumination and transmitted light.

8. Drawing and Measurements

All nematode drawings were made with the aid of the camera lucida, using the Leitz Ortholux microscope. Except for Fig.4A where a magnification of 750 diameters was used, all drawings were made at a magnification of 900 diameters.

For nematode measurements, W.C. Clark's methods recommended by Goodey (1963) were used throughout. All length measurements were made at a magnification of 250 diameters, using the lead fuse wire method (Goodey, 1963). Body widths, stylets, annules, cuticle thickness, spicules were measured at a magnification of 900 diameters, using a Leitz screw micrometer eye piece. The measurements were recorded according to the de Man formula.

B. pH and Moisture content determination methods

1. Moisture content determination

100 ml beakers were used. Each was thoroughly cleaned, dried, labelled and weighed. The soil samples were each properly mixed. 50 grams of each soil sample was weighed out in appropriately labelled beakers and oven-dried to constant weight at 75° C.

Moisture content was recorded as :

$$\frac{\text{Wt. of water}}{\text{Total wt. of soil}} \times \frac{100}{1}$$

2. pH Determination (in Distilled Water).

A 1:2.5 soil suspension was prepared for each sample by weighing 14 grams of soil into 35 ml of distilled water in a 100 ml labelled beaker which had been previously washed, rinsed with distilled water and dried to prevent contamination. Each was stirred vigorously, using a clean glass rod and all were left overnight (Metson, 1956).

Metrohm E 280 A pH* metre was used for all samples measured. Each measurement was preceded by vigorous stirring with clean glass rods. The suspension was left under the combined glass electrode for one minute before each reading.

CHAPTER IV

PRELIMINARY INVESTIGATION

4.1. Method: The object of this investigation was to familiarise the author with the types and range of nematodes present in the orchard. The samples were taken on the 6th April, 1966. Detailed descriptions of sampling techniques and all other methods used here are given in Chapter III. The only deviation from the standard methods described in Chapter III is that samples were taken from two directions only (instead of four) and horizontal distances of sampling points from the tree were one metre, two metres and three metres (instead of zero metre, 1 metre and 3 metres).

All nematodes extracted were processed for permanent preparation by the Glycerol-ethanol method of Seinhorst, 1959. As all the nematodes could not be mounted due to the high numbers encountered, comparative population assessments were carried out and results are shown in Table 4 below. The different genera found are also shown in Table 5.

4.2 Discussion

No significant differences are observed between the total nematode populations at the three different horizontal zones (Table 4). This was not unexpected for the IM and the 2M locations where apple and grass roots are expected to co-exist with

TABLE 4

Total Nematode Populations at the Different Vertical and Horizontal Zones

Sample Nos.	Assessed Grades/250 grms. soil.	Average per horizontal distance.
1M 0-10	B	
1M 10-20	A	B^+
1M 20-30	B	
2M 0-10	A	
2M 10-20	B^+	B^+
2M 20-30	B	
3M 0-10	A	
3M 10-20	B	B^+
3M 20-30	B	

A = 3000-4000/ 250 grms. soil.

B^+= 1000-2000/ 250 grms. soil.

B = 50-1000/ 250 grms. soil.

little or no differences in quantity. A decrease in nematode population with increasing distance away from the 2M zone (away from tree trunk) would, however, be expected, due to an expected reduction in numbers of available apple roots. Such a decrease would mainly result from the plant parasitic nematodes which are known to fluctuate with plant root availability (Dieter, 1959; Wehunt, 1957; Fisher, 1961). Sampling closer to the base of the

TABLE 5

Genera identified

TYLENCHIDA	RHABDITIDA	DORYLAIMIDA
1. Pratylenchus	10. Cephalobus	15. Dorylaimus
2. Tylenchus	11. Eucephalobus	16. Eudorylaimus
3. Helicotylenchus	12. Acrobeloides	17. Alaimus
4. Paratylenchus	13. Rhabditis	18. Mononchus
5. Aphelenchus		19. Cobbonchus
6. Aphelenchoides		
7. Nothotylenchus	AREOLAIMIDA	
8. Pseudohalenchus	14. Plectus	
9. Heterodera (larva)		

tree would probably yield an interesting result especially since this zone is devoid of ground cover at this time of the year, because of the shading effect of the tree canopy.

In the 2M and 3M samples, total nematode population declined with increasing depth. This agrees with some earlier findings (Overgaad Nielsen, 1949; Winslow, 1960; Yuen, 1966). At 1M, however, the highest figure was obtained for the 10-20 cm zone. This could be due to a number of factors, but since it is unadvisable to draw valid conclusions from a single sample, more samples or perhaps a change in the sampling technique, would be necessary to confirm this observation. A local favourable condition such as a high concentration of a particular root type might induce an unusually high multiplication of a single genus or species which boosts the overall figure. Composite sampling would probably neutralise such micro-habitat effects and give a better overall picture.

In quantity, the total population varies between a maximum of about 3000 to 4000 nematodes per 250 grams of soil at the top soil and a minimum of about 50 to 100 per 250 grams of soil at the 20 - 30 cm zone. The Tylenchids on one hand and the 'Rhabditids' plus the 'Dorylaimids' on the other hand seem to occur in roughly equal numbers. Prominent among the Tylenchida are the *Pratylenchus* spp. most of which were juveniles. *Helicotylenchus* also was prominent, but to a lesser degree. Only a few *Tylenchus* species were found. Other genera such as *Paratylenchus* were relatively few, while some were represented by a single specimen only, e.g., *Heterodera*, *Pseudohalenchus*.

4.3. Conclusions

From the above observations, it was concluded that the following should be adopted for all future samplings:

1. Composite sampling - to neutralise any microhabitat effects.
2. Horizontal distances would be OM, 1M and 3M instead of the 1M, 2M and 3M used in this experiment.

CHAPTER V

RESULTS AND DISCUSSIONS

This Chapter is reported under five main sub-headings as follows:

5.1. Quantitative Distribution of the Nematodes.

5.2. Horizontal Distribution: Relative Abundance of the Top Soil (0-10 cm) Nematodes at OM, 1M and 3M.

5.3. Vertical Distribution.

5.4. Effect of the Seasons on the Population Dynamics of the Nematodes.

5.5. Correlation of Populations with pH. and Soil Moisture Conditions.

5.1. Quantitative Distribution of the Nematodes.

Table 6 shows all the nematode genera and some of the species encountered in this study. A total of 39 genera, representing 5 orders were found. The 35 identified genera are composed of 12 genera of the order Tylenchida, 12 of the Dorylaimida, 6 of the order Rhabditida, 3 of the Aerolamida and 2 of the order Monhysterida.

Only the Tylenchids were identified to the species. Because most of the species did not fit into the existing taxa, they are represented in this table by numbers. Thirty four Tylenchid species were recognised and these form about 50-60% of the total nematode population. While the genera Scutellonema and Hemicycliophora appeared in appreciable numbers only temporarily, Tylenchus, Pratylenchus and Helicotylenchus were present in fair numbers throughout the course of this study. The genus Tylenchus was the most plentiful, constituting about 97% of the Tylenchid population in some samples. Paratylenchus and the Aphelenchids were relatively few.

The Rhabditids were fairly numerous, comprising about 25-30% of the total nematode population, while the Dorylaimids represent about 10-12% only. The order Areolaimida was represented mainly by species of the genus Plectus and a few Anaplectids while the Monhysterida compose a negligible part of the total fauna.

TABLE 6

Quantitative Distribution of Nematodes:
Generic and Specific List

TYLENCHIDA

Tylenchus Bastian

Tylenchus (Aglenchus) costatus

Tylenchus (Aglenchus) neozelandicus n. sp.

Tylenchus (Aglenchus) areolatus n. sp.

Tylenchus (Aglenchus) whitus n. sp.

Tylenchus (Filenchus) 1

Tylenchus (Filenchus) 2

Tylenchus (Lelenchus) 1

Tylenchus (Lelenchus) 2

Tylenchus (Lelenchus) 3

Tylenchus (Tylenchus) 1

Pratylenchus Filipjev.

Pratylenchus pratensis

Pratylenchus crenatus

Pratylenchus coffeae

Pratylenchus (?) scribneri

Pratylenchus penetrans

Scutellonema Andrassy

Scutellonema 1

Scutellonema 2

TABLE 6 (Contd)

Hemicycliophora de Man 1 spp.

Helicotylenchus Steiner

Helicotylenchus 1

Helicotylenchus 2

Helicotylenchus 3

Paratylenchus Micoletzky 1 spp.

Nothotylenchus Thorne

Nothotylenchus 1

Nothotylenchus 2

Nothotylenchus 3

Nothotylenchus 4

Aphelenchus Bastian 1 spp.

aff. Cryptaphelenchoides (Fuchs) Skryabin et al.

Aphelenchoides Fischer

Aphelenchoides 1

Aphelenchoides 2

Aphelenchoides 3

Aphelenchoides 4

Heterodera Schmidt (Larvae, probably H.trifolii)

Pseudohalenchus Raski

Pseudohalenchus minutus

TABLE 6 (Contd)

RHABDITIDA

Cephalobus Bastian

Eucephalobus Steiner

Acrobeloides (Cobb)

Rhabditis Dujardin

Aff. *Diplogasteroides* de Man

Monochoides Rahn

AREOLAIMIDA

Plectus Bastian

Anaplectus de Coninck & Schuurmans Stekhoven

Anonchus Cobb

DORYLAIMIDA

Dorylaimus Dujardin

Eudorylaimus Andrassy

Nygolaimoides Meyl

Pungentus Thorne & Swanger

Aporcelaimus Thorne & Swanger

Diphtherophora de Man

Anatonchus (Cobb)

Iotonchus (Cobb)

Cobbonchus Andrassy

Mononchus Bastian

TABLE 6 (Contd)

Miconchus Andrassy

Alaimus de Man

MONHYSTERIDA

Monhystera Bastian

Prismatolaimus de Man

5.2. Horizontal Distribution: Relative Abundance of the Top Soil (0-10 cm) Nematodes at OM, 1M and 3M.

5.2.1. Introduction.

Counting was done as described in Chapter III and results are presented in Table 7 and 8 and illustrated in Fig. 5. The totals given at the bottom of Table 7 includes the other Tylenchids comprising mainly of some Aphelenchids and *Heterodera* larvae. Also included are a few species representing the orders - *Areolaimida* and *Monhysterida* - all included under "Others." Figures are given and graphs composed for each plant separately so as to elucidate any differences or similarities in the behaviour of each nematode group in relation to the individual trees. Figures recorded for *Tylenchida* include those for *Pratylenchus*, *Helicotylenchus* and *Tylenchus*, while the 'Dorylaimids' also include figures for *Alaimus*.

An important feature worth noting here is that an area of about 50-70 cm. radius around each experimental tree is almost completely devoid of the usual grass cover nearly all the year round. This zone starts to lose its grass cover from late summer and it remains bare until the latter part of spring. This provides an important ecological difference between OM and the other sampling areas used in this work.

5.2.2. Observations.

1. Horizontal distribution of total nematodes

No constancy is observed in the behaviour of the total nematode

TABLE 7

Relative Abundance of Top Soil (0-10 cm) Nematodes per 250 grams of soil at OM, 1M, and 3M on 7.5.66

	N1			N2			N3			N4		
Taxonomic Groups	OM	1M	3M	OM	1M	3M	OM	1M	3M	OM	1M	3M
Tylenchida	380	425	695	760	1830	1815	1600	1100	920	700	1890	1420
Pratylenchus	20	90	80	0	960	460	20	165	15	0	650	110
Helicotylenchus	0	205	370	0	640	955	20	295	265	20	1055	875
Tylenchus	340	55	70	720	215	235	1440	620	430	580	170	310
'Rhabditids'	500	185	160	560	365	490	960	415	110	1160	550	445
'Dorylaimids'	340	110	50	280	190	155	280	230	45	800	820	80
Alaimus	80	40	0	160	55	25	200	90	0	580	610	5
"Others"	10	190	175	20	20	165	130	20	220	50	20	135
TOTALS:	1670	1300	1600	2500	4275	4300	4650	1935	2005	3890	5765	3380

TABLE 8

Relative abundance of top-soil' (0-10 cm) populations per 250 grams soil of Pratylenchus Helicotylenchus and Tylenchus on 15.10.66

TAXONOMIC GROUPS	N3		N4	
	OM	1M	OM	1M
Pratylenchus	5	325	5	485
Helicotylenchus	15	1,120	15	560
Tylenchus	1,315	1,075	885	150

figures both in Table 7 and in Fig. 5. While the centre of maximum density lies at OM in N1 and N3, it is at 3M in N2 while at N4, it is at 1M. The minimum population area varies from 1M in N1 to OM in N2 and 3M in N3 and N4.

2. Horizontal distribution of Pratylenchus and Helicotylenchus.

There is a striking uniformity in the population variation patterns of Pratylenchus and Helicotylenchus as shown in Table 7 and 8 and in Fig. 5. While these genera were completely absent at OM in two plants and only poorly represented in the others, their

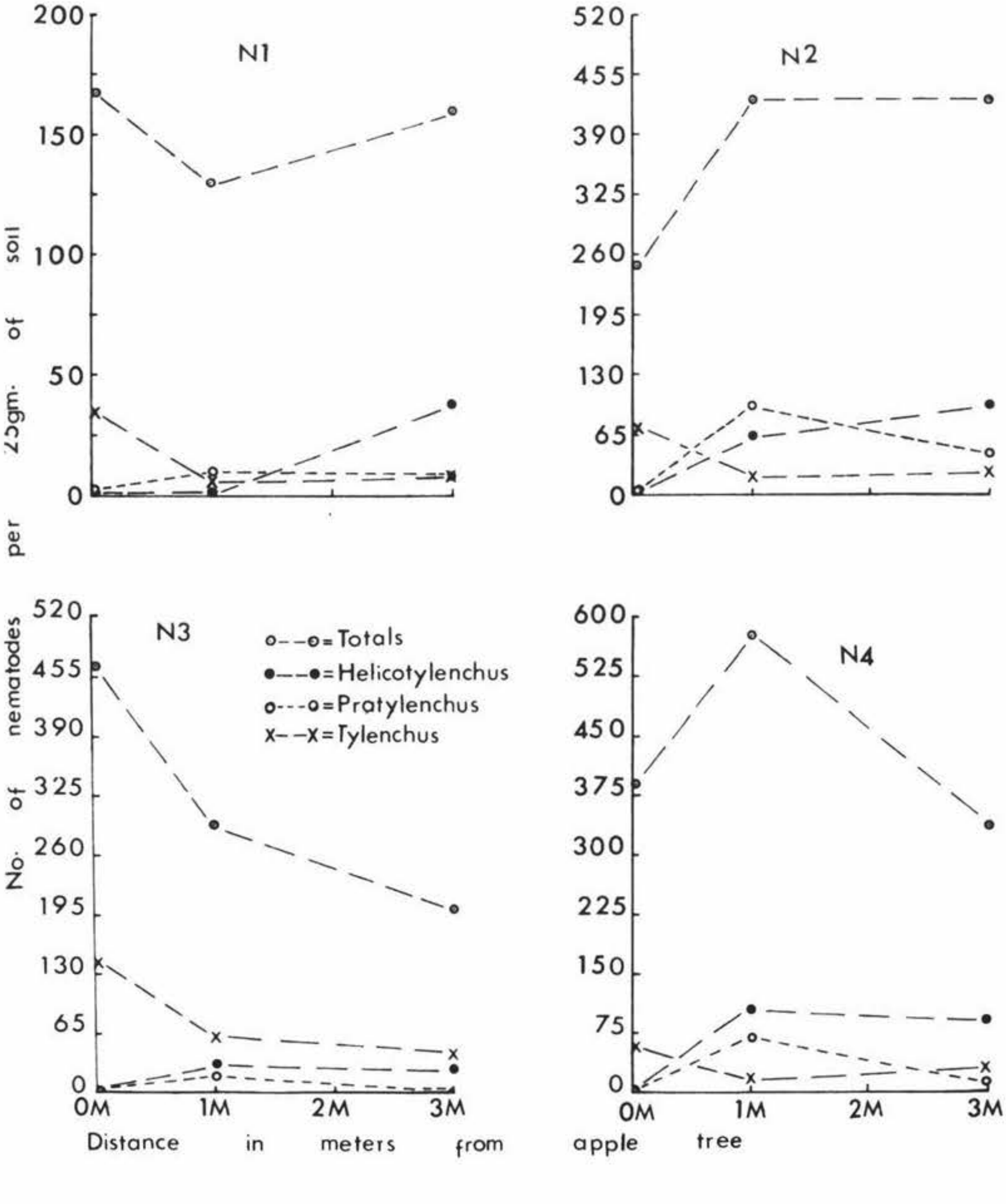

Fig. 5. Horizontal distribution: Relative abundance of the top soil (0-10 cm) nematodes at OM, 1M and 3M in relation to the apple trees on 7.5.66.

population together comprises about 75-90% of the high Tylenchid populations at 1M and 3M. In N2 where both were absent at OM, about 96 Pratylenchus species per 25 grams of soil were recovered at 1M, while Helicotylenchus at 3M was represented by about 95 specimens.

