ROOT-FEEDING NEMATODES OF CEREALS,

ESPECIALLY *HELICOTYLENCHUS* SPECIES

by

Robin Keith Jones

A thesis submitted for the degree of Doctor of Philosophy
of London University.

R.K. Jones,
Imperial College Field Station,
Ashurst Lodge,
Silwood Park,
Sunninghill,
Berkshire.

January, 1976.

ABSTRACT

The role of ectoparasitic nematodes in plant disease has been largely ignored, but an insidious loss due to the accumulating effect of the feeding of these nematodes is widely suspected to occur. Thus field experiments were made to test whether the ectoparasitic nematodes in arable soils affected yields of cereals. The results indicated that no accumulative effect occurred, and that Helicotylenchus nematodes, in typical population sizes, do not contribute to yield losses. Tylenchorhynchus dubius, when present in large numbers, was suspected as causing a decline in yield, but no definite link was demonstrated.

Helicotylenchus species were also shown to have no effect on the rate of development of cereals, and no indication of a threshold population was found. In addition, the feeding of these nematodes did not affect the loss of cortical cells of wheat roots.

Differences were found in the host preference and feeding site preference of three species, H. digonicus, H. pseudorobustus, and H. varicaudatus, but the cellular changes that occurred due to the feeding of these nematodes were similar.

The feeding of H. dihystera and H. varicaudatus on wheat roots differed from the suspected ectoparasitic habits, and the typical habit was of a sedentary endoparasite. A feed from a single cell lasted for several days, with no apparent lesion developing. The adult female nematodes, during feeds, laid eggs and often large egg clusters developed.

A histological and ultrastructural investigation of the feeding site revealed that ordered changes occurred in the cell from which the nematode fed. A lesion developed in which the cells contained increased numbers of organelles, and the cell cytoplasm was enlarged at the expense of the central vacuole. The nematode passed its stylet into the feeding cell, and a zone, presumed to be derived from the dorsal gland exudate, enveloped

the stylet in the cytoplasm. The zone appeared to be structureless, but associated with it, was a tube, also presumed to originate from the dorsal gland. The role of these structures in the feeding cell and the mechanism that controls the changes in the feeding lesion are discussed.

ACKNOWLEDGEMENTS

I should like to express my gratitude to Professor T.R.E. Southwood for allowing me the use of facilities at Imperial College Field Station, Silwood Park, during the course of this work.

I thank particularly Dr. A.A.F. Evans, my supervisor, for his interest, time and guidance in the planning and the carrying out of this thesis. I am grateful also to Dr. J. Bridge for the help he gave me in planning particular aspects of this work.

I should also like to thank

Dr. R.E. Sinden for allowing me the use of the Phillips 300 microscope, and guiding me through the many problems encountered,

Mr. J. Muirhead of Eastlands Farm, Essex, for extending to me the use of some of his land,

Ms. S. Page for aiding me during the harvesting and sowing of the experiment in Essex,

Mr. J. Smith for much and varied technical assistance.

I express my gratitude also to Dr. N.A. Croll, Miss J. McCulloch, Dr. G. Murdie and many others who either wittingly or unwittingly have helped me during my studies.

Finally, I thank my parents for their interest and help during my stay at Silwood Park.

This study was undertaken whilst in receipt of a grant from the Ministry of Agriculture, Fisheries and Food, to whom I am grateful for their support.

TABLE OF CONTENTS

Table of Contents (contd.)

Table of Contents (contd.)

SECTION 1.

INTRODUCTION

Cereal production in the United Kingdom makes a major contribution to the total agricultural output. In 1967, the area involved was 9.4 million acres out of a total arable and grassland area of 48.3 million acres. The distribution of cereal production (Fig. 1) indicates that the bulk is grown in the Eastern Regions of the United Kingdom, where the warmer, drier climate is more suited for growing barley and wheat.

During the last 20 years, cereal production has undergone major changes. These include,

1) a large increase in the barley acreage, associated with a decline in the oat acreage,
2) a steady and continuing increase in the total yields of barley and wheat. This has been achieved by the introduction of higher-yielding varieties, and a spread of cereal growing to areas other than those traditionally used.

Thus, cereals are now grown for direct cash return, and 60% of income from arable farming is derived from cereals. The increasing value of cereal products has led to a greater awareness of cereal diseases and the losses they induce.

These diseases can be catagorised as:-

1) seed-borne e.g. septoria, bunt, loose smut and leaf-strip
2) soil-borne e.g. eyespot and take-all, and
3) foliar diseases e.g. mildew, yellow rust, septoria and rhynchosporium.

Nematode diseases of cereals also fall into these same catagories, though the losses they induce do not rank with the losses induced by those diseases listed above. The range and importance of nematode induced cereal diseases in the United Kingdom has recently been reviewed by Jones (1972). The nematodes that have been implicated in causing

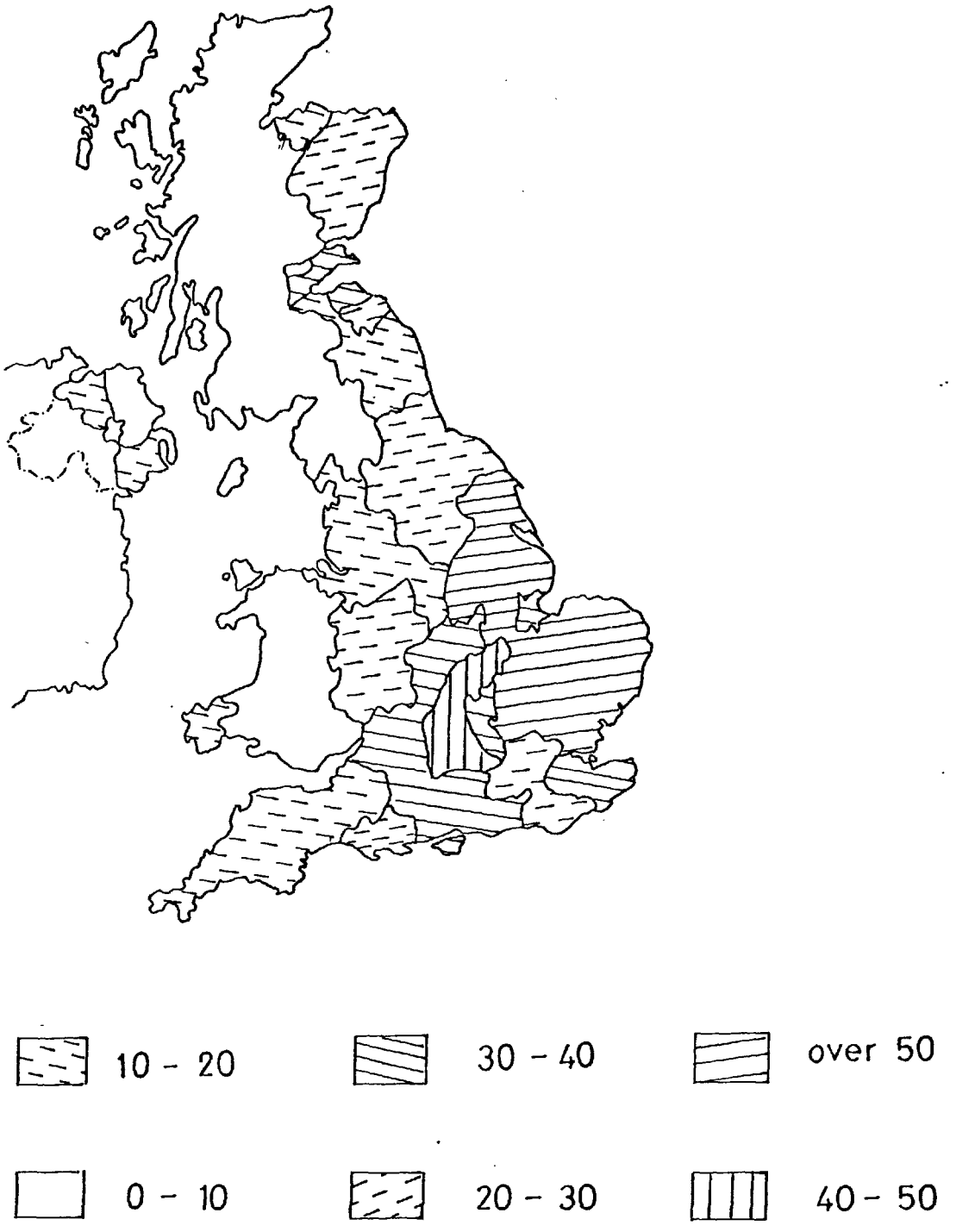


Fig. 1 : 1 Cereals, per 100 ha crops, grass and rough grazing, 1970

from Edward and Rogers 1974

damage in localised areas are:-

Heterodera avenae
Meloidogyne naasi
Pratylenchus spp.
Ditylenchus dipsaci

In addition to these nematodes, many other genera of plant parasitic nematodes have been found in arable soils. These include the so-called ectoparasitic nematode genera:-

Tylenchus spp.
Paratylenchus spp.
Tylenchorhynchus and Merlinius spp.
Rotylenchus and Helicotylenchus spp.
Trichodorus spp.
Longidorus spp.

Ectoparasitic nematodes occur in all soils, and the numbers of nematodes in this group generally comprise the bulk of all nematodes in the soil, (Banage, 1963). The term ectoparasitic nematode is a loose one, and is used to denote a wide range of feeding habits that include:-

1) nematodes feeding solely from the surface cells of host roots, (Bridge and Hague, 1974)
2) nematodes that feed on deep lying cells using a long onchiostyle, (Cotton, 1973), or stomatostyle, (McElroy and Van Gundy, 1968), inserted into deep lying cells, and
3) nematodes that feed from cells just beneath the root epidermis, in a more typically semi-endoparasitic manner.

Many of these ectoparasitic nematodes are pathogenic to their hosts directly, and disease complexes with other soil-borne pathogens have been demonstrated for the surface feeding and burrowing genera. Yet there is no firm evidence that the feeding of these nematodes, either in isolation or by the accumulated effect of their feeding interact to induce a decrease of yield of cereals in the United Kingdom. Jones (1972),

mentioned areas where further study of cereal nematodes are needed, which included that there should be an investigation of "The incidence and pathogenicity of root-ectoparasitic nematodes to cereal root system."

Thus the objectives of this study were to investigate:-

1) The relationships that exist between numbers of the commonly occurring ectoparasitic nematodes and yield of cereals,
2) Whether the control of these nematodes leads to an increased yield,
3) The distribution with depth, of the soil populations, and
4) The effects of soil disturbance on the survival of the nematodes.

The genus Helicotylenchus is one of the ectoparasitically grouped genera, and it occurs commonly in soils in South-East England, (Bridge, 1971). This genus was selected for further study of its ability to cause disease to cereals.

Pot experiments were made to test:-

1) The host preference of four species to a selection of cereal and grass hosts, and
2) The histological changes that result from the feeding of these nematodes on the cereal roots.

Experiments were also made to determine the pathogenicity of this genus on wheat, and the role feeding by the nematodes has in inducing root cortical necrosis of wheat.

The feeding behaviour of three species on wheat was studied and in conjunction with these observations, a detailed histological and ultrastructural investigation of the feeding site of H. dihystera on wheat was made so that the relationship that exists between these nematodes and cereal hosts could be better understood.

SECTION 2.

FIELD EXPERIMENTS

2:1 Literature review and Introduction.

An exhaustive list of nematodes associated with cereals in the United Kingdom has not been assembled, though certain genera have repeatedly been recorded from arable soils. Surveys in the U.S.A. by Farrar (1957), Norton (1959), Taylor and Schleder (1959), and in the U.S.S.R. by Lengvenyte (1961), Skuodyte (1962), Rasalov (1970), showed that the main genera of plant parasitic nematodes in cereals were:-

Tylenchus spp.	Helicotylenchus spp.
Ditylenchus spp.	Paratylenchus spp.
Aphelenchus	Heterodera spp.
Aphelenchoides spp.	Hemicycliophora spp.
Tylenchorhynchus spp.	Rotylenchus spp.
Merlinius spp.	Trichodorus spp.
Pratylenchus spp.	Longidorus spp.
Pratylenchoides spp.	Xiphenema spp.

All these genera were found in grassland soils in South-East England, (Bridge, 1971), where from 16 sites sampled 19 genera of plant-parasitic nematodes were identified. In this survey the most commonly found genera were:-

(a) Tylenchus and Tylenchorhynchus spp. present in all the sites, and
(b) Helicotylenchus, Paratylenchus and Pratylenchus spp. present in 15 of the fields sampled.

Studies of the seasonal population fluctuations of ectoparasitic nematodes in cereal fields (Winslow, 1964; Corbett et al., 1968), revealed that these same five genera were abundant. The maximum populations of nematodes occurred in later summer and early autumn, and was followed by a decline in numbers that commenced from late autumn through to early spring, and continued until a minimum population was

reached in June/July. Then a build up of numbers occurred to another autumn peak.

Collen (1972), followed the changes in nematode numbers when cereal crops followed grass and found that the changes varied with the age of the grassland and the individual genera and species of nematodes. When permanent pasture was ploughed and cereals sown, a decline in the total number of plant parasitic nematodes occurred, whereas the number of nematodes generally increased after ploughing of ley pastures. Paratylenchus spp., usually P. microdorus, always declined under cereals, whereas numbers of Pratylenchus spp. always increased. The changes in numbers of Helicotylenchus spp. were variable, with populations increasing occasionally.

Ploughing, or any soil disturbance, has been reported to cause changes in nematode numbers (Saynor, 1972), and numbers of Helicotylenchus spp. declined due to such disturbance. Yet, in comparing the effects of conventional cultivation techniques and direct drilling, Corbett and Webb (1970), found that migrating plant parasitic nematodes, including Helicotylenchus vulgaris, were greater in fields that had been ploughed.

The effects observed in these fields were related to the size of the nematodes, in that small nematodes, Paratylenchus spp. were less abundant in fields that had been ploughed, whereas large nematodes, Longidorus spp. and Trichodorus spp., favoured such fields. These changes were linked to the effects of the treatments on pore size, and availability of suitable pores in the soils for the various sizes of nematodes, (Corbett and Webb, 1970).

The use of nematicides to control ectoparasitic nematodes of cereals has, on occasions, resulted in an increase of yield, (Langdon et al., 1961); (Perez et al., 1970), whilst there are various reports where no yield

response has occurred (Norton, 1959; Saynor, 1972a). Sechler et al. (1967), achieved good control of ectoparasitic nematodes using methyl bromide, and a 40% increase in yields of small grains resulted. Yet factors besides nematode control, e.g. changes in soil fertility or the control of fungal or bacterial pathogens, were thought to have caused the bulk of the yield increase.

Three of the five genera that occur most commonly in pasture soils in South-East England, have been implicated in causing reduced yields of cereals. These are Pratylenchus spp., Tylenchorhynchus spp. and Helicotylenchus spp.

Pratylenchus spp. have most frequently been demonstrated to reduce yield, P. thornei (Perez et al., 1960), P. minyus (Benedict and Mountain, 1956). In the latter case, P. minyus caused greatest losses in association with Rhizoctonia Solani, and this disease complex caused a root rot of wheat which affected over 32% of the wheat acreage of South-West Ontario.

In England and Wales, Pratylenchus spp. are the most abundant plant parasitic nematode encountered in cereal fields (Corbett, 1970). Five species were found:-

P. minyus, P. crenatus, P. thornei, P. fallax and P. pinguicaudatus. The populations of these nematodes in cereal fields are variable, but large populations were recorded, e.g. 16,700 P. minyus per litre of soil. P. minyus was the most widespread of these species, but in field trials, (Gair et al.,, 1969; Cotton, 1970), and in pot experiments (Saynor, 1972a), this nematode has repeatedly been shown not to affect yields of cereals. P. fallax, recorded as occurring only in barley fields (Corbett, 1970), has been associated with causing patchy growth of barley in sandy soils in the Midlands, and thus certain species of this genus in localised areas do cause yield losses.

Tylenchorhynchus spp. together with the related genus Merlinius, have been shown to reduce the growth of cereals in field and glasshouse experiments, T. dubius on wheat (Sharma, 1971; Skarbilovich, 1972) and M. brevidens on wheat and barley (Langdon et al., 1961).

Bridge and Hague (1974) showed that, in agar plates, nematodes of these genera fed on surface cells around the growing region of roots of Lolium perenne. Epidermal cells and root hairs were also fed from, but only one species, T. maxinus retarded root growth, despite the sensitive nature of the feeding site of many of these nematodes.

Bridge (1971) recorded ten species of these nematodes in pasture soils of South-East England, six which occurred commonly, yet information on the species present in cereal fields, their abundance and their effect on cereal growth, is very limited. Saynor (1972a) found that T. dubius occurred commonly in cereal fields in East Anglia, but the populations found, of variable sizes up to 2,500 per litre of soil, were not implicated in reducing yields of spring barley.

Helicotylenchus spp. have only once been reported to reduce yields of cereals (Griffin, 1964). Yet large populations have been reported to reduce the growth of various host plants in field experiments, e.g. bananas (Minz et al., 1960) and pot experiments e.g. cacao (Tarjan and Jiménez 1973).

Helicotylenchus spp. induce small necrotic lesions in surface cortical cells of host roots (Davis and Jenkins, 1960; Blake, 1966) and are generally thought to feed ectoparasitically, (Yeates, 1971). Yet many species have been found as endoparasites in the roots of various hosts, e.g. H. varicaudatus in cereal and grass roots (Collen, 1972) and H. digonicus in Poa pratensis roots (Perry et al., 1959).

Bridge (1971) recorded six species in pasture soils, of which four were common, H. digonicus, H. pseudorobustus, H. varicaudatus and

H. vulgaris. Winslow (1964) and Corbett et al. (1968) recorded the population sizes and seasonal fluctuations of the genus in arable soils, and Collen (1972) noted that large populations of H. varicaudatus invaded the cortex of cereal roots which lead to the collapse of these cells. But no specific information is available on the effect these nematodes have on cereal growth despite the occurrence of large field populations, e.g. 5,000 per litre of soil (Winslow, 1964).

Other genera of migratory nematodes have been shown to cause damage to cereals. These include Longidorus spp. (Semkinä, 1971) and Trichodorus spp., (Russell and Perry, 1966). These genera are usually found in small populations (Winslow, 1964; Bridge, 1971), but on sandy soils large populations especially of Trichodorus spp. can build up (Whitehead and Hooper, 1970). The nematodes feed on root tips, but unlike Tylenchorhynchus spp., the cells fed from, lie deep in this sensitive zone. Trichodorus spp. aggregated around the root tips of barley and wheat and induced the characteristic stubby-root symptoms. Likewise, L. elongatus caused the galling of barley and wheat root tips, (Whitehead and Hooper, 1970). In sugar beet plots, these nematodes were controlled by D-D (dichloropropane, dichloropropene) but in subsequent years, when barley was sown in these plots, yields were 15% greater in the treated plots; this difference was attributed to the effects of nematode control.

Available information on ectoparasitic cereal nematodes shows

(a) the changes in populations of the nematodes that occur within a growing season, and

(b) the influence soil disturbance has on their survival.

In addition, some indication of their pathogenicity has been gained from experiments but these experiments have not been designed

primarily to indicate the response of cereals to these nematodes.

Thus field experiments were planned with this aim. Three experiments were made.

A) Population changes in permanent and ley pasture fields were recorded following ploughing. This trial was performed at Hayes Hill Farm, Twyford, Berkshire. The invasion of the roots of the new host crop, spring wheat, by semi-endoparasitic and endoparasitic nematodes was recorded. Two adjacent fields were chosen for study, and the soil type of each field was:-

1) ley pasture, silty loam and
2) permanent pasture, silty clay loam.

The initial sample was taken one day prior to ploughing and subsequently at three-weekly intervals.

B) This trial used experimental plots laid out in November, 1969 by N. Collen at Silwood Park, Ascot, Berkshire. N. Collen used these plots to follow the population changes of plant parasitic nematodes under various grass, cereal and fallow treatments. The plots were originally laid out with two replicates and the plot treatments from 1969 - 1971 were as laid out in Table 2:1.

Table 2:1

Plot Treatments 1969 - 1971 at Silwood Park

Plot	Treatment	Plot	Treatment
1	Grass	7	Spring Oats
2	Winter Wheat	8	Fallow
3	Winter Wheat	9	Spring Barley
4	Grass	10	Spring Wheat
5	Spring Oats	11	Spring Wheat
6	Spring Barley	12	Fallow

The plots were kept free from weeds from harvest 1972 through to

spring 1973, when I first sampled them. The nematode genera Tylenchus, Paratylenchus, Tylenchorhynchus, Pratylenchus and Helicotylenchus were all present. The latter four genera were present in populations that ranged from zero up to several thousand per litre of soil. Thus a trial was planned in which the yields of winter wheat from each plot were correlated with the various nematode populations.

C) The third trial involved an attempt to control a patchy early yellowing of cereals in a field on Eastland Farm, Bradwell-on-Sea, Essex. A.D.A.S., Cambridge, whilst investigating the cause of this disease, found large populations of Longidorus spp. (Table 2:2) in the region of the field where the early yellowing occurred. Thus in November, 1973, in conjunction with A.D.A.S., experimental plots were staked out in this field. The nematicide used was Vydate, which was broadcast onto the existing barley crop.

This initial trial proved inconclusive and thus a repeat trial was made in 1974/1975 when the same host crop and nematicide were used.

Table 2:2

Nematodes in soils from Bradwell-on-Sea, Nov. 1973

Numbers of nematodes per litre of soil, extracted by the Whitehead tray technique and counted in a Doncaster dish (Winfield pers.comm. Nov. 1973)

Sample depth (inches)	Area of early yellowing		Healthy area	
	Tylencho-rhynchus	Longi-dorus	Tylencho-rhynchus	Longi-dorus
0 - 5	890	15	1,099	0
5 - 10	665	40	266	0
10 -15	1,050	500	211	0

2:2 Materials and Methods.

2:2:1 Soil and Root sampling.

The apparatus used for taking soil and root samples is shown in Plate 1. Soil cores were taken by:-

1) A cylindrical tube corer 1 inch in diameter. This auger, later referred to as the tube auger, was graduated in one inch lengths, allowing the soil cores to be divided into defined lengths from the corresponding depths. Samples were obtained by pushing the auger into the soil, twisting it prior to removal from the ground, after which the soil was removed from the open side of the auger.

   This method of soil sampling tended to compact the soil, and frequently waterlogged soil could only be removed with difficulty. The depth from which samples could be consistently taken depended on the soil profile, but 9 inches was found to be the maximum effective sampling depth.

2) A Jarret auger. This auger was used to take samples from a greater depth than was possible with the tube auger. The auger consisted of a short cylinder 13 cm. long, mounted on a handle graduated in 13 cm. intervals. On the tip of the cylinder, blunt teeth were shaped such that, as the auger was rotated into the soil, the soil was lifted into the cylinder of the auger. The soil was removed from this cylinder using a sharp poker. The auger was then re-inserted into the original hole and another 13 cm. length of soil obtained.

   All soil samples were placed in small labelled polythene bags for transport and storage.

3) Root sampling. Plant roots were sampled so that the species and numbers of endoparasitic and semi-endoparasitic nematodes within the roots, could be counted. A divot of soil, containing the roots of an entire plant, was collected using a trowel and placed in a polythene bag pending nematode extraction.

2:2:2 Extraction of nematodes.

Soil extraction.

The apparatus used for extracting nematodes from soil is shown in Plate 2. Prior to extraction, stones and large organic debris were removed by sieving the soil through a 2 cm. aperture sieve. The soil from each sample was then mixed and a 200 c.c. subsample was removed for extraction. Two methods of extraction were used.

1) The Whitehead Tray modification of the Baerman funnel technique of Whitehead and Hemming (1965). This was used to extract the active tylenchid nematodes. The volume of water used was always 1 litre, and the nematodes were extracted for 24 hours at room temperature.

2) The Flegg flotation technique was used for extracting long nematodes (Flegg, 1967). A 90 μ mesh sieve placed in a funnel, and left for 24 hours, was used in the final extraction of these nematodes.

The extract derived from the Whitehead tray method was poured into a litre beaker and after allowing 24 hours for the nematodes to settle, the supernatant was siphoned off as described by Bridge (1971).

Root extraction.

To measure root infestation, 0.1 grm. (fresh weight) of roots was thoroughly macerated and the numbers of nematodes of each species were counted directly in a Doncaster (1962) counting dish.

2:2:3 Identification and Counting of nematodes.

The species of nematode present in the soil samples were identified by examining fresh and fixed specimens mounted on glass slides. A range of techniques for making permanent mounts of the nematodes were tested

Plate 1. Soil sampling equipment.

Plate 2. Nematode extraction equipment.

Plate 1.

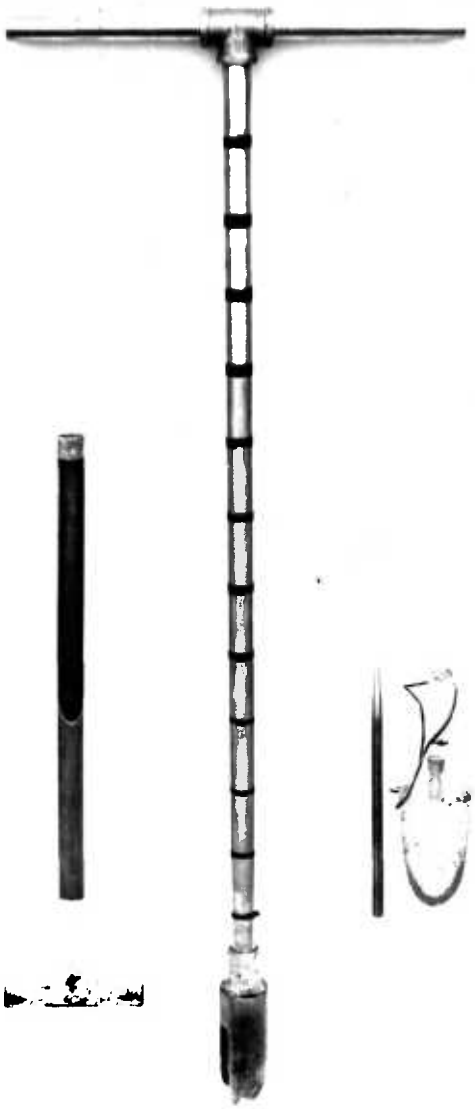


Plate 2.

before killing and fixing in hot F.A. fixative and Baker's (1953) method was chosen.

The nematodes were counted in a 1 ml. Hawksley slide under a x10 objective. Between sample counts, it was essential to clean the slide, due to retention of nematodes in the slide on dirt particles sticking to the glass surface. The extract volume was adjusted so that approximately 100 nematodes were counted per sample. This represented between 1% and 3% of the nematodes extracted per sample.

2:3 Results and Discussion.

2:3:1 The effects of ploughing on nematode numbers.

Soil populations.

Plots measuring 10 metres x 2 metres were staked out in each field and soil samples were taken from these plots at three-weekly intervals. The tube auger was used for soil sampling and the cores were taken to a depth of 9 inches. Each core was divided into lengths each of 3 inches and 20 cores were taken per sample and the respective core depths bulked. The nematodes were extracted by the Whitehead tray method and two aliquots of the extract were counted. The data on the numbers of nematodes found at each sampling are shown in Appendix A, Tables 1 - 6.

The species of nematodes present were:-

Pratylenchus crenatus, *P. minyus* and *P. penetrans*.
Tylenchorhynchus dubius and *Tylenchorhynchus* sp. (unidentified)
H. varicaudatus

Also present were *Tylenchus* spp.
Paratylenchus spp.

and a few individuals of *Pratylenchoides* sp. (unidentified)

Figs. 2:1 to 2:5 show the numbers of nematodes recovered from each sampling depth. More nematodes were recovered from the field that had previously been a ley pasture at all the sampling times, but the changes that occurred in the populations of the total plant parasitic nematodes, and the separate genera were similar in both fields. No sudden decline occurred following ploughing and in fact the numbers of *Paratylenchus*, *Pratylenchus* and *Helicotylenchus* increased in the first three weeks, and then declined through to week 12, the final sampling. Numbers of *Tylenchorhynchus* spp. remained nearly constant in both fields at all samplings.

Fig. 2:1 Depth Distribution Paratylenchus spp.

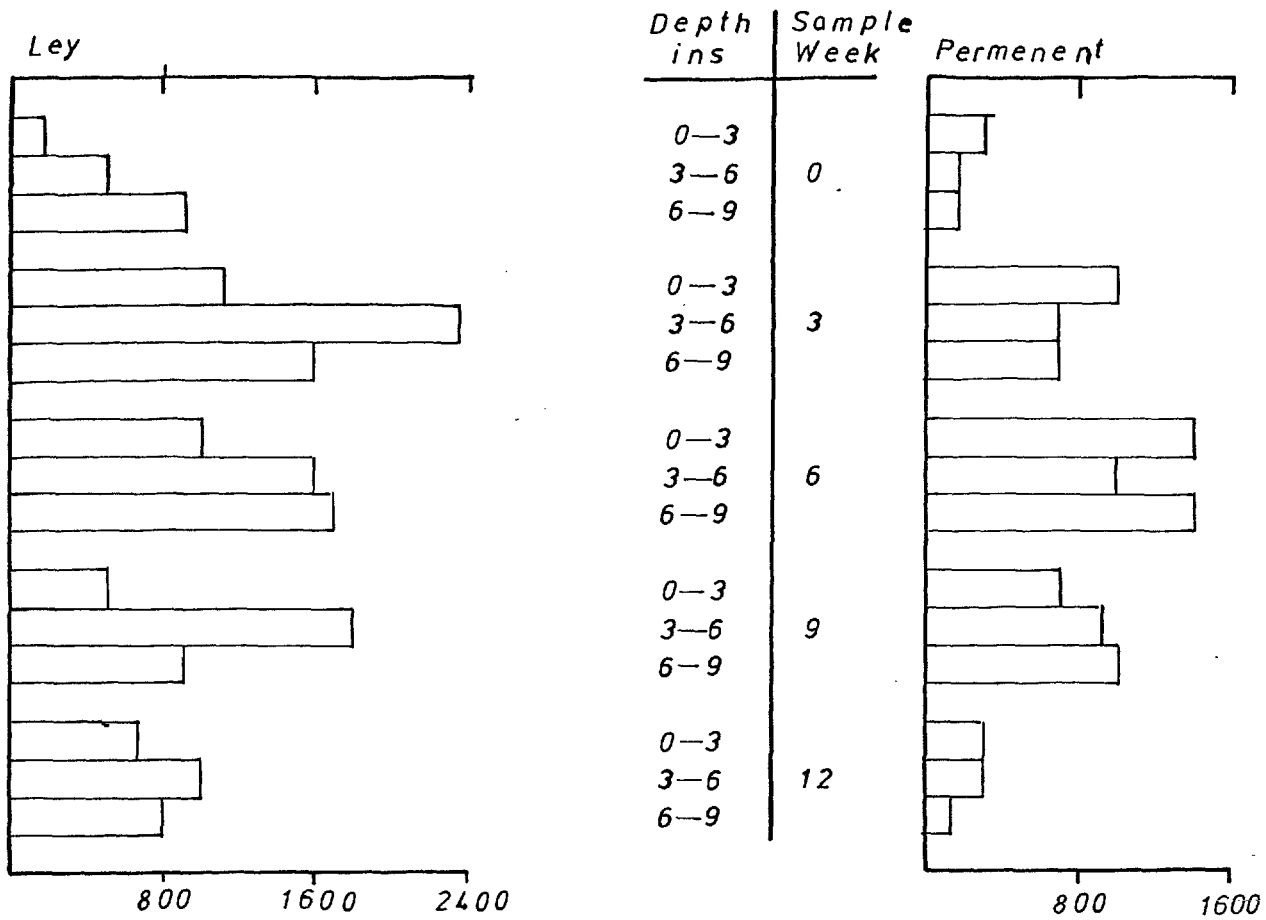


Fig. 2:2 Depth Distribution T. dubius.

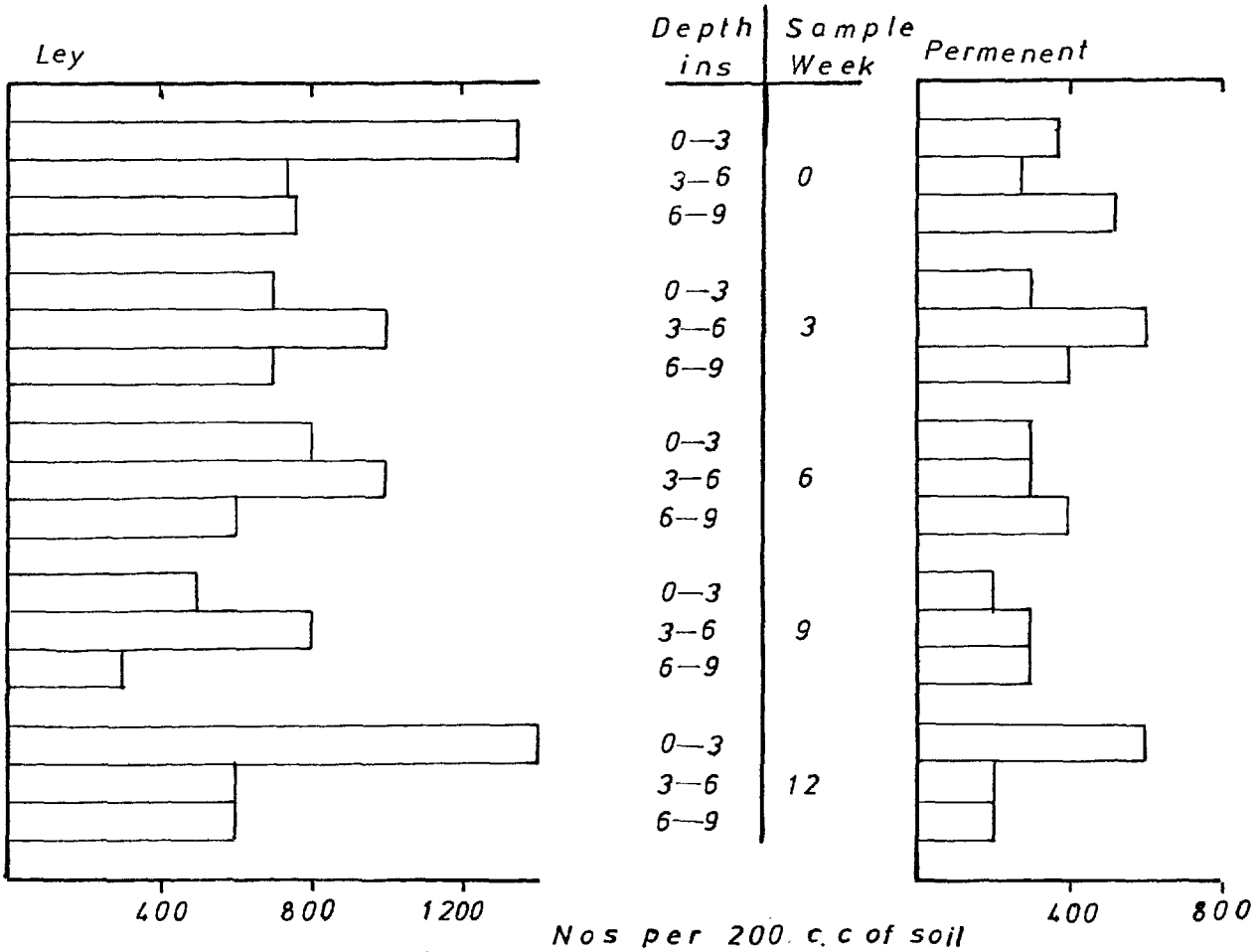


Fig. 2:3 Depth Distribution Pratylenchus spp.