In all four plants, Pratylenchus had a maximum density at 1M while Helicotylenchus, though very high at 1M and 3M, does not show constant differences in figures obtained for these two zones.

3. Horizontal distribution of Tylenchus and Alaimus

The distribution pattern of these two genera is the reverse of that described above (2). Tylenchus is constantly highest at OM where it represents about 89-94% of the Tylenchid population (Table 7 and Fig. 5). Alaimus follows closely this same pattern (Table 7). The gradual decline in numbers from OM to 3M is better defined in Alaimus than it is in Tylenchus which is also well represented at 1M and 3M.

4. Horizontal distributions of the 'Dorylaimids' and the 'Rhabditids.'

The trend of changes in the population levels of the 'Dorylaimids' seems to be mainly affected by the Alaimus population which often form a great percentage (about 50% or more) of this group. Thus, except in N4 where 1M population was slightly higher than that at OM, this genus is often most abundant at OM, gradually thinning out with the distance (Table 7). This is also true of the 'Rhabditids' which, however, are proportionately higher than the 'Dorylaimids' at 1M and 3M (Table 7).

5.2.3. Discussions and Conclusions

Although the total nematode figures obtained display a rather contradictory pattern of distribution, it is obvious from the analysis that the nematodes in the different sampling areas differ quantitatively. The fact that the distribution pattern of the total nematodes tends to camouflage that of the composite genera, seems to point attention to the limitations of total nematode counts in population ecology. Thus, while notable changes occur in the population of the constituent genera, the overall figures might remain constant.

The striking uniformity exhibited by each of the different genera in their horizontal zonation relative to the four different trees examined is not unrelated to the ecological factor pointed out in 5.2.1. above. Since OM was almost completely devoid of plants at the time of this sampling, and since very little or no apple roots are expected at this area owing to the age of the trees, the population pattern of Pratylenchus seems to agree with the findings of the earlier workers, such as Winslow, (1955); Endo, (1959); Kleyburg and Oostenbrink, (1959); Henderson & Katznelson, (1960); and Fisher (1966). The great necessity of host plants to the existence and distribution of plant parasitic nematodes stressed by these authors can be summed up in Oostenbrink's (1961) own language, "The population of nematodes in a place at a particular time is influenced by the type of plant growing there .....". It might be added here that the presence or absence and the numbers of a particular nematode in a particular place at a given time depends on the presence or absence of plants in such a place and the duration of this presence or absence.

It can be deducted from the constantly maximum density of Pratylenchus at 1M that this genus is both parasitic on grass and apple roots since it is reasoned that young apple roots would be most abundant about this area. (cf. Chapter VI). Contrary to this, however, Helicotylenchus horizontal zonation seems to suggest that it is mainly parasitic on grass roots.

Although the genus Tylenchus has been described as plant parasitic (Yuen, 1966), its centre of maximum density at OM in this work does not seem to support this idea. This almost bare, damp area of decaying organic matter where they mostly concentrate suggests that they might be saprozoic. As this habitat also makes an ideal substratum for fungal growth, they might also be fungivorous as Winslow (1964) suggested. However, the possibility of some species being plant parasitic cannot be ruled out as their distribution was not strictly limited to this zone only. A detailed examination of specific differences between specimens from the different habitats might throw some light on this question.

5.3. Vertical Distribution

5.3.1. Introduction and Results

All the 36 results obtained for total nematode vertical zonations are shown in Table 9. Table 10 shows results obtained in autumn for the Tylenchida, Tylenchus spp. Alaimus spp., the 'Rhabditids' and the 'Dorylaimids'. The means of all the results as shown in Table 10 are illustrated in Fig. 6, A, B, C and G, respectively. The total nematode vertical zonation pattern illustrated in Fig. 6, A, is based on the means for all OM samples obtained in autumn.

Tylenchus, Alaimus, the 'Rhabditids' and the 'Dorylaimids' were all counted from OM samples where they are known to be most abundant. For the same reason, Pratylenchus and Helicotylenchus counts were done on the 1M samples and illustrated in Fig. 6, F., where two independent results from N2 and N4 were plotted together to show the significant similarity in the zonation patterns displayed by these two genera even when widely separated plots are examined. Fig. 6, E, represents the 1M pattern of the total nematodes at N2 and N4 to afford a fair comparison with the Pratylenchus and Helicotylenchus spp. counted from these locations and also to show if there are any differences in pattern between the OM and 1M samples.

All illustrated figures, except those for Hemicycliophora and Scutellonema were results of samples obtained in autumn. For comparison purposes, the season was kept constant to avoid any complications by a possible seasonal effect. The two exceptions

TABLE 9

Total Nematodes Vertical Zonation: Figures per 25 gms Soil

Plant No.	Soil depths	Autumn			Winter			Spring			Summer		
		OM	1M	3M	OM	1M	3M	OM	1M	3M	OM	1M	3M
	0-10	135	92	96	87	171	183	-	-	-	-	-	-
N1	10-20	83	81	69	15	3	50	-	-	-	-	-	-
	20-30	67	80	18	7	3	4	-	-	-	-	-	-
	0-10	252	325	353	41	260	105	-	-	-	-	-	-
N2	10-20	48	75	39	52	30	46	-	-	-	-	-	-
	20-30	26	24	29	27	24	23	-	-	-	-	-	-
	0-10	323	237	150	191	392	136	345	72	179	199	265	89
N3	10-20	78	62	61	20	88	50	162	21	88	64	58	67
	20-30	34	5	20	19	12	31	5	16	21	16	41	56
	0-10	295	393	239	270	177	41	144	20	91	98	461	176
N4	10-20	62	91	56	38	96	90	67	60	25	41	55	72
	20-30	72	66	61	13	25	2	29	34	2	24	44	64
	0-10	251	206	207	147	250	116	244	46	135	148	363	132
$\bar{x}$	10-20	67	77	56	31	54	59	114	40	56	52	56	69
	20-30	49	43	32	14	16	15	17	25	12	20	42	60

TABLE 10

Vertical Zonation: Figures per 25 Grams Soil (7.5.66)

Plant	Soil Depths (in cm)	Tylenchida	Tylenchus	Alaimus	'Rhabditids'	'Dorylaimids
N1	0 - 10	38	34	8	50	34
	10 - 20	60	58	0	14	2
	20 - 30	36	36	0	2	2
N2	0 - 10	76	72	16	56	28
	10 - 20	24	14	0	8	6
	20 - 30	10	6	0	2	2
N3	0 - 10	160	144	20	96	28
	10 - 20	34	22	0	6	4
	20 - 30	14	10	0	4	0
N4	0 - 10	70	58	58	116	80
	10 - 20	28	26	4	2	4
	20 - 30	44	42	4	6	12
$\bar{x}$	0 - 10	86	77	26	80	43
	10 - 20	37	30	1	8	4
	20 - 30	26	24	1	4	4

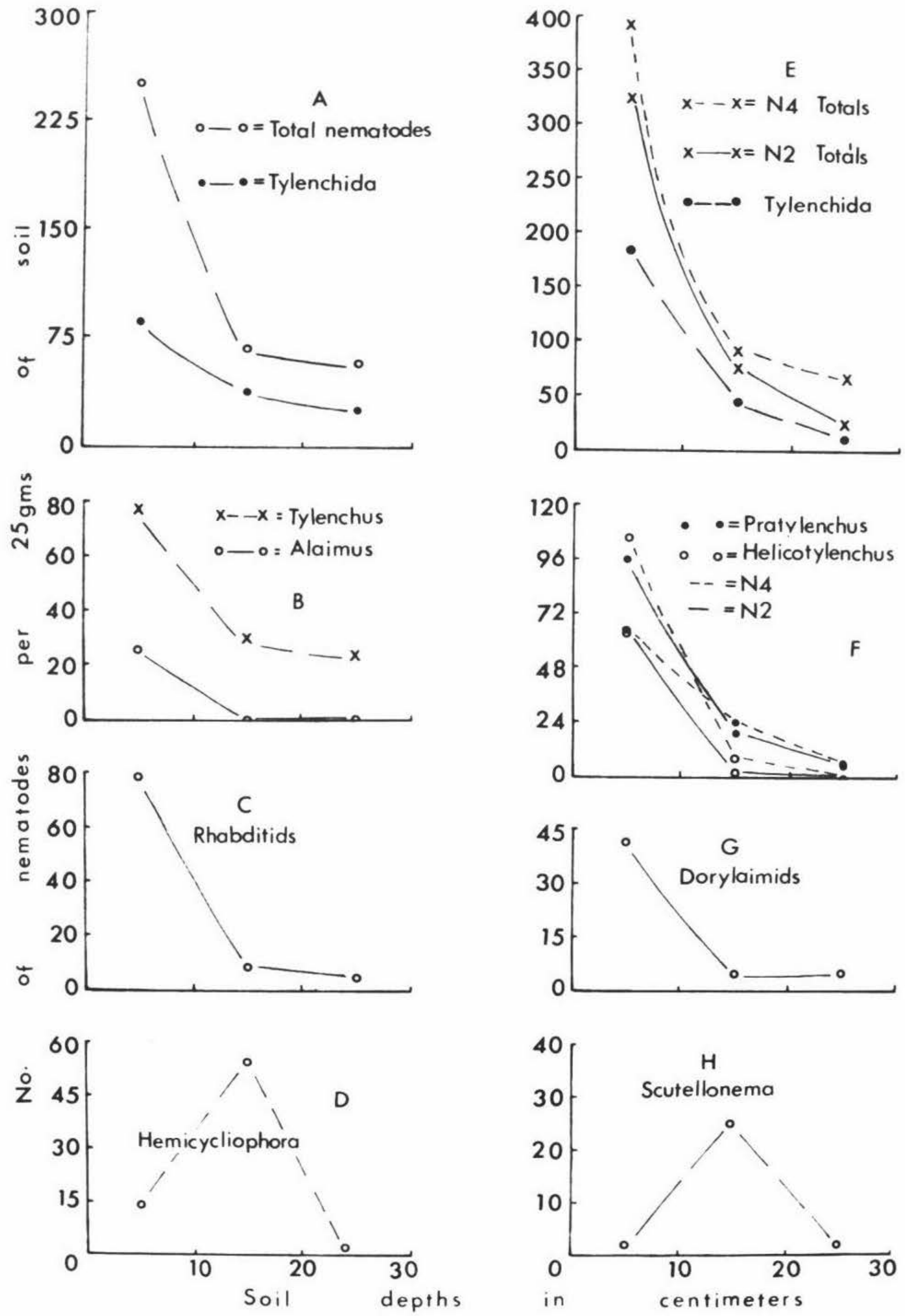

Fig. 6. Vertical distribution of the nematodes.

A, B, C, D and G from OM; E, F and H from 1M.

were illustrated from results obtained in spring as they were not recovered in autumn.

Because of the differences in composition observed (cf. 5.2.) between OM samples and those from 1M and 3M, no attempt has been made here to find means between results from 1M and OM (or 3M). Rather, results from these two ecologically different zones have been treated on comparative bases. Thus, all illustrations in Fig. 6, except E, F and H, were for results obtained from OM samples.

Fig. 7 represents the means for each season of all OM and 1M results (for N3 and N4) to show the seasonal effects, if any, on the vertical zonation patterns of the total nematodes.

5.3.2. Observations.

32 out of the 36 results obtained for the total nematodes show a fairly sharp fall between the top 10 cm. nematode populations and those for the next 10 cm., where the latter constitutes just about $\frac{1}{4}$ or less of the former. The differences between the bottom two zones are not so well defined. Generally, the bottom zone population ranges between $\frac{2}{3}$ and $\frac{1}{2}$ of the 10-20 cm. population. Occasionally, it is higher or less, but such variations are not observed in the mean figures shown in Table 9, which conforms to the pattern described above for most of the results (Fig. 6, A and F).

Although differences occur in the composition of nematodes at OM and 1M, no significant differences are observed in the

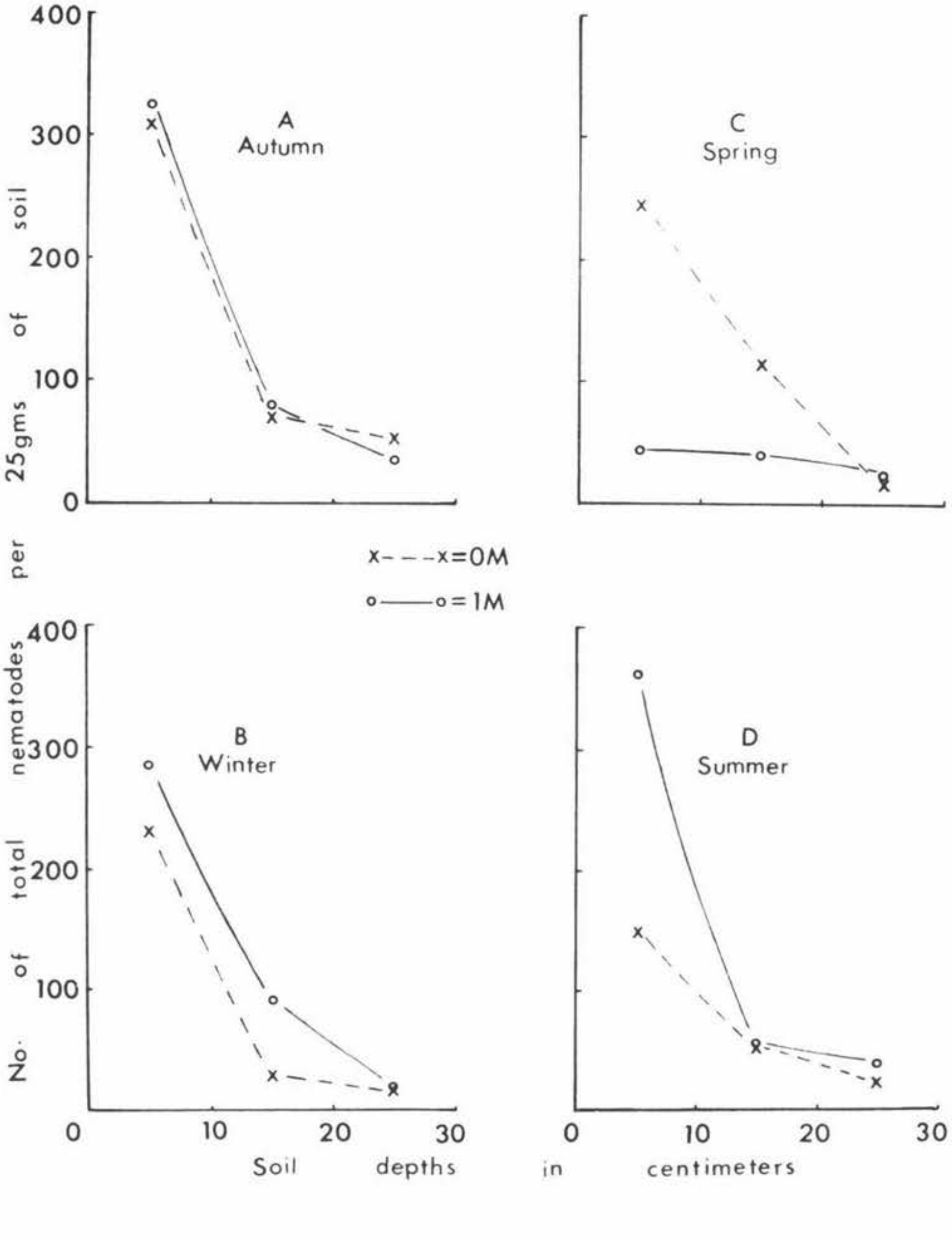

Fig. 7. The effect of the seasons on the vertical distribution patterns of the total nematodes.

vertical distribution patterns of the total nematodes in these two areas (Fig. 6, A and E, Table 9 and Fig. 7, A).

The distribution of Pratylenchus is closest to the above described for total nematodes (Fig. 6, F). The 10-20 cm. population ranges between one-third and one-fifth of those for the top soil.

However, those for Helicotylenchus, Alaimus, the 'Rhabditids' and the 'Dorylaimids' show a sharper decline from the top soil to the 10-20 cm. zone. For example, Helicotylenchus top soil populations are about 11-14 times those for the 10-20 cm. ones; those for the 'Rhabditids' are about 10 times, while those for Alaimus are about 14-20 times as many at the top soil, as those at the 10-20 cm. zones. There is little or no difference in numbers between the last two depths.

The distribution pattern of Tylenchus is less constant than the rest. However, the 10-20 cm. populations, although usually lower than the top soil ones, is constantly proportionately higher than recorded for other groups, and it is, in one result, actually higher than the top soil population. Compared with all the other genera examined, it has the highest representation at the 20-30 cm. zone.

Only Scutellonema and Hemicycliophora present a different picture where the centre of maximum population lies at the 10-20 cm. zone. Because these were collected in spring, they would be discussed under the seasonal effects.

Fig. 7 shows that the vertical zonation patterns of the total nematodes are not constant all the year round. In other words, the seasons do affect the distribution patterns of the total nematodes.

5.3.3. Discussions and Conclusions.

1. Total nematodes.

Results obtained for the total nematodes in this work only confirm a long known fact that nematode populations vary with soil depths (Godfrey, 1924; Chitwood & Feldmesser, 1948; Nielsen, 1949; Steiner, 1952). At OM, (Fig. 6, A), the fall in the curve at 10 cm. level in the total nematode figure is roughly intermediate between those for the 'Rhabditids', (Fig. 6, C), Alaimus (Fig. 6, B) and the 'Dorylaimids' (Fig. 6, G) on one hand and that for Tylenchus (Fig. 6, B) on the other. At 1M (Fig. 6, E and F), where Pratylenchus and Helicotylenchus form an important percentage of the total nematode population, the N4 total curve is higher than that for N2 (Fig. 6, E) partly because both Pratylenchus and Heliotylenchus populations at N4 are higher than those at N2 (Fig. 6, F). Similarly, the curve obtained for the 'Dorylaimids' (Fig. 6, G) is closely similar to that for Alaimus (Fig. 6, B), which constitutes a high proportion of this group. The Tylenchida curve at OM (Fig. 6, A) follows closely the pattern for Tylenchus which forms about 85-90% of this group. But at 1M (Fig. 6, E) where both Pratylenchus, Helicotylenchus and Tylenchus occur together in fair numbers, the curve cannot be considered as particularly close to any of these groups. Thus, it seems obvious that the vertical distribution pattern of total nematodes is determined by those of its component groups.

But the vertical gradient of the individual groups is determined by a variety of biotic and edaphic factors, which again differ according to the type of nematodes in question. The factors operating in this particular orchard have not been investigated in the course of this study, but the following suggestions can be made:

(i) Food type and feeding habits

(ii) Soil type

(iii) Although soil moisture content was examined (cf 5.5) this is not considered a very important factor here, since the rainfall is almost equally distributed throughout the year.

i. Food type and feeding habits:

It was noticed that the top soil, less than 10 cm. deep was rich in plant debris and decaying organic matter. This sort of environment would likely constitute an ideal home for saprophagous, fungivorous and bacteria feeding nematodes such as Alaimus, 'Rhabditids', some Tylenchus species and other groups. The predators like the Mononchids would probably also concentrate in this area, attracted by a greater concentration of prey.

ii. Soil type:

It was also noticed that the top soil was rather loose and lighter textured than the bottom layer below the 10 cm level which was often much heavier and more compact. The importance of soil texture and aeration to nematode survival in general had been pointed out by a number of workers and emphasised by Wallace (1963). One would expect large nematodes like the 'Dorylaimids' and some 'Rhabditids' to concentrate in light soil in preference to heavier ones for locomotory purposes. Even small nematodes like the Pratylenchus spp. are known to prefer light soils to heavier ones (Oostenbrink, 1961; Winslow, 1964; Wallace & Winslow, 1964; Parker & Mai, 1956; Sher & Bell, 1965).

From these considerations, the total number of nematodes would be expected to be highest in the top 10 cm soil layer.

2. Pratylenchus and Helicotylenchus.

When dealing with phytophagous nematodes such as Pratylenchus and Helicotylenchus, the vertical gradient would be expected to vary with the zone of maximum root concentration if food is as important a distribution factor as the above seems to suggest. This, in fact, has been found to be the case by several workers (Decker, 1959; Baines et al, 1959; Luc & Hoestra, 1960; Fisher, 1961; Wallace & Greet, 1964). Thus where host roots are shallow as in grass, most nematodes would be expected at the top soil. The results obtained for Pratylenchus and Heliotylenchus in this work therefore raise the question as to whether they are really parasitic on grass or on apple roots or more on one than the other.

Since Helicotylenchus is only barely represented below 10 cm layer (170 at top 10 cm to 10 at 10-20 cm) it could be conveniently concluded that it is mainly associated with grass roots, if not entirely. But for Pratylenchus with a more gradual slope (Fig. 6, F) the same conculsion cannot be reached.

This slope seems to suggest an association with both apple roots and grass roots. But the predominance at the top soil seems to suggest a closer association with grass rather than apple roots. The distribution pattern of apple roots varies with soil type and moisture content. With this type of soil (Chapter II) and the moisture content (see 5.5.), the distribution here is probably similar to that described by Gardner et al (1962) for the Hood River Valley Orchard, where feeding roots of apple trees occur at the top 6-25 cm soil depth. With this in mind, Pratylenchus would certainly be expected to be higher at the top 10 cm soil level if it were equally parasitic on grass and apple roots. But the expected value at 10-20 cm and 20-30 cm would also be much higher than the observed ones. The physical nature of the soil itself might be responsible for this observation. But besides this, the only other factor that can easily produce these results is if there were, in fact, more Pratylenchus spp. attracted to the grass roots than the apple roots (cf Chapter VI).

That P. penetrans populations in and around the tree roots can be significantly influenced by the cover crops grown beneath them was pointed out by Hoestra & Oostenbrink (1962), who found that Tagetes sp. would supress P. Penetrans populations in and around the apple roots, while red clover would increase them. If grass is a better host for the Pratylenchus than apple trees, it would be expected to attract more of the Pratylenchus populations to its root zones, resulting in a much higher figure at 0-10 cm - as obtained in this result.

Although the vertical zonation pattern of Helicotylenchus suggests a main association with grass rather than apple, a suggestion strengthened by its horizontal zonation pattern (5.2.6.), there is no evidence to suggest that it is not parasitic on apple roots, especially since it has been reported around apple roots earlier (Anderson, 1966). It might, therefore, be said that the grass roots offer better food than the apple roots do if Helicotylenchus is also parasitic on apple trees.

In conclusion, it might be said that if the above deductions are true, the grass pasture in this orchard has probably been of great economic advantage in reducing the possible degree of nematode infestation on the apple trees.

3. Tylenchus. The vertical zonation pattern of this genus does not seem to suggest a specific feeding habit. Its greater numbers at the bottom two zones than other examined genera

suggests that it might be plant parasitic. Its predominance at OM (5.2) and the great numbers in the top soil, however, offer strong reasons for thinking that it might be fungivorous or saprophagous. As concluded in 5.2., further work would be needed to throw more light on this question.

4. Scutellonema and Hemicycliophora: Only these two genera appear to have a closer association with apple roots. But the presence of the latter at OM alone does not seem to give strong support to this idea. Also, the restriction of their presence to a single plant each does not seem to provide sufficient evidence for serious deductions.

5. The effect of the seasons on the vertical zonation patterns of the total nematodes.

The total nematode curves for OM and 1M in autumn were remarkably similar except for a slight difference at the 30 cm point where the OM value was higher than that at 1M due to the higher numbers of Tylenchus at this depth. The significant difference in the 1M curve in winter can be explained in terms of the general reduction observed in the top soil values obtained for this season (cf. 5.4.). At OM, this reduction seems slightly greater and also affected the 10-20 cm population, probably because of the complete lack of grass cover in this area, thus exposing the soil to a greater influence of the external environmental factors. A similar explanation holds for the spring curves, which show the greatest deviation from the initial ones. This is particularly true for the 1M curve. With the Helicotylenchus and Pratylenchus populations composing about one half or more of the top soil total population, drastically reduced in spring (cf. 5.4.) and with the increase in numbers of the Scutellonema spp. in the 10-20 cm zones, the gap between the initial top soil population and that at10-20 cm zones as observed in autumn and winter was almost bridged. This results in the almost parallel graph in Fig. 7, C. At OM, the Tylenchus population increased at the expense of the Alaimus spp. This means that while there was an increase in Tylenchus population in the top soil, the loss due to Alaimus tends to neutralise the resulting effect to some extent. But with the vertical zonation pattern of Tylenchus (cf. 5.3.2.), there would also be an increase in the 10-20 cm population level where Alaimus population is almost nil. Besides, the Hemicycliophora multiplication at this time also increases the 10-20 cm population so that the usual fall at this level is almost totally eliminated. The overall result is the almost straight line graph obtained for OM in spring. In summer when Pratylenchus and Heliocotylenchus spp. have increased to the autumn level, Tylenchus has undergone a considerable reduction - giving an autumn-like curve for 1M, while OM, reduced by the Tylenchus population fall has again deviated from the autumn pattern.

These results illustrate the important influence of the seasons on the vertical distribution patterns of total nematode populations. A similar observation was made by Yuen (1966) when she found that the centre of maximum population for the total nematodes shifted from the usual 0-2 cm to 2-4 cm in August. The results also emphasise the differences brought about by the same external environmental conditions on populations in different ecological regions.

5.4 Seasonal Changes in Nematode Populations.

5.4.1. Introduction.

For this purpose, four sets of samples were collected between April, 1966, and February, 1967. Each sampling date was fixed as near the middle of each season as possible. Total nematodes were counted at the three soil depths separately. Except for Hemicycliophora, which was counted at 0-20 cm, the generic counts were limited to the top 10 cm samples. All results obtained are fully recorded in tables 11 and 12, each figure being the mean of four sub-samples. The means of all results obtained per season, regardless of soil depths and horizontal zonations, are plotted in Fig.8, A. The means of the total nematode figures for OM and 1M are separately plotted in Fig.8, B and D at the different soil depths. Figures obtained for the genera examined are also illustrated in Fig.8, C and E, each point based on the means for N3 and N4, and therefore composed of eight sub-samples. Fig.8, F and G, show temperature and rainfall distributions for the 'relevant period', with the monthly curves superimposed upon them, to show any correlations between these factors and the namatode population changes. The means of 30 years figures (cf. Chapter II) for the months of May, July, October and January are plotted in F and shown in form of a histogram in G for the 'relevant period.'

5.4.2. Discussions and Conclusions.

TABLE 11

Quantitative Distribution of Total Nematodes per 250 grms. Soil per season.

Plant No.	Soil Depth.	Autumn	Winter	Spring	Summer
	0-10	3,280	1,910	3,450	1,990
N3 OM	10-20	780	200	1,620	640
	20-30	340	185	50	160
	0-10	2,370	3,915	720	2,650
N3 1M	10-20	620	875	210	410
	20-30	50	115	160	580
	0-10	1,500	1,355	1,790	560
N3 3M	10-20	610	500	880	890
	20-30	200	310	210	670
	0-10	2,950	2,700	1,440	980
N4 OM	10-20	620	380	670	410
	20-30	720	125	290	240
	0-10	2,930	1,765	200	4,610
N4 1M	10-20	910	960	600	440
	20-30	660	250	340	550
	0-10	2,390	405	910	1,760
N4 3M	10-20	560	895	250	720
	20-30	610	20	40	640
Means per 250 gms. Soil:	-	1,980	935	768	1,050

TABLE 12

Quantitative Distribution of Some Nematode Genera per Seasons: Figures per 25 Grams Soil.