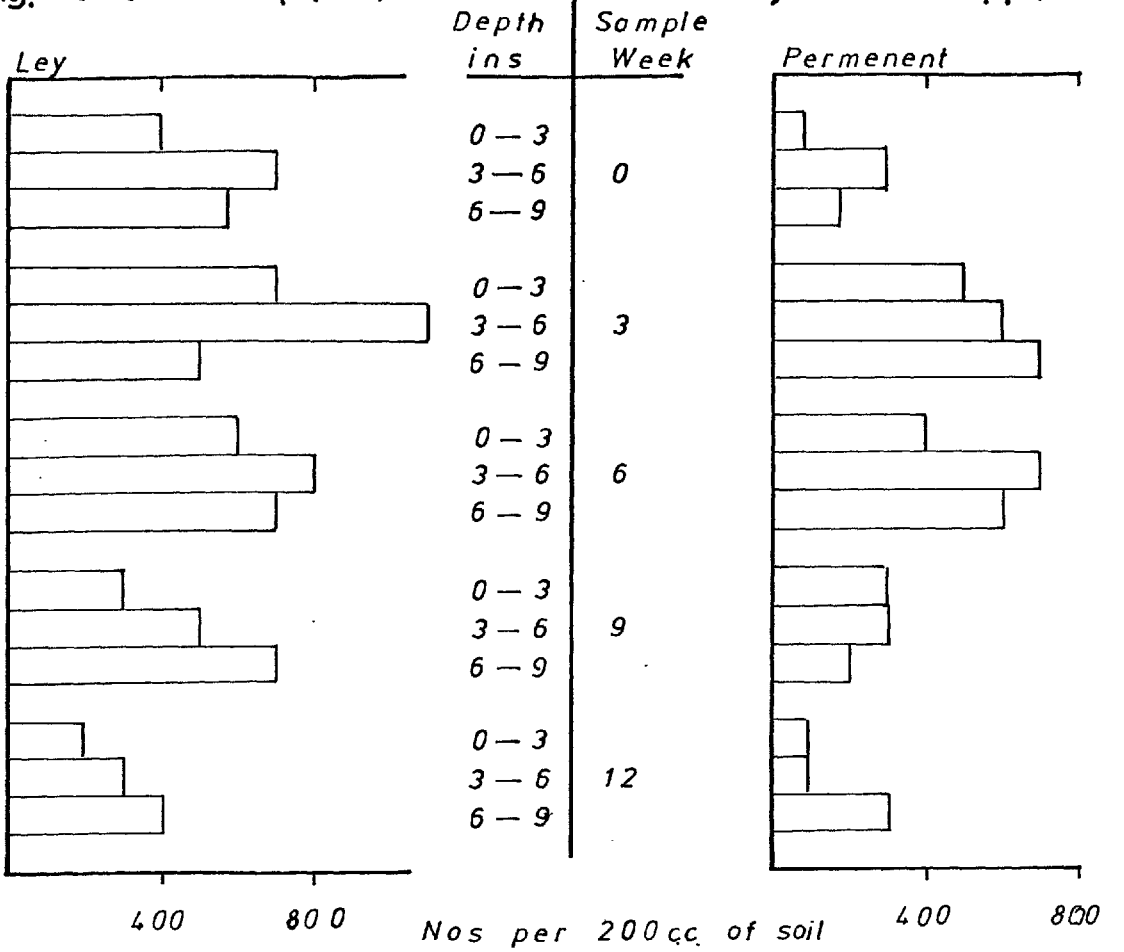


Fig 2:4 Depth Distribution H. varicaudatus.

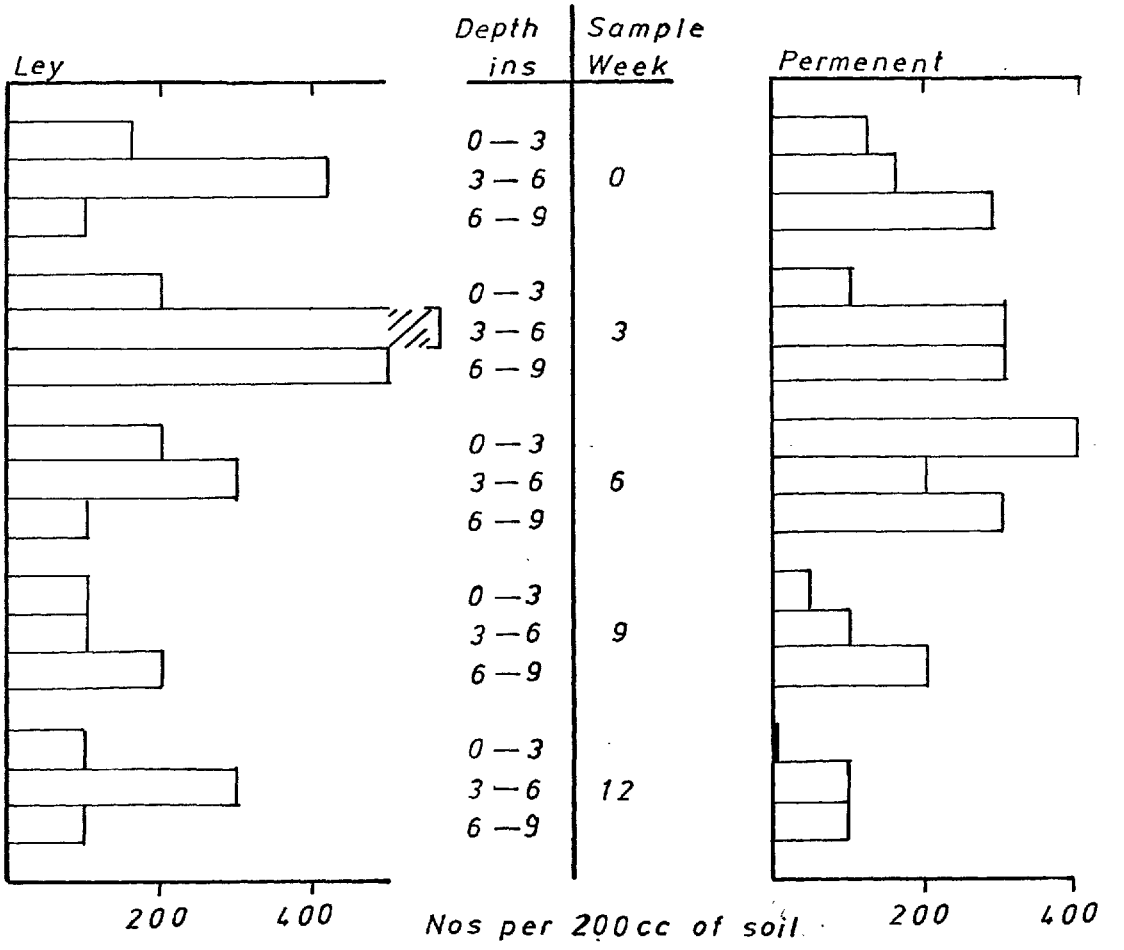
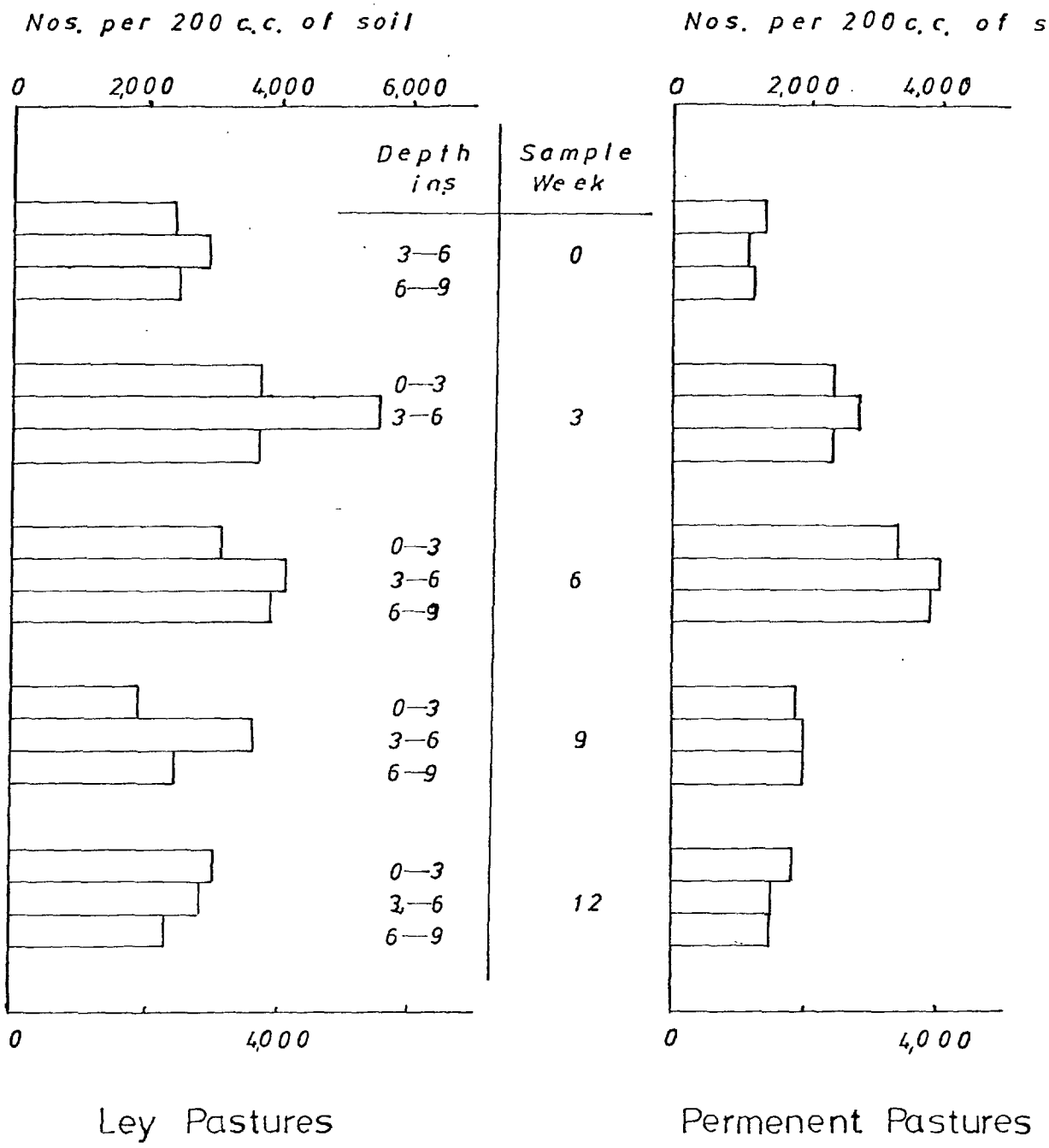


Fig. 2:5 Depth Distribution Total Plant Parasites



Ley Pastures

Permenent Pastures

The cause of the increase of numbers of Paratylenchus, Pratylenchus and Helicotylenchus could represent an increase induced directly by ploughing, and the resultant changes in soil structure, but both Pratylenchus spp. and H. varicaudatus have been shown to be endoparasites (Corbett et al., 1968; Collen, 1972) and thus the increased soil population may have been caused by the nematodes leaving the grass roots as these roots die.

The vertical distribution of Tylenchorhynchus dubius (Fig. 2:2) showed that initially these nematodes were most abundant in the surface soil layers, that after ploughing a more uniform spread occurred, but that by week 12, these nematodes were again most common in the top 3 inches. Paratylenchus spp., Pratylenchus spp. and H. varicaudatus were consistently present in greatest numbers at either 3 - 6 or 6 - 9 inch depth.

The uneven distribution of the latter three genera was consistent with the distribution of these genera reported from grassland by Bridge (1971) but again no obvious cause for this distribution was found. The initial distribution of T. dubius was also similar to the distribution of these nematodes in pastures, where the cause of the preference for the surface layer of soil, correlated with the abundance of grass roots. The decline that occurred in numbers in the top three inches between weeks 3 and 9 inclusive, could have been caused by simply mixing of the soil due to ploughing, but Wallace and Greet (1964) reported a similar trend for M. macrurus on pastures subject to intense drying of the surface layer of the soil. Thus, the decline and later recovery of populations of T. dubius in the surface layer of the soil probably resulted from this layer drying out initially, due to the lack of vegetative cover.

Root Invasion.

Two genera of nematodes, Pratylenchus and Helicotylenchus were

consistently found in roots. The data (Table 2:3 and Appendix A, Table 7) showed that Pratylenchus species had invaded the wheat roots in both fields by week 3. H. varicaudatus also invaded the wheat root in the ley field by week 3, but invasion of roots in the field previously permanent pasture was delayed until the ninth week after ploughing. This delay corresponds to a comparable delay in the decline of numbers of H. varicaudatus in the soil (Fig. 2:4).

Table 2:3

Root invasion by Pratylenchus spp. and H. varicaudatus.

Treatment	Numbers of nematodes per grm. of root			
	Week 3	Week 6	Week 9	Week 12
Pratylenchus spp.				
Ley Pasture	20	28	38	30
Permanent Pasture	18	62	54	40
H. varicaudatus				
Ley Pasture	24	68	20	18
Permanent Pasture	0	0	28	24

The change of soil populations of Pratylenchus spp. and H. varicaudatus was attributed to the endoparasitic habit of these nematodes, but the equally sudden increase and decrease in soil numbers of Paratylenchus spp. cannot be due to such a habit, but may have resulted from quiescent pre-adults in the grassland soils becoming active after ploughing, and causing a subsequent increase in the soil population. Paratylenchus spp. have been reported to decline in numbers when cereals follow the ploughing-in of grass (Collen, 1972) and one of the causes of such a decline could be the adverse effect, reported by Corbett and Webb (1970) that ploughing has on these nematodes. But on this occasion, ploughing favoured a short-term increase and then

equal decrease in numbers of these nematodes.

H. varicaudatus has previously been reported as feeding endoparasitically on cereal roots (Collen, 1972) and these observations confirm this, but Saynor (1972) suggested that Helicotylenchus spp. decline following soil disturbance. The fact that in this study H. varicaudatus invaded wheat roots within three weeks of sowing indicates that at least a portion of the population was not adversely affected. The reason for the delay of invasion by H. varicaudatus in the previously permanent pasture field, is unknown.

2:3:2 Relationships between nematode numbers and yield of Winter Wheat.

The plots laid out in Nov. 1969 by N. Collen each measured 3 metres by 1.20 metres. Between each plot was a 60 cm. guard row and around the outside of the plots was another guard strip 1 metre wide.

The site was first sampled on the 11th June, 1973 for the current investigation. Ten cores were taken per plot to a depth of 20 cm. using a tube auger at all samplings. The soil was a sandy loam.

The nematodes present in the plots were:-

Tylenchus spp. 4 spp.
Paratylenchus spp. 2 spp.
Tylenchorhynchus dubius
Pratylenchus minyus
P.crenatus
Helicotylenchus pseudorobustus

Also present, but in comparatively small numbers were:-

Merlinius nothus, the numbers of these nematodes were included with the counts for T. dubius
Rotylenchus robustus
Paratrichodorus atlantodorus
Criconemoides sp.

The nematodes were extracted from 200 c.c. of soil by the Whitehead tray method and the counts of the nematodes found at the various samplings are listed in Appendix B, Tables 1 to 6.

Between June and September 1973, four of the plots were sown with spring wheat in an attempt to enhance differences in the population densities between these and the other plots. The four plots chosen, numbers 3, 5, 9 and 10 represented one plot from each of the four cereal treatments used by N. Collen (Table 2:1). Wheat, var. "Cardinal", was sown on the 12th June, 1973, and harvested on the 20th September, 1973, after which all twelve plots were dug, hoed and raked. Three days later soil samples were taken and used for the estimation of the initial nematode population. The following day, 24th September, 1973, winter wheat, var. "Maris Huntsman", was sown in drills 6 inches apart at a rate of $1\frac{1}{2}$ cwt. per acre. Undressed seed was used. Fertilizer was added to all plots on the 12th March, 1974 (3 cwt. per acre I.C.I. No. 3).

Soil samples were taken on the 12th March, 1974 and on the 23rd July when the plots were also harvested. A final soil sample was taken on the 22nd September, 1974, a year after the experiment was begun.

The rainfall and soil temperature at 10 cm. depth in the Ascot area during the experiment is shown in Fig. 2:6.

Since the growth of wheat during the trial was uneven within as well as between plots, at harvest each plot was divided into four equal sub-plots measuring 1.5 metres by 60 cm. Soil sampling and the estimation of plot yields was carried out on this sub-plot basis.

The plots were netted from sowing till harvest to avoid bird damage, but despite these precautions from mid-June on, the plots suffered severe bird damage, making any estimation of grain yield impossible. Thus the assessment of yield (Appendix B, Table 7) was

made from

1) straw weight and
2) the surface area of the top two leaves of 25 plants.

The leaf area was measured ny passing pressed leaves through a planimeter and recording the total leaf area of the 50 leaves from each sub-plot.

The data obtained was used to assess whether any correlations existed between nematode numbers and yield.

The initial populations of H. varicaudatus in the plots ranged from 40 to 1,600 per 200 c.c. of soil but despite the very large populations present no correlation existed between numbers and yield (Fig. 2:7). The initial population of Pratylenchus spp. ranged from zero to 800 per 200 c.c. of soil and tended to be positively related to yield, though this relationship was not significant. Saynor (1972a) reported a significant positive correlation between initial numbers of P. minyus and yield. These results suggest that plant growth is increased where P. minyus is present in large numbers.

The initial populations of T. dubius ranged from 0 to 1,032 per 200 c.c. of soil, and these populations were significantly, at 5%, negatively correlated with the leaf area yield estimate, such that a yield reduction of 20% occurred at the highest population of T. dubius (Fig. 2:9). T. dubius has previously been shown to cause stunted growth of wheat in pots (Sharma, 1971) but Saynor (1972a) found no correlation between populations of T. dubius of up to 250 per 200 c.c. of soil and yield of spring barley.

The contradiction that exists between these results could be due to:-

1) The two hosts having varying susceptibility to T. dubius, i.e. spring barley is more tolerant to T. dubius than winter wheat, or

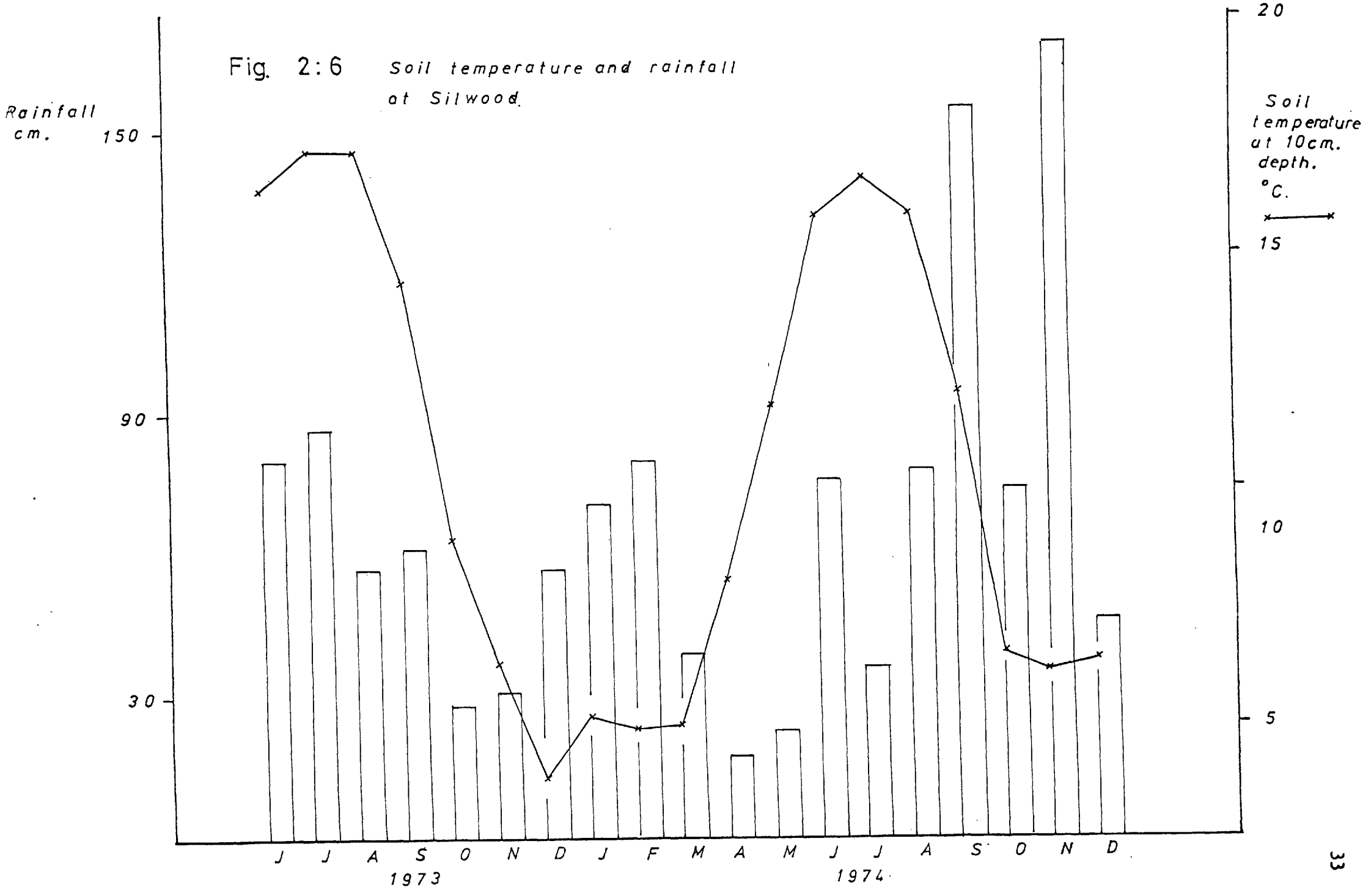
2) The larger populations of T. dubius caused a breakdown of the tolerance of winter wheat to T. dubius.

The relationship between final populations of nematodes in the plots and leaf area (Figs. 2:10 to 2:12), and between final populations in the sub-plots and sub-plot yield (Figs. 2:13 to 2:15) show that no correlations existed between these populations and yield of T. dubius or Pratylenchus spp., but that a negative correlation, significant at 5%, existed for numbers of H. pseudorobustus in the main plots and leaf area (Fig. 2:12). A similar relationship existed between final numbers of Heterodera schachtii and yield of sugar beet (Jones, 1956) and both relationships are caused by relic populations. In the two plots, numbers 1 and 9, large populations of H. pseudorobustus were still present at the final sampling even though each had declined to less than a half of the initial population, and if these two populations are excluded then no correlation existed.

The relationships that exist between initial and final nematode populations showed that T. dubius (Fig. 2:16) approached a final population of 257 per 200 c.c. of soil, whilst H. pseudorobustus (Fig. 2:18), if the relic population of plots 1 and 9 are ignored, approached a final population of 87 per 200 c.c. of soil. The changes in populations of Pratylenchus spp. (Fig. 2:17) showed no obvious trend and fluctuated around the maintenance population.

If the changes of populations that occurred within plots are compared to the cropping history of the plot, a similar picture to the overall interpretation occurs. The population changes of T. dubius on the plots that had been cereals from 1969 fluctuated around the final population of 250 per 200 c.c. of soil, whilst the populations from the grass plots, numbers 1 and 4, declined to the 250 per 200 c.c. level and populations from the fallow plots tended to increase to

Fig. 2:6 *Soil temperature and rainfall at Silwood.*



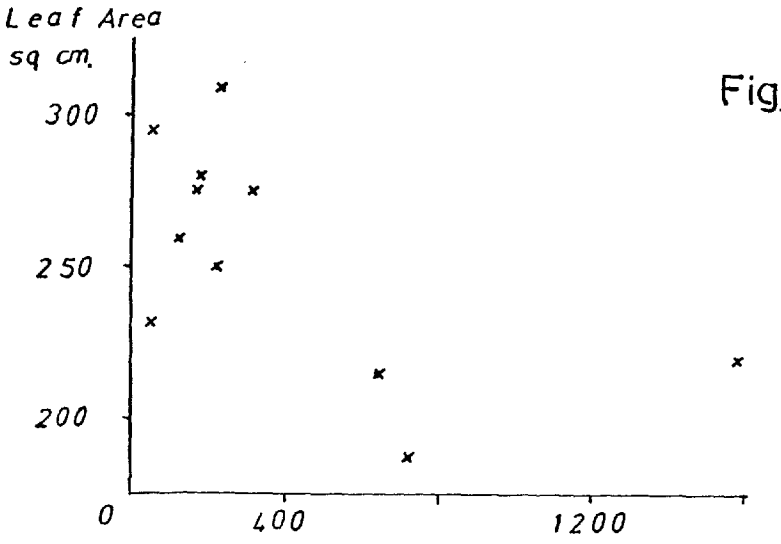


Fig. 2:7 H. pseudorobustus

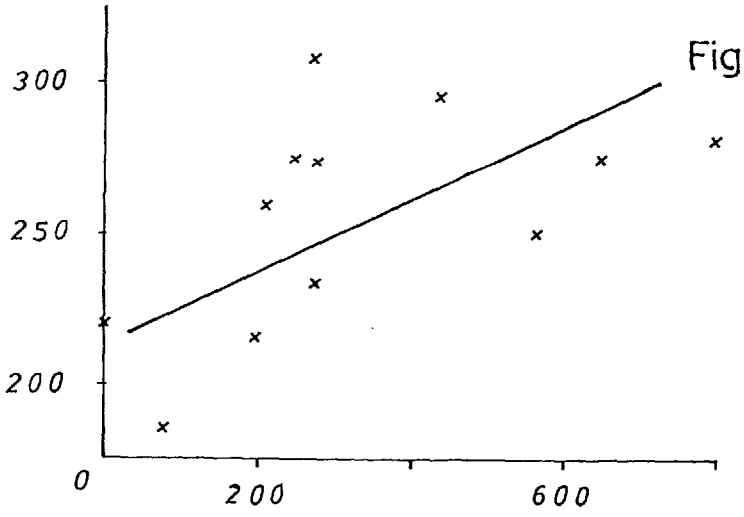


Fig 2:8 Pratylenchus spp.

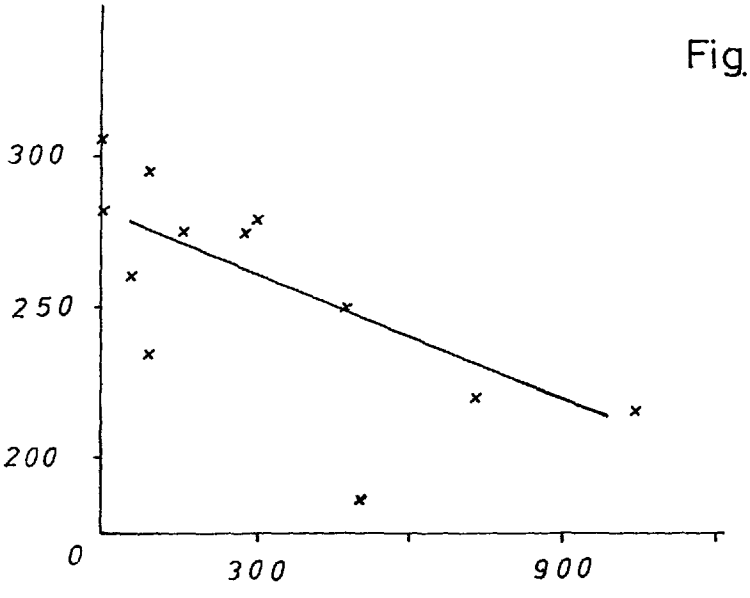


Fig. 2:9 T. dubius

Figs. 2:7 — 2:9 Relationship between iniaial population and yield.

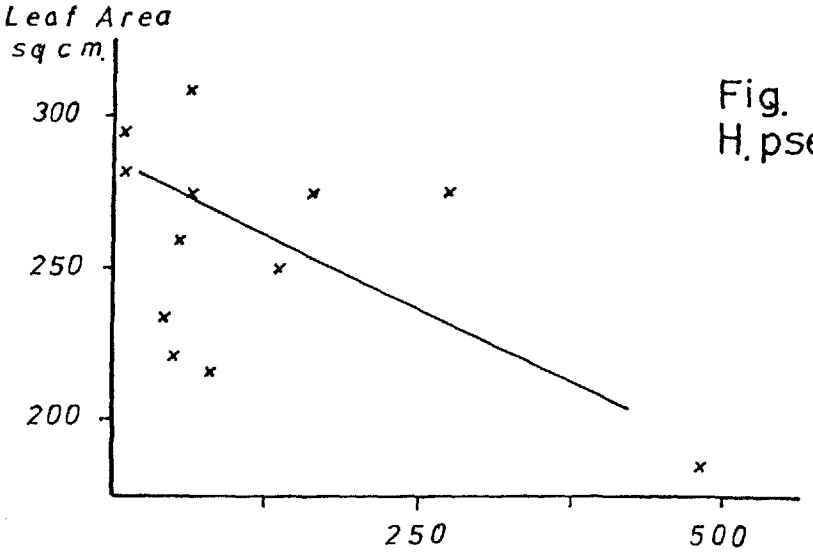


Fig. 2 : 12
H. pseudorobustus.

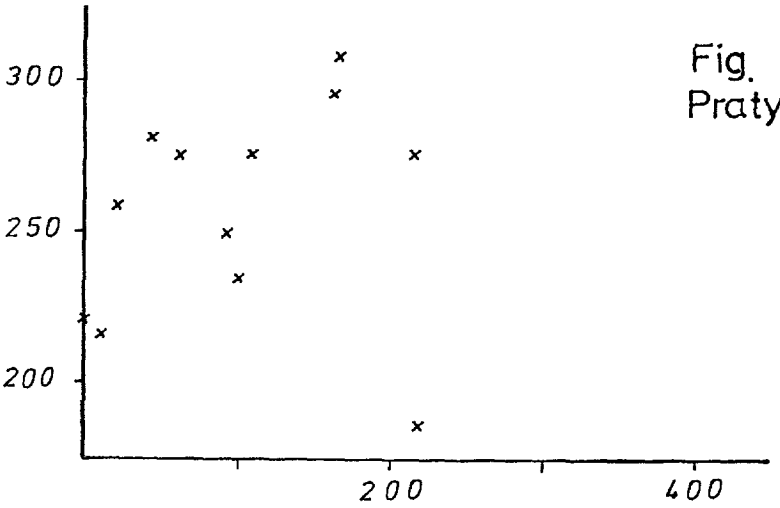


Fig. 2 : 11
Pratylenchus

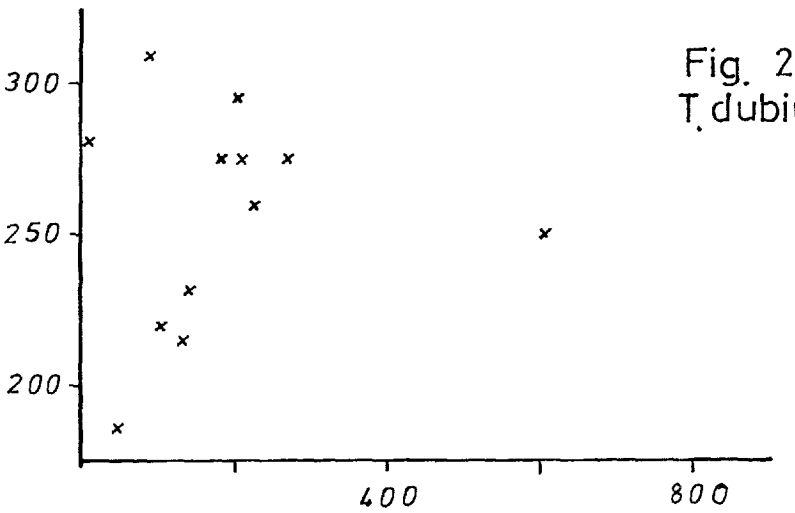


Fig. 2 : 10
T. dubius

Nos. per 200 c. c. of soil

Figs 2 : 10 — 2 : 12 Relationships between final population and yield

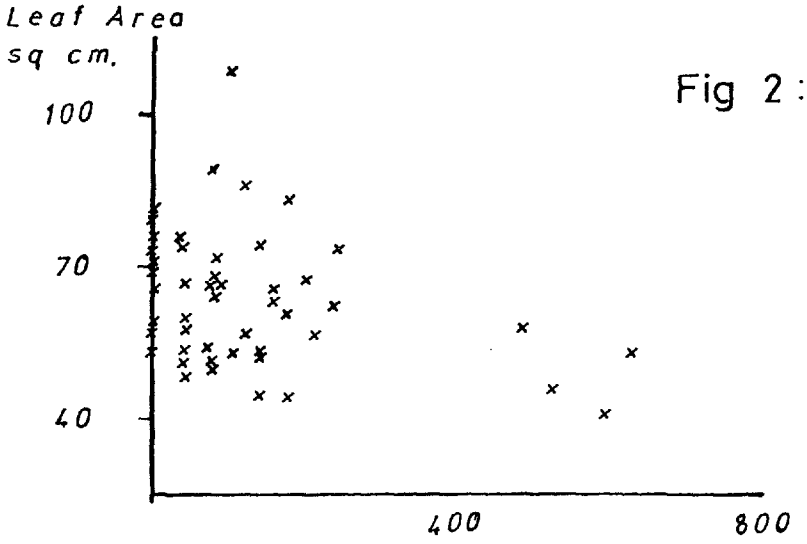


Fig 2 : 15 H. pseudorobostus.