Generic Name.	Sample No.	Autumn	Winter	Spring	Summer
Tylenchus	N3 OM 0-10	144	132	183	96
	N4 OM 0-10	58	89	58	34
$\bar{x}$ per 25 gms. Soil		101	110	120	65
Alaimus	N3 OM 0-10	20	45	4	6
	N4 OM 0-10	58	38	6	1
$\bar{x}$ per 25 grms. Soil		39	21	5	4
Hemicyclio-phora	N3 OM 0-10	0	0	14	0
	N3 OM 10-20	0	1	55	2
$\bar{x}$ per 25 gms. Soil		0	0.5	35	1
Helicotylen-chus	N3 1M 0-10	37	112	6	74
	N4 1M 0-10	105	56	5	111
$\bar{x}$ per 25 gms. Soil		71	84	6	93
Pratylenchus	N3 1M 0-10	17	33	4	14
	N4 1M 0-10	64	48	4	41
$\bar{x}$ per 25 gms. Soil		41	40	4	28

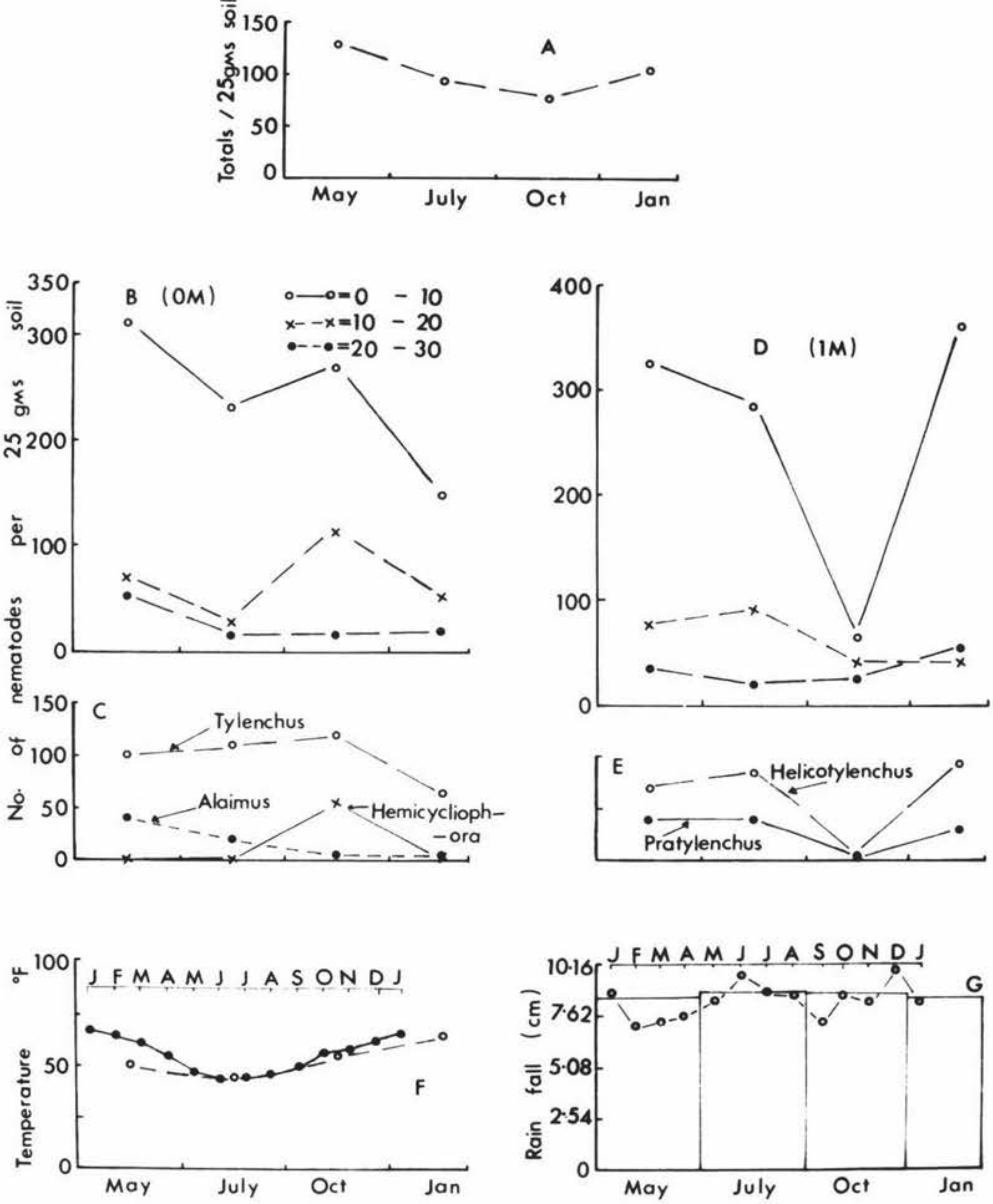

Fig. 8. Seasonal changes of the nematode populations. A. Overall average of total nematodes; B. Total nematodes at OM; D. Total nematodes at 1M; C. from OM; E. from 1M.

There were two peaks in the seasonal distribution of the overall totals, as shown in Fig.8, A, viz., one in autumn (May) and the other in summer (January). The lowest figure was in spring (October). But comparison of the totals for OM and 1M (Fig.8, B and D) show interesting discrepancies. While the latter (D) is similar to A in possessing an autumn and a summer peak with a spring minimum, the former (B) has autumn and spring maxima with a summer minimum. These differences are not unrelated to the ecological differences as well as the differences in generic composition of these two zones pointed out in 5.2. and 5.3. above. As shown in C and E, the main different genera associated with these different localities seem to possess major differences in their seasonal population patterns. Thus, while the OM total curve could be said to owe much of the spring increment and summer decline to the Tylenchus and Hemicycliophora species, the fall and rise at 1M for these seasons could be strongly linked with Heliocotylenchus and Pratylenchus species whose populations were reduced to about 2½% and 5% respectively of their autumn figures in spring and whose increase in summer was quite significant (E).

As much as these generic differences offer some explanation for the striking differences in total nematode seasonal patterns at OM and 1M, they do not seem able to account for all the observed changes throughout the seasons. Thus, the winter fall at both OM and 1M cannot be explained in terms of the behaviour patterns of these genera except Alaimus (C), which, however, constitutes only a small fraction of the OM totals. The winter depression is, perhaps, due largely to the free living groups such as the Areolaimids, the Monhysterids and especially the Dorylaimids, whose populations were much lower in winter.

Only Helicotylenchus possessed a winter and summer population peak. Pratylenchus appears to have maintained a steady population between autumn and winter, while the summer population became slightly lower than both (E). Alaimus was most abundant in autumn from whence it underwent a steady decline in numbers with a minimum in summer. Of all the genera examined, Hemicycliophora was the only one with a temporary appearance, mainly in spring. It was sparsely represented in winter and summer.

The seasonal variation pattern obtained for the total nematodes in the grass covered area (1M) resembles that recorded for nematodes in various meadow, pasture and non-agricultural soils of Switzerland by Burkhalter (1928), where he found that numbers were generally minimal in May (spring), increased to high numbers in July (summer) and this high population was maintained till October (autumn) and then fell steadily again until the following spring. The grass cover seems to be the only important common factor between the 2,000 meters high lands of Switzerland and the site of this work. The unvegetated OM area with the autumn and spring peaks and the winter and summer minima is closest to Winslow's 1964 records for Woburn Six-course rotation site in England, but differs from this in having a winter depression while the latter had a winter peak. As far as soil type and vegetation are concerned, there seems to be no similarities between the two sites. In the Broadbalk Wilderness grassland in England, Yuen (1966) reported two peaks for *Helicotylenchus vulgaris* - one in spring (March) and the other in summer (July and August). Although *Helicotylenchus* possess a summer maximum in this work, a spring minimum was also found - contrary to the above results for *H. vulgaris*. Similarly the spring minimum recorded for *Pratylenchus* in this work does not agree with the findings of many earlier workers (Decker, 1960; Edwardo, 1961; Jensen, 1950; Mountain & Boyce, 1958).

Disagreements between seasonal results obtained by different workers is not uncommon. For example, while Edwardo (1961) recorded a winter minimum for *Pratylenchus penetrans* in New Jersey, Jensen (1950) found a winter maximum in California for *P. vulnus*. Yuen's results for *H. vulgaris* in England was similar to that of Norton (1963) for *Xiphinema americanum* - a different genus altogether. Such differences are not unexpected, with the realisation that the seasonal curves for nematode population are products of many factors interacting together. Important among such factors are soil type, rainfall and moisture content (Norton, 1959; Wallace, 1962; Wallace & Greet, 1964; Winslow, 1964) and vegetation types. As far as phytophagous nematodes are concerned, most workers seem to agree on the fact that the period of maximal plant growth corresponds to the period of maximal nematode populations (Sasser & Nusbaum, 1955; Goheen & Williams, 1955; Wehunt, 1957; Dickerson, Darling & Griffin, 1964; Ferris & Bernard, 1961; Yuen, 1966). Although crop becomes second in importance to soil type in qualitative distribution of the polyphagous species like Pratylenchus, it is nevertheless very important in the quantitative distribution (Jensen, 1953; Townshend & Davidson, 1960). Although other climatic factors might be of significant importance, the similarities between results obtained here for the 1M totals and that recorded by Burkhalter can be attributed mainly to the vegetation types in the two different areas.

All the total nematode seasonal curves found in this work cannot be correlated with any of the two factors examined - viz., temperature and rainfall. However, Helicotylenchus curves show some positive correlation with rainfall as shown in E and G. The autumn population of this genus was fairly high, following a two months period of slight increase in rainfall. In winter, with four months of rains culminating in a June peak, population became higher, only to fall in October, following a month of comparatively low rainfall. This was followed by an increase to a maximum in summer after two months of rains, including December, the month of maximal rainfall.

Vegetation growth and root availability can also be correlated with the 1M nematode population dynamics if viewed as follows: Since ground frosts still occurred till the spring sampling date (a grass temperature of 26.1° F. was recorded for the 14th October), it might be deducted that the vegetation did not fully recover from the winter dormancy until late spring or summer, when growth was apparently vigorous. The effect of such dormancy was therefore most reflected in the spring results. It might also be explained that in July when the winter samples were taken, the effect of the season on the plant roots did not yet seriously impair the already existing roots to an extent very detrimental to the nematodes. Also, since most of the autumn population of *Helicotylenchus* and *Pratylenchus* were juveniles, a reduced reproductive activity would probably not have an apparent influence on the populations of these genera. Since no sampling was done in June, it is not impossible that the observed population in July was actually built up in late autumn and only maintained till winter. It must, however, be specified that, until otherwise proved, the possibility of these *Helicotylenchus* spp. (mainly composed of a single species) actually multiplying and increasing in winter cannot be ruled out, even though such changes seem narrow.

As already pointed out (5.2.) the distribution patterns of *Tylenchus* and *Hemicycliophora* do not suggest a strict phytophagous habit. Hence, their low summer populations cannot be explained as above. Lownsberry (1961) suggested that soil moisture fluctuations may be responsible for populations of Criconemoides xenoplax decreasing in some California peach orchards during summer. This seems unlikely in this case where December is obviously one of the wettest months and January rainfall is also quite high. However, their high population period seem to coincide with the period of low temperatures below 58°F. (C & F). Mountain & Boyce (1958) attributed the low summer populations of Pratylenchus penetrans to the limiting high summer temperatures. This might also be true of these genera. But changes in temperatures within the limits recorded here do not seem to affect the populations of the plant parasitic nematodes recorded above.

5.5. Correlation of nematode population with soil moisture levels and pH.

5.5.1. Introduction.

The soil moisture content and pH values were determined for all samples as described in Chapter II. An attempt was made in Fig.9 to show any relationships between these factors and:

(1) Seasonal variations in nematode numbers, (A,B,C & D);

(2) Vertical zonation of nematodes (D,F,G & H); and

(3) Differences between samples from different trees (P,Q,K & L).

For reasons already stated (5.3.1.) OM and 1M results are treated separately. Except for A,B,C & D, all figures were taken from the autumn (7.5.66) countings and except for E,F,G & H, all were for the top 0-10 cm soil layer. All figures in E,F,G & H represent the means of values obtained for N2 and N4 trees.

5.5.2. Discussions and Conclusions.

Neither the soil moisture content nor the pH values is apparently correlated with the seasonal fluctuations of nematode numbers, both at OM and 1M (Fig. 9, A,B,C & D). At first, Helicotylenchus and Pratylenchus seem to be roughly related to both factors (C & D), but the factors fail to account for the summer maximum figure for Helicotylenchus.

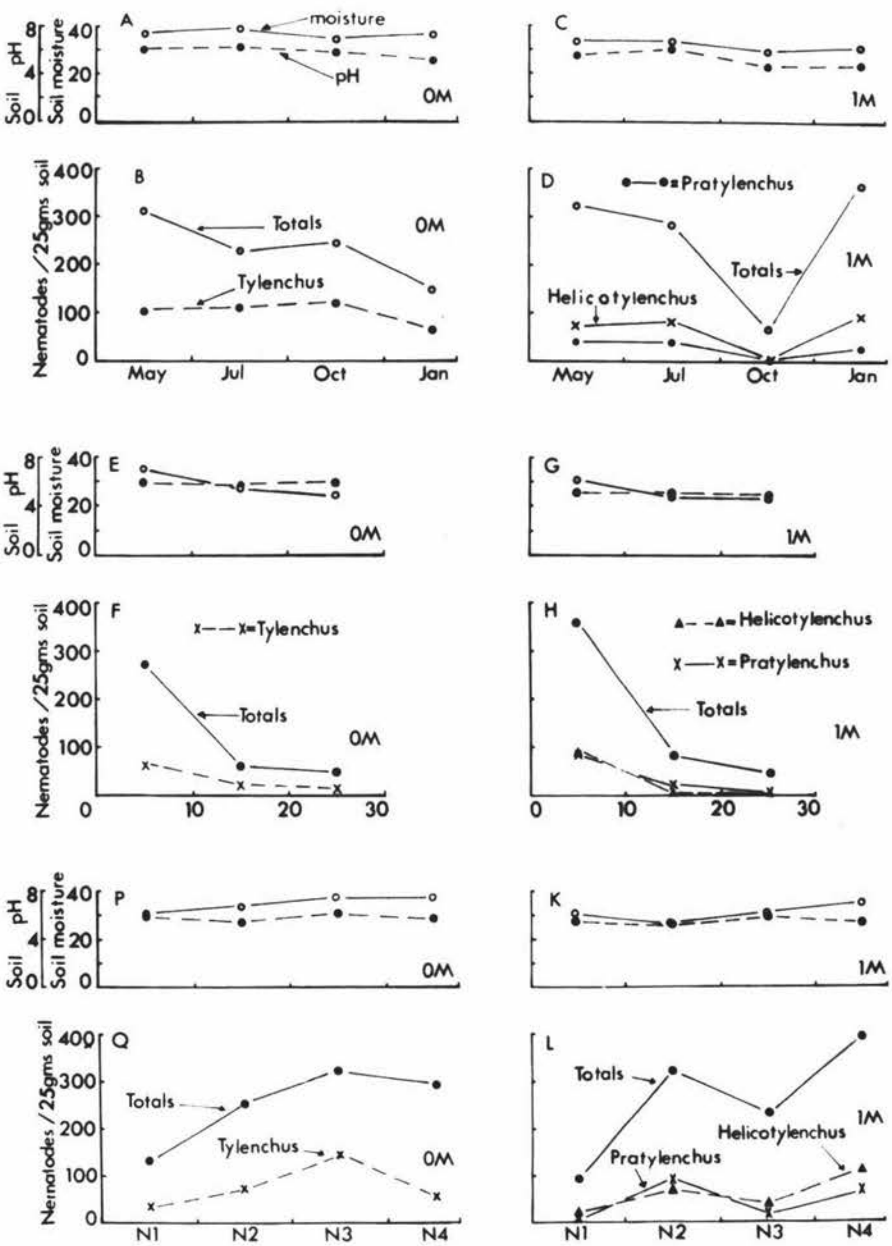

Fig. 9. Correlation of pH and soil moisture levels (% weight) with nematode population changes. A, B, C & D. Correlation with seasonal population changes; E, F, G & H. Correlation with nematode vertical distributions; P, Q, K & L. Correlation with inter plant sampling variations.

The lowest values for both moisture and pH were obtained for October, corresponding to the nematode minimum figures at 1M. To be able to bring about such a significant decrease in nematode numbers, one or both of the values must be close to the lethal low value. But in N2 (K & L) where lower values were recorded for both factors, population of both genera were much higher than the October numbers. The slight similarity in the curves thus appear to be a coincidence rather than correlation.

At both OM and 1M, soil moisture decreases gradually with soil depth. Although the total nematodes also decrease with depth, the pattern is less gradual (E & F, G & H). This is also true of Helicotylenchus, but Tylenchus and Pratylenchus vertical zonations appear to be related to the soil moisture levels. No correlation is observed between the pH and any of the genera examined.

Similarly, there is no correlation between the pH values and the variations in samples from different trees (P & Q, K & L). At OM, differences between the first three plants seem to be related to differences in soil moisture levels (P & Q), but this does not explain the decrease in N4 figures where the moisture content is just as high as in N3. Fisher (1961) also found that soil samples from around the individual apple trees showed extreme variations in nematode numbers. He was able to show that root distribution accounted for about 50% of such variations. This would probably be true of the 1M samples, where roots are present. Soil moisture might, however, be more important at OM where vegetation constitutes a less important factor than at 1M.

From field observations, it is often difficult to distinguish which of the several biotic and climatic factors exerts the greatest influence in producing the observed effect. Thus, in this result, unequivocal statements cannot be made concerning the role of soil moisture or pH on nematode populations. In all probability, the inter plant variations observed at OM (P & Q) is determined by a number of factors of which soil moisture levels play a part. Just how important that 'part' is can only be elucidated by a more controlled experiment which is beyond the scope of this work. Vegetation and root distribution patterns are probably more important in the vertical zonations of Pratylenchus than soil moisture in this work. The importance of soil type on nematode populations cannot be considered in isolation from the actual moisture level. Most nematodes are known to be adversely affected by extreme moisture conditions (Brown, 1933; Hollis & Johnston, 1957; Johnston, 1958; Peacock, 1957; Ward, 1960; Wallace, 1963). As shown in Fig.9, A & D, variations with seasons of the moisture levels is very small, most falling between 30 and 38. Within these limits, soil moisture would probably make little difference to most nematodes. Hence, the lack of correlations in A & B, C & D.

Besides the fact that moisture levels and pH are generally slightly higher at OM than 1M, very little or no differences can be seen between the OM and 1M patterns of these two factors.

CHAPTER VI

INVESTIGATIONS AND COMPARISON OF PRATYLENCHUS SPP. POPULATION DENSITIES IN GRASS AND APPLE ROOTS.

6.1. Introduction.

In trying to explain the vertical distribution pattern of the genus *Pratylenchus* in the last Chapter (5.3.3.), it was suggested that grass might be a better host for this genus than is the apple tree. This investigation was designed to check experimentally the validity of this hypothesis.

6.2. Methods

Early in spring (6.9.66), some apple and grass roots were collected from around the N2 experimental tree. These were thoroughly washed several times and 25 grams of each was cut into small pieces and the nematodes from each sample were extracted as follows: The Bearmann funnel method was used, substituting a nylon sieve, inlaid with "snowtex" tissue papers for the original butter muslin. The funnel was filled with water to a level when the contents of the sieve was just submerged. A few millilitres of water was run off from the rubber tube into a small 50 ml beaker at twelve-hourly intervals. The water in the beaker was then examined for *Pratylenchus* which were counted as described in Chapter III. After each few millilitres had been run off, the funnel was refilled with water to the original level.

The experiment was continued for 72 hours when no more nematodes were recovered. Results are recorded in Table 13, A, and illustrated in Fig.10,A.

During the spring soil sampling (15.10.66), the second lot of grass and apple roots were collected, this time from around N1 and N2 trees. These were treated as described above, but the modified Bearmann funnel method of Schindler (1961) was used for the nematode extraction, substituting Seinhorst's 8 cm nylon sieves for Schindler's wire gauze ones. Water was run into each petri dish until the contents of the sieves were just submerged. The sieves were removed every twelve hours and all the water in each petri dish separately examined, counting all Pratylenchus present. After each counting, the sieves were returned into the petri dishes which were then refilled with fresh water to the original levels. The twelve-hourly counting was continued for 84 hours after which the experiment was continued only to determine the approximate total nematodes present in each of the four samples. The experiment was therefore left standing for a further 5 days during which counting was done at irregular intervals. Results were recorded in Table 13, B, and illustrated in Fig.10, B.

The third samples were taken on the 19.11.66, this time, not only to confirm the differences observed above between the grass and apple root populations, but also to increase the number of samples for N1 and N2 trees so as to check whether the rather low figures obtained for N1 apple roots in the last two experiments truly indicated some degree of resistance in

TABLE 13

The Comparison of Pratylenchus spp. Population Densities in Grass and Apple Roots: A.(6.9.66), B.(15.10.66) and C.(19.11.66).

A

TIME IN HOURS	N2	
	GRASS	APPLE
12	25	5
24	32	5
36	32	5
48	33	12
60	33	12
72	33	12
TOTAL FOR 72 hrs.	33	12

B

TIME IN HOURS	N1		N2	
	GRASS	APPLE	GRASS	APPLE
12	20	1	28	21
24	48	3	58	37
36	64	3	74	46
48	89	4	93	54
60	113	5	119	66
72	124	5	129	72
84	136	5	135	79
TOTAL FOR 5 days	280	11	187	120

C

TIME	REP. I.		REP. II.		PLANT No.
	GRASS	APPLE	GRASS	APPLE	
TOTAL READING FOR 8 DAYS	452	52	469	78	N1
	-	213	-	228	N2

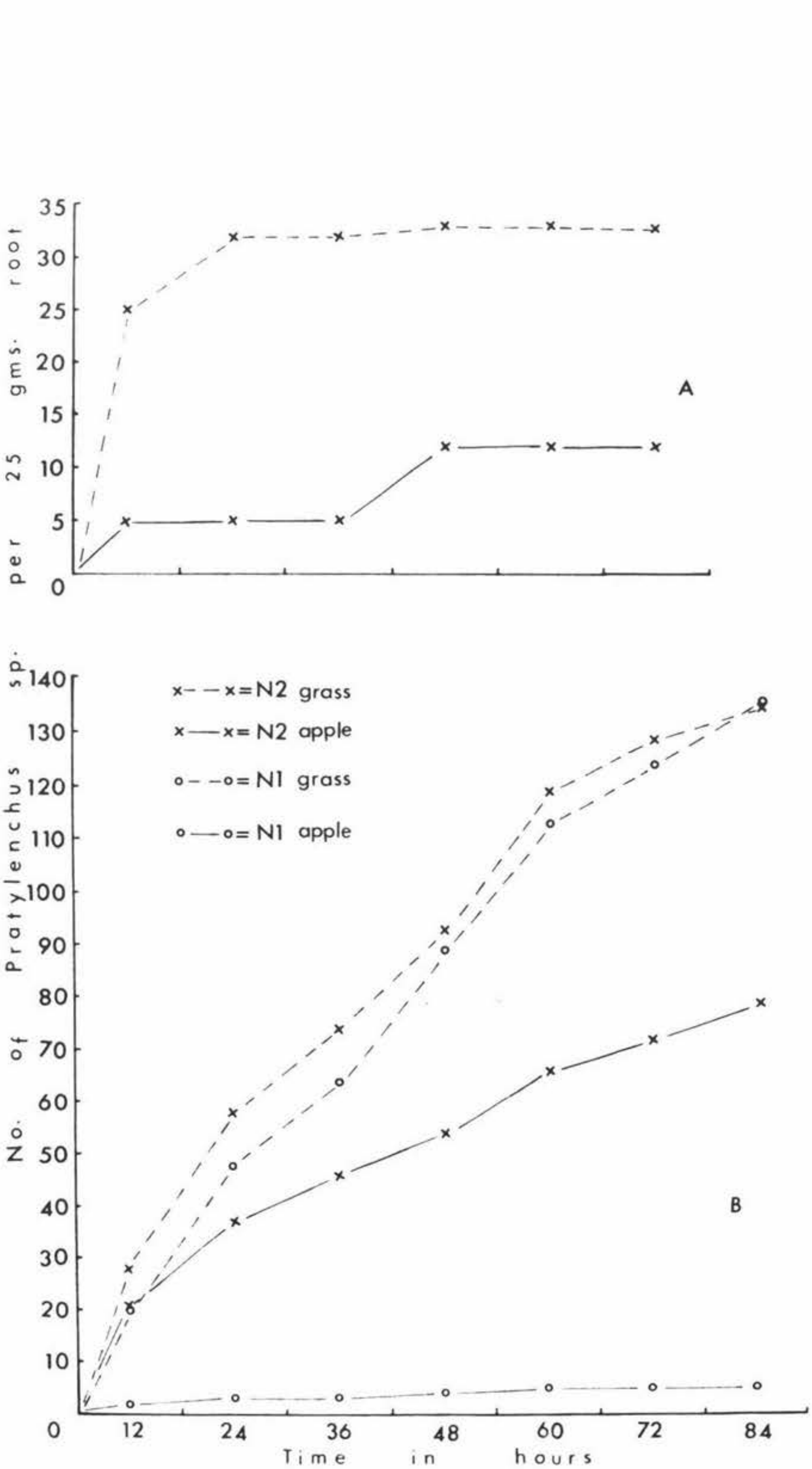

Fig.10. Comparison of Pratylenchus spp. population densities in grass and apple roots. A. Collected on 6.9.66; B. Collected on 15.10.66.

the N1 apple tree or it results from errors in the experiments. Two 25 gram replicates each of apple and grass roots from each tree were separately extracted as described for the second samples above. In this case, counting intervals were quite irregular and only the approximate total Pratylenchus numbers were recorded in Table 13, C.

6.3. Discussions and Conclusions

(a) All the four results obtained indicate a significant predominance of the grass root populations over those of the apple roots (Table 13, A-C, and Fig.10, A-B). These results agree with those obtained in the Netherlands by Hoestra and Oostenbrink (1962) for similar orchards. From a survey of several orchards with permanent pastures, they concluded that "A low population density in the apple roots is often associated with a high population density in grass roots." Some earlier work also indicate that grasses in general support heavier populations of Pratylenchus species than some other tree crops. Henderson and Katznelson (1961) reported a greater population of Pratylenchus sp. in the rhizospheres of grains than in those of legumes. Yuen (1966) also noticed that the genera Pratylenchus, Pratylenchoides and Rotylenchus, so commonly found in the grass land of the Broadbalk wilderness were conspicuously absent in the wood land. Thus it appears as if, besides apple trees, other fruit trees and leguminous plants might be less efficient hosts of Pratylenchus spp. and probably other phytophagous nematodes than are grasses.

From the economic point of view, this finding seems to have an advantage as well as a disadvantage. With reference to this particular orchard, it might be seen as an advantage because it appears as if the possible *Pratylenchus* infestation level on the apple trees has been reduced by the concentration of most of the phytophagous nematodes in the top soil layers around the grass roots and out of the reach of most apple roots. It can also be considered a disadvantage because the possibility of the actual presence of the grass increasing the soil population and hence the infestation level of the apple trees by the very virtue of its being a more efficient host than the apple trees cannot be overlooked. However, earlier work does not seem to suggest that the latter* is true. Hoestra and Oostenbrink (1962) found that *P. penetrans* populations within apple roots were *very high* in clean cultivated orchards on light soils, with barely detectable soil populations. In heavy soils, soil populations were higher than the root ones. But in their conclusion that low *P. penetrans* populations were often recovered from apple roots where grasses harbour significantly high numbers, no soil type was specified. Therefore, it might be concluded that apple roots are generally more heavily infested with *P. penetrans* in clean cultivated orchards than in ones with cover crops, regardless of soil type.

Thus, there seems to be more evidence in support of the suggestion that the grass pasture in this orchard has probably

* latter refers to the infestation level.

been of economic advantage rather than a disadvantage, with regards to Pratylenchus infestation, which is widely known to constitute a most serious problem to the apple economy (Colbran, 1953; Bosher & Newton, 1957; Decker, 1959; Goss, 1961; Palmiter & Braun, 1962). The suggestion made in 5.3. above that grass might also be a better host for Helicotylenchus than the apple trees, and the results of Yuen (1966) and Henderson & Katznelson (1961) cited above also suggest that other fruit trees and leguminous plants could perhaps benefit in a similar way from a grass pasture.

It is not here suggested that grasses would reduce the soil nematode population. On the contrary, it would be expected to increase rather than decrease it. The work of Pitcher et al (1966) where it was found that the apple land which had been under a grass sword for many years had much higher Tylenchid population than the cherry land which was originally clean cultivated, but contained some grass weed in its later years, indicate that this might, in fact, be the case. This seems to warn that the possibility of using grass pasture to reduce nematode infestations of apple trees can only be applied with great caution, and further investigation is necessary before such can be recommended. While a grass pasture might achieve this aim in orchards with some initial nematode populations and with apple roots distributed mainly below the grass root zones, it could, in cases where the original level of phytophagous nematodes are very low, increase rather than reduce infestation by encouraging a rapid population build up, especially if the trees were young and the roots superficially distributed. The fact that the vertical zonation pattern of Scutellonema (5.3.) suggests a closer association with apple rather than grass roots seems also to warn that any conclusion reached here concerning Pratylenchus in relation to grass and apple trees is not necessarily true of all other nematodes.

(b) The conspicuously low populations recovered from N1 apple roots (Table 1, B) as compared with the grass populations (grass : apple = 280 : 11) appear to suggest that this tree might be resistant to Pratylenchus spp. to some extent. So also does the singularly lower figures usually obtained from its soil samples. The fact that the tree is also much bigger than the others tends to lend weight to this postulation. Later results (Table 13, C) seem to confirm that although the tree is not absolutely resistant to this nematode, it does possess some degree of resistance to it. This seems apparent when its populations are compared with those of N2 (N2 : N1 = 120 : 11 in B and 220 : 65 in C).

Differences have been observed in the degree of susceptibility of different apple stocks to nematode attacks (Bosher, 1959; Hoestra & Oostenbrink, 1962). This observation might, therefore, be closely related to differences in the root stocks used. Unfortunately, all attempts to find out the root stock used for this plant failed, since no such records were kept at the time of planting, 24 years ago. This search was abandoned to avoid misleading statements due to contradictory suggestions obtained.

(c) It might also be mentioned that an appreciable increase was observed in the root population levels of Pratylenchus both in grass and apple roots over the three months of sampling. Although a complete picture of the seasonal changes within the roots cannot be drawn here, it is worth noting that the population, corresponding with that in the soil, was quite low in September (grass = 33/25 grms; apple = 12/25 grms.) and was still on the upward trend early in summer (grass = 455/25 grms; apple = 220/25 grms.) when the last sample was taken.

CHAPTER VII

TAXONOMY

There are 3 predominant Tylenchid genera encountered in this work - viz., *Pratylenchus*, *Helicotylenchus* and *Tylenchus*. The last mentioned attracted more attention because of its greater number of species (about 10 or more).

7.1. A revised key to the subgenera of the genus *Tylenchus* Bastian, 1865.

Since 1954, when Andrassy last reviewed this genus, the number of subgenera has increased from four to seven. Andrassy (1959) himself has since erected another sub-genus - *Miculenchus*. Goodey (1962) established a new subgenus *Cephalenchus* to receive *Tylenchus hexalineatus* Geraert, described that year by both Geraert and Goodey. In 1966, Jairajpuri separated one of the nonodelphic species of the genus *Psilenchus* de Man, *P. tumidus* Colbran, 1960, in a separate subgenus *Clavilenchus*.

In 1960, Meyl raised Adrrassy's 1954 subgenera *Tylenchus*, *Aglenchus*, *Filenchus* and *Lelenchus* to generic level, but these were retained as subgenera under the genus *Tylenchus* by Goodey (1963). Andrassy (1963) thought that only the subgenus *Aglenchus* possesses morphological differences distinct enough to merit the status of a separate genus. While agreeing with Andrassy that the *Aglenchus* species possess characters more outstanding than the rest of the subgenera, I do not feel that the presence of the vulval plates which form the key diagnostic feature of these species constitutes enough reason for the creation of a new genus.

No attempt is being made here to review this genus, but in the key provided below, Anglenchus is included as a subgenus of the genus Tylenchus as proposed by Andrassy (ibid) and supported by Goodey in 1963.

A revised key to the subgenera of the genus Tylenchus Bastian, 1865. (Revised after Andrassy, 1954).

1. Cuticle strongly annulated, stylet well developed with prominent knobs, median bulb rounded............... 2
   Cuticle delicately annulated, stylet weakley developed, median bulb oval 3

2. Head slightly offset, tail ventrally curved, females without lateral vulval plates............. Tylenchus.
   Head well offset, annulation remarkably prominent, females with lateral vulval plates .... Anglenchus.

3. Tail terminally clavate................... Clavilenchus.
   Tail not terminally clavate 4

4. Head well offset, not annulated, lateral field with six incisures............................... Cephalenchus.
   Head not well offset, lateral field with less than six incisures 5

5. Head clearly annulated, males without bursa...Miculenchus.
   Head not clearly annulated, males with bursa 6

6. Medium sized species (L = 0.481 - 1.7 mm), annules clearly defined, bursa slightly developed ... Filenchus.
   Small species (L = 0.293 - 0.71 mm), annules very delicate, bursa rudimentary .... Lelenchus.