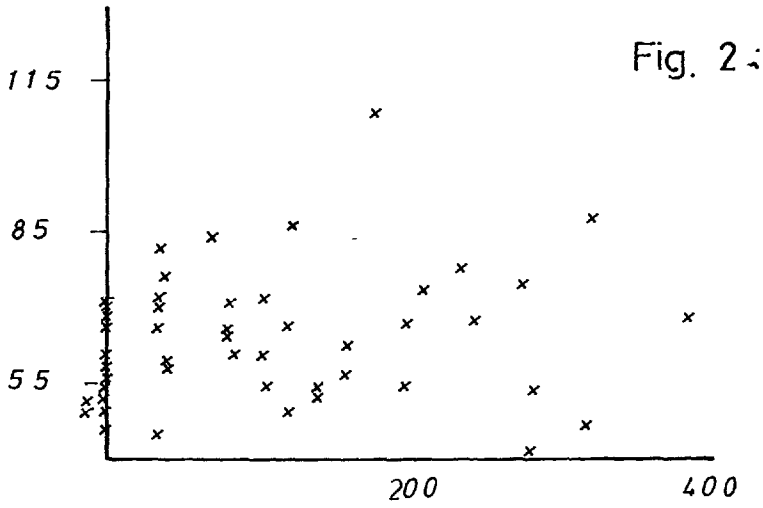


Fig. 2 : 14 Pratylenchus spp.

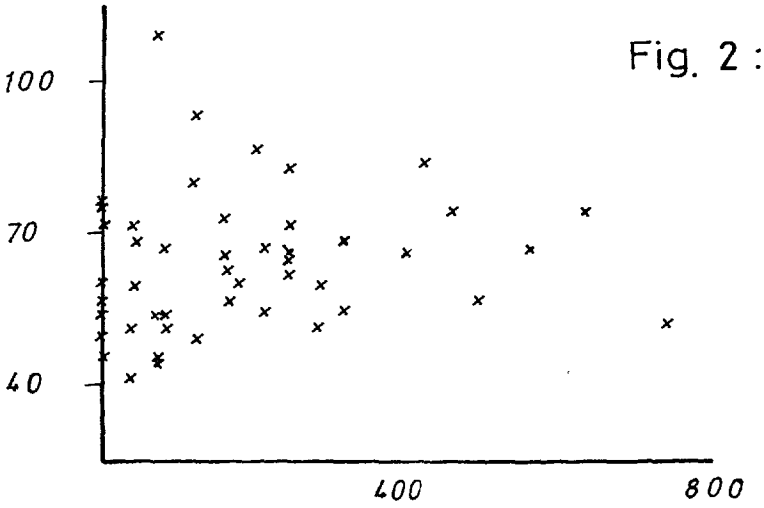


Fig. 2 : 13 T. dubius.

Figs 2 : 13 — 2 : 15 Relationships between final sub-plot population and yield.

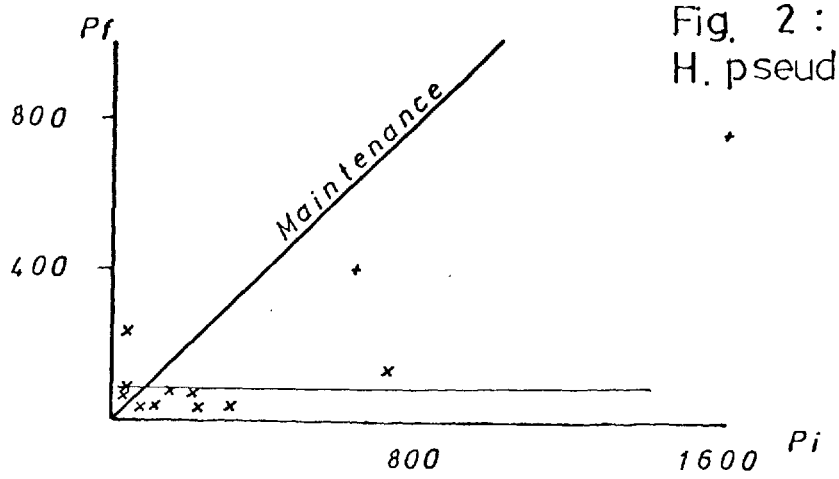


Fig. 2 : 18
H. pseudorobustus.

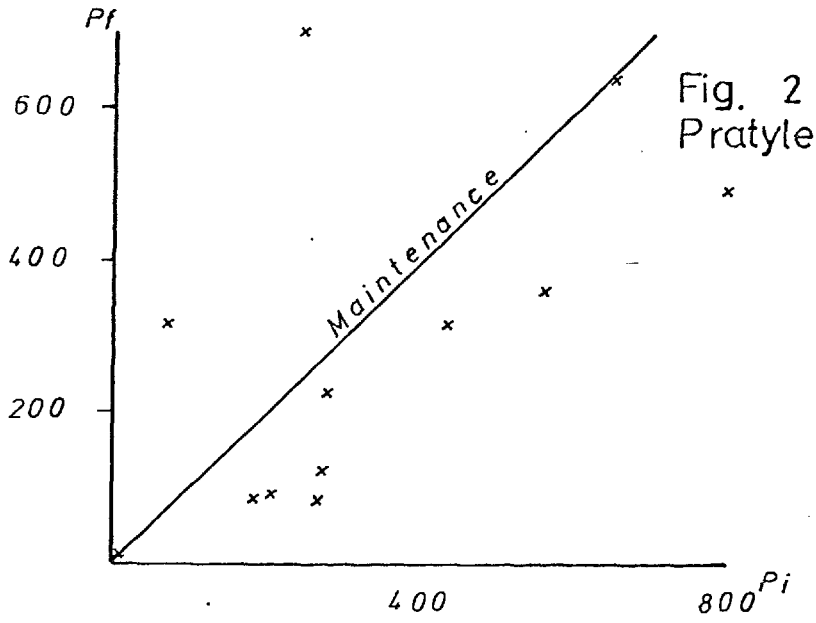


Fig. 2 : 17
Pratylenchus spp.

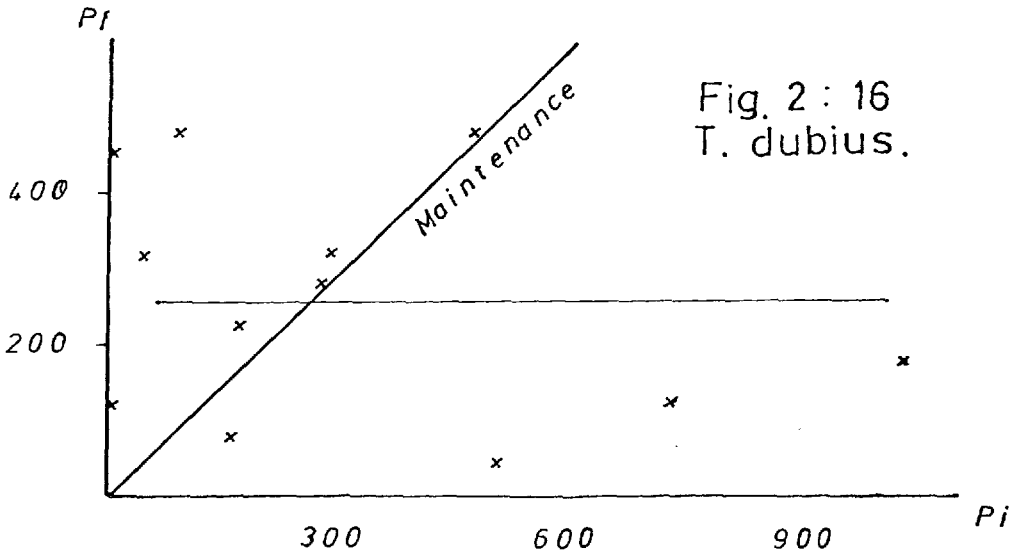


Fig. 2 : 16
T. dubius.

Figs. 2 :.16 —2 : 18 Relationships between initial and final population.

this level. The initial populations of H. pseudorobustus on all the cereal and grass plots showed a marked decline whereas the numbers from the fallow plots increased. The population changes of Pratylenchus spp. again showed no marked tendency to increase or decrease. The lack of a population increase of Pratylenchus spp. even on the fallow plots conflicts with earlier reports since large populations of P. minyus and P. crenatus occurred in cereal fields (Gair et al., 1969; Cotton, 1970; Corbett, 1970) and Collen (1972) observed that these nematodes increased in cereal fields after grass.

The final relationships tested were between the separate genera within plots. Both Helicotylenchus spp. (Collen, 1972) and Pratylenchus spp. (Corbett et al., 1968) are endoparasites and the similarity of feeding site may lead to competition between these species. Conversely conditions favouring one genus may favour another genera, and thus each genus of the five common tylenchid nematodes was tested to see if any correlation existed between the genera. No such relationships were found between the abundance of the nematodes in the plots.

2:3:3 Nematicidal Experiments.

The first trial 1973/1974 season, was laid out with two control and two treated plots in the region of even growth and the region of patchy growth of the field. The nematicide used was Vydate, 5 lbs. active ingredient per acre, and this was broadcast onto the recently emerged barley on 27th November, 1973. The experimental plots measured 12 ft. by 12 ft. and were separated by an 8 ft. guard row.

Soil cores were taken to a depth of 20 cm. using the tube auger and divided into the top and bottom 10 cm. Ten cores were taken per

sample and the respective sample depths were bulked. At the initial sampling only the control plots were sampled, but at subsequent samplings taken on 1st April, 1974 and at harvest on 31st July, 1974, all plots were sampled. The nematodes were extracted from the soil by the Whitehead tray method and Flegg's method.

The nematodes present in the field were:-

Tylenchus spp. 3 spp.
Paratylenchus sp.
Tylenchorhynchus dubius
Pratylenchus minyus and *P. penetrans*
Helicotylenchus digonicus
Longidorus macrosoma and *L. leptocephalus*

Also present were a few individuals of

Merlinius nothus and *M. icarus*
Longidorus goodeyi

An estimate of yield from this first trial was made by cutting 2 metres by 1 metre of barley with a sickle from the centre of each plot, and recording the total weight of grain at 9.7% moisture, and the total weight of straw (Table 2:4). Yield from the region of even growth was

Table 2:4

Yields of Grain and Straw from Barley plots 1974

Plots	Grain weight at 9.7% moisture. grms.		Straw weight. grms.	
	Patchy Growth	Even Growth	Patchy Growth	Even Growth
Treated 1	856	609	2,863	1,105
2	790	1,171	2,353	1,388
Control 1	477	875	1,360	1,247
2	Lost	Lost	Lost	Lost

not markedly affected by the treatment, but in the region of patchy growth the yields were greater in the treated plots than in the single contol plot harvested. Though the yields are very different, little reliance could be placed on these results, and thus in 1974 a further trial was made.

The experimental plan was the same four treatments, control and Vydate treated plots in both the patchy and even growth region, but each treatment was replicated five times. The plots measured 10 ft. by 10 ft. and each plot was surrounded by a 3 ft. guard strip. The entire experimental areas were sown with winter barley var. "Astrix", broadcast at 1½ cwt. per acre. The seed was dressed and fertilizer (I.C.I. No. 3 at 3 cwt. per acre) was broadcast also over the entire experimental area. Both the seed and the fertilizer had to be broadcast onto the soil, because high rainfall in the preceding two months had waterlogged the soil (Fig. 2:19).

Vydate at 7 lbs. active ingredient per acre was broadcast onto the treated plots, after which all the plots were raked to incorporate the seed, fertilizer and nematicide into the soil.

Soil samples were taken from each plot using a Jarret auger on 18th November, 1974, 17th March, 1975, and after harvesting the plots on 29th July, 1975. Cores from the three separated depths were bulked from the five replicates of each treatment, and the nematodes were extracted by the Whitehead tray method and Flegg's technique. The numbers of nematodes found at each sampling are shown in Appendix C, Tables 1 - 8, and the plot yields in Appendix C, Table 9.

The nematicide effectively controlled all nematodes in the 1973/1974 growing season to a depth of 20 cm., and its effect persisted to the final sampling of these plots. Yet in the 1974/1975 season the treatment

was not as effective, reducing the total tylenchid nematodes by only a half. The nematicide was least effective in reducing the population of T. dubius, but the numbers of Tylenchus spp., Paratylenchus spp. and H. pseudorobustus were reduced to populations below half the control populations. Tylenchorhynchus spp. and Pratylenchus spp. have been reported as being susceptible to small doses of the nematicides D-D and ethylene dibromide, (Whitehead et al., 1970) and the failure of Vydate to control the nematodes in this trial is most likely related to the virtual waterlogged state of the soil at sowing, which prevented the incorporation of the nematicide into the soil. The waterlogging of the soil may also have been responsible for the low populations of nematodes including T. dubius (Fig. 2:23) in the surface layers of the soil at this sampling.

Low nematode populations were recovered at the final sampling. During June and July, the total rainfall was very low (Fig. 2:19), less than half the monthly means, and this, associated with the high temperatures, resulted in the soil becoming very dry and the low nematode populations found at the final sampling.

At harvest, the standing crop on each plot was cut and tied in a sheaf. The grain from each sheaf was threshed and weighed. In addition, "1,000 grain weight" for each plot was measured. These grain weights were calculated to a 15% moisture weight. The plots suffered no bird damage due to the use of electronic bird scarers.

The means of total grain yield (Table 2:5) did not differ significantly, though again an increase occurred in grain yield on the nematicide treated plots in the patchy region. The means of 1,000 grain weights were significanly different at the 1% level, but the differences between control and treated plots in the two regions were

not significantly different.

Table 2:5

Total grain weight and 1,000 grain weights mean per treatment

Plots	Total grain weights grms.		1,000 grain weights grms.	
	Even Growth	Patchy Growth	Even Growth	Patchy Growth
Control	5,730	5,301	54.9	51.9
Treatment	5,219	5,993	55.7	52.2
	n.s.		** L.S.D. at 5% of 1.8 grms.	

These two experiments were made to determine whether Longidorus spp. or T. dubius were causing the patchy early-yellowing of cereals. The numbers of Longidorus spp. (Fig. 2:20) at the original sampling date were 500 per litre of soil extracted by the Whitehead tray method. At subsequent samplings, only the odd individual was extracted by the Whitehead tray method, and only low numbers recovered by Flegg's technique. In addition, the bulk of the Longidorus spp. were found consistently at the lower sampling depths, where they are less likely to severely affect root growth. The nematicide had no effect on Longidorus numbers due to the poor incorporation of the nematicide to the depth at which the bulk of these nematodes were present.

H. digonicus numbers (Fig. 2:21) were controlled by the nematicide in both seasons, and it appears that since no yield increase occurred, populations of H. digonicus up to 600 per 200 c.c. of soil are tolerated by winter barley. Similarly, Pratylenchus spp. (Fig. 2:22) in populations up to 3,000 per 200 c.c. of soil do not appear to reduce the yield of winter barley.

Whether T. dubius is involved in the disease symptom is still in

doubt. The nematicidal treatment of 1974 affected these nematodes only slightly but the population of T. dubius in the region of patchy growth remained consistently greater than that in the region of even growth (Fig. 2:23). In the 1973/1974 growing season, the population differential between the regions was 400 per 200 c.c. of soil and yield increased from 500 to 875 grms. whilst in 1974/1974 a population differential of 300 per 200 c.c. of soil resulted in a yield difference of between 5% and 6%.

The observations might be explained either by soil characteristics that favour reproduction of T. dubius while adversely affecting cereal growth, or alternatively the direct effect of feeding by T. dubius on the roots of barley. But since the populations of T. dubius were not reduced in the treated plots, despite the increased yields of areas with low populations T. dubius cannot be firmly implicated with causing patchy growth.

Fig. 2 :19. Rainfall and soil temperature at 10 cm depth, Essex.

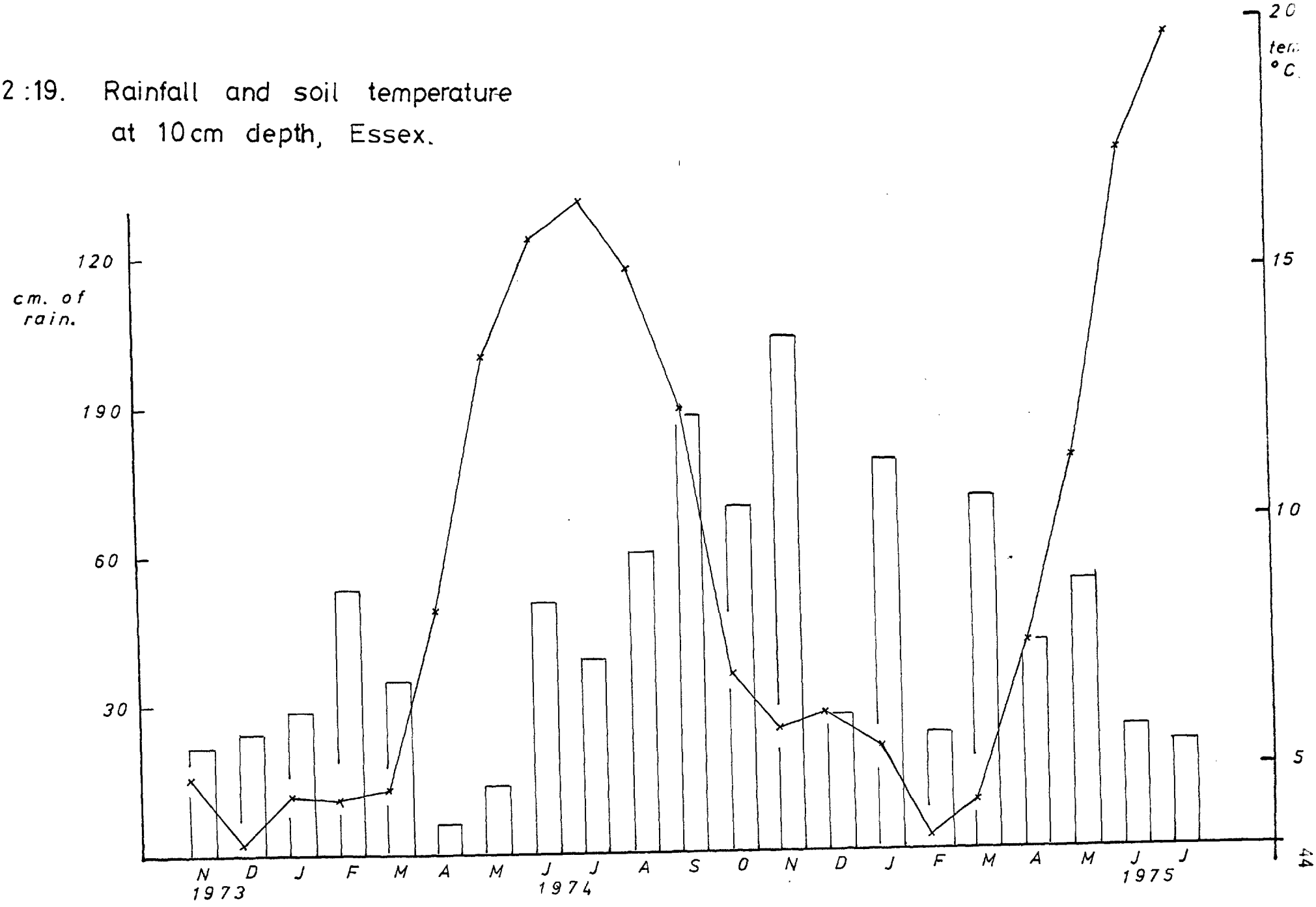


Fig 2:20 Longidorus spp.

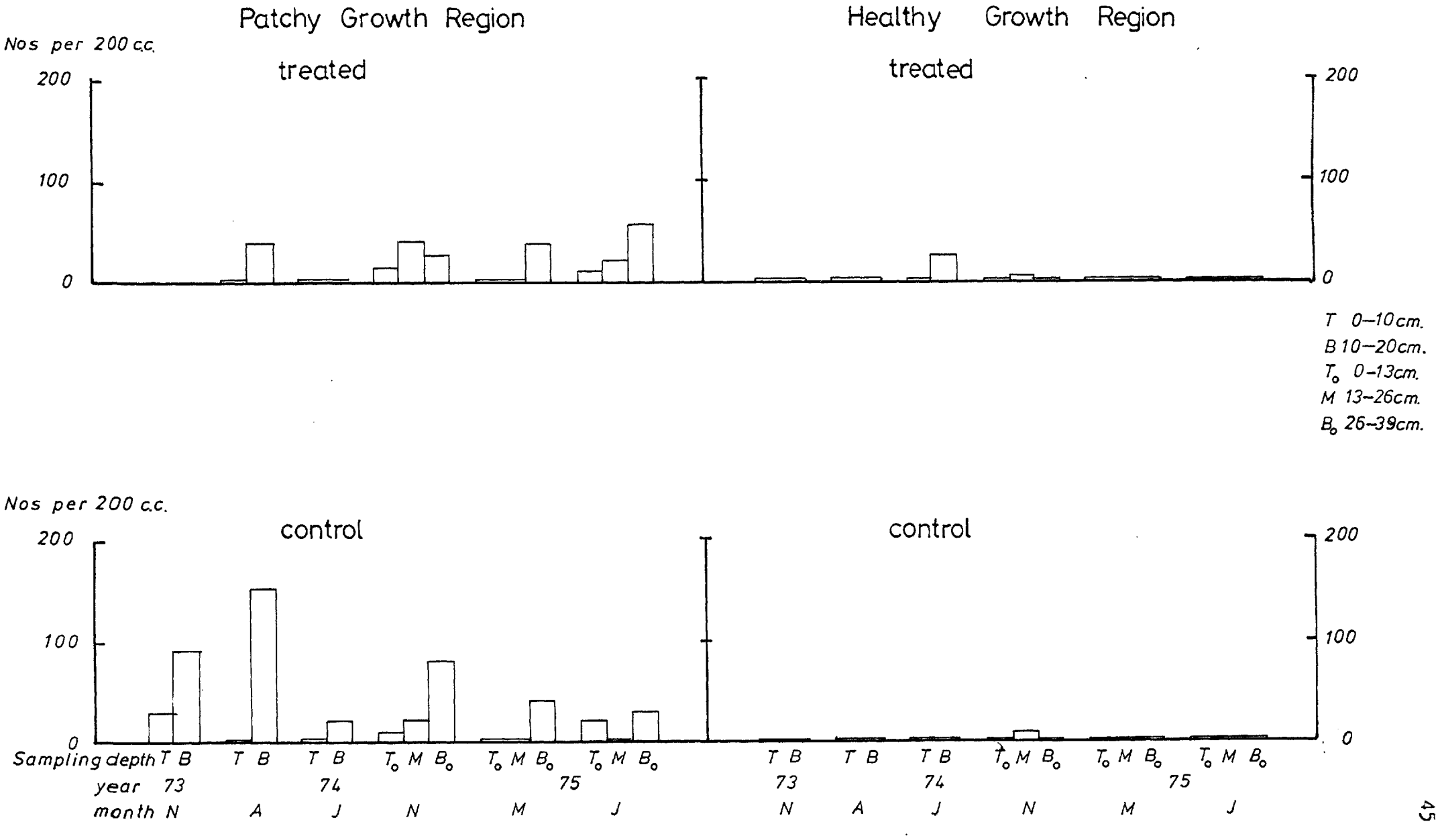


Fig. 2 : 21 H. digonicus.

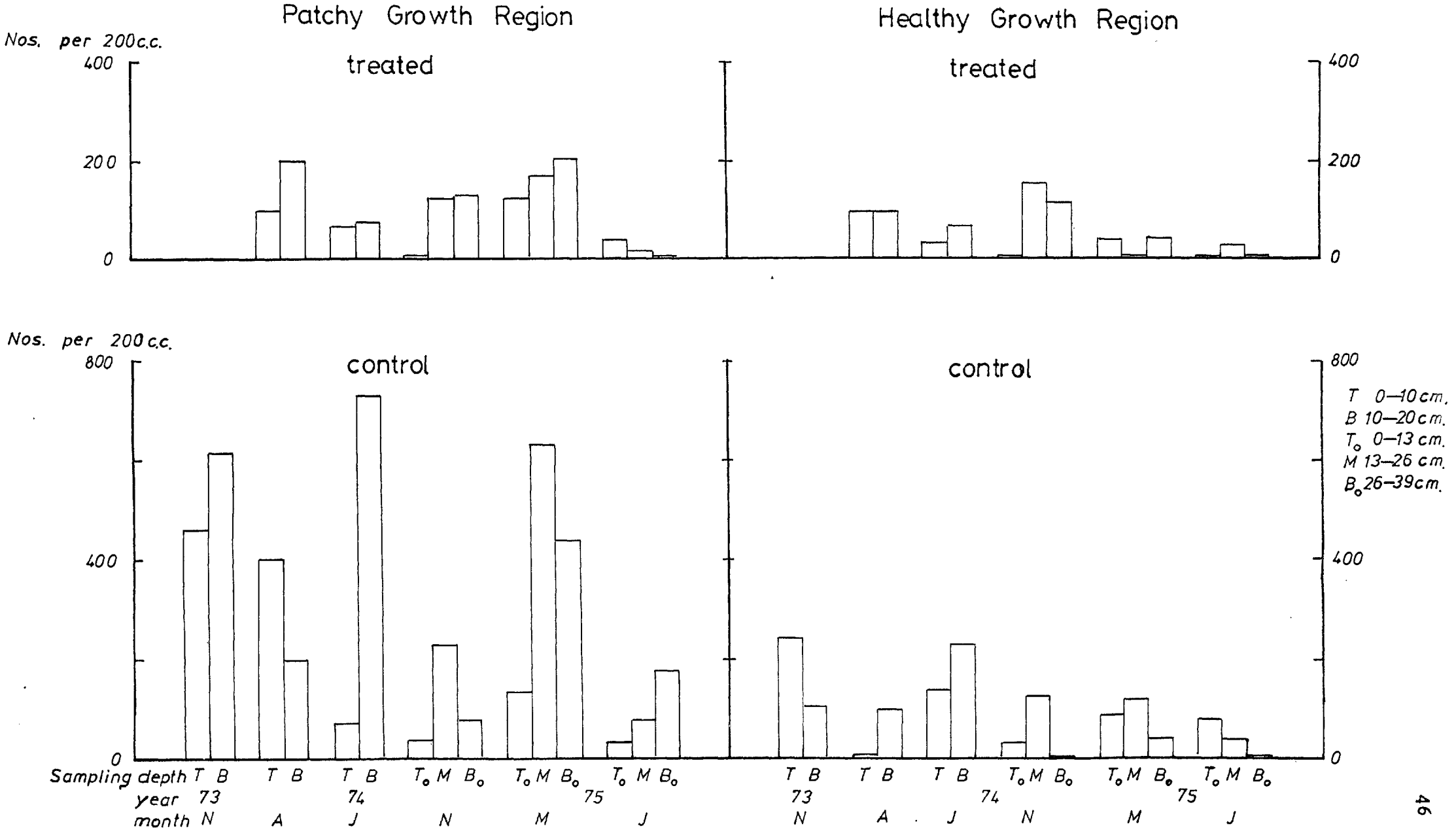


Fig 2: 22 Pratylenchus spp.

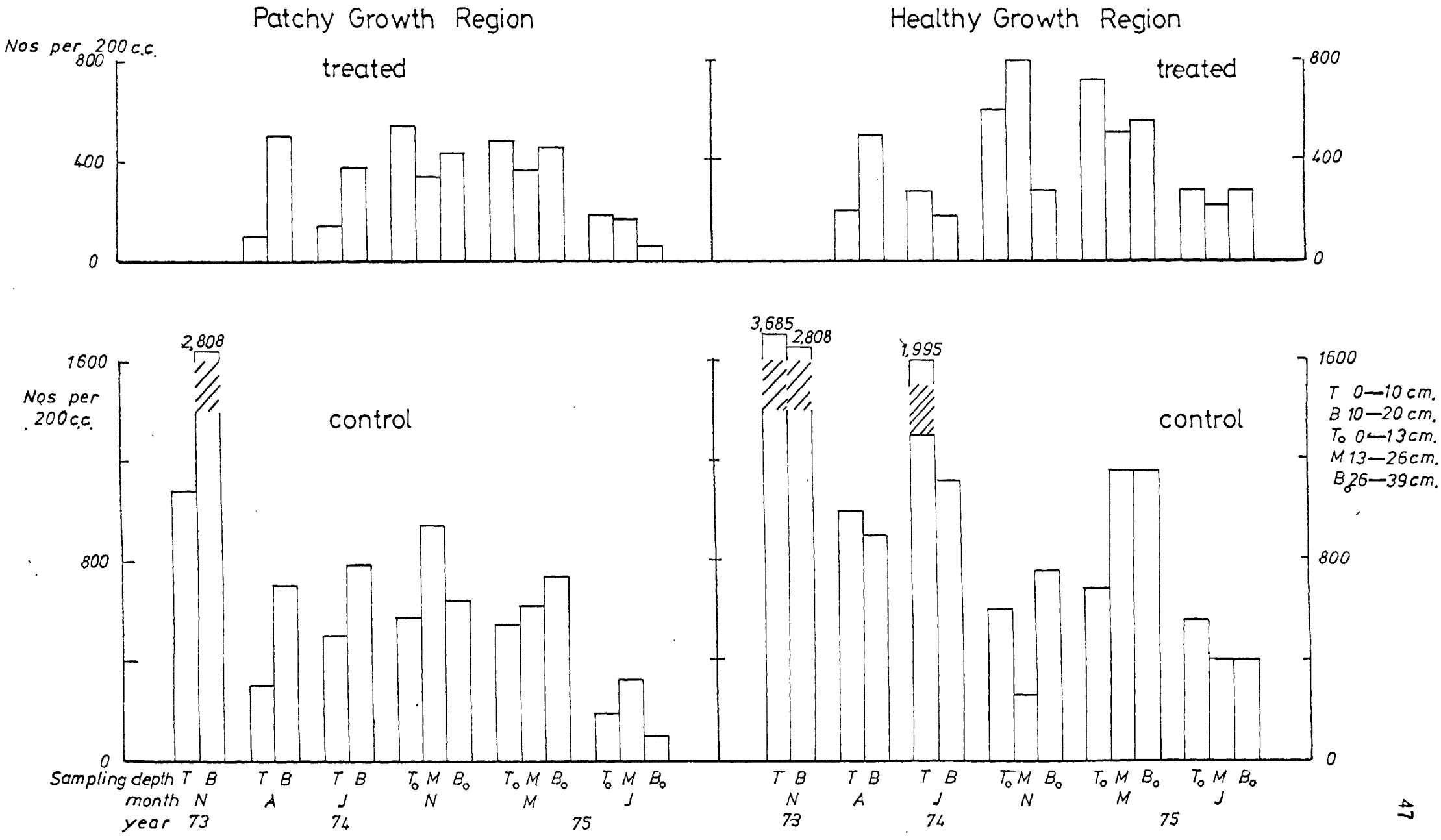
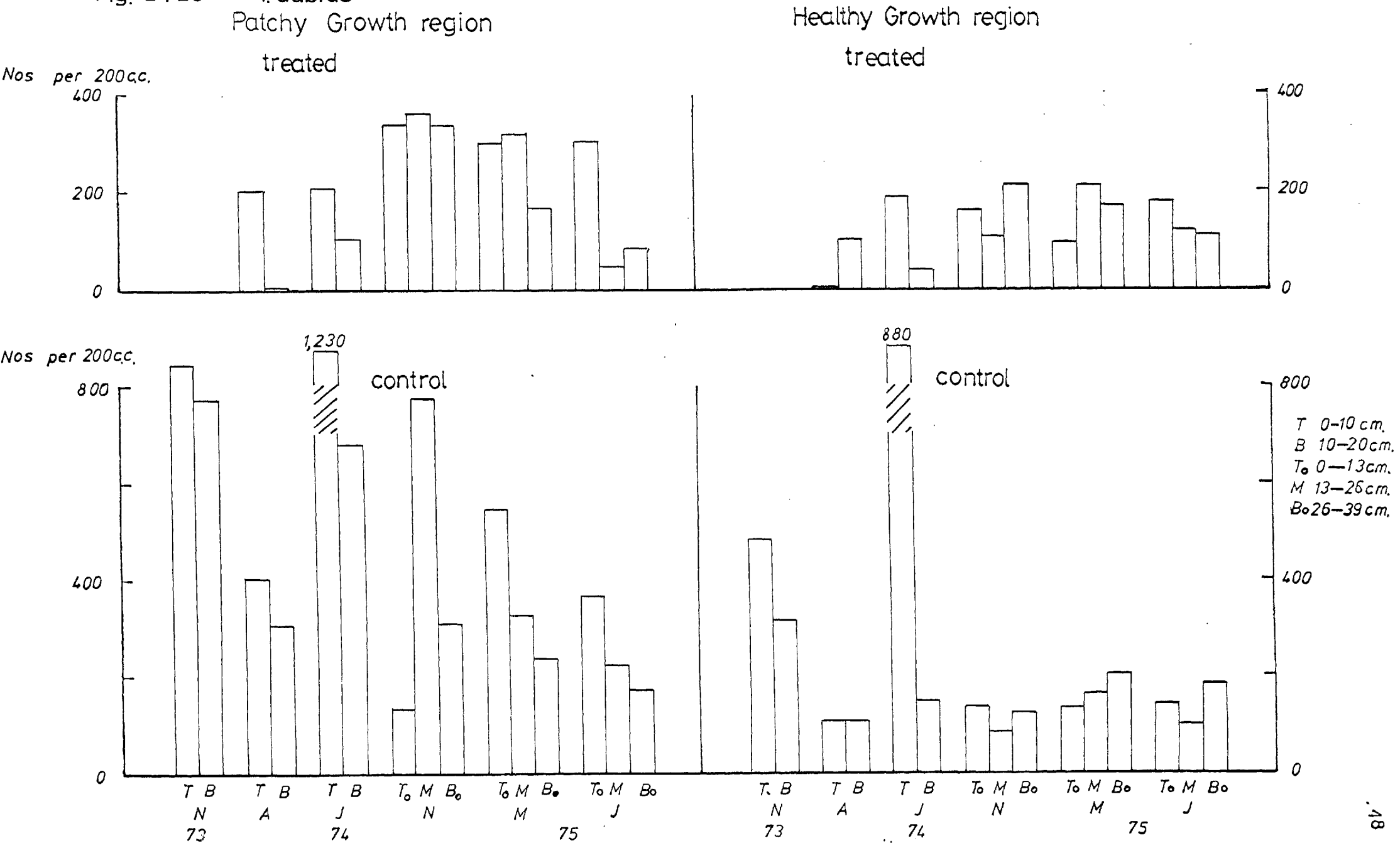


Fig. 2 : 23 T. dubius



2:4 Concluding Discussion of Field Experiments.