7.2. Description of four new species of the genus Tylenchus. Bastian, 1865.

Three of the four species described here belong to the subgenus Aglenchus. Two of these with diagnostic longitudinal ridges are most closely related to the two authentically known species of this genus with such ridges - viz., T. (A) costatus de Man, 1921, and T. (A) sachsi Hirschmann, 1952, (Andrassy, 1954). They, however, differ from these in characters discussed in the diagnosis. They are therefore described under the new names of T. (A) neozelandicus n. sp. and T. (A) areolatus n sp. T. (A) whitus n sp., also belongs to this subgenus. It is unique in possessing seven incisures and a rounded, offset, non-annulated head. The fourth species - T. (Filenchus) ruatus n sp. belongs to the subgenus Filenchus and is most closely related to T. (F) Filifornus Butschli, 1873, from which it differs as shown in the diagnosis.

Tylenchus (Aglenchus) neozelandicus n. sp.

(Fig.11, A-E)

Holotype ♀: L = 0.51 mm, a = 38.2; b = 2.7; G1 - 18.6;
V = 82.5; Spear 17 µ.

Paratypes: n = 5; L = (0.523) 0.48 - 0.59 mm; a = (29.1) 19.2 - 38.2; b = (5.26) 4.9 - 5.6; C = (4.1) 4.1 - 4.8; G1 = (23.6) 20.4 - 30.3;
V = (63.9) 62.4 - 65.3; Spear (16.50) 16 - 17 µ.

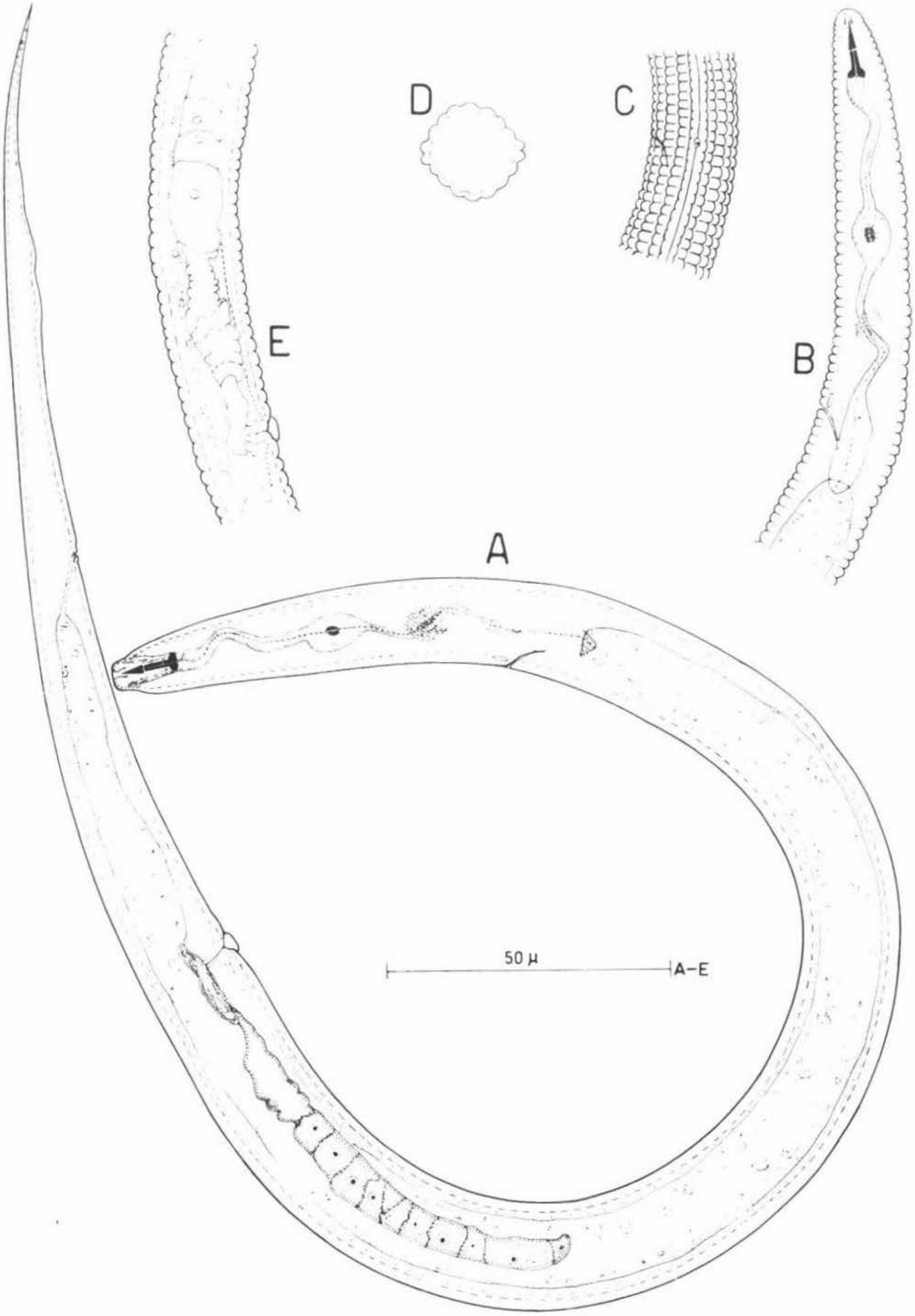

Fig. 11. A-E. Tylenchus (Aglenchus) neozelandicus n. sp.
A. Female; B. Anterior end of female;
C. Lateral field at the excretory pore region;
D. Cross section at mid-body; E. Vulval region.

Small nematodes. Body filiform, tapering towards both ends. Annules distinct and coarse (about 3 μ wide about middle of body and 1.8 μ wide near tail). Greatest body width about 20 μ. Cuticle about 2.5 μ thick with fourteen distinct longitudinal ridges about middle of body, amalgamating at head and tail ends. Head narrower than body, but offset, with four annules, about 4 μ high and 6.5 μ broad. Tail tapering to a fine point, about eleven anal body widths long and annulated to tip.

Lateral field about one-quarter greatest body diameter, with three distinct incisures, outer edges crenate. Incisures begin as two just anterior to median bulb, become three about the level of nerve ring and amalgamate just beyond anus, to disappear about middle of tail. Deirids present in each lateral field at level of, or just slightly posterior to excretory pore.

Spear well developed, about 17 μ long, anterior tapering part about one-half of total length; posterior cylindrical shaft arising from three rounded basal knobs, measuring about 3 μ across.

Procorpus relatively wide. Median bulb roundish - oval, about 10 μ long and 9 μ wide, with distinct crescentric valve plates in the centre. Isthmus slender and longer than procorpus, ending in an oblong, egg-shaped terminal bulb which abuts on the intestine without overlap; short cardium present.

Nerve ring about middle of isthmus. Excretory pore opposite anterior end of terminal bubl; hemizonid just anterior to this, about two body annules long. Intestine with large granules, rather transluscent in some specimens. Rectum about one and a quarter times anal body width.

Vulva posterior, in a depression with prominent, non-annulated lateral membranes. Vagina very strongly thickened, leading into a fairly thick-walled uterus with short posterior branch. Ovary single, prodelphic, outstretched; cells usually in a single row. No spermotheca present. Males not found.

Diagnosis: This species is most closely related to the two authentically known Tylenchus species with longitudinal ridges: *T.(A.) costatus* de Man, 1921, and *T.(A.) sachsi* Hirschmann, 1952. (Andrassy, 1954). It differs from both in having fourteen longitudinal ridges (not 18-24 or 10) and three incisures. The absence of males and the presence of a short post uterine branch further differentiates it from *T.(A.) sachsi* while its longer stylet (17 μ in *neozelandicus* : 11-14 μ in *costatus*) and the single row of cells in the ovary (double in costatus) further distinguish it from *T.(A.) costatus*.

Type Slides: Holotype and paratype slides in nematode collection, Entomology Division, D.S.I.R., Nelson, New Zealand.

Type Locality: Under the canopy of apple tree (*Malus pumila* (Mill) var Sturmer), Batchelar Orchard, Massey University, Palmerston North, New Zealand; altitude 27 metres; Map reference N.Z.M.S. No.149 108325.

Tylenchus (Aglenchus) areolatus n. sp.

(Fig. 12, A-E.)

Holotype ♀ L = 0.53 mm; a = 28; b = 5.4; C = 4.8;
G1 = 27.1; V = 63.2; Spear = 10.4 µ.

Paratypes ♀ L = 0.56 mm; a = 24.7; b = 5.8; c = 4.2;
G1 = 27.61; V = 63.2; Spear = 9.4 µ.

Body filiform, about 0.54 mm long and 16 - 22 µ greatest body width, tapering more posteriorly than anteriorly. Cuticle strongly annulated, each annule about 2.2 µ wide at middle of body and about 2 µ wide near tail; with 22 longitudinal ridges which amalgamate into about eighteen at level of metacorpus and at tail end, commencing from immediate anterior to vulva. No lateral lines seen.

Head narrower than body, with four annules about 6 µ wide and 3 µ high. Spear well developed, about 9 - 10 µ long, with rounded basal knobs measuring about 3 µ across.

Procorpus slightly wider anteriorly. Median bulb rounded, with clearly visible central crescentric valve plates. Isthmus long, slender, ending in a pear-shaped terminal bulb which abuts on the intestine. Nerve ring about one third total isthmus

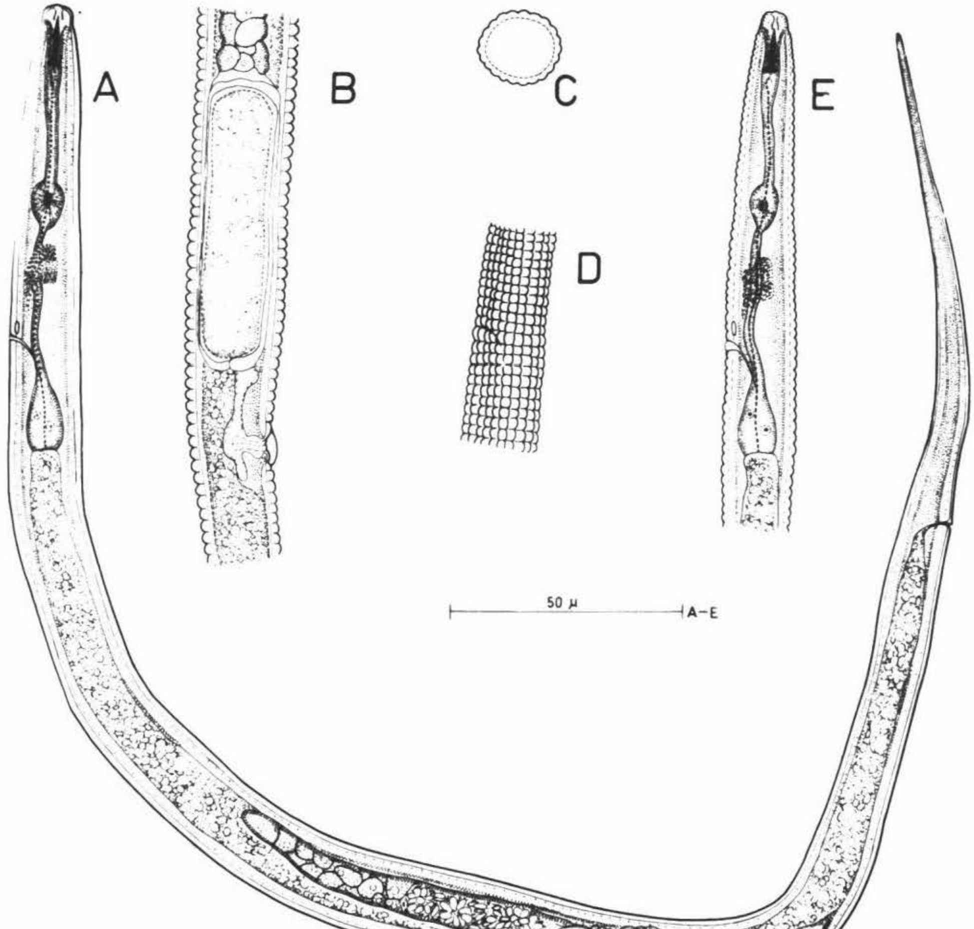

Fig.12, A-E. Tylenchus (Aglenchus) areolatus n. sp.
A. Female; B. Gonad variation; C. Cross section at mid-body; D. Surface view of cuticle; E. Anterior end of female.

length below metacorpus. Excretory pore about two thirds total isthmus length below metacorpus; hemizonid just anterior to excretory pore, measuring about one and half body annules in length. Intestine with large granules; anterior wall thicker than the rest. Rectum bent, about three fifths anal body width. Vulva recessed, with conspicuous non-annulated lateral membranes. Vagina thick-walled, about three sevenths to three quarters vulval body width. Uterus with a posterior branch measuring about three sevenths to one quarter vulval body width. A prodelphic gonad present; ovary with cells in more than one row. A vaguely defined spermatheca seems to be present. A single, large egg measuring about 56 μ x 14 μ present in paratype (Fig.12, B).

Tail about ten times anal body width, ending in a rounded tip.

Diagnosis: T.(A.) areolatus n sp. is most similar to T.(A.) neozelandicus n. sp. It is distinguished by the absence of incisures in the lateral field, the number of longitudinal ridges present (22 instead of 14) and the presence of a vaguely defined spermotheca.

Type Slides: Holotype and paratype slides in the nematode collection, Entomology Division, D.S.I.R. Nelson, New Zealand.

Type locality: Around the roots of apple tree (Malus pumila

(Mill) var. Kidd's Orange Red), Batchelar Orchard, Massey University, Palmerston North, New Zealand; altitude 27 metres, map reference N.Z.M.S. No.149, 108325.

Remarks: The name areolatus is derived from the areolae-like sections into which the cuticle is divided by the striae and the longitudinal ridges.

Tylenchus (Aglenchus) whitus n. sp.

(Fig.13, A-E)

Holotype ♀: L = 0.4 mm; a = 26.2; b = 4.91; C = 5; G1 = 24.2; V = 67.5; Spear = 16.4 μ

Paratypes: n = 6; L = (0.42 mm) 0.4 - 0.45 mm; a = (30.2) 26-34.6; b = (4.8) 4.4 - 5.7; c = (4.7) 4.3-5; G1 = (28) 20-36; V = (65.2) 64.3 - 67.4; Spear = (16.4) 15-17 μ.