Populations of nematodes in pastures and cereal field soils decline from a winter peak through to a minimum in the summer (Bridge, 1971; Corbett *et al.*, 1968) and thus the changes in nematode numbers that occurred in the soil after ploughing must be viewed with this in mind. Yet after ploughing the numbers of three genera increased. The endoparasitic habit of *Pratylenchus* spp. and *Helicotylenchus* spp. was thought to have caused these changes with these genera. An increasing root population in the initial growth stages of cereals has been recorded for *Pratylenchus* spp. by Corbett *et al.*, (1968) and Saynor (1972a). The similar trend that occurred for *H. varicaudatus* has not been reported before. The finding of fewer *H. varicaudatus* than *Pratylenchus* spp. in roots is probably a reflection of the lower soil population of *H. varicaudatus*.

The trend for populations of *T. dubius* above 300 per 200 c.c. of soil to decline, and populations below 200 per 200 c.c. of soil to increase, suggests that on winter wheat a ceiling population of approximately 250 per 200 c.c. of soil existed. Similarly for *H. varicaudatus* if the relic populations of plots 1 and 9 are ignored a ceiling population of around 100 per 200 c.c. of soil existed. The cause of the rather low ceiling population of *H. varicaudatus* is unlikely to be a density dependent factor since a population of 1,600 per 200 c.c. of soil built up on spring wheat var. "Cardinal". Prevailing climatic factors (Fig. 2:6) though variable, appeared not to cause a similar decline on other species of nematodes, nor are altered soil characteristics likely to have induced such a decline only in this species. Thus it appears likely that winter wheat var. "Maris Huntsman" was just a poor host for *H. pseudorobustus* in the conditions of this experiment.

The populations of *T. dubius* in plots in which cereals were grown

from 1969 to 1972, and on all the plots after winter wheat, fluctuated around a ceiling population of 250 per c.c. of soil. Thus a change of cereal host or variety did not alter this ceiling population, yet on plots that were previously grass, the population declined, indicating that a higher ceiling population occurred on these hosts. T. dubius feeds on actively growing root-tips (Bridge and Hague, 1974) and thus the reason that a uniform ceiling population occurred on cereals, below the ceiling population that occurred on grass hosts, may be accounted for by the greater availability of feeding sites. In cereals, root growth declines after the spring flush (Troughton, 1962) but in a mixed grass plot, a longer growing cycle and greater volume of roots would provide a greater number of feeding sites. The ceiling population of H. schachtii (Jones, 1956) and other Heterodera species is thought to be caused by intraspecific competition for feeding sites and although the availability of feeding sites may limit the increase in numbers of T. dubius, an alternative limiting factor may be the inability of a percentage of the population of nematodes to find these sites.

Seinhorst (1966) derived the equation

$$Pf = aE\ Pi \left\{ (a - 1)\ Pi + E \right\}^{-1}$$

to describe the relationship between population increase and the density of low populations of migratory plant parasitic nematodes. Pf refers to the final population, Pi to the initial population, E the equilibrium population and a the rate of increase of the population. Accurate estimation of low values of Pi are difficult and Seinhorst achieved it by extracting soil with a high Pi and then diluting the soil with a sterilised soil to obtain known low values of Pi. In estimating low values of Pi for field populations, unless very large quantities of soil are extracted, it is inevitable that any population below 100 per 200 c.c.

of soil is a poor estimate of the population. In the second field experiment, the values of *a* for *H. pseudorobustus* and *T. dubius* were sufficiently large to bring all values of *Pi*, even the values that were recorded as zero, up to *E* in one growing season. Both *E* and *a* vary independently with the host type and environmental conditions (Seinhorst, 1967) and Seinhorst defined a good host as having a high value of *E* and *a*, and conversely a poor host a low value of *E* and *a*. A non-host he defined as having a value of *E* approaching 0 and a value of *a* less than zero. Thus as *H. pseudorobustus* increased on fallow plots from a low *Pi*, *a* was greater than 1 but *E* was very low. Thus winter wheat in these conditions was a poor host. The value of *E* for *T. dubius*, approximately 250 per 200 c.c. of soil was low when compared to the grass plots, and populations found on other hosts (Seinhorst, 1967). But, it represents a population greater than found typically in East Anglia (Saynor, 1972a). Also on fallow plots with a low *Pi*, a value of *a* greater than 1 occurred, and thus winter wheat in these conditions was an intermediaty or good host of *T. dubius*.

The suggestions that ectoparasitic nematodes induced root growth by the accumulative effect of their various feeding habits was not substantiated in this study. Only the genus *Tylenchorhynchus* seems likely to cause a yield loss in the populations that are found in field soils. In the trials with winter wheat and winter barley *T. dubius* was implicated in causing a reduction of yield. This result supports the finding by Sharma (1971) that a 50% reduction in growth of wheat occurred when 1,000 *T. dubius* were inoculated into 150 grms. of soil yet these results are contradicted by Bridge (1971) and Saynor (1972a) who recorded no effect on root growth of *Lolium perenne* or spring barley respectively. In addition Whitehead and Frazer (1972) found that *T. dubius* did not cause stunted growth of barley.

H. digonicus and H. pseudorobustus, at initial populations of 600 per 200 c.c. of soil and 1,600 per 200 c.c. of soil respectively, caused no stunting of cereal growth in the field trials. Likewise, the Pratylenchus spp. produced no disease symptoms, and thus it appears that vermiform endoparasitic nematodes that feed from the differentiated regions of roots, do not cause stunted growth in the field populations typically encountered.

Helicotylenchus spp. though they cannot be demonstrated to be pathogenic to cereals can build up to large populations. In addition, H. varicaudatus was demonstrated to be an endoparasite of wheat roots, at least in the initial growth stages of these roots. Therefore it was decided to investigate whether this is a typical habit for these nematodes on cereal and grass hosts, and to determine the effects feeding does have on the growth of cereal roots.

SECTION 3.

HOST PREFERENCE OF AND HISTOLOGICAL CHANGES INDUCED BY FOUR HELICOTYLENCHUS SPP. ON CEREALS AND GRASSES

3:1 Introduction.

Helicotylenchus nematodes are found in a wide range of soils, associated with the roots of many plants. In grasslands in South-East England, H. digonicus, H. pseudorobustus, H. varicaudatus and H. vulgaris were found commonly in clay and sandy soil (Bridge, 1971).

The catholic host range of the genus is indicated by H. pseudorobustus syn. microlobus, which has been shown to feed from the roots of 94 host plants ranging from Picea glauca, white spruce, to a selection of cereal and leguminous plants (Taylor, 1960). In addition H. pseudorobustus maintained a high population density in a rotation involving maize, oats, soybean and wheat (Ferris and Bernard, 1971a).

H. varicaudatus has been found in grassland soil where typically large populations were maintained (Bridge, 1971) and in woodland soil where numbers were locally high (Yuen Pick-Hoong, 1966). H. digonicus, H. varicaudatus and H. vulgaris have also been found in wheat rotations, where, in one soil, they were the most numerous plant parasitic nematodes (Corbett and Webb, 1970).

Thus, at the onset of this study, attempts were made to set up single species cultures of the four common Helicotylenchus nematodes, using wheat as a host plant, so that later, their pathogenicity to cereals could be assessed in pot experiments. These attempts were unsuccessful, and the cause of these failures was thought to be either:-

1) Incorrect soil type. Initially a coarse sandy soil, partially sterilised, was used. When this failed various other soils were used (e.g. a soil/peat/sand mixture) but these also were unsuccessful.

2) The repeated disturbance of the soil to monitor the population changes of the nematodes, or

3) The failure of the host plant, wheat, to act as a good host for these nematodes.

The first possibility was soon disccunted due to the failure of the various soils, and thus it was decided to set up a trial using soils already infested with one of the species to determine:-

1) The comparative suitability of cereal and grass varieties to act as hosts, and

2) The period taken for the nematodes to recover from the effects of soil disturbance, associated with the setting up of the experiment.

In conjunction with testing for host preference, the feeding habits, and relationships with grass and cereal hosts of these nematodes were studied. Helicotylenchus spp. are generally considered to be ectoparasites (Jones, 1972; Apt and Koike, 1962; Ferris and Bernard, 1971b) and have been described as feeding from these surface cells in a browsing manner (Yeates, 1971). Yet there are many reports of endoparasitic populations (Taylor, 1961; Blake, 1966; Churchill and Ruehle, 1971).

On cereal and grass hosts H. varicaudatus has been reported as feeding endoparasitically (Collen, 1972) and H. digonicus also fed endoparasitically on Poa pratensis (Perry et al., 1959). In both these instances, a cortical necrosis resulted from the feeding of the nematodes.

Thus the same four temperate nematodes studied in Section 3:2 for host suitability, were selected to determine:-

1) The position of the feeding nematodes in cereal and grass roots,

2) The extent of the lesion that develops in the cortex around the nematodes, and

3) The histological changes that can be associated with feeding.

3:2 Host Preference.

3:2:1 Materials and Methods.

Soil was collected from sites where known single species populations of the nematodes had been found. This soil was sieved using a 2 cm. sieve to remove coarse debris, and nematode numbers were assessed by extracting 200 c.c. of soil by the Whitehead tray method. Following a 24 hour extraction period, nematodes were collected by passing the extracts through three 53 μ copper sieves, then washing the nematodes into a plastic beaker. This method was used for all subsequent extractions. The nematodes were then counted using a 1 ml. aliquot of the extract in a Hawksley slide.

The sites, where the soils were obtained, were:-

1) Royston Golf Course, Cambridgeshire:- *H. digonicus* under mixed coarse grass.

2) Orchard Field, Silwood Park, Ascot:- *H. pseudorobustus* under turf grass.

3) Broombill Farm, Camber, Sussex:- *H. varicaudatus* under grazed pasture, and

4) Broadbalk Wilderness, Rothamsted, Herts.:- *H. vulgaris* under ungrazed mixed pasture.

The original population densities of *H. digonicus*, *H. pseudorobustus* and *H. varicaudatus* (Table 3:1) were reduced to a level from which it was considered increases would be more evident. This was achieved by incorporating into these original soils, sterilised sandy loam in amounts necessary to produce a population of between 200 - 500 nematodes per 200 c.c. of soil. The soils with the reduced nematode populations (Table 3:2) were used to assess host preferences. No soil mixing

was attempted with the H. vulgaris population, because the clay content of this soil would have prevented a thorough incorporation of the sterilised soil. The soil was measured into 100 c.c. amounts and placed in individual disposable plastic pots, "Vacopots". The experimental treatments were:-

1) Fallow,
2) Poa trivalis,
3) Lolium perenne S.24,
4) Festuca pratensis,
5) spring wheat var. "Cardinal",
6) winter wheat var. "Maris Huntsman",
7) spring barley var. "Maris Mink",
8) winter barley var. "Maris Otter",
9) spring oats var. "Mostyn",
10) winter oats var. "Pendrum".

The ten treatments were replicated five times except for the trial involving H. varicaudatus where four replicates were used.

The seeds of all hosts were soaked overnight to promote germination, and two cereal seeds were sown per treatment. The grass seeds were sown thickly on the soil surface. Following germination, the cereal seeds were thinned to one seedling per pot. The intervals between sowing and ending of the growth periods were 3, 6 and 9 months, and for each nematode, each time interval was performed as a separate fully randomised experiment. The trial involving H. vulgaris was started later than the others and thus only a 3 month experimental period was possible. The spring cereals, in the trials lasting 6 and 9 months, had to be re-sown after each 3 month period, whilst the grass and winter cereals were left undisturbed for the entire experiment. In the 9 month trial many of the winter cereals had died prior to harvest, but the pots were left undisturbed until the end of the experiment period. At the end of the experiments, the individual pots were cut open, and the entire contents, soil and cut roots were used for assessing final nematode numbers. The nematodes were extracted by the Whitehead tray method.

3:2:2 Results.

A comparison of the numbers of nematodes extracted from the original soils (Table 3:1, Appendix D, Table 1) and the tested soils (Table 3:2, Appendix D, Table 2) showed that the effects of soil mixing were unpredictable. The numbers of H. digonicus and H. pseudorobustus were reduced to the calculated values, but numbers of H. varicaudatus were reduced to a population size below the expected value. Effects on the remaining genera were also unpredictable with populations of Tylenchus spp. not declining in either of the soils containing H. digonicus and H. pseudorobustus. The soil containing H. varicaudatus showed an unexpected decline of all nematodes. This was attributed to physical damage of the nematodes during the sieving and mixing of the soil. These unpredictable changes were a further reason for avoiding soil mixing in the case of H. vulgaris populations.

Numbers of H. vulgaris recovered after 3 months were very low for all treatments (Table 3:3; Appendix D, Table 3). The highest recovery was from Festuca pratensis where less than 10% of the original population was recovered. The poor survival of H. vulgaris was caused by the repeated waterlogging of this soil in the small pots. This would have had the effect of washing the nematodes out of the soil, as well as preventing active root penetration of surviving nematodes.

In some of the other treatments, no Helicotylenchus nematodes were recovered (Table 3:3; Appendix D, Tables 4 - 6). This was particularly true for H. varicaudatus. This could have been due to unsuitable hosts, but the irregular occurrence of this failure (e.g. H. varicaudatus on winter wheat, where at 9 months no nematodes were recovered, yet at 6 months, a population four times the size of the fallow treatment occurred) suggests that the cause may have been the poor mixing of the sterile and

Table 3:1

Original soil populations. Nos./200 c.c. of soil

Sample site and sp. of Helocotylenchus	Tylenchus sp.	Tylenchorhynchus	Paratylenchus	Pratylenchus	Helicotylenchus	Rotylenchus	Non Plant Parasites
Royston							
H. digonicus	242	172	1,086	-	2,042	-	7,445
Ascot							
H. pseudorobustus	235	415	3,380	107	1,552	-	3,085
Broomhill Farm							
H. varicaudatus	755	68	550	-	526	-	4,258
Broadbalk							
H. vulgaris	67	57	370	32	485	205	1,142

Table 3:2

Tested soil populations. Nos./100 c.c. of soil

Sample and sp. of Helicotylenchus	Sterile: Infested	Tylenchus	Tylenchorhynchus	Paratylenchus	Pratylenchus	Helicotylenchus	Rotylenchus	Non Plant Parasite
H. dignonicus	4:1	125	31	169	-	217	-	941
H. pseudorobustus	2:1	207	199	719	15	314	-	844
H. varicaudatus	1:1	126	55	92	-	64	-	590
H. vulgaris	0:1	33	28	1,885	16	242	102	571

Table 3:3

Host preference of Helicotylenchus spp.
on Cereals and Grasses. Mean Nos./100 c.c. of soil

Treatments	Sampling Dates Months.									
	H. digonicus			H. pseudo-robustus			H. vari-caudatus			H. vulgaris
	3	6	9	3	6	9	3	6	9	3
Fallow	54	35	32	34	48	108	35	22	20	6
Poa trivalis	64	279	752	164	412	504	20	57	142	8
Festuca pratensis	30	191	214	42	532	696	30	50	40	0
Lolium perenne	80	231	374	114	556	814	30	0	120	28
Spring Wheat	72	30	126	300	400	748	42	55	12	0
Winter Wheat	128	118	244	312	382	946	27	80	0	6
Spring Barley	154	53	152	188	174	1,892	102	20	95	8
Winter Barley	88	202	224	104	274	588	45	89	80	16
Spring oats	90	23	130	110	84	450	30	26	50	24
Winter oats	138	272	686	142	432	1,080	25	27	30	0
Grass Hosts	58	234	447	107	500	671	27	36	101	
Cereal Hosts	112	116	260	193	291	951	45	49	44	
Signif-icance	N.S.	x	x	x	N.S.	xx	N.S.	N.S.	x	N.S.
L.S.D. at 5%	-	105	274	112	-	406	-	-	44	-

and infested soil.

The populations recovered from the fallow treatments (Table 3:4) showed that after a rapid decline of between 50% and 90% in the first

Table 3:4

Means of nematodes in Fallow treatments

Nematode	Extraction Counts, nos. per 100 c.c. of soil			
	Initial population	3 months	6 months	9 months
H. digonicus	217	54	25	32
H. pseudorobustus	64	35	22	20
H. varicaudatus	314	34	48	108
H. vulgaris	242	6	-	-

3 months, the populations then stabilised. This stabilisation suggested that the decline up to 3 months was not caused entirely by starvation of the nematodes, and thus this initial decline was attributed again to soil disturbance. This disturbance could affect the nematodes either by direct physical damage or by disrupting the soil aggregates so that the nematodes are deprived of suitable pore spaces.

The stabilisation of numbers that occurred at 6 months indicated that the nematodes are able to survive extended periods of starvation, and in fact after 9 months numbers of H. pseudorobustus increased threefold from the 3 month population. A similar increase has been reported previously, when H. pseudorobustus increased tenfold in nine months (Churchill and Ruehle, 1971). If such increases actually occurred in the individual pots, it indicates that either dormant eggs existed in the soil, that later hatched, or adult non-feeding females laid eggs that subsequently hatched. No evidence exists for either of these possibilities, and the explanation of this apparent increase is

probably that poor soil mixing resulted in pockets of soil containing large numbers of H. pseudorobustus.

The initial decline in the fallow treatments was mirrored in the treatments containing plant hosts, but the decline was not as great. If the decline was caused by physical disruption, an even decline would be expected and thus the slight increases of the plant treatments when compared to the fallow treatments indicates that the populations had started to feed and reproduce by 3 months.

After 3 months, numbers of Helicotylenchus nematodes were greater on cereal hosts (Table 3:3) and this earlier recovery was linked with a quicker growth of cereal roots allowing the soil populations to start building up. After 6 and 9 months, numbers of H. pseudorobustus were still greater on the cereals yet numbers of H. digonicus and H. varicaudatus had built up to higher numbers on the grass hosts (Table 3:3).

The numbers of H. digonicus and H. pseudorobustus recovered after 9 months indicated that feeding and reproduction had occurred on all plant treatments, and thus all are hosts for these nematodes. The recovery of H. varicaudatus from all treatments was very variable, but population increases did occur for all plant hosts at either the 6 or 9 month sampling, and thus all plants did act as hosts. Significant differences did exist between treatments for the three species at the 9 month sampling, and also at the 3 month sampling for H. pseudorobustus and the 6 month sampling for H. digonicus (Table 3:3). Yet when the plant treatments are compared to the fallow treatments at 9 months, only 3 plant hosts for H. digonicus, four hosts for H. varicaudatus and 7 hosts for H. pseudorobustus are significantly different from the fallow treatment. This does not mean that the other plants are non-hosts, since at some time all hosts supported a population build up, but it clearly shows that there are a range of preferences for the

Table 3:5

Host preference. 3 most favoured and 3 least favoured hosts

Nematode	Favoured Hosts	Final Population per 100 c.c. soil	Disfavoured Hosts	Final Population per 100 c.c. soil
H. digonicus	*Poa trivalis*	752	Spring Wheat	72
	Winter Oats	686	Spring Oats	90
	Festuca pratensis	314	Spring Barley	154
H. pseudorobustus	Spring Barley	1,892	Spring Oats	450
	Winter Oats	1,080	*Poa trivalis*	504
	Winter Barley	946	Winter Barley	588
H. varicaudatus	*Poa trivalis*	142	Winter Wheat	0
	Festuca pratensis	120	Spring Wheat	12
	Spring Barley	95	Winter Oats	30

separate hosts. If the three most favoured and three least favoured hosts are compared for the three species (Table 3:5), the similarity of *H. digonicus* and *H. varicaudatus* in favouring the grass hosts *Poa trivalis* and *Festuca pratensis* is evident, whereas *Poa trivalis* is one of the least favoured hosts of *H. pseudorobustus*. *H. pseudorobustus* in fact, shows a marked preference for cereal hosts. There is no evidence that any species either favours or does not favour spring or winter cereal varieties, or that a species has a preference for one of the cereal types, be it barley, oats or wheat.

The number of nematodes of other genera in the soils are listed in Appendix D, Tables 7 - 10. In the soil from Royston containing H. digonicus, the numbers of Paratylenchus and Tylenchus spp. declined up till 3 months (Fig. 3:1; Fig. 3:2). Thereafter on specific hosts, numbers stabilised and increased. Both genera favoured grass hosts particularly Poa trivalis and Lolium perenne. In addition Paratylenchus spp. favoured winter oats as a host. The numbers of these nematodes on the remaining hosts and the fallow treatment continued to decline up till the 9 months sampling.

The nematodes in the soil from Broomhill Farm containing H. varicaudatus had the same initial decline and for Tylenchus spp. (Fig. 3:3) and Tylenchorhynchus spp. (Fig. 3:4) the decline continued on to the 9 month sampling. Thus for the nematodes in the conditions offered in these experiments, the plants acted as non-hosts. Paratylenchus spp. (Fig. 3:5) declined only slightly on the fallow treatment after 9 months and all plant treatment at one sampling produced populations greater than the fallow treatment, yet no one plant was consistently favourable. There was a trend however for grass hosts to support larger populations than cereal hosts.

In the soil from Silwood Park containing H. pseudorobustus, Paratylenchus spp. declined up to 3 months and only very low populations survived to 6 and 9 months. The Tylenchus spp. (Fig. 3:6) and Tylenchorhynchus dubius (Fig. 3:7) increased on the majority of plant treatments when compared to the fallow treatments. No plant was consistently favoured by Tylenchus spp., though again grass hosts supported larger populations than cereal hosts. The hosts on which the largest populations of T. dubius built up were Poa trivalis, winter oats and spring oats.

If all the nematodes are considered, most plants acted as hosts to most nematodes, but in many cases certain nematodes showed definite preferences. Broadly, grass hosts supported larger populations than cereal hosts, except for H. pseudorobustus. But the individual preferences indicated, implies only that such preferences existed in the experimental conditions. In field conditions, many variables may interact to produce a very different host preference, and in grassland situations where a variety of root types are available, a specific preference may be only transitory.

Fig. 3 : 1 Tylenchus spp. Royston.

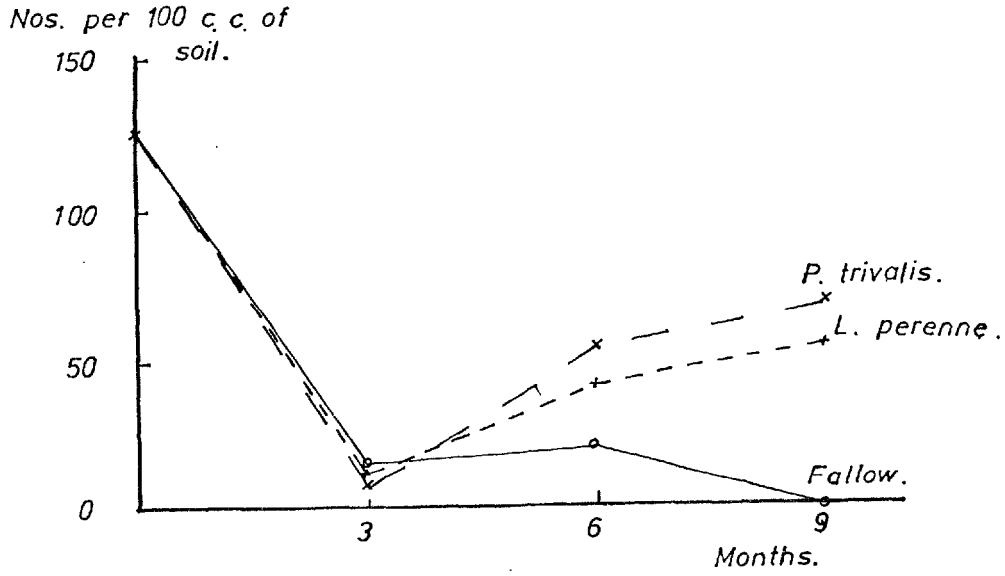


Fig. 3 : 2 Paratylenchus spp. Royston.

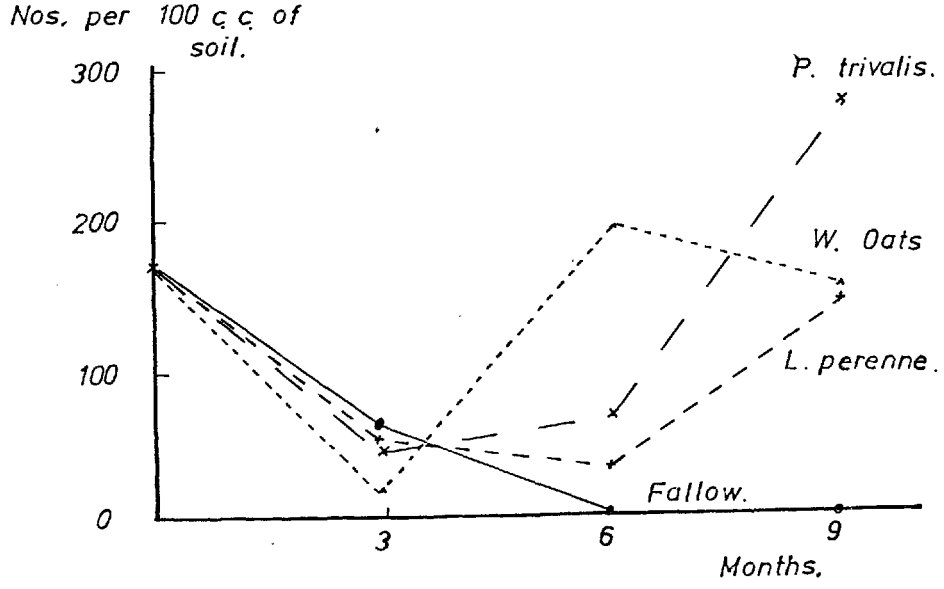


Fig. 3 : 3 Tylenchus spp. Broomhill.

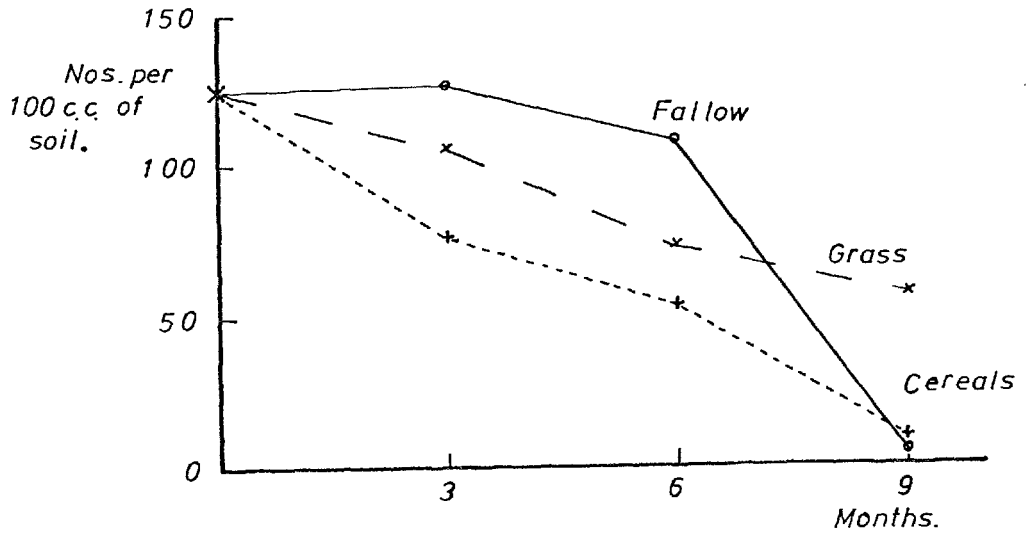


Fig. 3 : 4 Tylenchorhynchus spp. Broomhill.

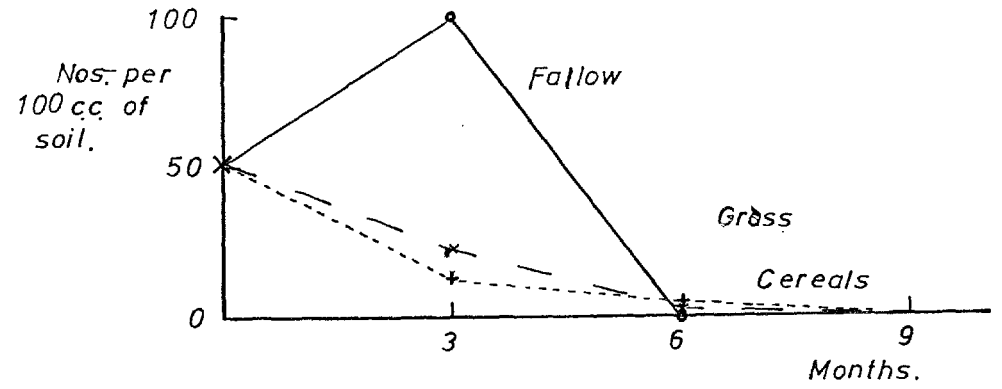


Fig. 3 : 5 Paratylenchus spp. Broomhill.

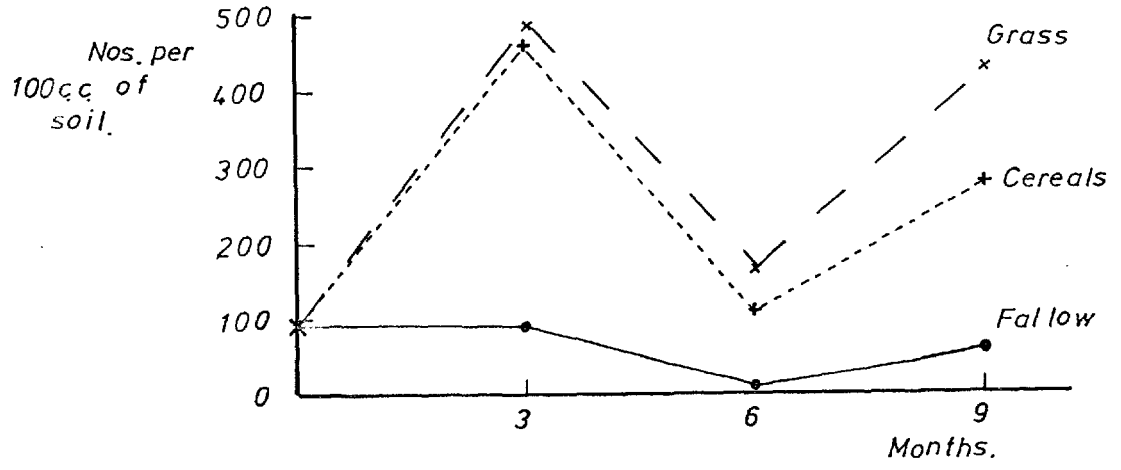


Fig. 3 : 6 Tylenchus spp. Ascot.

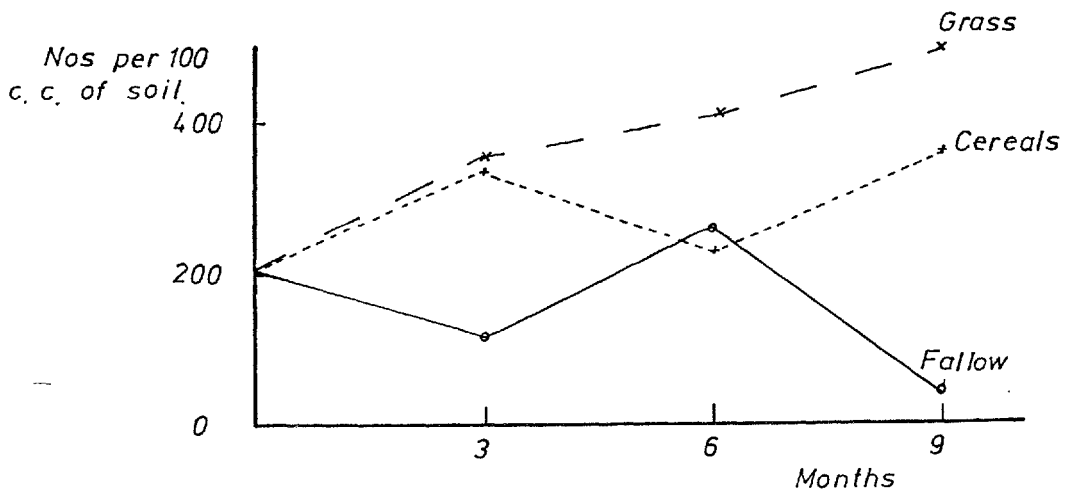


Fig. 3 : 7 Tylenchorhynchus dubius Ascot.