Small nematodes with rather slender body, tapering towards both ends; greatest body diameter about 14 μ. Cuticle about 1.1 - 1.8 μ thick, distinctly annulated; annules about 1.7 - 2.1 μ wide. Lateral field about two-fifths greatest body width, with seven clear incisures which amalgamate at both ends, outer edges not crenate. Head rounded and offset by a constriction in neck region, non-annulated, about 2 - 3 μ high and 4.9 - 5.3 μ broad. Spear well developed, about 16 μ long; spear knobs rounded, measuring about 3 μ across. Dorsal oesophageal opening about 1.5 μ behind basal knobs. Tail relatively short, about nine times anal body width, ending in a rounded tip. In

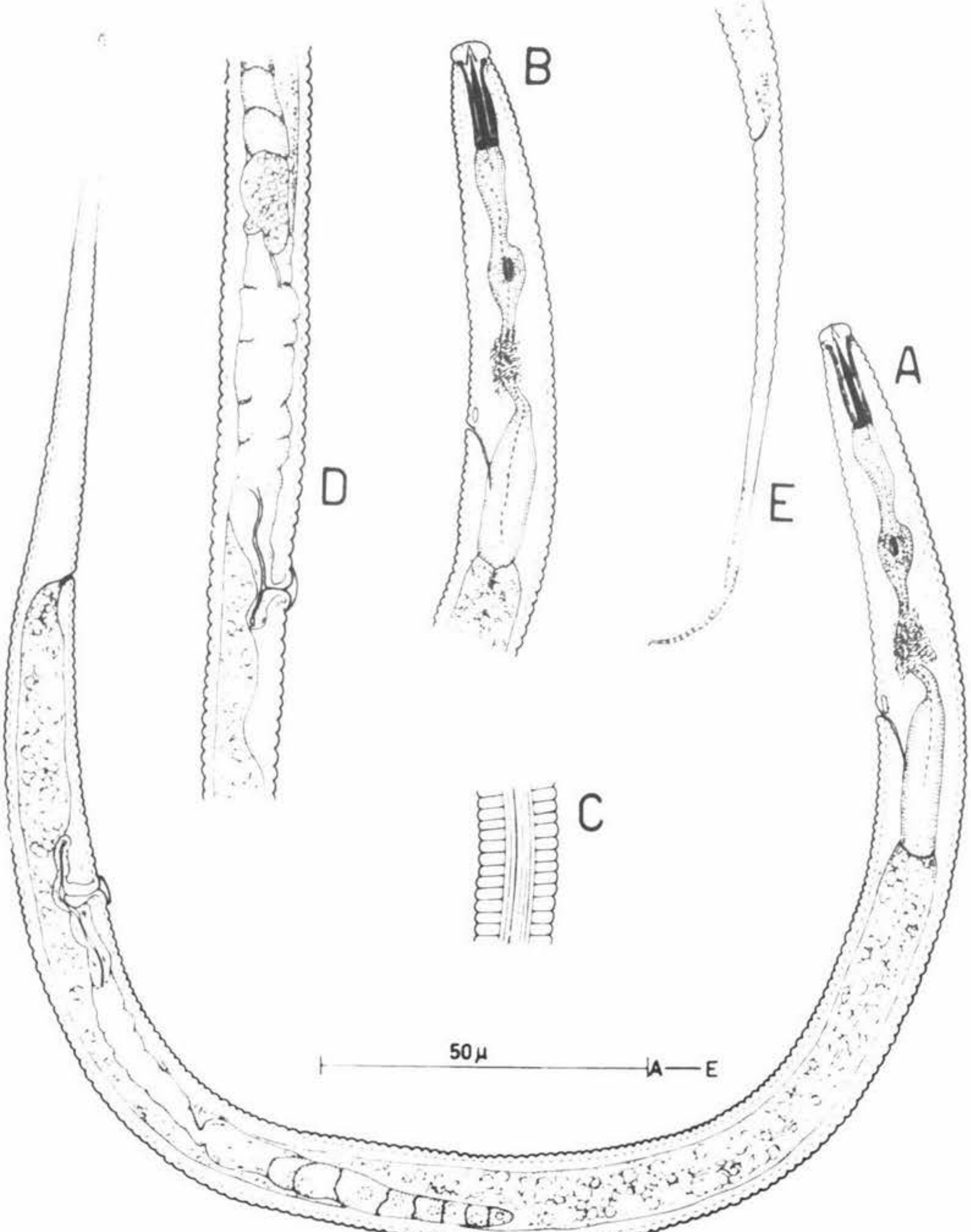

Fig.13, A-E. Tylenchus (Aglenchus) whitus n. sp. A. Female; B. Anterior end of female; C. Lateral field; D. Gonad variation; E. Tail variation.

a few paratypes, tail tip is less bluntly rounded (Fig.13, E). Annules extend to about half tail length.

Procorpus short, about as long as the stylet. Median bulb well defined and roundish, with central valve plates. Isthmus longer than procorpus and slender, swelling into a longish, egg-shaped terminal bulb which abuts on intestine. No cardium present.

Nerve ring half way between the two bulbs. Exretory pore slightly posterior to the junction of isthmus and median bulb. Intestine with large, rounded granules. Rectum short and difficult to see.

Vulva posterior, with non-annulated lateral membranes. Vagina very thick-walled, about one half vulval body width, opening into thick-walled uterus with a posterior branch measuring just over half vulval body width. Gonad prodelphic, outstretched, with a spermatheca. Ovary short, with about eight to thirteen cells arranged in a single row. Males not found.

Diagnosis: T. (A) whitus n. sp. is characterised by its rounded, offset, non-annulated head and the presence of seven incisures in the lateral field. It is most closely related to Tylenchus (Cephalenchus) hexalineatus Geraert, 1962. It differs from this in having lateral vulval membranes, seven incisures (not 6), rounded tail tip and in absence of males.

<u>Type slides</u>: Holotype and paratype slides in nematode collection, Entomology Division, D.S.I.R., Nelson New Zealand.

<u>Type locality</u>: Soil around roots of apple tree *Malus pumila* (Mill) var. Kidd's Orange Red), Batchelar Orchard, Massey University, Palmerston North, New Zealand; altitude 27 metres, map reference N.Z.M.S. No.149, 108325.

<u>Remarks</u>: The specific name is a Maori word (whitus - seven) which refers to the seven incisures in the lateral field - a character, which seems to be unique among the species of *Tylenchus* known so far.

<u>*Tylenchus (Filenchus) ruatus*</u> n. sp.

(Fig.14, A-E)

Holotype ♀: L = 1.2 mm; a = 31; b = 6.2; c = 3.4;

G1 = 26.1; V = 55.8; Spear = 17.4 μ.

Paratypes ♀♀: n = 5; L = (1.24 mm) 1.1 - 1.5 mm;

a = (28.5) 28-29; b = (5.5) 3.8 - 6.1; c = (3.4) 2.9-3.9;

G1 = (30.1) 23.8 - 37.2; V = (56.4) 54.2 - 59;

Spear = (12) 10.4 - 17 μ.

♂: L = 0.86 mm; a = 27.3; b = 5.2; c = 3.1; T = 43.5;

Spear = 10.6 μ.

Allotype ♂ : L = 0.9 mm; a = 23; b = 4.6; c = 3.1; t = 46.

Body long, filiform, tapering gradually towards both ends. Body contour continuous with a narrower, non-annulated head.

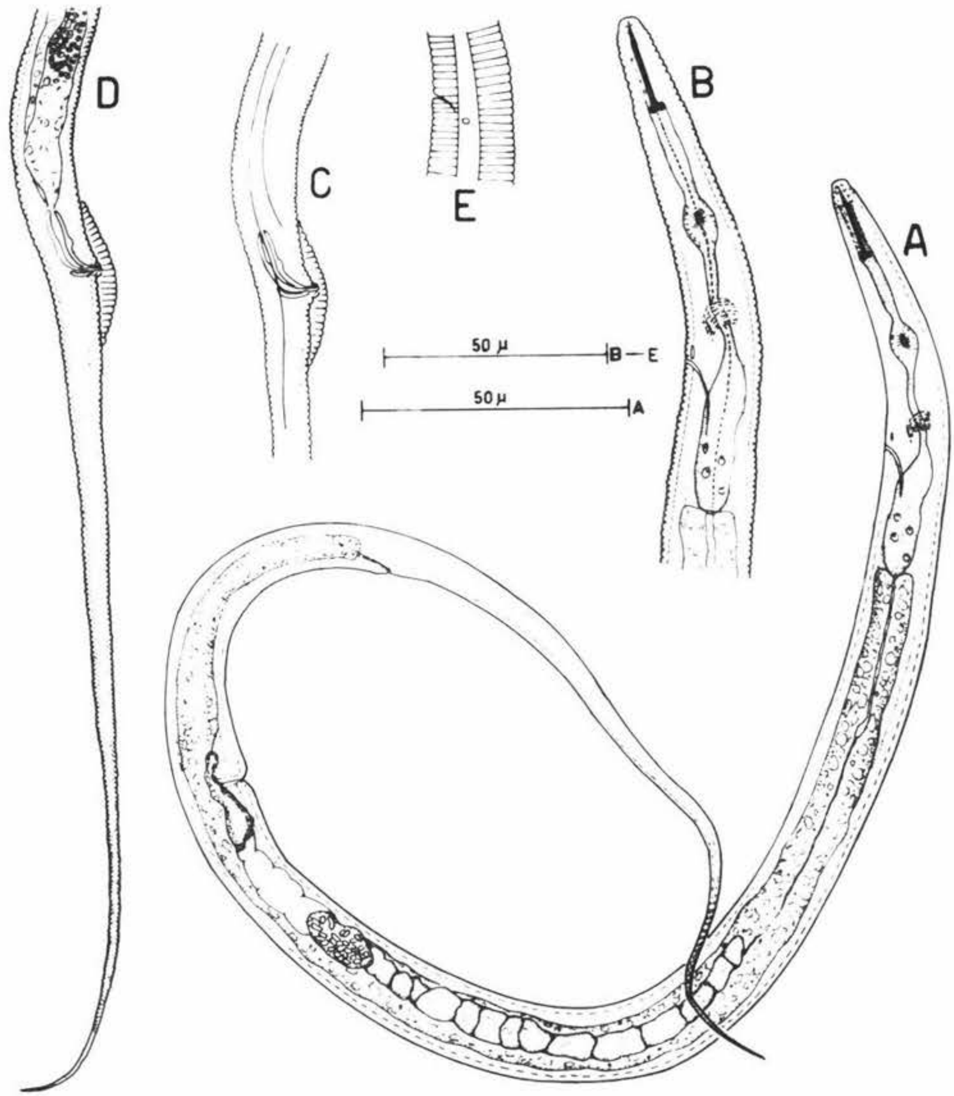

Fig.14, A-E. Tylenchus (Filenchus) ruatus n. sp. A. Female; B. Anterior end of female; C. Male posterior end; D. Male tail; E. Lateral field.

Fairly large nematodes, about 1 - 1.5 mm long; greatest body width about 10 -13 µ. Cuticle about 1 - 1.3 µ thick, with distinct annules, about 0.9 - 1.2 µ wide. Lateral field about one quarter body width, with two clear incisures, originating from just in front of metacorpus and disappearing at about one sixth of tail length from anus. Tail long, filiform, tapering to a fine point, about seventeen times anal body width, annulated to the tip.

Head about 2.6 - 3.9 µ high and 5 - 6 µ wide. Spear delicate, weakly developed, of various lengths between 10 and 17 µ; anterior part only about four fifteenths of total length. Basal knobs rounded, about 2 - 2.8 µ across. Isthmus longer and narrower than procorpus. Nerve ring about two thirds total length of isthmus being metacorpus. Metacorpus ovate, with indistinct valve plates. Terminal bulb egg-shaped and long, with very short cardium. Excretory pore at beginning of terminal bulb. Deirids easily seen, posterior to excretory pore. Hemizonid immediately anterior to excretory pore about two and a half body annules long. Intestine granular, with easily visible lumen. Rectum about the same length as anal body width.

Vulva a depressed slit in the last half of body. Vagina about one third vulval body width, with slightly thickened wall. Uterus with short posterior branch which measures about one quarter vulval body width. Gonad single, anteriorly outstretched, with an oval spermatheca containing sperms. Ovary with about thirteen cells in a single row.

Males similar to females in general body form, but slightly smaller, about 0.88 mm long. Bursa moderately developed, adanal, about three anal body widths long, with clear transverse striae. Spicules tylenchoid, paired, about 14 - 17 µ long. Gubernaculum a small, bent structure about 5 µ long. Testis with cells in a single row.

Diagnosis: This species bears an obvious resemblance to T. (F) filiformis Butschli, 1873, but differs in having two incisures (4 in T. (F) filiformis), excretory pore at level of isthmus - terminal bulb junction (not half way between the two bulbs), deirids posterior to excretory pore (not anterior to it) and in its usually larger size (♀ = 1.1 - 1.5 mm long; as opposed to 0.542 - 0.635 mm).

Type slides: Holotype, allotype and paratype slides in nematode collection, Entomology Division, D.S.I.R., Nelson, New Zealand.

Type locality: Around roots of apple tree Malus pumila (Mill) var. Sturmer), Batchelar Orchard, Massey University, Palmerston North, New Zealand; altitude 27 metres; map reference N.Z.M.S. No.149, 108325.

Remarks: The specific name is coined from a Maori word (rua -

two) to denote the two characteristic incisures in the lateral field.

7.3. Key to the Species of the Subgenus *Aglenchus*.

1. Cuticle with longitudinal ridges........................ 2
   Cuticle without longitudinal ridges 3

2. Lateral field not defined, 22 longitudinal ridges present .... *areolatus* n sp.
   Lateral incisures well defined 4

3. Lateral field with 7 incisures .... *whitus* n. sp.
   Lateral field with less than 7 incisures 6

4. Cuticle with 10 longitudinal ridges; lateral incisures with crenate edges, males present.......*sachsi* Hirshmann, 1952
   Cuticle with more than 10 longitudinal ridges, males unknown 5

5. Lateral field with 4 incisures, the two inner ones closer together, 18-24 longitudinal ridges .....*costatus* de Man, 1921.
   Lateral field with 3 incisures, 14 longitudinal ridges ....*neozelandicus* n. sp.

6. Post uterine branch absent .... *agricola* de Man, 1884.
   Post uterine branch present 7

7. Cuticle delicately annulated, head slightly offset ....*thornei* Andrassy, 1954.
   Cuticle strongly annulated, head well offset 8

8. Head markedly offset, lateral field plain, vulval plates not projecting beyond the body contour.......*machadoi* Andrassy, 1963.
   Head not so markedly offset, lateral field with crenate edges, vulval plates projecting beyond the body contour ....*bryophilus* Steiner, 1914.

CHAPTER VIII

GENERAL DISCUSSIONS AND SUMMARY

General Discussions

The object of this work was dual. One aimed at giving some ecological information of some orchard soil nematodes, the other aspired to investigate whether or not nematodes constitute a potential threat to apple trees in this country, as they do in many others (United States of America, United Kingdom, Netherlands, Germany, South Africa, Italy, etc.).

Information contained in this work does give some idea about both, but probably not all that one would want to know. From the ecological point of view, it has but scratched the subject on the surface. Whatever results have been reported here is true of the particular orchard, with the particular soil described and for the period of the investigation, but might or might not hold for other orchards. This indicates a need for further investigation, preferably on monthly bases as this would leave little or no room for speculations.

One of the difficulties encountered in this work is the inconsistency in results obtained and the variations in samples from different plants. Thus, the horizontal distribution patterns of the total nematodes relative to the trees were not constant for the same tree for both autumn and winter (cf. appendix). It was also found that sometimes the seasonal behaviour of the same genus varies from one tree to another. These observations could be due to a number of factors, important among which might be the species composition of the individual genus, which may differ from one tree to another. Because different species of the same genus might differ in their reaction to the same environmental factor (Wallace, 1963; Yuen, 1966) such discrepancies can easily be related to differences in the species composition. Besides this, some unusual external factors might be responsible. The mat and the chemical sprays (Chapter II) associated with this orchard constitute such unusual factors, which might form a part of a possible interacting complex. So also does the scattered water accumulations encountered during the winter sampling. Because the mat is not uniformly distributed, it might form a sort of insulating barrier between the soil and the atmospheric factors or shield the soil and prevent the penetration of chemical sprays in places where it is thick, while leaving the soil vulnerable to their effects in places where it is thin or absent. This partial protedtion might result into differences in populations in exposed and protected parts. Although some work has been done on the effect of decaying vegetable matter on nematode prpulations (Hutchinson et al, 1960), the influence of a mat does not seem to have been investigated.

The number of plant parasitic nematodes recovered from the orchard soil, and, in particular, the presence of Pratylenchus spp. within apple roots, leaves no room for doubt as to whether or not apple trees are infested with nematodes in this country. The results obtained in Chapter VI and the closer association of Helicotylenchus spp. with grass roots discussed in 5.3. are very interesting from the economic view point. These findings seem to offer explanations as to why the apple trees have presented a satisfactory growth in the face of high nematode populations. With the soil populations recorded for Pratylenchus spp. and Helicotylenchus spp. and other plant parasitising species, the orchard seems well equipped for a replant problem.

As taxonomy forms an important corner stone of any nematological investigations, more taxonomic work, particularly on the Tylenchid nematodes would make an important contribution to nematode research in this country. Although four new species of the genus Tylenchus Bastian, 1865, have been described in this work, more still remain open to investigation. Like Andrassy (per. com.), I believe that more costatus - like species of this genus still remain either to be synonymised with some of the already described ones or to be described under new names.

SUMMARY

Of the 35 nematode genera found in the orchard soil, 12 belong to the Tylenchida order. These 12 genera, represented by about 34 different species, constitute about 50-60% of the total nematode population. Although no obvious qualitative differences exist between the Tylenchid compositions of the unvegetated OM and the grass-covered 1M areas, distinct quantitative differences are found where Pratylenchus spp. and

Helicotylenchus spp., only barely represented at OM, are apparently predominant at 1M. Although the genus *Tylenchus* occurs in both areas, it is constantly much higher at OM. Total nematode figures for both areas show no significant differences.

Nematodes were most abundant at the top 10 cm soil layer and the density decreases with depth. For total nematodes, this vertical zonation pattern is very similar at both OM and 1M. *Helicotylenchus* spp., *Alaimus* spp., the 'Rhabiditids' and the 'Dorylaimids' all show a sharp fall in populations below the 10 cm soil level. The curves for *Tylenchus* spp. and *Pratylenchus* spp. were more gradual. Only *Scutellonema* spp. and *Hemicycliophora* spp. had a maximum density at the 10-20 cm zone. The effect of the seasons on the vertical zonation patterns of the total nematodes is illustrated and discussed.

The mean values of the total nematodes recovered had two peaks, one in autumn and one in summer. The seasonal pattern at OM with an autumn maximum and a spring peak, differs from that at 1M which had an autumn peak and a summer maximum. There was a winter fall in both, but the significant spring fall at 1M corresponds to the high summer reduction at OM.

The seasonal pattern of the genus *Tylenchus* is very close to that for OM totals. *Pratylenchus* and *Helicotylenchus* both had a spring minimum, but while the former remained constant between autumn and winter, the latter had a winter increase. *Alaimus* had a maximum population in autumn with a gradual decrease over the months to a minimum in summer.

The seasonal fluctuation patterns of Tylenchus and Hemicycliophora appear to be correlated with the period of lower soil temperatures below 58° F. There was also a correlation between rainfall and Helicotylenchus population changes. The possible influence of vegetation growth on the seasonal patterns of the phytophagous nematodes is discussed.

The seasonal variations of the total nematodes, Helicotylenchus, Pratylenchus and Tylenchus were not related to either pH or soil moisture differences, neither are these factors responsible for the inter plant sampling variations although at OM, a slight correlation was observed between the soil moisture and such variations in the total nematodes and the Tylenchus populations. There was also a negative correlation between the vertical zonation patterns of the total nematodes and Helicotylenchus and both pH and soil moisture, although Pratylenchus and Tylenchus showed a positive correlation with the soil moisture levels.

The Pratylenchus population densities on apple roots were significantly lowerthan those on grass roots in September, October and November, 1966. The horizontal distribution and vertical zonation of Helicotylenchus also suggest a closer association with grass roots than apple roots. A possible economic advantage of these findings are discussed.

Four new species of the genus Tylenchus Bastian, 1865, are described from New Zealand for the first time. A revised key for the identification of the seven known subgenera is provided as well as a new key to the species of the subgenus Aglenchus.

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Appendix

Detail results of all total nematode counts obtained are fully recorded here. The appendix also include the seasonal group counts, but the group counts for the vertical zonations which have already been recorded and illustrated in the thesis are not included here. Dashes (-) in some of the tables indicate that the nematode group concerned was not counted at the particular location in question.

APPENDIX A

AUTUMN COUNTS: TOTAL NEMATODES

Plant No.	Sample No.	Aliquot No.	5% Counts	5% Averages	Totals/ 250 grms Soil	Total/ Horizontal Zone
N1	OM 0-10	1	74)	67.5	1,350)	
		2	61)			
	10-20	1	40)	41.5	830)	2,850
		2	43)			
	20-30	1	32)	33.5	670)	
		2	35)			
	1M 0-10	1	49)	46	920)	
		2	43)			
	10-20	1	36)	40.5	810)	1,810
		2	45)			
	20-30	1	3)	4	80)	
		2	5)			
	3M 0-10	1	49)	48	960)	
		2	47)			
	10-20	1	33)	34.5	690)	1,830
		2	36)			
	20-30	1	7)	9	180)	
		2	11)			

APPENDIX A (ctd.)

Plant No.	Sample No.	Aliquot No.	5% Counts	5% Averages	Totals/ 250 grms Soil	Total/ Horizontal Zone
N2	OM 0-10	1	108	126	2,520	3,260
		2	144			
	10-20	1	26	24	480	
		2	22			
	20-30	1	10	13	260	
		2	16			
	IM 0-10	1	163	162.5	3,250	4,240
		2	162			
	10-20	1	41	37.5	750	
		2	34			
	20-30	1	12	12	240	
		2	12			
	3M 0-10	1	156	176.5	3,530	4,210
		2	197			
	10-20	1	20	19.5	390	
		2	19			
	20-30	1	18	14.5	290	
		2	11			

APPENDIX A (ctd.)

Plant No.	Sample No.	Ali-quot No.	5% Counts	5% Averages	Totals/ 250 grms Soil	Total/ Horizontal Zone
N3	OM 0-10	1	170)	161.5	3,230)	4,350
		2	153)			
	10-20	1	40)	39	780)	
		2	38)			
	20-30	1	18)	17	340)	
		2	16)			
	IM 0-10	1	112)	118.5	2,370)	3.040
		2	125)			
	10-20	1	34)	31	620)	
		2	28)			
	20-30	1	4)	2.5	50)	
		2	1)			
	3M 0-10	1	94)	75	1,500)	2,310
		2	56)			
	10-20	1	30)	30.5	610)	
		2	31)			
	20-30	1	8)	10	200)	
		2	12)			

APPENDIX A (ctd.)

Plant No.	Sample No.	Ali-quot No.	5% Counts	5% Averages	Totals/ 250 grms Soil	Total/ Horizontal Zone
N4	OM 0-10	1	145)	147.5	2,950)	
		2	150)			
	10-20	1	32)	31	620)	4,290
		2	30)			
	20-30	1	39)	36	720)	
		2	33)			
	1M 0-10	1	190)	196.5	3,930)	
		2	203)			
	10-20	1	41)	45.5	910)	5,500
		2	50)			
	20-30	1	37)	33	660)	
		2	29)			
	3M 0-10	1	113)	119.5	2,390)	
		2	126)			
	0-10	1	30)	28	560)	3,560
		2	26)			
	20-30	1	27)	30.5	610)	
		2	34)			

APPENDIX B

WINTER COUNTS: TOTAL NEMATODES

Plant No.	Sample No.	Aliquot No.	10% Counts	10% Averages	Totals/ 250 grms Soil	Total/ Horizontal Zone
N1	OM 0-10	1	81	86.5	865	1,080
		2	92			
	10-20	1	13	14.5	145	
		2	16			
	20-30	1	7	7	70	
		2	7			
	1M 0-10	1	160	170.5	1,705	1,765
		2	181			
	10-20	1	3	3	30	
		2	3			
	20-30	1	4	3	30	
		2	2			
	3M 0-10	1	181	182.5	1,825	2,360
		2	184			
	10-20	1	45	49.5	495	
		2	59			
	20-30	1	3	4	40	
		2	5			

APPENDIX B (ctd.)

Plant No.	Sample No.	Aliquot No.	10% Counts	10% Averages	Totals/ 250 grms Soil	Total/ Horizontal Zone
N2	OM 0-10	1	41)	40.5	405)	
		2	40)			
	10-20	1	49)	51.5	515)	1,190
		2	54)			
	20-30	1	27)	27	270)	
		2	27)			
	1M 0-10	1	241)	259.5	2,595)	
		2	278)			
	10-20	1	28)	30	300)	3,120
		2	32)			
	20-30	1	27)	23.5	235)	
		2	20)			
	3M 0-10	1	98)	105	1,050)	
		2	112)			
	10-20	1	47)	46	460)	1,735
		2	45)			
	20-30	1	26)	22.5	225)	
		2	19)			

APPENDIX B (ctd.)

Plant No.	Sample No.	Aliquot No.	10% Counts	10% Averages	Totals/ 250 grms. Soil	Totals/ Horizontal Zone
N3	OM 0-10	1	199)	191	1,910)	2,295
		2	183)			
	10-20	1	21)	20	200)	
		2	19)			
	20-30	1	10)	18.5	185)	
		2	18)			
	1M 0-10	1	393)	391.5	3,915)	4,905
		2	400)			
	10-20	1	89)	87.5	875)	
		2	86)			
	20-30	1	13)	11.5	115)	
		2	10)			
	3M 0-10	1	149)	135.5	1,355)	2,165
		2	122)			
	10-20	1	48)	50	500)	
		2	52)			
	20-30	1	31)	31	310)	
		2	31)			

APPENDIX B (ctd.)

Plant No.	Sample No.	Aliquot No.	10% Counts	10% Averages	Totals/ 250 grms Soil	Totals/ Horizontal Zone
N4	OM 0-10	1	287)	270	2,700)	
		2	253)			
	10-20	1	39)	38	380)	3,205
		2	37)			
	20-30	1	10)	12.5	125)	
		2	15)			
	1M 0-10	1	174)	176.5	1,765)	
		2	179)			
	10-20	1	92)	96	960)	2,975
		2	100)			
	20-30	1	23)	25	250)	
		2	27)			
	3M 0-10	1	37)	40.5	405)	
		2	44)			
	10-20	1	84)	89.5	895)	1,310
		2	95)			
	20-30	1	2)	2	20)	
		2	2)			

APPENDIX C

SPRING COUNTS: TOTAL NEMATODES

Plant No.	Sample No.	Ali-quot No.	10% Counts	10% Averages	Totals/ 250 grms Soil	Totals/ Horizontal Zone
N3	OM 0-10	1	357)			
			)	345	3,450)	
		2	333)		)	
					)	
	10-20	1	167)		)	
			)	162	1,620)	5,120
		2	156)		)	
					)	
	20-30	1	5)		)	
			)	5	50)	
		2	5)			
	1M 0-10	1	90)			
			)	72	720)	
		2	54)		)	
					)	
	10-20	1	20)		)	
			)	21	210)	1,090
		2	22)		)	
					)	
	20-30	1	16)		)	
			)	16	160)	
		2	16)			
	3M 0-10	1	185)			
			)	179	1,790)	
		2	172)		)	
					)	
	10-20	1	86)		)	
			)	88	880)	2,880
		2	90)		)	
					)	
	20-30	1	21)		)	
			)	21	210)	
		2	21)			

APPENDIX C (ctd.)

Plant No.	Sample No.	Aliquot No.	10% Counts	10% Averages	Totals/ 250 grms Soil	Totals/ Horizontal Zone
N4	OM 0-10	1	145)	144	1,440)	2,400
		2	142)			
	10-20	1	73)	67	670)	
		2	61)			
	20-30	1	31)	29	290)	
		2	26)			
	1M 0-10	1	16)	20	200)	1,140
		2	24)			
	10-20	1	54)	60	600)	
		2	66)			
	20-30	1	31)	34	340)	
		2	38)			
	3M 0-10	1	90)	91	910)	1,205
		2	92)			
	10-20	1	24)	25	250)	
		2	26)			
	20-30	1	5)	4.5	45)	
		2	4)			

APPENDIX D

SUMMER COUNTS: TOTAL NEMATODES

Plant No.	Sample No.	Aliquot No.	10% Counts	10% Averages	Totals/ 250 gms. Soil.	Totals/ horizontal zone.
N3	OM 0-10	1	205)	199	1,990)	2,785
		2	192)			
	10-20	1	55)	64	640)	
		2	72)			
	20-30	1	16)	15.5	155)	
		2	15)			
	1M 0-10	1	264)	265	1,650)	2,635
		2	266)			
	10-20	1	50)	58	580)	
		2	65)			
	20-30	1	42)	40.5	405)	
		2	39)			
	3M 0-10	1	91)	89	890)	2,115
		2	87)			
	10-20	1	53)	67	670)	
		2	80)			
	20-30	1	55)	55.5	555)	
		2	56)			

APPENDIX D (Contd)

Plant No.	Sample No.	Aliquot No.	10% Counts	10% Averages.	Totals/ 250 gms. Soil	Totals/ horizontal zone.
N4	OM 0-10	1	95)	98	980)	
		2	101)			
	10-20	1	40)	41	410)	1,630
		2	42)			
	20-30	1	16)	24	240)	
		2	31)			
	1M 0-10	1	443)	461	4,610)	
		2	480)			
	10-20	1	49)	55	550)	5,600
		2	50)			
	20-30	1	43)	44	440)	
		2	45)			
	3M 0-10	1	168)	176	1,760)	
		2	183)			
	10-20	1	71)	72	720)	3,120
		2	73)			
	20-30	1	60)	64	640)	
		2	68)			

APPENDIX E

GROUP COUNTS PER 25 GRAMS SOIL

(Each figure represents the mean of two countings).

AUTUMN:

Sample No.	Pratylenchus.	Helicotylenchus.	Tylenchus.	Other Tylenchids.	Rhabditids.	Dorylaimids.	Alaimus.	Other Groups.
N1 OM 0-10	2	0	34	4	50	34	8	2
1M 0-10	9	21	6	8	19	11	4	11
3M 0-10	8	37	7	18	16	5	0	0
N2 OM 0-10	0	0	72	4	56	28	16	2
1M 0-10	96	64	22	2	37	19	6	1
3M 0-10	46	96	26	17	49	16	3	0
N3 OM 0-10	2	2	144	12	96	28	20	2
1M 0-10	17	37	62	2	42	25	9	0
3M 0-10	2	27	43	22	11	5	0	1
N4 OM 0-10	0	2	58	10	116	80	58	4
1M 0-10	65	105	17	2	55	82	61	1
3M 0-10	11	88	31	13	56	8	1	1

APPENDIX E (Contd)

WINTER:

Sample No.	Pratylenchus.	Helicotylenchus.	Tylenchus	Other Tylenchids.	Rhabditids.	Dorylaimids.	Alaimus.
N1 1M 0-10	30	32	28	14	86	16	7
N1 3M 0-10	24	103	6	14	16	2	1
N2 1M 0-10	55	20	36	11	85	26	14
N2 3M 0-10	14	28	25	8	38	8	1
N3 OM 0-10	1	2	132	2	48	17	5
N3 1M 0-10	33	112	107	2	48	36	11
N4 OM 0-10	1	2	89	1	114	56	38
N4 1M 0-10	49	56	15	2	48	6	2

APPENDIX E (Contd)

SPRING:

Sample No.	Pratylenchus	Helicotylenchus	Tylenchus	Alaimus
N3 OM 0-10	-	-	183	4
N3 1M 0-10	4	6	-	-
N4 OM 0-10	-	-	58	6
N4 1M 0-10	4	5	-	-

SUMMER:

Sample No.	Pratylenchus	Helicotylenchus	Tylenchus	Alaimus
N3 OM 0-10	-	-	96	6
N3 1M 0-10	14	74	-	-
N4 OM 0-10	-	-	34	1
N4 1M 0-10	41	111	-	-