3:3 Histological Changes.

3:3:1 Materials and Methods.

The histological changes due to feeding of these temperate Helicotylenchus species were examined by preparing whole mounts and sections of infested roots. The infested roots were obtained by growing the hosts in soil from the same sites as used for the host preference assessment. The nematodes present in these soils (Table 3:1) and the hosts used were also the same.

Seeds, soaked overnight, were sown singly in pots containing 100 c.c. of soil. Four pots of each host type were prepared, but the roots of one plant generally provided sufficient infested material. The plants were allowed to grow for one month in a heated glasshouse, after which the roots of the host plants were carefully separated from the soil and washed free of adhering dirt. These roots were then dropped into boiling fixative, F.A.A.,

95% ethanol	20 ml.
Formalin	6 ml.
Glacial acetic acid	1 ml.
Distilled water	40 ml.

to kill and fix the nematodes in their feeding positions. The roots of the cereal hosts were then examined, immersed in F.A.A. by using a dissecting microscope, and root pieces containing semi-endoparasitic nematodes were cut away and placed in fresh F.A.A., for completion of fixation.

Lactophenol staining.

The remaining roots of the cereal hosts and all the roots of the grass hosts were stained to differentiate the remaining nematodes. The stain used was cotton blue, and the method used involved placing the roots in a small muslin bag that was carefully lowered into nearly boiling 0.1%

cotton blue in lactophenol. The bag, containing the roots, was left in the stain for 3 minutes when it was removed and the excess stain washed out. The roots were then removed from the bag and given a further washing before they were placed in a beaker of clear lactophenol. The roots were left in the clear lactophenol for 2 days to destain the roots, when the differentiated namatodes could be clearly seen.

The whole of the root system of each host was then examined under a dissecting microscope and the typical feeding positions of the nematodes recorded. Root pieces containing stained nematodes were cut and placed on microscope slides in clear lactophenol as a permanent record of the feeding sites.

Root Sectioning.

Further root pieces of the cereal hosts were added to the unstained segments, if insufficient numbers of unstained semi-endoparasitic nematodes had been found. At least five separate nematodes within roots were sectioned for each species on each of the six cereal hosts.

These root pieces were then processed for serial sectioning and staining. The procedure used was:-

1) Fixation in F.A.A. 24 hours.
2) Dehydration in firstly Methyl cellusol followed by Absolute Alcohol.
3) Infiltration and embedding in 56° Paraffin wax or Paraplast.

Paraplast was the preferred embedding material, and was used for all sectioning, except for a few initial blocks. The paraplast was preferred due to the smaller crystals that this material formed, allowing thinner sections to be cut. Paraffin blocks were cut at 12 μ, whereas paraplast blocks were cut at 8 μ on a Reichert rotary microtome.

The sections were stained in Weighert's haematoxylin as outlined by Feder and O'Brien (1968), and mounted in Canada Balsam.

3:3:2 Results and Discussion.

The roots grown in the H. vulgaris infested soil, were not invaded by the nematodes, despite careful attempts not to waterlog the soil.

Whole root mounts.

The nematodes, H. digonicus, H. pseudorobustus and H. varicaudatus fed as endoparasites and semi-endoparasites on primary and lateral seminal roots. The nematodes generally fed singly, but on occasions a group of nematodes fed from a limited area of root (Plate 3.). The nematodes had invaded the roots of all hosts except:-

1) Poa trivalis, which was not parasitised by H. varicaudatus or H. pseudorobustus, and
2) Lolium perenne which was not parasitised by H. digonicus.

These results conflict with the host preference results and are thought to have arisen from the immaturity of these grass roots after one month's growth.

The nematodes invaded the roots in all fully developed regions; no nematodes were found in the root-tip, the zone of differentiation or elongation. In cereals mainly the seminal roots were invaded, as after only one month, the nodal roots were only partially developed. The nematodes were typically lying completely within cells of cereal hosts though on all hosts some nematodes were found in a semi-endoparasitic position.

The numbers of nematodes found in the grass roots were less than in the cereal roots, probably also due to the slow growth of these roots, and thus several plants of each host were examined to find feeding individuals. Again nematodes fed as endoparasites (Plate 4.) on the fully developed areas of the grass roots, but semi-endoparasitic nematodes

were more frequent than on cereal roots. The cause of this was felt to be that the grass roots had a thinner cortex than cereal roots, and nematodes are thus more likely to have their body protruding from the root.

Eggs were found within cortical cells of both cereal and grass roots, frequently in clusters, referred to as "nests" (Plate 5.). These "nests" were found either free in cells, or associated with one or a group of females. Associated with nematodes, and their eggs, the root cortex was occasionally swollen (Plates 4 and 5.). In addition, cortical lesions were found around nematodes, and these were evident as areas of cell walls that retained the cotton blue stain. These lesions were present around approximately half the feeding nematodes.

The typical position of nematodes in the cortex was for the bulk of the nematode's body to be coiled within one cell, and the head end to pass from that cell through adjacent cortical cells towards the central stele of the root (Plate 6.). The cell adjacent to the nematode's head was on occasions filled with a granular material (Plate 7.), but the actual type of cells from which a nematode fed, could not be discerned from these mounts of root pieces.

Sectioned Cereal Roots.

The positioning of the nematode's body in cortical cells was confirmed by the root sectioning (Plate 8.). The posterior regions of the nematode generally laid coiled in the outer cortical cells, or outstretched along a series of such cells. Occasionally, the cell walls of adjacent cells collapsed, so that the nematodes were positioned in small cavities in the cortex. Frequently eggs were found in such cavities. The anterior region of the body passed from this area inwards so that the head of the nematode was positioned next to the feeding cell (Plates 8, 12 and 14.). The feeding cell was either a cortical cell adjacent to

the endodermis (Plates 9 and 10.), an endodermal cell (Plates 8, 11 and 12.), or a pericyclic cell (Plates 13 and 14.). The cell walls of the cells through which the anterior end of the nematode passed, were ruptured and contracted away from the nematode's body but no cell wall thickening, nor an extensive cortical cell collapse occurred (Plates 8, 12 and 14.). These cells generally maintained their shape despite the presence of the nematodes.

The nematode's head was always observed outside the feeding cell (Plates 8 to 14.), with only the stylet protruded through the cell wall into the cytoplasm of the feeding cell (Plate 13.). The changes in these feeding cells ranged from cells with no apparent alteration in contents, to cells filled either partially or wholly with a dark staining material. The latter distinction, between cells partially or wholly filled, was more a reflection of the origin of the feeding cell, in that pericyclic feeding cells (Plates 13 and 14.), produced a more marked reaction than either endodermal or cortical feeding cells.

The cellular changes extended beyond the feeding cell, and involved a group of 2 or 3 cells around the central cell. This spread only involved pericyclic cells when the feeding cell was in the pericyclic layer, but the spread of the lesion from endodermal and cortical cells was laterally andinwardly and thus did involve other cell types. The dark material in the feeding cells appeared to be derived from the cell cytoplasm, and thus represents an enlargement of this normally thin layer at the expense of the central vacuole. On some preparations though (Plate 14.), this material could not be distinguished from the material surrounding the nematode's body and which obviously was not within the feeding cell. Nuclei were occasionally seen in the cytoplasm of the feeding cell and adjacent cells, but no nuclear or cellular enlargement was seen.

In some feeding cells, a knob like swelling of the cell wall occurred that surrounded the stylet where it passed into the feeding cell (Plate 13.). This was only seen in pericyclic cells. The nematodes did not normally enter pericyclic cells, and thus invasion was limited by the inner endodermal cell wall, but once the head of a H. varicaudatus nematode, in spring barley roots, laid in either a pericyclic cell, or a protoxylem pole cell (Plate 14.). The actual feeding cell in this instance was also unclear, but it appeared to be an adjacent pericyclic cell in which a cell wall knob occurred. At no time were nematodes observed to feed from either phloem or xylem cells.

Endodermal cells were most frequently used as the feeding cells of all three species, but H. digonicus and H. pseudorobustus fed more frequently from cortical cells than H. varicaudatus, and conversely, H. varicaudatus had a greater tendency to penetrate into the central stele and feed from the pericyclic cells.

Differences also existed between the host response to the presence of the nematodes. Both oat varieties, "Mostyn" and "Pendrum", developed a greater cortical lesion than the other cereal varieties. The lesion, that was still only evident as an altered staining reaction of cell walls, was not restricted to the outer cortical cells, but also surrounded the nematode's anterior end in the inner cortical cells. Also the barley variety "Maris Mink" had a greater frequency of pericyclic feeding cells than the other hosts. These differences can only be considered as tentative conclusions in that only a limited number of nematodes were sectioned on each host.

Plate 3.

H. varicaudatus nematodes in spring oat roots. The nematodes are present in a localised group with lesions around some nematodes and no lesion around others. (x120)

Plate 4.

H. varicaudatus in *Festuca pratensis* roots. Note the slight swelling of the cortex and the two eggs lying within coils of the nematodes' body. (x300)

Plate 5.

Egg "Nest". Ten eggs around a female, with a slight swelling of the cortex. (x350)

Plate 3.

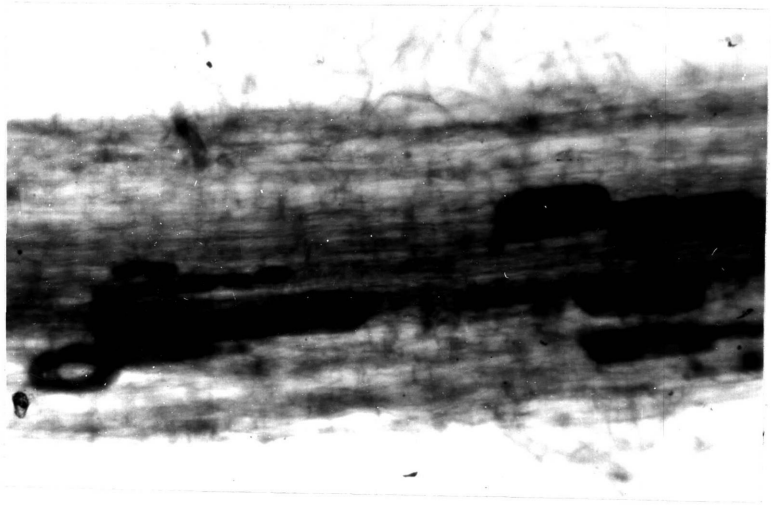


Plate 4.

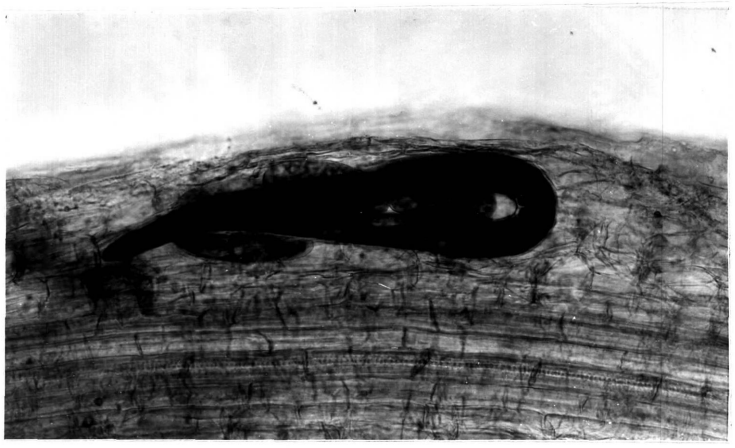


Plate 5.

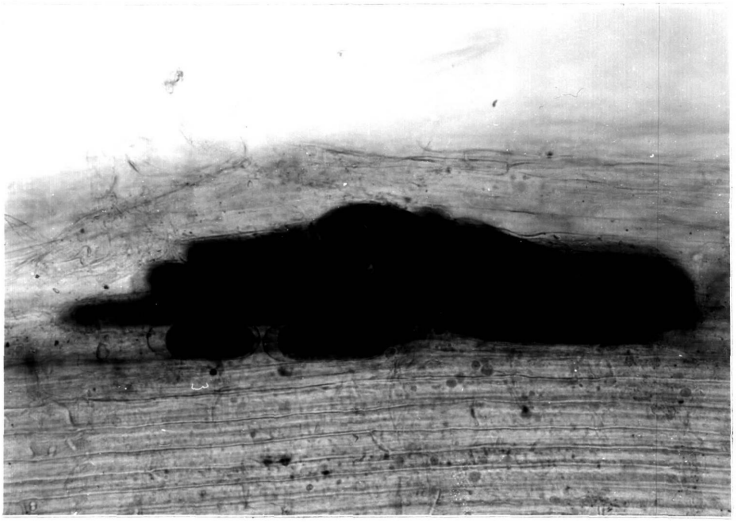


Plate 6.

H. pseudorobustus endoparasitic in winter oat root. Note the line of eggs within the roots, and the lack of any lesion around the nematode's body. (x1,100)

Plate 7.

Head end of H. pseudorobustus in winter oat roots. Note the granular contents of the cell adjacent to the nematode's head. (x3,500)

Plate 6.

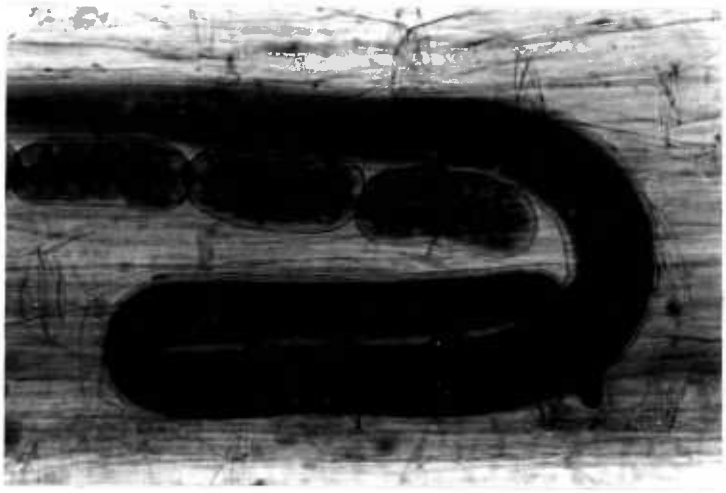


Plate 7.

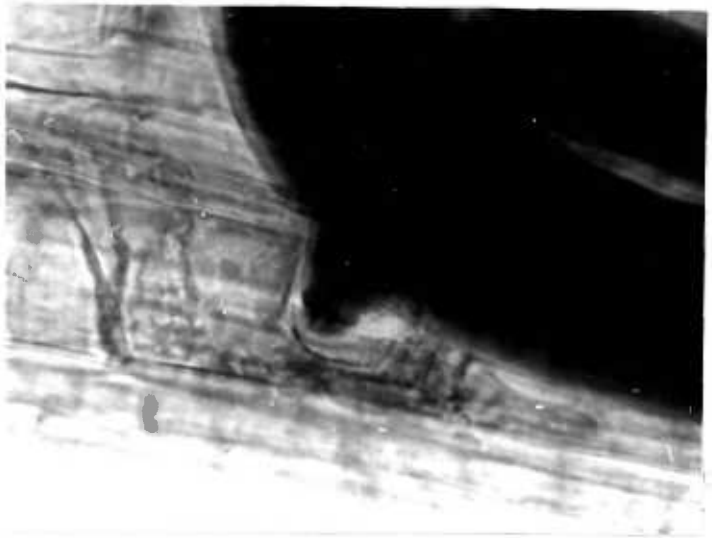


Plate 8.

H. pseudororobustus endoparasitic in winter oat roots. The nematode has penetrated into a cortical cell, and the endodermal feeding cell contains a region of dark staining material. The nematode's body lies in coils in a cortical cell. No alteration other than cell wall rupturing is evident in cortical cells. (x1,500)

Plate 8.

Plate 9.

Cortical feeding cell of *H. digonicus* in spring oats. The cortical cell contains a granular material assumed to be an enlarged cytoplasmic layer. (x1,400)

Plate 10.

Cortical feeding cell of *H. digonicus* in winter oats. The cortical cell contains a region of dense cytoplasm, surrounded by bead like material (compare Plate 7.). (x1,400)

Plate 9.

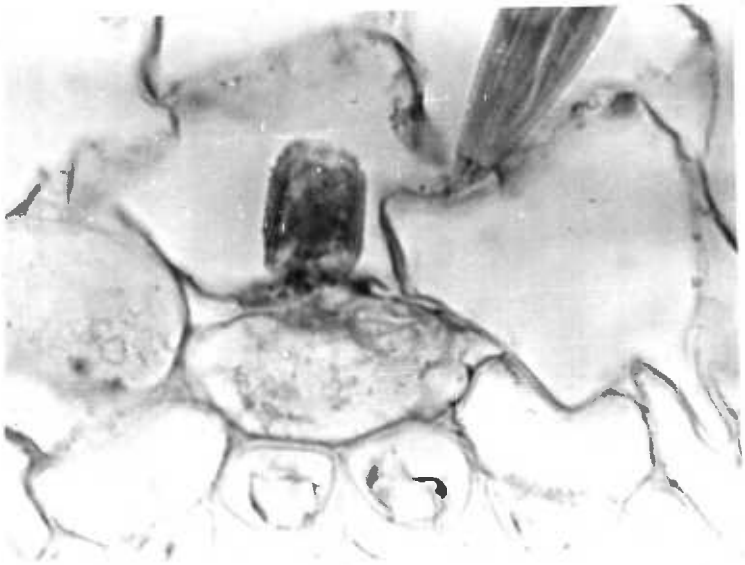


Plate 10.

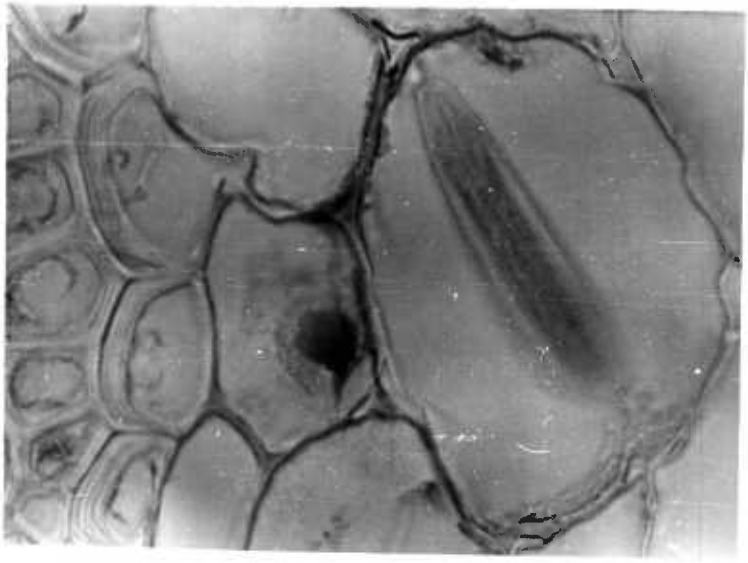


Plate 11.

Endodermal feeding cell in lateral root of winter wheat. The nematode's head lies in a cortical cell, and the adjacent endodermal cell is partially filled with an enlarged cytoplasm. (x500)

Plate 12.

Endodermal feeding cell, H. digonicus in spring oats. Note the dark staining cell walls around the nematode's body. These areas represent deposits on the cell wall and not a local thickening of cell walls. (x1,400)

Plate 11.

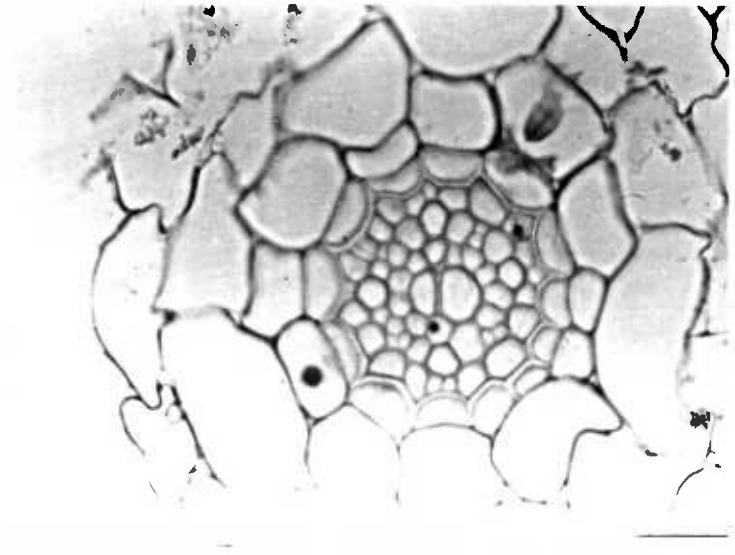


Plate 12.

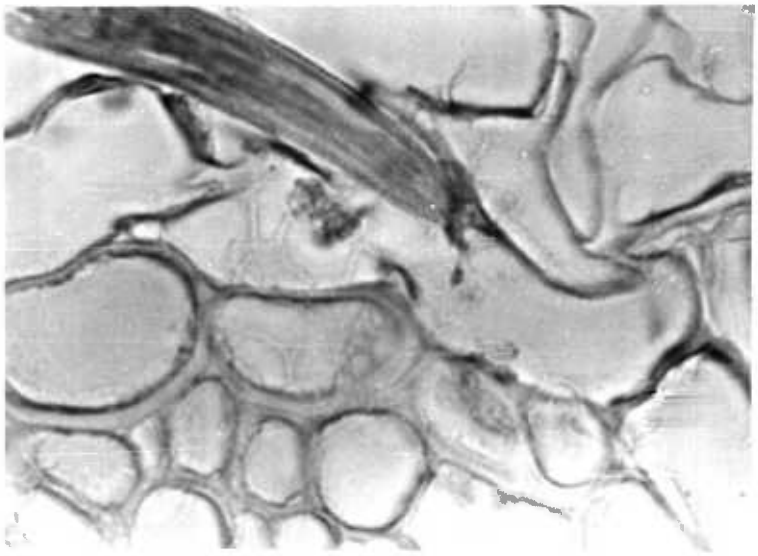


Plate 13.

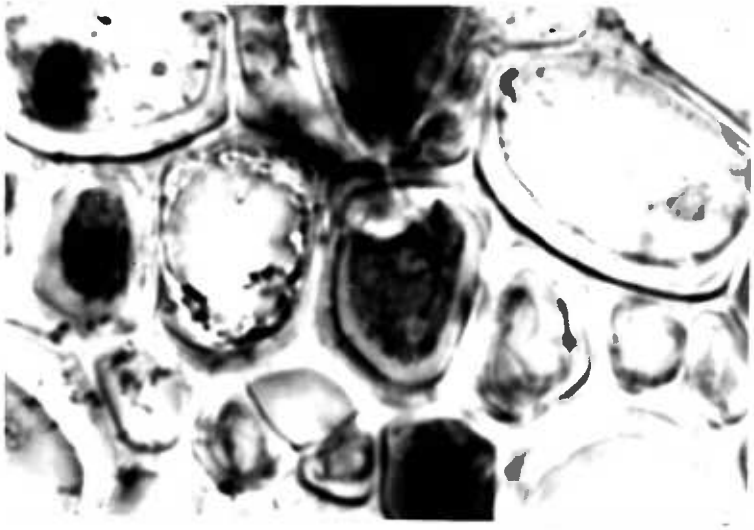
Pericyclic feeding cell. H. varicaudatus in spring barley. The pericyclic cell is filled with cytoplasm and the nematode's stylet passes through the cell wall, and the cell wall knob into this cytoplasm. (x3,000)

Plate 14.

Pericyclic feeding cell. H. varicaudatus in spring barley. The nematode's head appears to lie in the protoxylem pole cell, but the adjacent pericyclic cell appears to be the feeding cell. Note the extensive deposits on cell walls around the nematode's body. (x1,600)

Plate 13.

Plate 14.



3:4 Discussion.

After nine months, the numbers of the Helicotylenchus spp. recovered from the various treatments, differed significantly. If the final soil populations are compared to the initial population, the greatest gain was a sixfold increase of H. pseudorobustus on spring barley. The greatest gain of H. digonicus was fourfold and of H. varicaudatus only a doubling of the original population occurred. In comparison to other reports, these increases are low. H. pseudorobustus, on sycamore increased 350 fold in 9 months (Churchill and Ruehle, 1971) and on tomato a tenfold increase in 40 days at 90°F. occurred (Taylor, 1961).

If the increases of nematode numbers over 9 months is related to the fall in population that occurred in the first three months, then the cause of the failures in culturing are evident. It was simply that the nematode populations were not allowed to recover from the effects of one handling, before the soil was again disturbed. Thus the low fecundity of the nematodes in the conditions offered, prevented a build up of high numbers.

"Nests" of eggs around feeding females have been observed on bananas, H. multincinctus (Zuckerman and Strich-Harari, 1963) and on cereals and grasses, H. varicaudatus (Collen, pers. comm.). On this occasion, clusters containing up to ten eggs were seen, and thus it is clear that in one month, one female is able to produce at least this number, if not more, of eggs. If all these eggs hatched and developed through to adults in approximately a further month, (the typical generation time of Helicotylenchus species at 25°C. ranges from 25 -40 days (Saxena et al., 1973; Taylor, 1961)), then a population increase many times that observed in the host preference trial is possible, in spite of the poor survival of nematodes at the onset of these trials. The failure of this potential increase to occur, could

have been because:-

1) many of the eggs did not hatch, or that there was a high larval death rate,

2) larval development in these conditions was slower than observed for other species in other conditions,

3) a large fraction of the soil population remained inactive in the soil, not attracted to the hosts.

The first two possibilities seem unlikely, whereas if the latter reason applied, the higher population build-up of H. pseudorobustus on sycamore and tomato, could only occur if these roots attracted a larger fraction of the population to feed and reproduce.

The roots containing egg "nests" of H. multicinctus were discoloured (Zuckerman and Strich-Harari, 1963). In unstained cereal and grass roots no discolouration was seen, but lesions evident as cell walls retaining the cotton blue stain occurred. Such lesions were found mainly around the posterior regions of the nematodes, and did not spread into the deeper cortical cells except in the oat hosts. In serial sections, this cell wall lesion appeared as a deposit on the cell wall, though superficially it resembled a thickening of the cell wall. In fact no general wall thickening occurred as was reported for H. dihystera in cortical cells of soybean roots (Orbin, 1973).

The slight swelling of the cortex around nematodes and their eggs, also occurred in maize roots parasitised by H. pseudorobustus (Taylor, 1961). The cause of this swelling in cereal roots was neither hyperplasia nor hypertrophy, but was due to the formation of cavities in the cortex that contained single or groups of nematodes and their eggs. The local abundance of nematodes led to a physical enlargement of the cells and cavities, thus resulting in a swelling of the cortex. H. digonicus (Perry et al., 1959) and H. varicaudatus (Collen, 1972) have been observed

to cause necrosis and loss of the cortex of grasses. Groups of these nematodes were seen associated with areas of cereal roots where the cortex was shrivelled, but there was no indication that the feeding of these nematodes induced the cortical loss. The greatest disruption seen in the sectioned roots was the formation of cavities around the namtodes.

The changes in the feeding cell differed from the changes involved in the cell wall lesion around the posterior regions of the nematode. The granular material seen in a whole root mount (Plate 7.) reflected the changes that were evident in the root sections (Plates 8 - 14.). The feeding cells contained an enlarged cytoplasmic layer and a consequently reduced central vacuole. The nematode's stylet passed through the cell wall into the feeding cell cytoplasm. The knob like enlargement of the cell wall surrounding the stylet in pericyclic cells (Plate 13.), has been seen in cells fed from by Pratylenchus fallax (Corbett, 1972) and Rotylenchulus reniformis (Razak, 1975) though in neither of these reports was the nematode's stylet seen to pass through the cell wall knob, into the host cell cytoplasm.

H. digonicus was reported to feed from phlcem and possibly xylem cells of Poa pratensis (Perry et al., 1959). At no time was this species, or H. pseudorobustus and H. varicaudatus positively identified as feeding directly from phloem or xylem cells. It is likely though that cells of the phloem or xylem were involved in the feeding lesion.

If the host preferences, and the histological changes at the feeding sites of these three species are compared, (Table 3:6) H. varicaudatus differed from H. digonicus and H. pseudorobustus in that it more commonly induced a pericyclic feeding lesion. Since the reaction associated with pericyclic feeding cells was greater than occurred in cortical or endodermal feeding cells, H. varicaudatus produced a more pronounced lesion

Table 3:6

Summary of Feeding Characteristics of H. digonicus, H. pseudorobustus and H. varicaudatus

Nematode Characteristic	H. digonicus	H. pseudorobustus	H. varicaudatus
Endoparasite	Typical	Typical	Typical
Semi-endoparasite	Uncommon	Uncommon	Uncommon
Egg nests	Common	Common	Common
Cortical feeding cell	Common	Common	Uncommon
Endodermal feeding cell	Typical	Typical	Typical
Pericyclic feeding cell	Uncommon	Uncommon	Common
Occurrence in mixed populations	Frequent	Rare	Frequent
Host preferences	Grasses	Cereals	Grasses

than either of the other two species. This difference may be due to H. varicaudatus penetrating to pericyclic cells earlier than the remaining species, or that H. digonicus and H. pseudorobustus are able to feed and reproduce from endodermal and cortical cells without the need to penetrate through the thickened endodermal inner cell wall. Though H. digonicus feeds from similar cells to H. pseudorobustus, H. digonicus has a similar host preference as H. varicaudatus. Also these two species are commonly found as mixed populations whereas H. pseudorobustus is generally found as a single species population (Bridge, 1971). These conflicting results may be due to the low population of H. varicaudatus in the host preference tests, but still in terms of occurrence in field soils, H. digonicus and H. varicaudatus are linked. Thus it does seem that the conflicting results are real, and

that *H. digonicus* and *H. varicaudatus* do favour similar soils and hosts, but once in host roots, the two species feed in different sites and therefore do not occupy the same ecological niche.

SECTION 4.

POT EXPERIMENTS ON PATHOGENICITY

4:1 Introduction.

In field experiments (Section 2), the growth of winter cereals was not adversely affected by populations of either H. digonicus or H. pseudorobustus. Yet some species of this genus have been implicated in causing disease symptoms in field crops. Various Helicotylenchus spp. have been found around banana and plantain roots, and in sub-optimal growing regions e.g. Israel, Cyprus and Canary Islands, they have been shown to cause yield losses (Strover, 1972). In the Jordan Valley, Israel, H. multincinctus built up to large populations on bananas and caused a decline in productivity of banana plantations, such that after 3 years an uneconomic yield was produced (Minz et al., 1960). On cacao H. erythrinae was associated with unthrifty growth (Tarjan and Jimenez, 1973) and high densities of H. dihystera were correlated with patchy growth of turfgrass (Wallace, 1971).

In pot experiments, there are many reports of species being pathogenic to plants. The typical symptoms of disease are shoot stunting and a reduced root volume. H. dihystera has most frequently been implicated and has been reported to damage sugarcane (Apt and Koike, 1962), carnations (Stewart and Schindler, 1956), tomatoes (Libman et al., 1964), olives (Diab and El-Eraki, 1968) and pines (Ruehle, 1975). H. digonicus on Poa trivalis (Perry et al., 1959), H. elegans on tomatoes (Saxena et al., 1973) and H. pseudorobustus on sycamore (Churchill and Ruehle, 1971) also caused stunted growth of either shoots or roots of their hosts.

As well as causing stunted growth, Helicotylenchus spp. have been reported to cause other disease symptoms. H. dihystera caused a delay in the development of maize in the first two weeks of growth, from which at

low inoculum levels the plants recovered (Sledge, 1956). H. elegans at a population of 1,000 per 500 c.c. of soil reduced flowering of tomatoes and at 10,000 per 500 c.c. of soil inhibited flowering completely (Saxena et al., 1973).

Conversely, an incoulum of 25,000 H. dihystera per 12 c.m. pot did not affect root or shoot growth of sycamore after four months, yet sycamore proved to be an excellent host (Churchill and Ruehle, 1971), and in glasshouse soils, Helicotylenchus spp. did not produce signs of disease on cucumbers or tomatoes (Johnson and Boekhoven, 1969). These conflicting results in which one species caused stunted growth on some hosts and not on others could be due to some hosts tolerating a population that on the other hosts would be pathogenic. Alternatively, biological races varying in their pathogenicity may exist.

In field soils, disease symptoms appear only when a susceptible host is introduced to a large soil population, or if a large population is able to build up on that host over a prolonged period. H. digonicus (Section 2) in the nematicidal trial had an initial population similar to that found in many arable soils, and this population remained relatively stable during the trial. Initial numbers of H. pseudorobustus in the winter wheat experiment (Section 2) represented a large population with which the tolerance of cereals could be tested. Unfortunately the wheat was a very poor host, and in the conditions of the trial seemed to be resistant to the nematode.

Thus pot experiments were made to test the pathogenicity of wheat to Helicotylenchus species, to determine whether wheat is able to tolerate large populations, or whether large populations reduce plant vigour.

4:2 Soil Inoculation Experiments.

4:2:1 Materials and Methods.

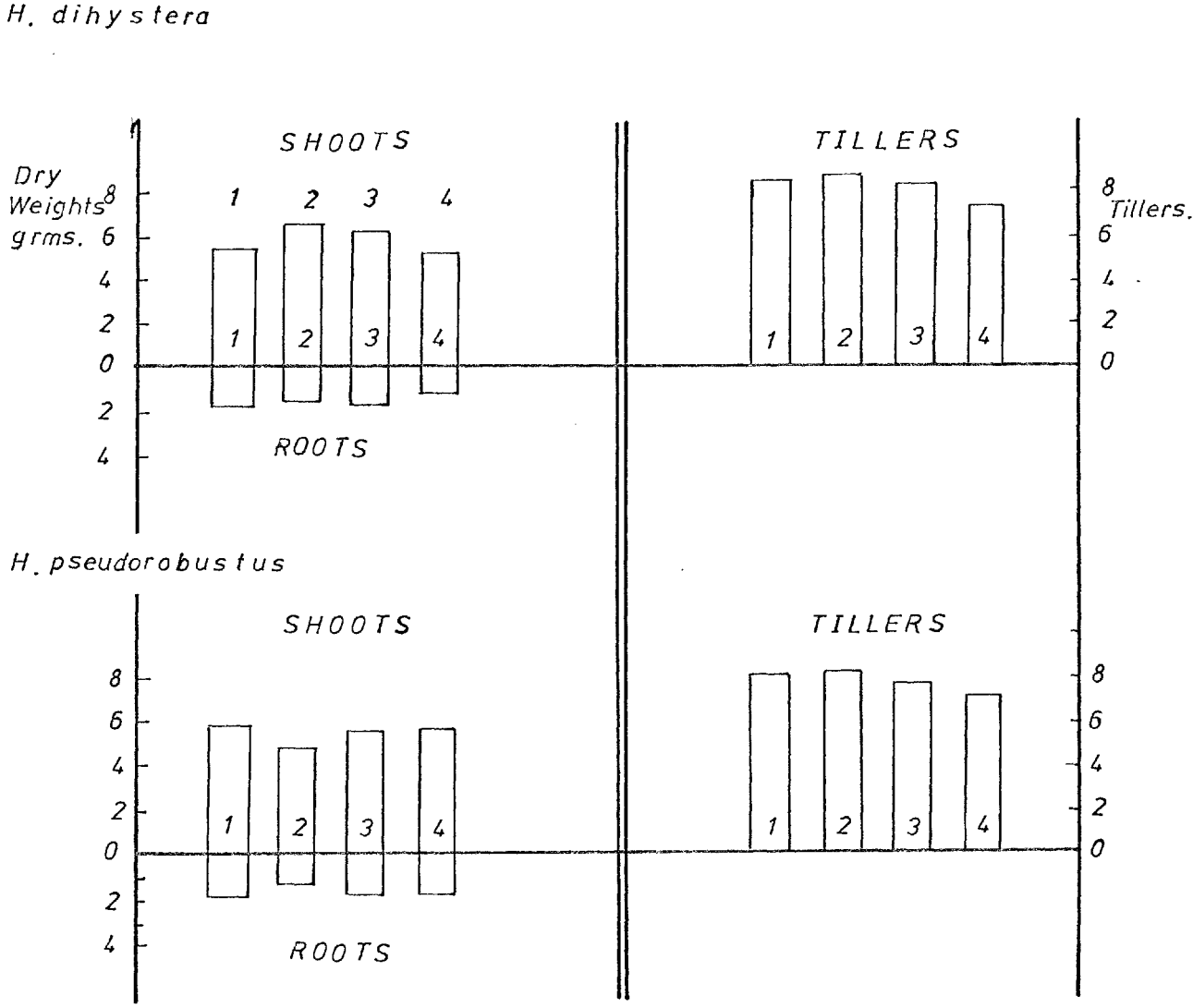
Two species, H. dihystera and H. pseudorobustus, were inoculated separately into sterilised soils, to determine the effect of population size on the growth of wheat var. "Cardinal". Four treatments, control, 1,000, 2,000 and 5,000 nematodes per 500 c.c. of soil in plastic pots, were each replicated four times in fully randomised experiments for both species. The soil used was a mixture of 7:3:2 parts soil, peat and sand mixed 1:1 with sand. A powdered fertiliser was added to this mixture. This soil mix was used because it had proved suitable for culturing nematodes and in it, wheat grew well.

At inoculation, the nematodes were pipetted onto the soil surface in 10 ml. of water and a similar volume of extract supernatant was added to the control pots. Wheat seeds, soaked overnight, were added directly after the nematode inoculum, and all pots were thoroughly watered. The plants became mildly infected with mildew after thirty-nine days, and were therefore immediately sprayed with 0.2% Calixin. Sixty days after inoculation the plant roots were carefully removed from the soil and the numbers of nematodes present in the soil were assessed by extracting nematodes from 200 c.c. of soil by the Whitehead tray method. The effect of the treatments on growth was assessed by counting the numbers of tillers per plant, and measuring the dry weights of roots and shoots after drying them in an oven at 60°C. for two days.

4:2:2 Results and Discussion.

No significant differences existed between the treatments for either trial, but the numbers of tillers per treatment (Fig. 4:1) showed a tendency to increase at the low inoculum, and decrease below the control at the

Fig. 4:1 Pathogenicity of soil inoculated nematodes.



high inoculum.

The numbers of nematodes recovered from the soil (Appendix E, Tables 1 and 2) were very variable, with no nematodes recovered from some treatments. The poor survival of nematodes, treatment means ranging from less than 10% up to 50% of the inoculum, could have been caused either, by extraction and handling damaging the nematodes, or that the nematodes did not become incorporated into the sterile soil. The latter reason seems most likely to have occurred, since if the nematodes had been damaged prior to inoculation, an even decline of nematodes would have resulted.

4:3 Population Increase and Pathogenicity of H. dihystera to Wheat.

4:3:1 Introduction.

The poor incorporation of the nematodes into the soil following inoculation, noted in the previous experiments, could have been caused by the drying out of the soil surface after inoculation. Thus to avoid this, a pathogenicity trial was planned using soil already infested with H. dihystera.

In June, 1974, fresh cultures of H. dihystera were prepared. The nematodes used were derived from a pure culture of H. dihystera on wheat, and 5,000 nematodes were introduced to a pot containing 10 litres of the soil mixture (Section 4:2:1). A second pot also containing 10 litres of this soil mixture was inoculated with an equal volume of the extract supernatant so that soil, uninfested with nematodes, but with a comparable cultural history was available to be used as a control treatment, and to reduce the soil population of the infested soil.

These cultures were then sown thickly with wheat, and at three monthly intervals, the old plants were removed, and the soil mixed and freshly potted, after which further seeds were sown.

4:3:2 Population Increase.

Materials and Methods.

An experiment to monitor the population increase of the main cultures, was made to determine the rate of increase of H. dihystera inoculated into sterile soil. 250 c.c. of the soil mixture (Section 4:2:1) was added to eighteen small plastic pots, and 500 H. dihystera were inoculated in 1.5 mls. of water to the soil surface. Two wheat seeds were planted in each pot. At intervals of 1, 4, 8, 16, 32 and 48 weeks three pots were removed and the pot contents, soil and finely cut roots were extracted by the Whitehead tray method and the nematodes ccunted.

After 3, 6 and 9 months, the old wheat plants were removed and fresh seeds planted in the remaining pots.

4:3:3 Population Increase.

Results and Discussion.

The nematodes recovered (Fig. 4:2; Appendix E, Table 3) indicated that after one week only 18% of the inoculated nematodes remained. This occurred despite care taken in introducing the nematodes, and means that either the nematodes are damaged during handling of the extract, or that a large percentage of the active nematodes were washed through the pot. The population remained at this level for a further three weeks, and only after eight weeks had elapsed did reproduction, and a build up in numbers begin. At eight weeks a threefold increase had occurred (Fig. 4:2), and thereafter the population continued to increase through to the end of the experiment. If the increases that occurred in each four week interval from week 4 to week 48 are calculated (Fig. 4:3) the reproduction rate declined from the first 4 week interval where a 300% increase occurred, to an increase of only 50% in each four week interval between weeks 32 and 48. The cause of this decline in reproductive rate was probably competition for feeding sites by the high populations present, 4,000 per 250 c.c. of soil.

The numbers of nematodes increased eightfold over the 48 weeks, but the final population built up from a population less than 1/5th of the inoculum. Thus in 44 weeks the observed population increase was fiftyfold, and is comparable to the seventyfold increase reported by Churchill and Ruehle, (1971) for H. dihystera on sycamore over a nine month period. But on sycamore, the seventyfold increase was from an inoculum level of 1,000 nematodes, and if a similar percentage of inoculum perished, it is evident that sycamore supported a greater reproductive rate than wheat. That this is so is indicated by the twentyfold increase in nematode numbers that occurred

Fig 4:2 H. dihystera, population increase.

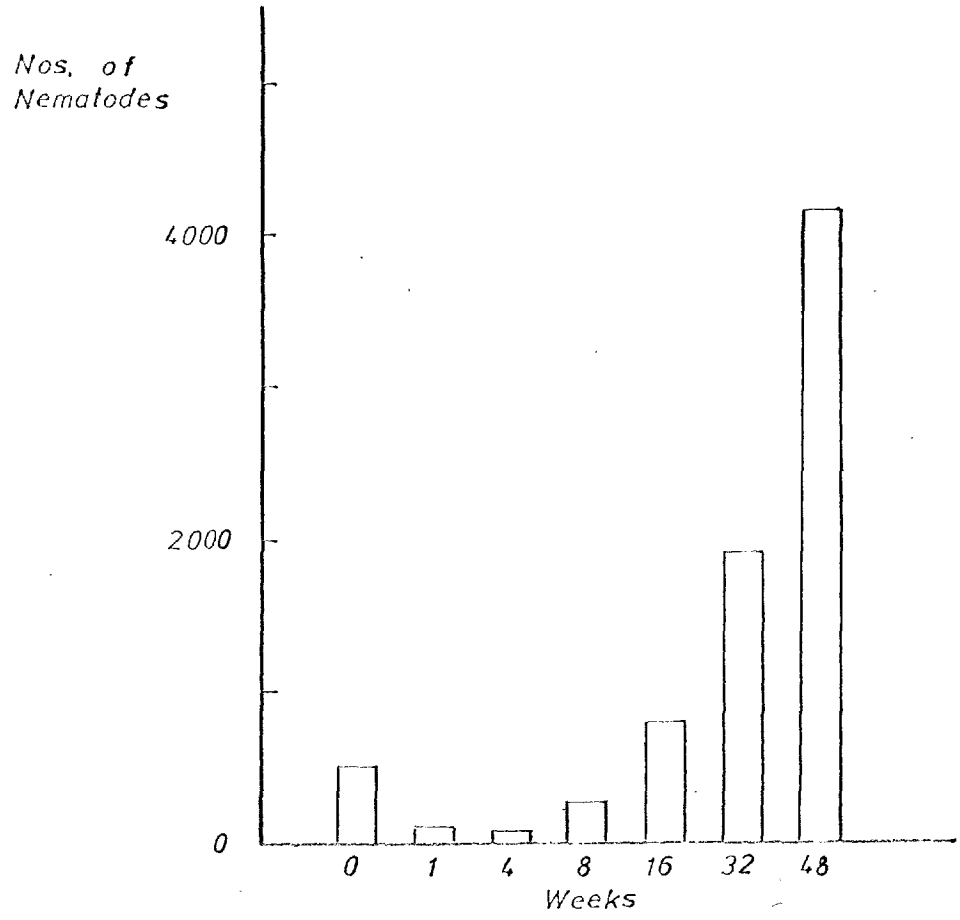
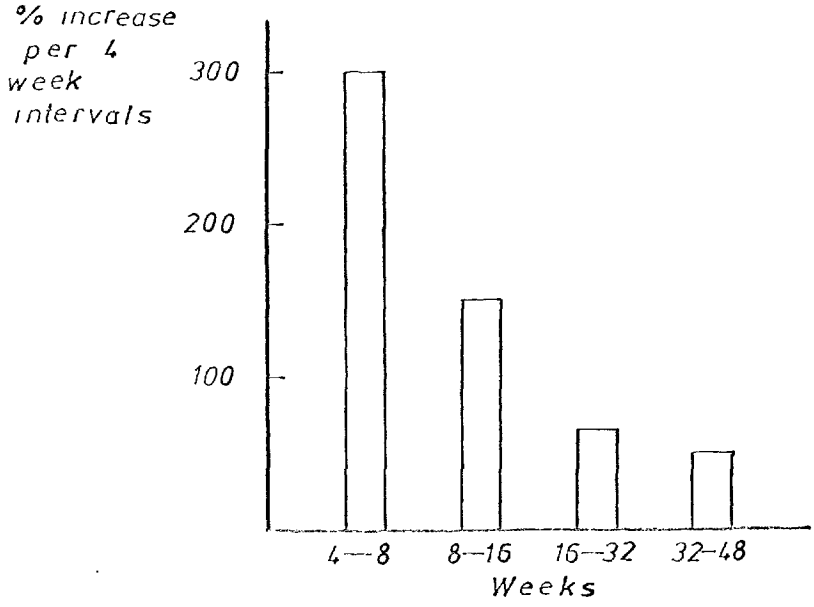


Fig 4:3 H. dihystera, rate of population increase.



in the last three months of the trial on sycamore, compared to the twofold increase that occurred in the last sixteen weeks on wheat.

4:3:4 Pathogenicity of H. dihystera on wheat. Materials and Methods.

The population recovered from the cultures prior to this trial, showed that an eightfold increase, from 500 per litre to 4,100 per litre of soil had occurred over the 48 weeks. This increase was less than anticipated, but did represent a population above that commonly found in field soils. Thus since a higher population size was not available, only two treatments were used, the control uninfested soil, and the treated infested soil. The treatments were replicated eighteen times in an attempt to maximise between treatment differences. In addition, wheat seedlings; selected for their uniformity three days after germination and a Latin square design, were used to minimise the experimental variability.

The three-day old seedlings were planted carefully in 500 c.c. of infested soil, and the experiment was terminated after a further sixty days growth. Each plant was then graded, using a scale based on Feeke's scale of cereal maturity (Large, 1954). The plants were then removed from the soil, and the shoots and washed roots were cut up and dried to a constant weight in a 60°C. oven to determine their dry matter weights. The initial and final nematode populations were counted from two 200 c.c. samples of soil extracted by the Whitehead tray method.

4:3:5 Pathogenicity of H. dihystera on wheat. Results and Discussion.

The nematodes present at the end of the experiment, had declined from the initial population (Appendix E, Table 4) and this decline was probably caused by the nematodes being damaged during the handling of the soil prior to the experiment.

Neither the shoot or root dry matter weights differed significantly between the treatments. These weights were very variable though, but despite this variability there were no differences in the final growth stages of treated and control plants. In addition, no pattern of uneven growth due to the treatments occurred at the initial or subsequent growth stages, and thus an initial soil population of 837 H. dihystera per 200 c.c. of soil had no visible effects on either the rate of development of wheat, or the size of the plant up to 60 days, (Appendix E, Table 5.).

4:4 The Development of Cortical Necrosis due to H. dihystera.

4:4:1 Introduction.

Two species of Helicotylenchus have been associated with the loss of root cortical cells. H. varicaudatus caused a breakdown of the cortex of of Lolium perenne when present in high numbers (Collen, 1972), and H. digonicus while feeding on cortical cells induced a necrosis that lead to the cortical sloughing of Poa pratensis (Perry et al., 1959).

Root examination during this work revealed that groups of all the Helicotylenchus spp. were associated with regions of roots where the cortex was necrotic. But the cortex of healthy cereal roots, particularly wheat roots, shrivels and turns brown with age, such that Troughton (1962) considers the cortex of wheat to be "early deciduous". Because the cortex of wheat grown in a glasshouse began to shrivel and slough away from the stele on one month old plants, a trial was derived to determine whether nematodes are involved in causing or hastening this loss of cortical cells.

During previous work, H. dihystera had been observed to feed as an endoparasite and primary roots were their preferred feeding site. Furthermore, where lateral roots were parasitised, a greater preponderance of semi-endoparasites seemed to occur. Thus in addition to assessing the effects of feeding nematodes on the cortex, the feeding position of the nematodes was recorded.

4:4:2 Materials and Methods.

The soil used was the soil mixture (Section 4:2:1), and the infested soil contained 125 H. dihystera per 100 c.c. of soil. The uninfested soil had been cultivated in a similar manner to the infested soil prior to this experiment. 100 c.c. of soil was measured into plastic pots and uniform

three-day old seedlings were planted. The experiment, a randomised block design, was carried out at 25°C. in a 16 hour day length, with the pots watered twice daily.

The treatments were infested and uninfested control soil, each replicated four times in five blocks. Each of the five blocks represented a growth period, and the intervals were of 5, 10, 15, 25 and 40 days. At the end of these intervals, each batch of plants were separately flooded with boiling 5% formalin so that the nematodes were fixed in their feeding positions in the roots. The pots were removed from the fixative after five minutes and the soil was carefully washed away from the roots in a water bath. The nematodes were differentiately stained in 0.1% cotton blue lactophenol, and their feeding positions in the roots, together with the extent of the cortical necrosis was noted. The roots were laid out in a perspex tray 20 cm. by 7 cm. for examination, and the whole root system of each plant was examined. The numbers of nematodes in the roots were recorded, and each nematode was grouped according to its feeding position. The groupings were:-

1) Primary root feeders	1) Ectoparasites
	2) Semi-endoparasites
	3) Endoparasites
2) Lateral root feeders	1) Ectoparasites
	2) Semi-endoparasites
	3) Endoparasites

Prior to day 25 all the nematodes were found in seminal roots, but by day 25 nodal roots had begun to develop and by day 40 these roots were well devoloped. The nematodes that had invaded these roots were thus included with the seminal roots in the same groupings as listed above.

Cortical shirvelling was assessed as the percentage length of root, separated into primary and lateral seminal roots, in which cortical cell

collapse had begun. No cortical cell loss occurred on nodal roots.

4:4:3 Results and Discussion.

The position of the nematodes in the roots (Fig. 4:4; Appendix E, Table 6) indicated that:-

(a) An endoparasitic habit predominated. Only a few semi-endoparasitic nematodes and no ectoparasitic nematodes were found.

(b) The impression that lateral roots had a greater proportion of semi-endoparasites was not shown to be true. Feeding nematodes generally on lateral roots were endoparasitic.

(c) The nematodes invaded only the areas of roots where cells were fully developed. Nematodes did not invade cells of the rootcap, or the zone of differentiation or elongation.

(d) The pattern of nematode invasion was limited to root development. Prior to day 5, the root growth had been restricted to elongation of the primary seminal roots, and as nematodes fed only on developed roots, invasion was low. The lateral roots had just begun to emerge by day 5 but the nematodes were not attracted to these regions of the cortex, as was P. fallax on cereals (Corbett, 1972). By day 10 the developed areas of primary roots were frequently invaded, whilst the lateral roots, that were still elongating were invaded by only a few nematodes.

The numbers of nematodes in the primary roots reached a plateau between day 15 and 25 (Fig. 4:4a) and then declined. By day 25, the seminal primary roots began to loose their cortical cells, and the decline in nematodes in primary roots by day 40 paralleled an increase in cortical cell loss. The size of this decline was masked due to the invasion of nodal primary roots by day 40, and thus the fall in numbers in seminal primary roots between day 25 and 40 is considerably greater than indicated in Fig. 4:4a.

(e) The rate of loss of cortical cells, and the percentage length of roots where this occurred was similar in infested and uninfested roots (Table 4:1). Also the cortical cells around individual or groups of nematodes, although frequently necrotic, showed no signs of collapse by day 15. By day 25 and 40, some nematodes were associated with regions where collapse had begun, but this

association appeared purely fortuitous, as many nematodes were present in areas where no collapse had occurred.

The total numbers of nematodes found in roots at day 25 and 40, averaged 32, and represented about 1/4 of the initial population. The remaining nematodes could have been injured during the setting up of the experiment or else represent and inactive fraction of the population.

Roots that had completely lost their cortical cells had no nematodes attached. The inability of nematodes to feed from these roots could be due simply to the lack of supporting cells, but in such roots the remaining endodermal, pericyclic and vascular parenchyma cells become heavily sclerotised (Percival, 1921) and this itself could prevent feeding by forming a physical barrier to stylet penetration.

Fig. 4: 4 H. dihystera, Root invasion.

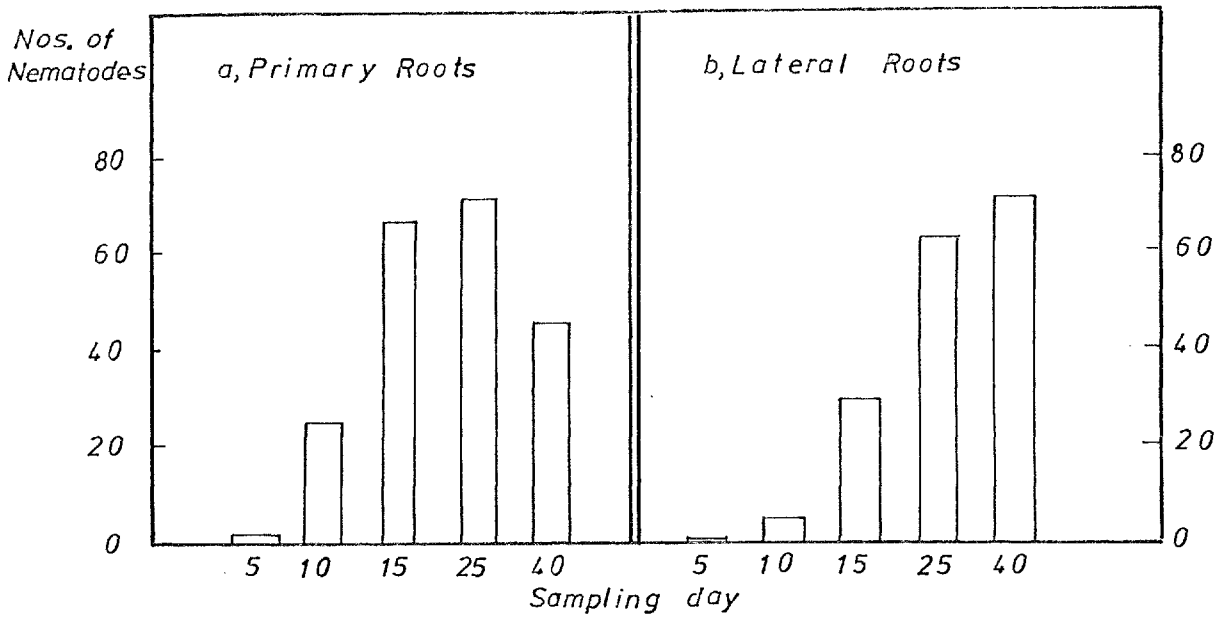


Table 4: 1 Percentage of root with cortical cell loss.

Sampling day	Control		Infested	
	Primary	Lateral	Primary	Lateral
5	0	0	0	0
10	0	0	0	0
15	0	0	0	0
25	20	0	20	0
40	50	20	50	20

4:5 Discussion of Pot Pathogenicity Experiments.

Disease is recognised when the population of a pest increases beyond the tolerance threshold at which a host plant can compensate for loss due to feeding. The maximum population that can be supported without loss is termed the threshold population. Threshold populations have been described for various Heterodera spp. on their hosts e.g. H. rostochiensis on potatoes (Jones, 1965). On cereals, H. avenae has a threshold population of 10 eggs per grm. of soil (Dixon, 1969), and Dixon has shown that for every 10 eggs of H. avenae per grm. of soil there is an increasing yield loss for:-

barley of between 0.4 - 0.8 cwt. per acre
wheat of up to 1.5 cwt. per acre, and
oats of up to 3.0 cwt. per acre.

The loss in yield typically reaches a maximum, after which any increase in nematode numbers causes little further decline in yield.

In the field trials, 3,000 H. digonicus per litre of soil and 9,000 H. pseudorobustus per litre of soil caused no reductions in either grain yield or leaf area respectively. Likewise in glasshouse pathogenicity trials, an inoculated population of 10,000 per litre of soil of both H. pseudorobustus and H. dihystera, and pre-infested soil with an initial population of 4,000 H. dihystera per litre of soil produced no stunting of shoot or root growth. Thus cereals can tolerate such populations of these Helicotylenchus spp. The failure of Helicotylenchus spp. to cause yield reduction is predicted when it is borne in mind that only larger populations of H. avenae cause yield reduction.

Some pathogenicity experiments have shown that populations of Helicotylenchus spp. have affected the intial stages of growth, after which the host is able to compensate for this injury and recover (Sledge, 1956). In the first two pathogenicity trials there were indications that development

was affected due to the differences in numbers of tillers produced but these effects on development were not seen in the trial involving soil pre-infested with H. dihystera.

On occasions, comparatively low populations of Helicotylenchus spp. have been shown to cause disease. 1,000 H. caribensis per litre of soil stunted the growth of garden canna (Jacob et al., 1969) whilst 2,000 H. elegans per litre of soil reduced flowering of tomatoes (Saxena et al., 1973). This variability of pathogenicity also occurs within one species and it is likely that the variability is due to either the existence of races of one species, or that tolerance to populations differs with the hosts. There is no evidence for the existence of biological races of Helicotylenchus spp., yet this does not preclude this possibility, but it does seem evident that hosts can tolerate populations that on some other host are pathogenic.

As well as there being no evidence of any sign of disease reflected in reduced shoot or root growth, the secondary effects of feeding of these nematodes, a promotion of cortical necrosis does not appear to occur on wheat. The only indication that feeding of these nematodes may promote cortical loss is the limited necrosis around some nematodes (Section 3:3). This necrosis was never extensive and appeared only in peripheral cortical cells whereas the cortical loss involved intact cells of the cortex peeling directly from the central cylinder of the root. Thus the two do not appear to be linked, and the only disease symptom due to feeding by these nematodes was peripheral necrosis which had no widespread effect on the plant.

The slow population increase of H. dihystera on wheat is comparable to the increases of H. digonicus, H. pseudorobustus and H. varicaudatus on the cereal and grass hosts (Section 3:2). The cause of this slow and declining population increase (Fig. 4:3) could have been either competition at feeding sites reducing the percentage of feeding nematodes with a resultant increase in the non-feeding population, or competition for nutrients at feeding sites

reducing the fecundity of the adult females. Since in localised regions of roots clusters of nematodes occurred, whereas in the major part of the length of the roots no nematodes were found, competition for feeding sites seems unlikely to be limiting, but also in the areas of roots where clusters of nematodes were found, adult females with egg clusters were common, and thus unless these egg clusters were reduced in size, competition for nutrients among nematodes at feeding sites is also an unlikely cause of the low population increase.

The influence root development has on nematode invasion was expected. The initial delay in invasion noted both in these soil experiments and later in agar plates (Section 5) is probably caused by a combination of an inability of nematodes to feed on developing roots, and a lag that would occur between the formation of roots and the attraction of nematodes to penetrate roots.

A further indication of the relationship that exists between host and nematode is the switch that occurs from feeding on seminal roots to nodal roots that results from the loss of the seminal root cortex or sclerotisation of the remaining cells. By the time the cortical cell loss is advanced, the nodal roots are well developed and thus provide a continuous food supply for the nematodes. The cause of this switch to the nodal roots is most likely initiated by the sclerotisation of the central stele cells, since if these cells remained unaltered after cortical cell loss, nematodes could still feed from the remaining endodermal and pericyclic cells. Since no nematodes remained attached to these roots, it seems that they no longer act as a suitable food source due to the cellular sclerotisation.

SECTION 5.

FEEDING BEHAVIOUR AND THE HISTOLOGICAL CHANGES IN HOST ROOTS DUE TO FEEDING OF HELICOTYLENCHUS SPP.

5:1 Feeding of Helicotylenchus spp. on Spring Wheat.

5:1:1 Introduction.

H. dihystera has been reported to feed ectoparasitically on root hairs of maize (Sledge, 1959), but histopathological investigations have revealed that a semi-endoparasitic or endoparasitic feeding position is common (Perry et al., 1959; Davis and Jenkins, 1960; Zuckerman and Strich-Harari, 1963; Blake, 1966; Churchill and Ruehle, 1971; Collen, 1972; Ruehle, 1975). Thus it seems evident that the observations by Sledge were atypical and that these nematodes feed within roots rather than from root surfaces (Winslow, 1956). Indeed, H. multicinctus was reported to penetrate into the piliferous zone of roots (Linford, 1939).

The feeding habits of related genera vary. Hoplolaimus indicus fed as an ectoparasite of tomatoes (Gupta and Atwal, 1971), where individual feeds lasted 10 to 15 minutes, whilst Rotylenchus robustus fed in one region of roots usually as a semi-endoparasite for up to five days (Klinkenberg, 1963) and during the feed a lesion, comprising a group of cells, developed around the nematode's head.

Detailed observations on the feeding methods of endoparasitic nematodes are hindered by surrounding cells, and for this reason information on Tylenchida feeding activities have been derived mainly from fungal or ectoparasitic root feeding species (Linford et al., 1949; Anderson, 1966; Doncaster, 1966; Wyss, 1973; Bridge and Hague, 1974). The feeding behaviour of Tylenchida has been reviewed by Doncaster (1971), who separated the phases of activity into Exploration, Penetration, Salivation and Ingestion.

In a later paper Doncaster and Seymour (1973) grouped salivation and ingestion into a single category - Feeding, due to the habit of some endoparasites to have cyclical periods of salivation and ingestion.

Thus observations were made on Helicotylenchus spp. to determine:-

1) whether they fed as endoparasites and if so,
2) whether an endoparasitic habit imposes fundamental changes on the basic pattern of ectoparasitic feeding behaviour.

5:1:2 Materials and Methods.

Three species of Helicotylenchus, H. dihystera, H. varicaudatus and H. pseudorobustus were selected for observations. H. dihystera was observed most frequently and the general description of the feeding habits refer to this species.

In vitro Plant Culture and Inoculation Techniques.

All preparations were made in a sterile laminor flow cabinet to minimise contamination. Seeds of wheat var. "Cardinal" were surface sterilised in 0.5% $HgCl_2$ for five minutes, then placed on sterile 1% water agar in 9 cm., petri-dishes. Two days later, a sterile seed was transferred to a fresh 1% water agar plate. The seedling was allowed to grow for a further three days, when fifty hand picked nematodes were pipetted onto the agar surface using a modified Pasteur pipette (Razak, 1975). Generally no steps were taken to sterilise the nematodes though on two occasions attempts were made to create a monoxenic culture. On the first occasion nematodes were sterilised for two minutes in 0.1% $HgCl_2$ and subsequently washed twice in sterile distilled water. On the second occasion the nematodes were hand picked through three washes of sterile distilled water.

After the inoculation of the nematodes, the surface of the agar was scored with a sterilised needle to promote the penetration of the agar by the nematodes. If this was not done the nematodes became trapped on the

agar surface. The plates were then sealed, and when not being observed, kept at laboratory temperature.

Methods of Observations.

The feeding nematodes were initially observed through the base of the petri-dish, but to facilitate observations with objective lenses higher than 10x magnification, a piece of the petri-dish base above feeding nematodes was removed using a hot dissecting needle and scalpel. A cover-slip was then lowered onto the agar surface, and this technique allowed feeding to be observed using a 100x oil immersion objective lens. Details of feeding of individual nematodes were then recorded over prolonged periods and the duration of the separate activities of feeding were recorded on a multiple event recorder. A video-tape recorder was also used to aid observations, but this was of limited value due to the need to alter focus regularly to record the separate events of feeding.

Root Staining.

The observations on one plate lasted up to three weeks, before contamination of the agar affected detailed observations. When a plate was no longer suitable for observations, root segments containing the feeding nematodes were cut from the agar plate, and placed in hot F.A. to kill and fix the nematodes *in situ*.

These root segments were embedded in paraffin wax and sectioned at 12 u (Section 3:3:1). The remaining roots were then removed from the agar plates and stained in 0.1% cotton blue lactophenol (Section 3:3:1) to determine the feeding positions of the remaining nematodes.

5:1:3 Observations and Results.

Following introduction to the agar, the sterilised nematodes remained

inactive, coiled in the typically helical shape of H. dihystera. The cause of the failure of the first batch was thought to be the toxic effects of the $HgCl_2$, but on the second occasion no such explanation occurred.

The non-sterilised plates had a low success rate and only a few nematodes were observed feeding in most plates, yet in some plates, up to half of the inoculated nematodes did feed. Only nematodes that fed on the surface of roots that had been positioned against the base of the petri-dish were suitable for detailed observations.

H. dihystera fed in deep lying cells of roots and its feeding habits can be separated into the same groups erected by Doncaster and Seymour (1973) for endoparasitic nematodes.

Exploration.

No nematodes penetrated or fed from roots in the first two days after inoculation. Initially the nematodes either moved freely over the entire root system or else they remained inactive in clusters on the root surface.

The clusters of nematodes that formed on the roots occasionally consisted of the majority of the nematodes but eventually the nematodes dispersed leaving one or two feeding individuals.

The active nematodes explored all regions of the root, and this activity continued for some individuals throughout the period of observations of a plate. Prior to penetration this general activity was replaced by localised exploration when the nematode ceased general body movement and began restricted movement of the anterior regions of the body over a specific area of the root. During localised exploration, the nematode's head was arched over the root, so that the nematode's lips were pressed onto the cell surface, and periodically the head was held static, when a few thrusts of the stylet would occur.

Localised exploration was followed by attempts at penetration, but frequently the explored site was abandoned and the nematode resumed general

exploratory activity. Endoparasitic nematodes, penetrating through cells also explored these cells in a similar manner to localised exploration of the cell surface and passage into and through a recently entered cell lasted up to one hour.

Penetration.

Cell wall penetration was achieved by continuous stylet action. The movement of the stylet was not regular but consisted of two distinctive types of protraction, associated with stylet re-alignment in the buccal cavity and alterations of the placement of the nematode's lips on the cell wall (Fig. 5:1).

The types of stylet protraction were:-

(a) A Thrusting Action, which consisted of an even protraction and retracting of the stylet resulting in either

1) indentation of the cell wall when the stylet hit the wall, combined with
2) a back thrust on the nematode forcing the nematode's lips off the cell surface,
3) the buckling of the stylet tip or
4) the successful penetration of the cell wall.

(b) A Probing Action, when the stylet was only partially protracted such that the stylet tip only just protruded from the nematode's lips. The stylet was quickly withdrawn after protraction.

The rate of stylet movement varied from 19 to 51 thrusts per min. ($\bar{x}$ = 34 per min. S.D. = 9.26 per min.) and the duration of continuous stylet action in successful penetration lasted from 30 to 600 minutes. ($\bar{x}$ = 190 mins. S.D. = 171 mins.).

The type of cell nematodes were attempting to enter could not always be identified, but nematodes appeared to be slower entering epidermal cells than other cells. One nematode continued stylet activity for 31 hours on an epidermal cell surface before abandoning penetration, and the cause of

Fig. 5:1 Rhythm of Stylet Action.

a) *Thrusting action.*
b) *Probing action.*

the difficulty in entering epidermal cells may be the lack of support given to the nematode from the agar. In the endoparasitic nematodes adjacent cell walls were used as a brace to reduce the back thrust of the nematode's head.

Stylet activity during penetration was only interrupted to move the angle of the stylet in the nematode's head, or to alter the placement of the nematode's lips. These changes were linked neither to an alteration of the thrusting to probing action of the stylet, nor to successful penetration of a cell wall, although after penetration, the position of the nematode's lips was altered. If a newly selected site co-incided with an already existing hole, only a few thrusts of the stylet occurred till the nematode again moved its head.

Successful entry to a cell was achieved by the combination of

a) they stylet puncturing and weakening the cell wall, and
b) a forward pressure of the nematode's body indenting the cell wall (Plate 15.).

These two effects resulted in cell wall rupture allowing the nematode to surge a short distance into the new cell (Plate 16.).

Following successful entry to a cell, localised exploration began in the newly entered cell (Plate 17.). The nematode typically moved slowly to the opposite end of the cell and resumed penetration, but occasionally the nematode either became inactive or else it abandoned the newly entered cell and attempted to penetrate into the other cells from the original cell.

Feeding.

Salivation and Ingestion.

Following successful penetration to a feeding cell, the nematode began feeding activity. H. dihystera fed in a similar manner to other

endoparasites (Doncaster and Seymour, 1973) with salivation and ingestion following each other in a cyclical pattern.

The salivation phase was defined as the period during which no medium bulb pulsation occurred and lasted from 2 mins. to 24 mins. ($\bar{x}$ = 9 mins. 20 secs. S.D. = 8 mins. 27 secs.)

The ingestion phase was characterised by the contraction of the musculature of the medium bulb dilating the valve plates of the bulb. The rate of muscular contractions ranged from 86 to 330 per min. ($\bar{x}$ = 187 per min. S.D. = 61 per min.) and continuous ingestion lasted from 4 to 65 mins. ($\bar{x}$ = 36 mins. S.D. = 20.5 mins.). The onset of ingestion was indicated by irregular un-co-ordinated contractions of the whole bulb musculature pulling the medium bulb to one side of the nematode's body. These contractions lasted for up to thirty seconds when they were suddenly replaced by co-ordinated pumping contractions.

During ingestion, granular materials was occasionally seen to move forward from the base of the dorsal gland behind the medium bulb, through a narrow length of the dorsal gland duct in the medium bulb, to the pro-corporeal region of the gland duct (Plate 18; Fig. 5:2). When medium bulb pulsation ceased, the start of salivation was marked by a sudden surge forward of this granular material from the pro-corporeal region to the ampulla which consequently filled with the granules (Plate 19; Fig. 5:3). With continued salivation, the granules moved around in the ampulla, but were not actually observed leaving the ampulla. However granular material did slowly disappear as granules apparently coalesced in a small area around the duct connecting the ampulla to the oesophageal lumen. The loss of granules continued till the end of the salivation phase when the ampulla was nearly emptied of granular material. No medium bulb movement or any muscular movement in the oesophageal regions were seen during salivation.

In some feeding cells, a cap of dark material was seen enveloping the stylet. The zone was only seen when nematodes had been feeding for a considerable period, and its size remained constant during salivation and ingestion.

The duration of feeds ranged from 40 mins., a single salivation and ingestion on a root hair, up to 19 days ($\bar{x}$ = 145 hours S.D. = 124 hours). When feeding in roots either as a semi-endoparasite (Plates 20 and 21.) or as an endoparasite (Plate 22.) the nematode coiled in a characteristic manner that differed from the helical shape of killed nematodes.

The nematodes laid eggs after prolonged feeding, (e.g. a nematode laid 2 eggs after seven days feeding, and during the next four days laid a further three eggs) and frequently large egg clusters developed (Plate 20.). The nematodes did not stop feeding during egg laying.

Feeding of H. pseudorobustus.

No feeding by H. pseudorobustus was seen on the twenty plates prepared. The nematodes were active in exploring the roots and this activity continued for all the time the plates were studied. Three individuals attempted to penetrate epidermal cells and the duration of stylet activity and speed of stylet movements were:-

1) 100 mins. at 25 thrusts per min.
2) 5 mins. at 24 thrusts per min.
3) 5 mins. at 20 thrusts per min.

At the end of these periods, the nematodes resumed normal exploratory activity.

Comparison of Feeding of H. dihystera and H. varicaudatus.

The feeding behaviour of these species was similar (Table 5:1) except for the marked variation in the duration of the salivation phase. This

difference was due to the prolonged initial salivation that followed penetration. This was only recorded during the feeding of H. varicaudatus and the mean duration of these salivations was 75 mins. The mean duration of subsequent salivations for these same nematodes was approximately 19 mins.

Table 5:1

Comparison of the Feeding Behaviour of H. dihystera and H. varicaudatus

Activity	H. dihystera		H. varicaudatus	
	Mean	Nos. of Nematodes	Mean	Nos. of Nematodes
1) Speed of stylet movement	34 per min.	25	43 per min.	5
2) Duration of stylet activity	190 mins.	25	325 mins.	12
3) Duration of salivation	9 mins. 20 secs.	25	46 mins. 50 secs.	6
4) Speed of medium bulb pulsation	187 per min.	25	105 per min.	5
5) Duration of ingestion	36 mins.	25	40.5 mins.	5
6) Duration of feeding	145 hrs.	25	164 hrs.	6

Stained Roots.

Examination of the roots stained in 0.1% cotton blue showed that most nematodes were positioned as endoparasites. The living roots did not appear to be altered by the nematodes feeding, but stained roots revealed lesions around the nematodes (Section 5:2).

One individual H. varicaudatus was migrated through several cells without feeding, but when this root piece was sectioned there was no evidence of any damage due to the nematode's passage.

Plates 15 and 16.

Plate 15. *H. dihystera* penetrating a cell wall with the cell wall indented. (x500)

Plate 16. *H. dihystera* after successful penetration. This nematode had only just ruptured the cell wall and had only moved into the cell due to the forward force of the nematode present during penetration. (x500)

Plate 17. *H. dihystera* beginning localised exploration in a recently entered cell. (x500)

Plate 15.

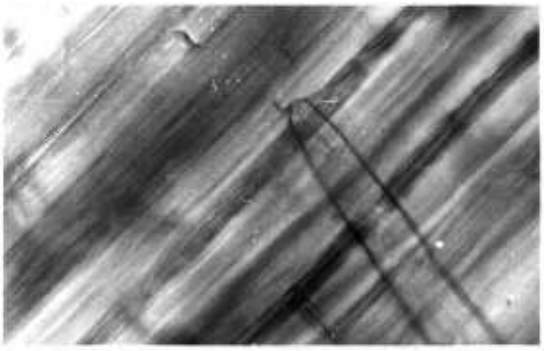


Plate 16.

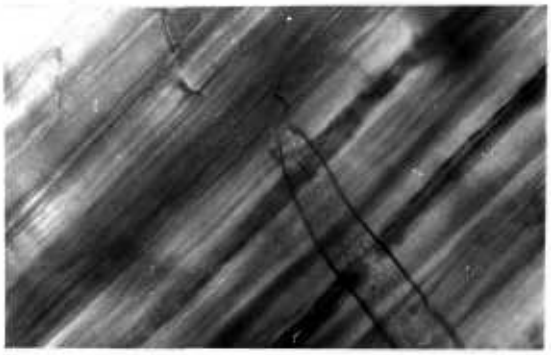


Plate 17.

Plates 18 and 19. Figs. 5:2 and 5:3.

Plate 18. Fig. 5:2.

A nematode during ingestion with the granular dorsal gland exudate in pro-corporeal region of the gland duct. (x3,000)

Plate 19. Fig. 5:3.

A nematode during salivation when the granules have passed to the dorsal gland ampulla. (x3,000)

A = ampulla

P = pro-corporeal region

G = granules of dorsal gland exudate

Plate 18.

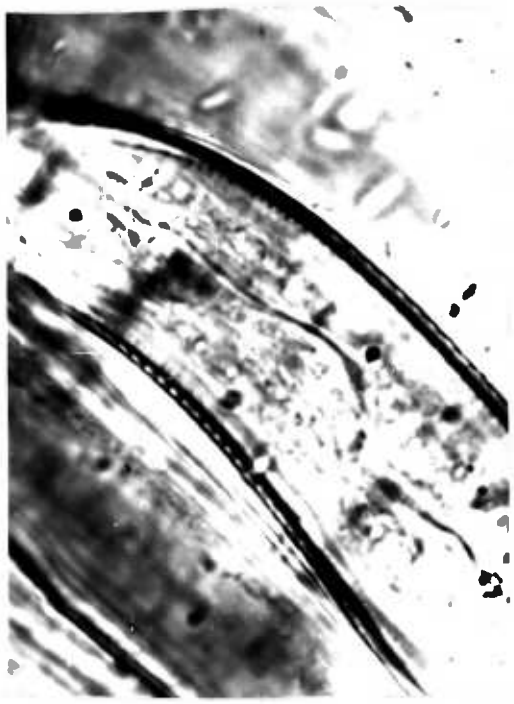
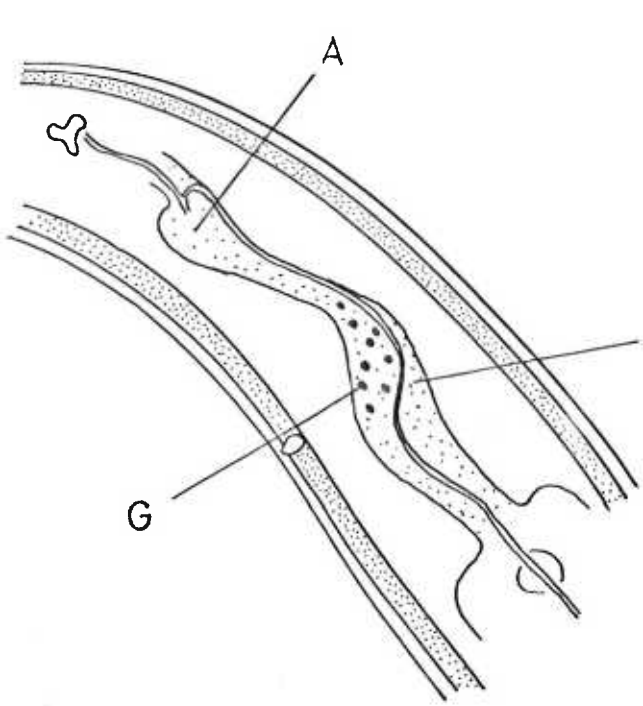


Fig. 5 : 2 Ingestion Phase

Plate 19.

Fig. 5 : 3 Salivation Phase



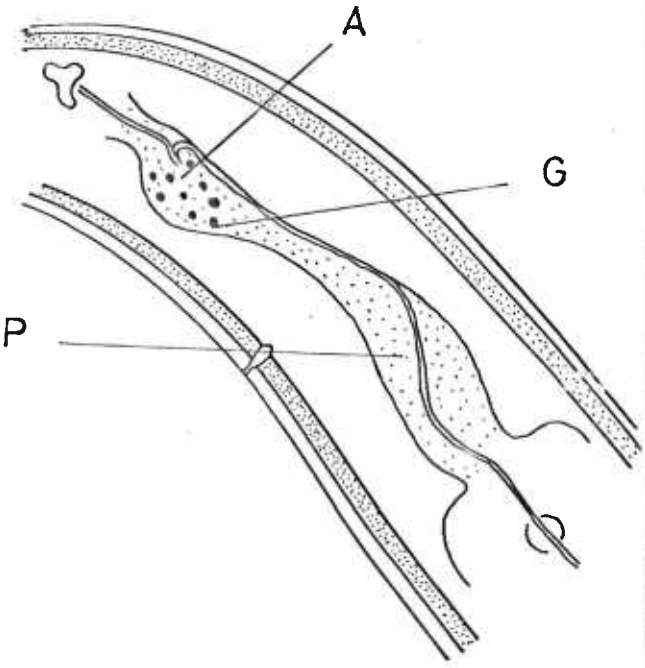


Plate 20.

Semi-endoparasitic nematode with egg cluster. Note the typically coiled shape of the nematode. (x1,200)

Plate 21.

Semi-endoparasitic nematode with eggs in which the initial stages of egg development have occurred. (x1,400)

Plate 22.

Endoparasitic nematode. (x1,200)

Plate 20.

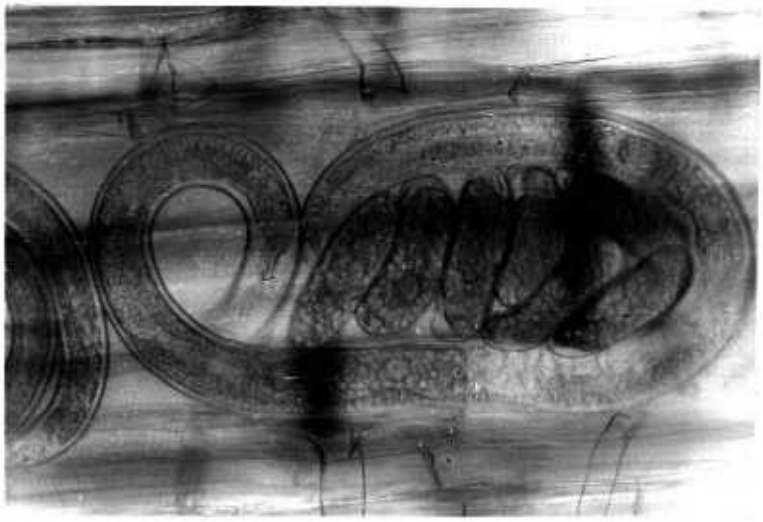


Plate 21.

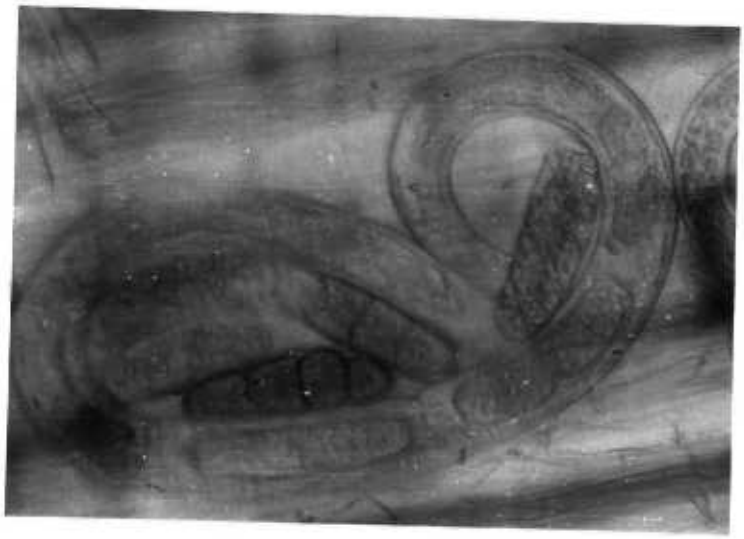
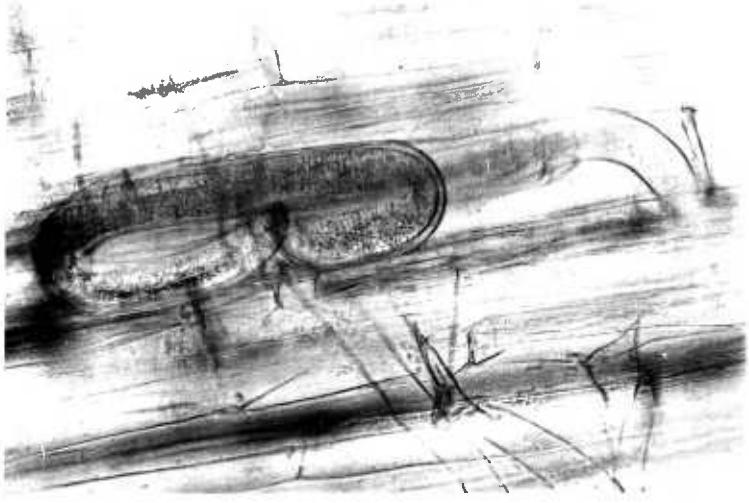


Plate 22.



5:2 Histological Investigations of the Feeding Site of H. dihystera.

5:2:1 Introduction.

Helicotylenchus spp. are typically thought to cause a superficial necrosis of epidermal and cortical of their host roots (Strover, 1972; Davis and Jenkins, 1960). In tomato roots, H. pseudorobustus caused a typical superficial lesion, and Taylor (1961) reported that cell walls around the nematode were disrupted. The bulb-shaped lesions that developed in soybean roots parasitised by H. dihystera were typical (Orbin, 1973) and in addition, cortical cell walls were thickened due to lignification, but Orbin also noted that the cytoplasm and nuclei of these remained unaltered. Conversely H. digonicus induced the coagulation of the cell cytoplasm of 7 to 10 cells in lima-bean roots (Perry et al., 1959) whereas the lesions that developed in Poa pratensis infected with H. digonicus extended from the cortex where the cells collapsed into the endodermis. In addition, phloem and possibly xylem tissues were fed upon and these phloem cells were considered to be the favoured food source (Perry et al., 1959).

Earlier observations of Helicotylenchus spp. (Section 3:3) indicated that feeding cells can be either cortical endodermal or pericyclic on cereal roots, and that changes ranged from a slight to an extensive increase in granular material in the host cells. Yet this failed to give a precise indication of the changes occurring within the feeding cell, that allowed a nematode to feed from one cell for up to 19 days. Thus wheat roots infested by H. dihystera were examined to determine the cellular changes that occur in parasitised roots.

5:2:2 Materials and Methods.

Source of Infested Material.

Initially root pieces containing feeding nematodes were removed from

agar plates, fixed in F.A.A. and embedded in paraffin wax (Section 3:3:1).

Subsequently infected roots were obtained from month-old wheat plants var. "Cardinal", grown in soil heavily infested with H. dihystera. The plants were carefully removed from the soil and washed gently in tap water. Roots were then stained using the cotton blue lactophenol procedure (Section 3:3:1) and root pieces were mounted in lactophenol to observe the nature and extent of any lesions, and the position of feeding nematodes.

Fixation.

The roots containing nematodes were placed in a petri-dish containing 3% gluteraldehyde in 0.025 M. phosphate buffer pH. 6.8 (Feder and O'Brien, 1968), and examined at low power magnification. Root segments, approximately 0.5 cm. long, containing semi-endoparasitic nematodes were removed and placed in fresh fixative. Various fixatives were tested separately and in combination but the procedure finally adopted was:-

1) 3% gluteraldehyde in 0.025 M. phosphate buffer pH. 6.8 at 0°C. overnight, followed by three washes in fresh buffer at 0°C.

2) 1% O_sO_4 in sodium cacodylate buffer pH. 7.2 at 0°C. for two hours, followed by three washes in fresh buffer at 0°C.

This combination was adopted because the gluteraldehyde fixative acted sufficiently quickly to inactivate the nematodes while still in their feeding positions. Also the osmium tetroxide stained the nematodes black, so that the position of the nematode's head could be clearly seen during block trimming and sectioning.

Dehydration.

This was originally carried out in an alcohol series, but eventually an acetone procedure was adopted. The root segments were left for 1/2 hour in each concentration, 30%, 50%, 75%, 90% and 100%, of acetone at 0°C.

Infiltration and Embedding.

Two embedding methods were used. These were glycol methacrylate (G.M.A.), Juniper et al., 1970 and Spurr's low viscosity resin (Spurr, 1969). The latter method was adopted because the G.M.A. sections when obtained did not take up stain despite the repeated use of a varied selection of stains.

The resin was progressively infiltrated into the root pieces at room temperature over two days, and fresh resin was then pipetted into block holders in which root pieces were orientated prior to polimerisation. The resin blocks were polimerised in a dry oven at 70°C. for sixteen hours. The blocks were then trimmed ready for sectioning.

Sectioning.

1/2 μ sections were cut on a L.K.B. ultramicrotome, and mounted on glass slides. The blocks were originally sectioned only at 1/2 μ, but following the initial results ultra-thin sections were cut for electron-microscope examination. Blocks sectioned for the electron-microscope were initially sectioned at 1/2 μ so that a comparison of changes seen at low and high magnification could be made. The ultra-thin sections were mounted on formvar coated copper grids.

Staining.

The 1/2 μ sections were stained for 10 - 30 seconds in drops of 2% toluidine blue in 2% borax heated gently over a bunsen. The stain was then washed off in running water, the slide dried, and the sections mounted in Canada balsam and examined.

The ultra-thin sections were first examined unstained at 40 K.V. in a Phillips 300 electron microscope. After this initial examination the grids were double-stained in saturated uranyl acetate in 50% ethanol pH. 4 - 5 and lead citrate (Reynolds, 1963). Following staining the grids were dried

and examined at 60 K.V. in the Phillips 300

5:2:3 Observations.

Whole Mounts.

H. dihystera fed in wheat roots in similar positions to H. digonicus, H. pseudorobustus, and H. varicaudatus in cereal and grass roots (Section 3:3). Nematodes fed as semi-endoparasites (Plate 23.) and as endoparasites (Plate 24.) and separate lesions developed in superficial cortical cells around the nematode, (Plate 23.) and in cells around the nematode's head (Plate 24.). Egg clusters were also evident adjacent to the nematodes, and occasionally non-feeding larval nematodes were found among the unhatched eggs, but the bulk of all nematodes seen in roots were adult females.

Wax Sections.

H. dihystera fed in cortical endodermal or pericyclic cells of wheat, and these cells became filled with a dense material assumed to be an enlarged cytoplasm (Plate 25.). The nematode's body was positioned in coils in cavities in the root cortex and eggs were also laid in these cavities (Plate 26.).

On one occasion a nematode feeding in a cortical cell had the tip of its stylet enveloped by a cap of material (Plate 27.) that closely resembled the salivary zone seen in feeding nematodes (Section 5:1). The uncertainty in interpreting the function of this and other structures was the reason why a more detailed investigation of the feeding cell was undertaken.

Resin Sections.

Light microscope sections.

The changes induced within the roots could be attributed to either penetration or feeding.

During penetration cell walls were ruptured and the torn ends hung

Plate 23.

Semi-endoparasitic nematode with the cell walls of the cortical cells indicating the necrotic lesion of superficial cells. (x600)

Plate 24.

Endoparasitic nematode and eggs within a cortical cell. Note the globular material in the feeding cell adjacent to the nematode's head. (x700)

Plate 23.

Plate 24.

Plates 25 and 26.

Plate 25. The feeding lesion of H. dihystera involving endodermal pericycle and protoxylem pole cell. (X1,400)

Plate 26. Coils of the nematodes body and eggs in a cortical cavity. Note the extensive cell wall rupture and lack of a necrotic lesion. (x1,000)

Plate 27.

A nematode with its stylet passing through a cell wall, and with its stylet tip enveloped in a cap of material. This material is presumed to be the product of the dorsal gland exudate. (x3,000)

Plate 25.

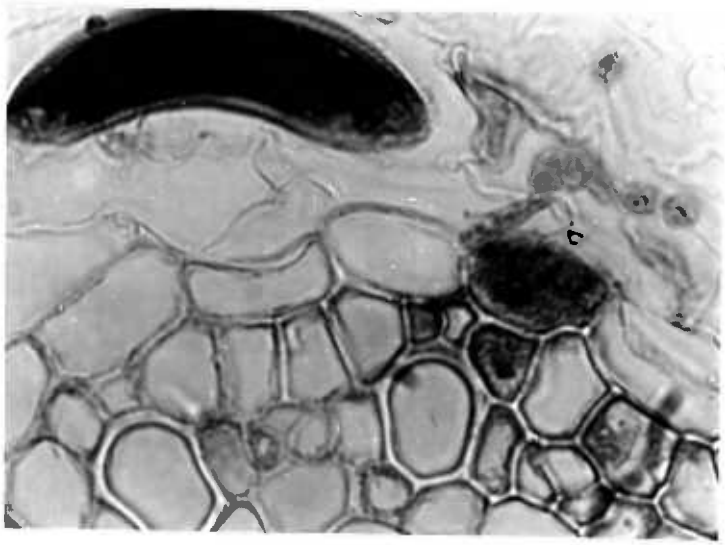


Plate 26.

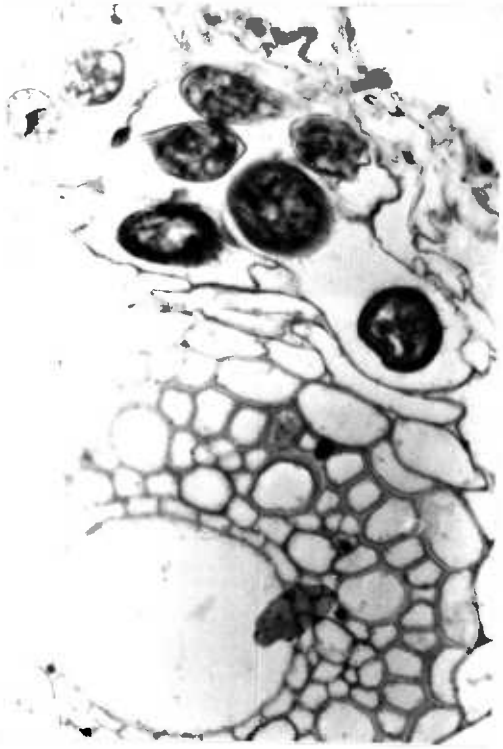
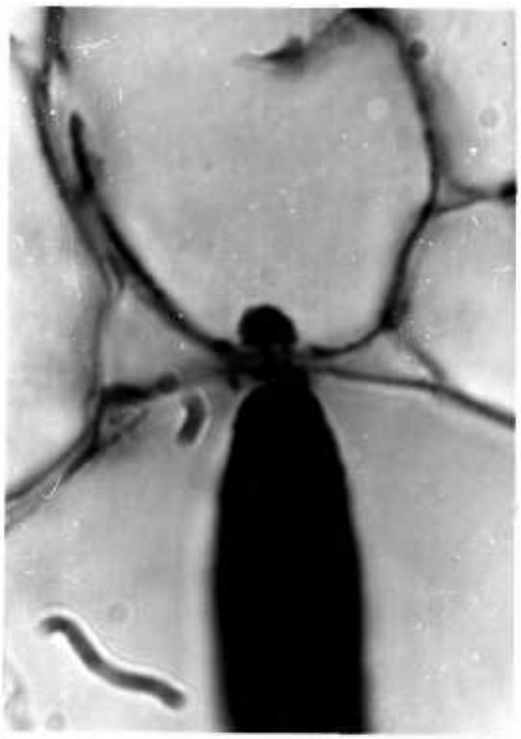


Plate 27.



free in the cell (Plate 28.). Occasionally the cell walls pinched in the nematode's body (Plate 28.) indicating that a general cell collapse did not result from the presence of the nematode.

Also during penetration, contaminants were introduced to cells (Plate 29.) and this material usually adhered to the torn ends of cell walls, though such material did also occur free in the cell.

The cellular changes due to feeding occurred in a group of cells around the nematode's head. The cellular changes in cortical (Plates 30 and 31.), endodermal (Plate 32.) and pericyclic feeding cells (Plate 33 to 35.) were similar. In unparasitised cells, the cytoplasm was pressed into a thin layer on the cell wall by a large central vacuole, whilst in parasitised cells, the cytoplasmic layer increased in volume at the expense of the size of the central vacuole.

Cells adjacent to the feeding cell occasionally also had an enlarged cytoplasm (Plate 32.) but these changes were less marked than in the feeding cell. Feeding cells, whether they were cortical, endodermal or pericyclic in origin, tended to be adjacent to a xylem vessel (Plates 30 to 33 and 35.) and in such situations the protoxylem pole cell was filled with a dense material (Plates 30 to 32 and 35.).

Cortical feeding cells were always adjacent to the endodermal passage cell (Plates 30 and 31.) and no nematodes were found that had fed from superficial cortical cells.

No cell wall thickening was seen in cells in which the nematode's body lay, yet in feeding cells a localised projection of the inner wall of a cortical (Plate 31.) and an endodermal cell (Plate 32.) were found. In the cortical cell, the nematode's stylet was embedded in this projection and the action of the stylet in penetrating the cell wall was thought to induce this wall defence response.

Occasionally the tip of the nematode's stylet was enveloped in a zone within the cytoplasm (Plate 35.). This zone was also seen in feeding cells from which the nematode had withdrawn its stylet (Plates 32 and 34.) and resembled the cap of material seen earlier surrounding the nematode's stylet in living (Section 5:1) and fixed nematodes (Plate 27). In some sections a coiled structure occurred (Plate 32.) that closely resembled the feeding tube of Rotylenchlus reniformis (Razak, 1975) though the origin and function of this tube could not be determined from these sections.

Electron-Microscope Sections.

Parasitised cells contained an enlarged cytoplasmic layer in which there were increased numbers of cell organelles (Plates 36 to 38.). That feeding cells were alive and not moribund was indicated by the presence of an intact phasmalemna (Plate 44.) and the presence of a nucleus with an intact nucleolus and nuclear membrane (Plate 39.). In the cytoplasm of feeding cells, mitochondria (Plate 38.) plastids (Plates 40 and 41.) and endoplasmic reticulum (E.R.) (Plate 43.) were common. Also present were amyloplast like structures (Plate 42.). Both granular and agranular E.R. occurred in the cytoplasm and free ribosomes were also present. The granular E.R. was associated with fibrillar proteinaceous like deposits (Plate 43.) and occasional lipid droplets were found in the cytoplasm (Plate 46.).

Thickening of the cell wall seen in cortical feeding cells (Plates 36, 44 and 45) were caused by a localised enlargement of the lignin layer of the cell wall. In one cortical feeding cell (Plate 36.) the thickening extended along the whole outer tangential cell wall.

Adjacent to the nematode's head in the cell cytoplasm a zone of material occurred. This was the salivary zone (Plates 45 and 46.) and it was positioned within the intact plasmalemna (Plate 47.), but was not separated from the remaining cytoplasm. The zone, that was devoid of all

cell organelles, occurred in all feeding cells and is thus considered to be the normal product of the dorsal gland exudate. The cytoplasm adjacent to this zone contained strands of granular E.R. (Plates 45 and 46.) that radiated away from the salivary zone and ended in dense globular deposits in the cytoplasm. Though the salivary zone was devoid of definite structures, within and surrounding the zone were small dense bodies (Plate 49.) that on close examination were derived from a collection of ribosomal-like particles. In addition the bulk of the salivary zone appeared to be made up from intertwining strands of this ribosomal-like material.

The tube seen in 1/2µsections (Plate 32.) in an endodermal cell, also occurred in a cortical (Plate 45.) and a preicyclic feeding cell (Plate 46.). In the pericyclic cell, the tube appeared as a coiled helix (Plates 46 and 47.) with an external diameter of 0.6 to 0.7 µ and an internal diameter of 0.2 to 0.3 µ. The tube also, appeared to consist of ribosomal-like particles (Plate 48.) and thus is likely to originate from the dorsal gland exudate.

Cortical cell walls ruptured by penetrating nematodes appeared as loose ends free in the cells. Around these ruptured cell walls and the nematode's lips, was a fine granular material which contained contaminating bacteria. It is these contaminants that are though to induce the cell wall necrosis seen around the body of feeding nematodes.

Plate 28.

Root penetration. The ruptured ends of cell walls are rolled back along the nematode's body and appear to indent the nematode's cuticle. (X1,200)

Plate 29.

Nematode feeding in an endodermal cell. Note the contaminants lying free in the cortical cell and adhering to the torn ends of cell walls. (x1,600)

Plate 28.

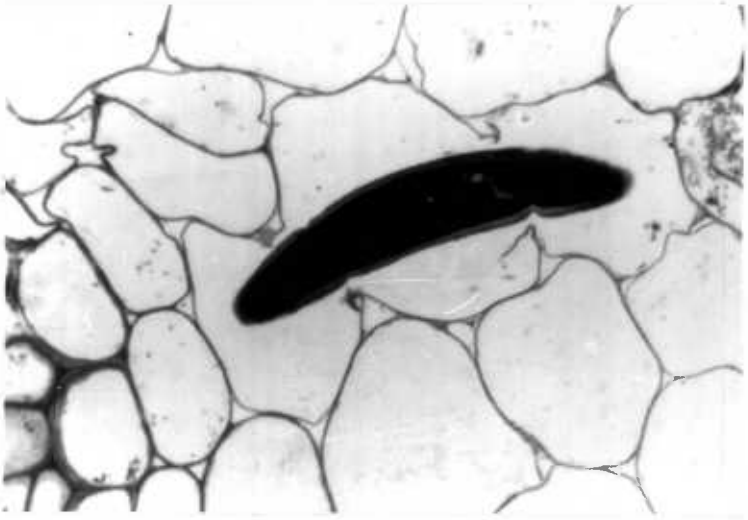


Plate 29.

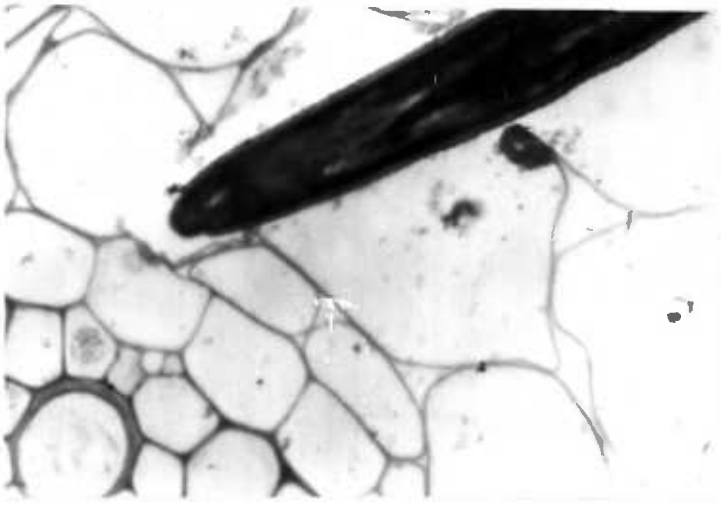


Plate 30.

Cortical feeding cell filled with cytoplasm, adjacent to an endodermal passage cell. (x3,000)

Plate 31.

Cortical feeding cell with only a partially increased layer of cytoplasm. The nematodes stylet is embedded in a localised projection of the cell wall. The protoxylem pole cell is filled with a dark stained material. (x3,000)

Plate 32.

Endodermal feeding cell. Note the partially reduced vacuole, and the involvement of pericyclic and protoxylem cells in the feeding lesion. In the endodermal cell, a localised cell wall thickening, a salivary zone, and a feeding tube occur. (x3,000)

Plate 30.

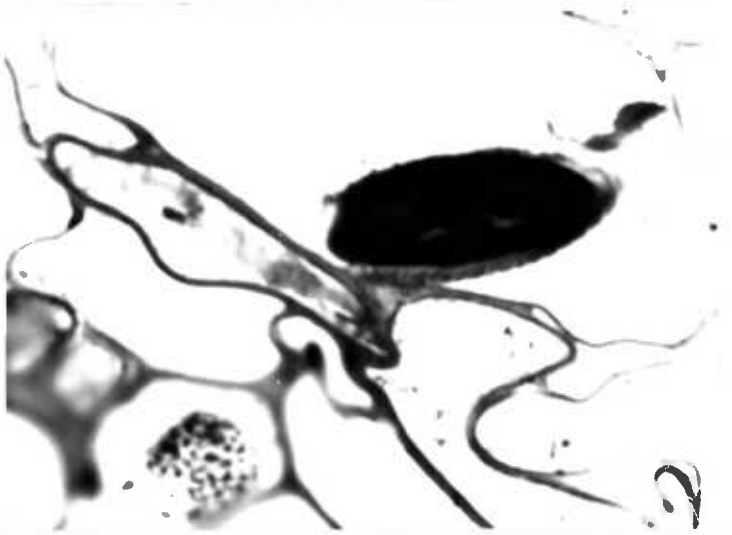


Plate 31.

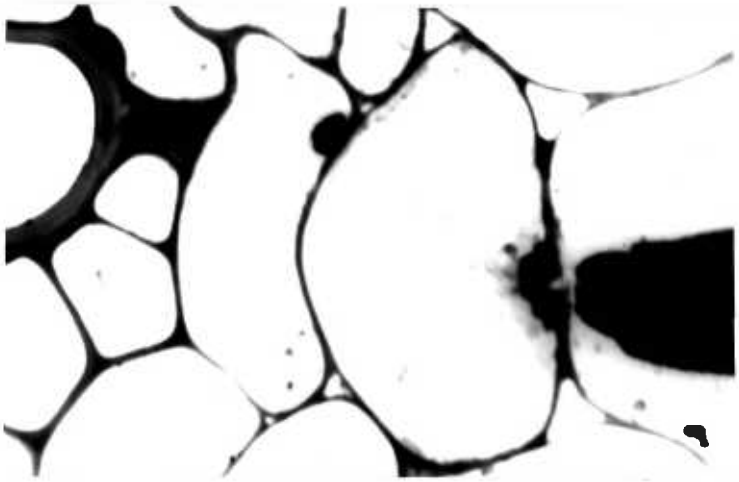


Plate 32.

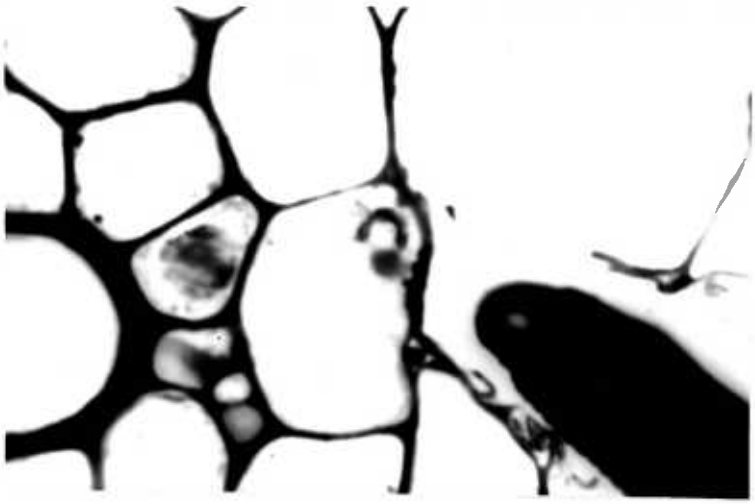


Plate 33.

Pericyclic feeding cell, with no recognisable cellular alteration. (x2,000)

Plate 34.

Pericyclic feeding cell, with reduced central vacuole, cell cytoplasm containing many organelles, a salivary zone, and feeding tube. (x3,500)

Plate 35.

Pericylic feeding cell. The nematode's stylet passes through the cell wall, and is positioned in the salivary zone. (x4,000)

Plate 33.

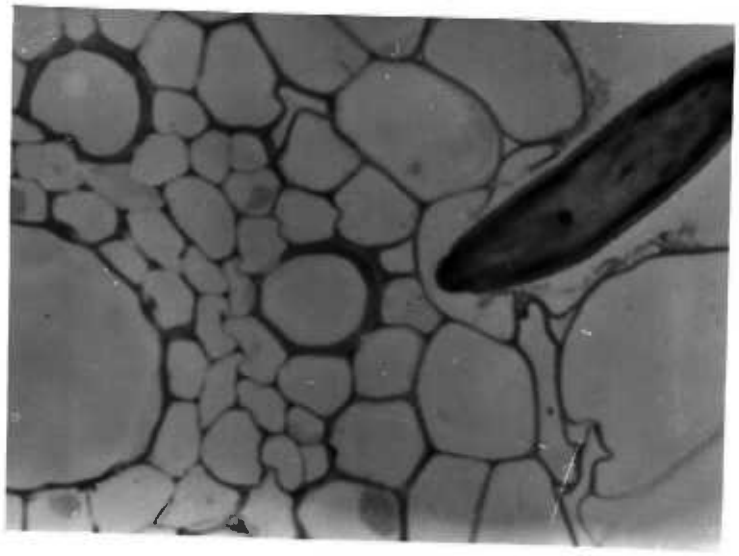


Plate 34.

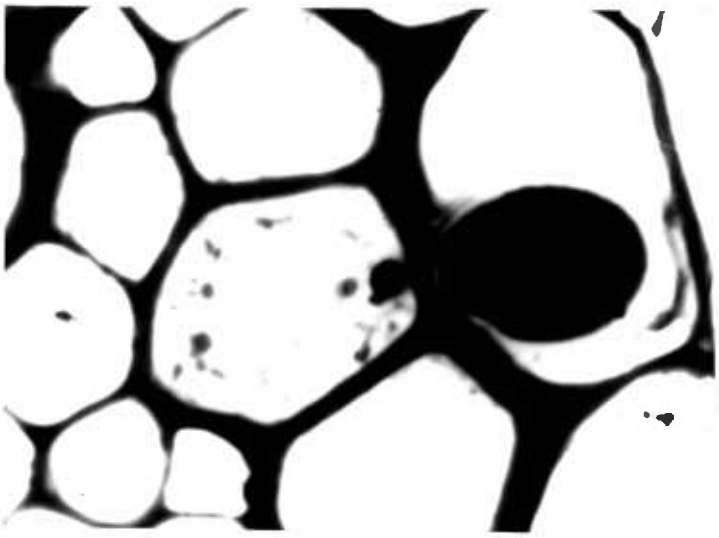


Plate 35.

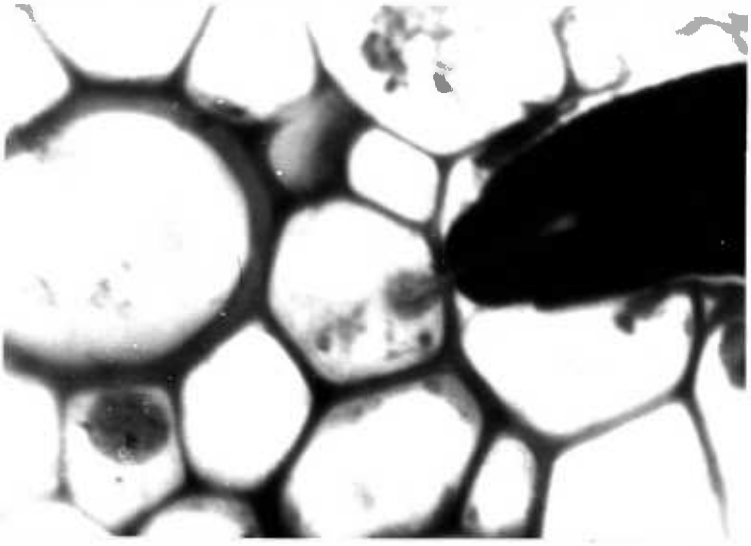


Plate 36.

Cortical feeding cell. (Compare Plate 30.).
The feeding lesion comprises a cortical cell with reduced vacuole and pericyclic and protoxylem cells. Note the localised thickening of the cell wall adjacent to the nematode and the thickening of this layer along the entire tangential wall of this cell. (x2,000)

Plate 37.

Endodermal feeding cell, with reduced central vacuole and increased numbers of cell organelles. (x4,500)

Plate 36.

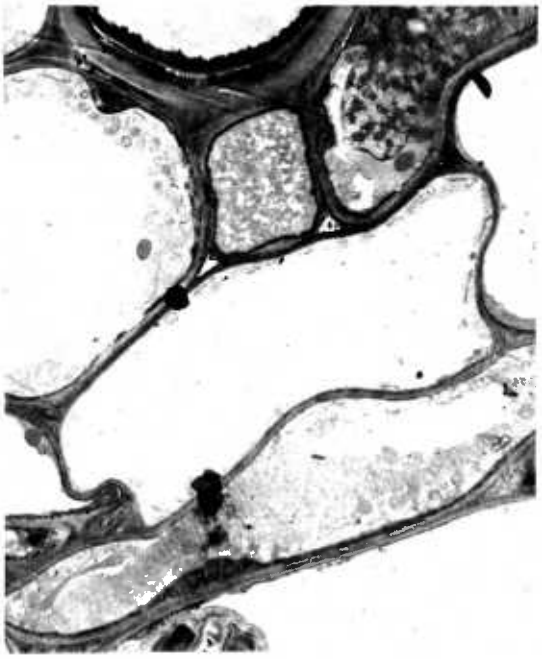


Plate 37.

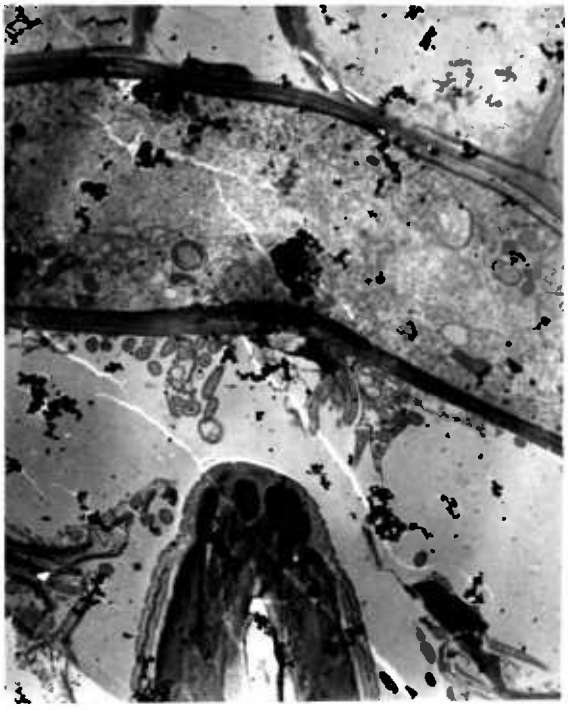


Plate 38.

Pericyclic feeding cell, with enlarged cytoplasm containing many mitochondria and plastids of irregular shape and size. (x6,000)

Plate 39.

Pericyclic feeding cell with intact nucleus, nucleolus, and nuclear membrane. (x6,000)

Plate 38.

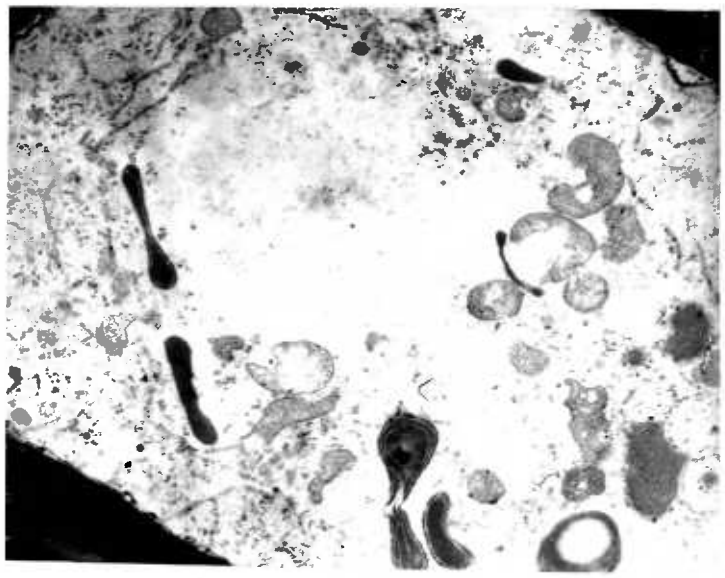
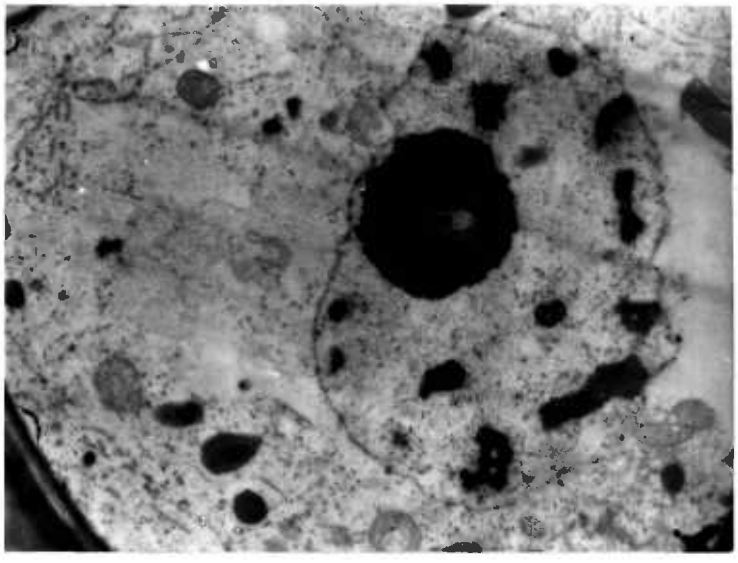


Plate 39.



Plates 40 and 41.

Plastids and mitochondria.

Plate 40. (x35,000)

Plate 41. (x54,000)

Plate 42.

Amyloplast-like structure adjacent to plastids and mitochondria. (x22,000)

Plate 40.

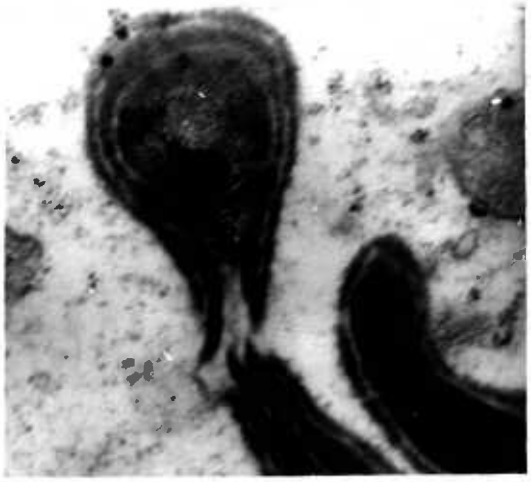


Plate 41.

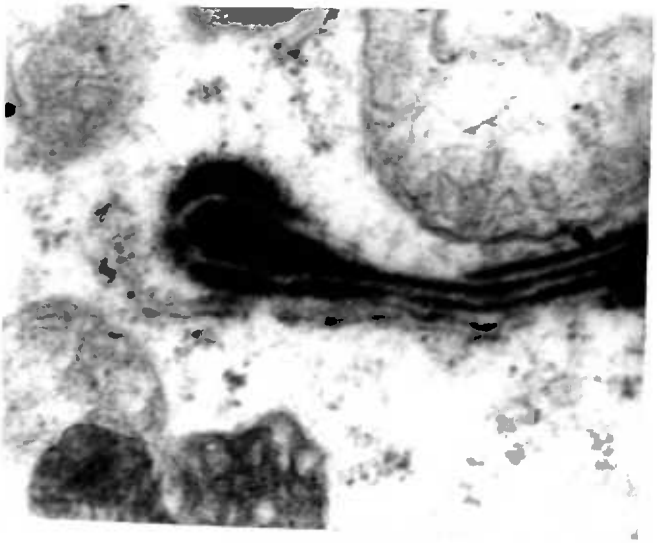


Plate 42.

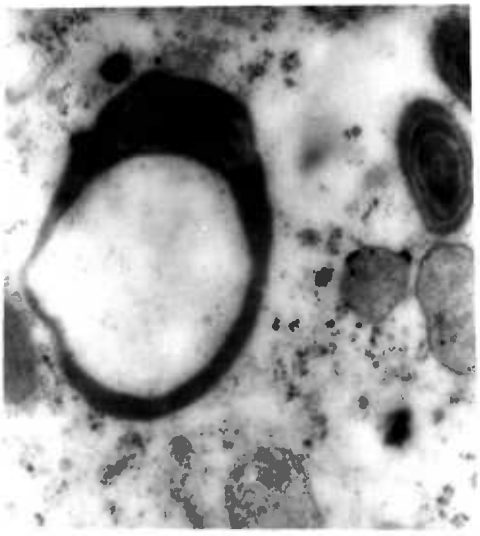


Plate 43.

Granular endoplasmic reticulum present in irregular strands adjacent to fibrillar deposits in the cytoplasm.
(x30,000)

Plate 43.

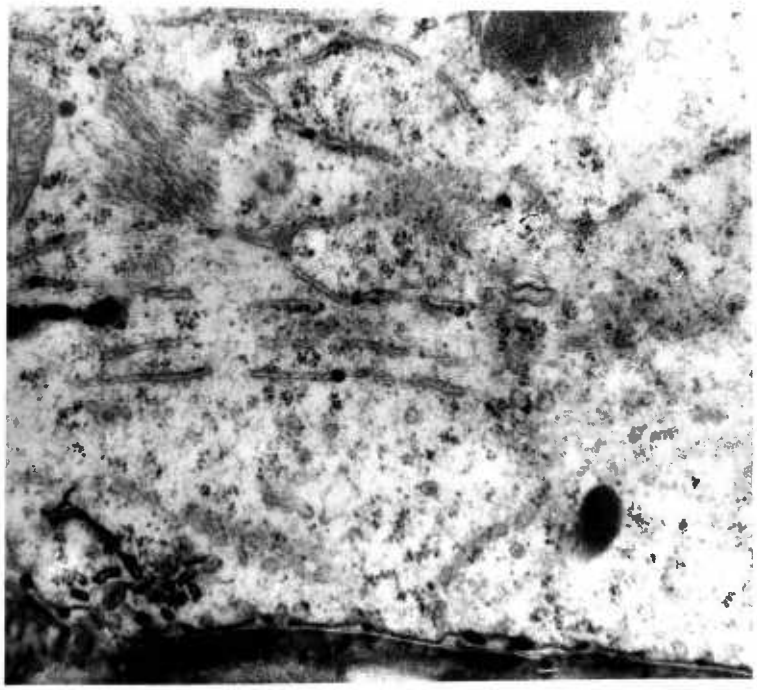


Plate 44.

Cortical feeding cell with intact plasmalemna. The inner layer of the cell wall (lignin) has thickened to form the localised projection. (x11,000)

Plate 45.

Cortical feeding cell (Compare Plate 31.)
Note the cell wall projection and the salivary zone adjacent to this projection in the cell cytoplasm. Strands of granular E.R. radiate from the salivary zone. (x6,500)

Plate 44.

Plate 45.

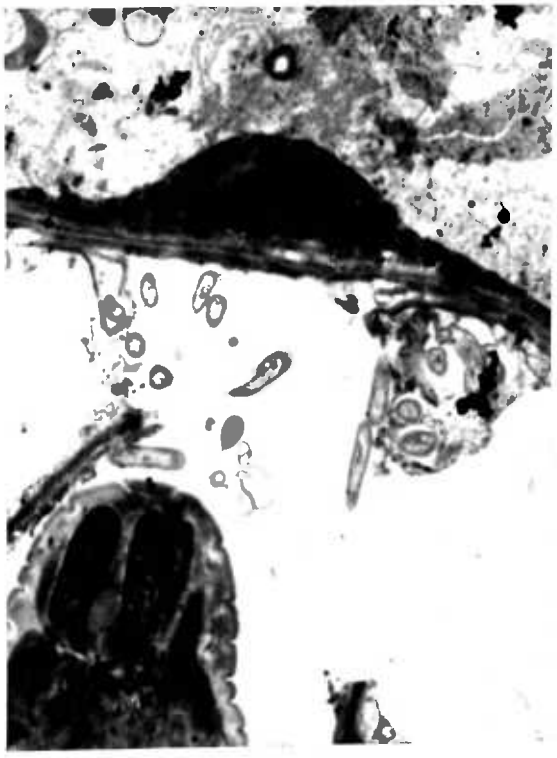


Plate 46.

Pericyclic feeding cell with a structureless salivary zone in the cell cytoplasm. (x11,000)

Plate 47.

Pericyclic feeding cell showing sections of the salivary tube and lipid droplet. (x14,000)

Plate 46.

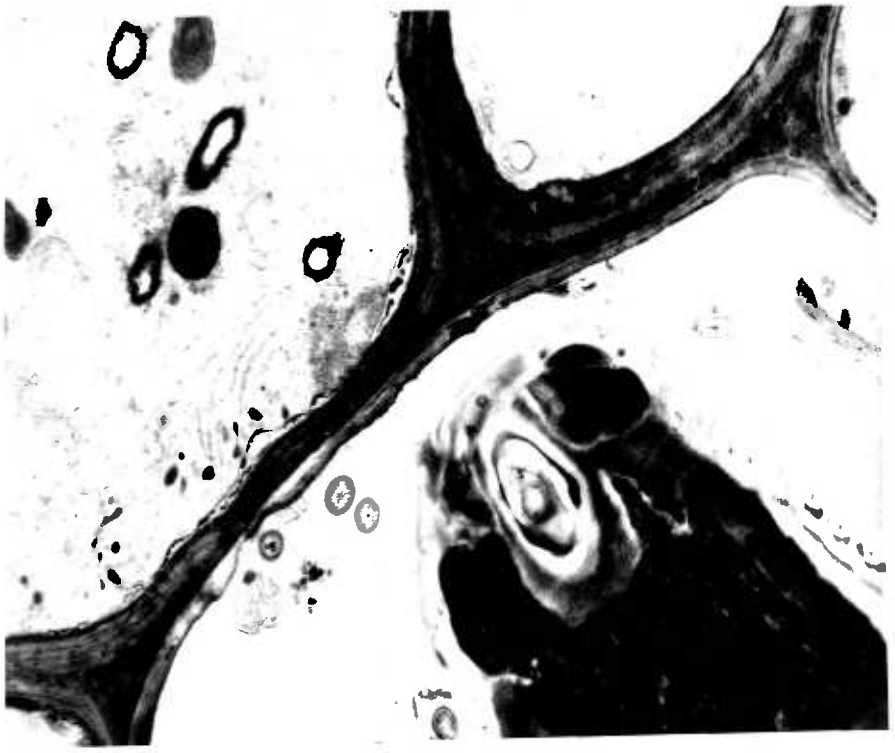


Plate 47.

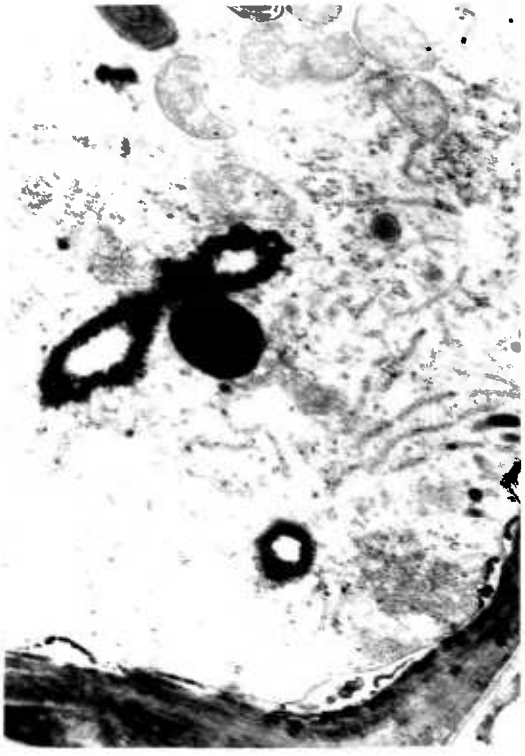


Plate 48.

Salivary tube surrounded and consisting of ribosomal-like particles. (x42,000)

Plate 49.

Salivary zone, containing dense bodies and strands of intertwining particles resembling ribosomes. (x60,000)

Plate 48.

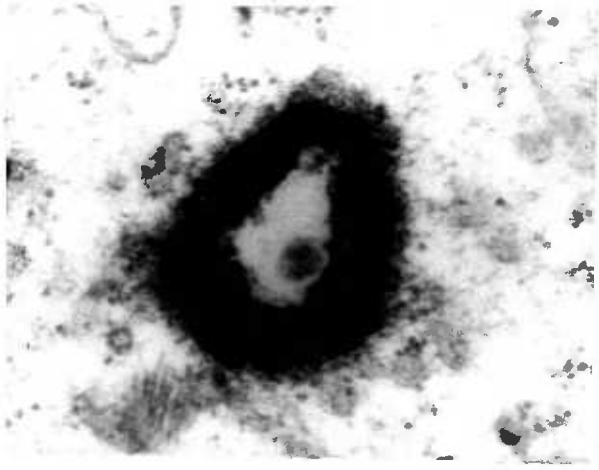
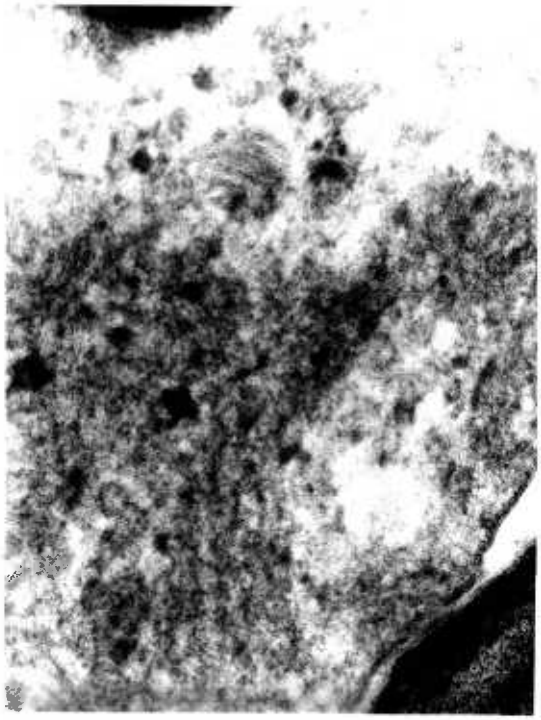


Plate 49.



5:3 Oesophageal Gland Secretions.

5:3:1 Dorsal Gland.

Introduction.

Two intracellular structures, the salivary zone, and the helically coiled tube, appeared to be of nematode origin, and were thus further investigated.

The salivary zone may be associated with the dorsal gland exudate by its similarity to the structure observed in cells fed on by ectoparasitic nematodes (Doncaster, 1966; Wyss, 1973), in ectoparasites the zone formed while granular material disappeared from the dorsal gland ampulla. This link was not seen for H. dihystera but both the loss of granules, and the salivary zone were seen on separate occasions. Bird (1969) showed that in Meloidogyne javanica females the granules in the dorsal gland broke down, before entering the oesophageal lumen, into ribosomal-like particles which then passed down the stylet lumen and formed the nematode's exudate. In addition Linford (1937) observed that Meloidogyne spp. teased out of pea roots, when placed in a solution of 2% peptone and 2% dextrose, produced an exudate that formed a string of beads or globules from the stylet tip, but, when the females were placed in water, the exudate produced "usually formed a compact refractive coiled filament of irregular diameter and shape". In sectional feeding sites of Meloidogyne spp. a salivary zone is present (Jones and Dropkin, 1975), thus these nematodes produce an exudate that in varied situations, formed either a globule or a filament.

Accordingly, H. dihystera females were removed from roots to determine the nature of the dorsal gland exudate.

5:3:2 Materials and Methods.

The nematodes were obtained from roots of sugarcane heavily infested with H. dihystera. Semi-endoparasitic nematodes were teased out of the roots

and immediately placed in drops of solutions. The solutions used were:-

1) distilled water
2) 2% soya peptone, 2% sucrose in distilled water
3) 3% gluteraldehyde in 0.025 phosphate buffer pH. 6.8
4) The supernatant of macerated roots. This was prepared by grinding 0.5 grms. of 10 day old wheat roots in 12 ml. of 0.025 M. phosphate buffer pH. 6.8. The mixture was then centrifuged at 2,000 r.p.m. for 5 minutes, and the supernatant pipetted off.

Up to six nematodes were placed on a slide, covered with a cover slip and sealed so that observations could be made with a 100x objective.

5:3:3 Observations.

The majority of the nematodes placed in the solutions immediately became fully active and were thus disregarded. Nematodes placed in fixative after a short period of normal activity slowly became moribund.

The nematodes that remained comparatively static in the solutions were observed and the various feeding activities were seen. All these nematodes moved the anterior end of the body in a slow waving manner as seen in localised exploration. Some nematodes began a thrusting action of the stylet, whilst on one occasion irregular contractions of the medium bulb were seen. Salivating nematodes were seen repeatedly and nematodes likely to salivate were immediately detectable due to the protrusion of the stylet (Plate 50.) and the presence of granular material in the dorsal gland ampulla (Plate 51.).

Nematodes that showed these characteristics eventually salivated but frequently an interval of up to 30 minutes elapsed before an exudate was produced. At the end of exudation, the gland ampulla was largely devoid of contents, and the nematode would withdraw its stylet and slowly resume normal body movement.

The reactions of the exudate to the solutions varied. In distilled water, the exudate formed a globule within which was some filamentous material (Plate 52.). In peptone dextrose solution, a similar product formed but in fixative no exudation was seen. The nematodes that appeared likely to salivate did not, and the gland ampulla remained filled with granular material. Yet on one occasion, a coiled filamentous tube was seen adhering to a nematode's stylet when the nematode was first observed (Plate 53.). In the supernatant of macerated roots the exudate consistently formed only as a globule of material enveloping the stylet tip (Plate 54.). The volume of this exudate was approximately 200 μ^3.

5:3:4 Ventral Gland.

At no time during observations on salivation were any changes seen in the ventral glands, yet in nematode extracts the ventral glands of approximately 20% of the nematodes contained a particulate secretion. The particulate nature of this secretion was lost when nematodes were fixed or heat-killed but it remained intact in nematodes narcotised in 2% propylene phenoxytol (Plate 55.).

Plate 50.

Pre-salivating nematode. H. dihystera with stylet protruded. (x3,000)

Plate 51.

Pre-salivating nematode. Dorsal gland ampulla containing granular secretion. (x3,000)

Plate 50.

Plate 51.

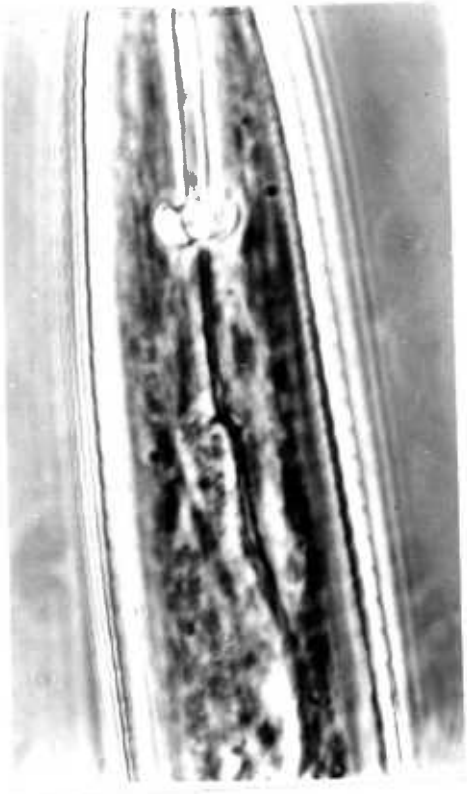


Plate 52.

Nematode in distilled water. Saliva produced as a globule containing filamentous material. (x3,000)

Plate 53.

Nematode in 3% gluteraldehyde. Saliva formed into a coiled tube. (x4,500)

Plate 52.

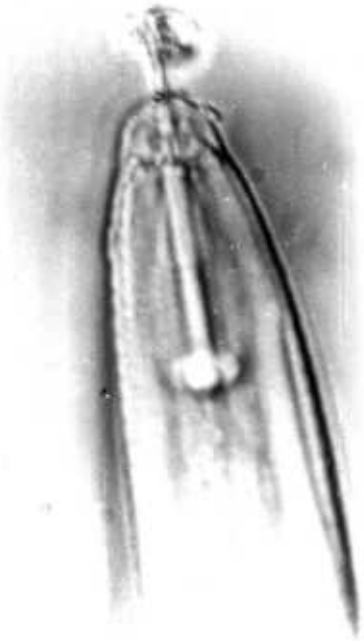


Plate 53.

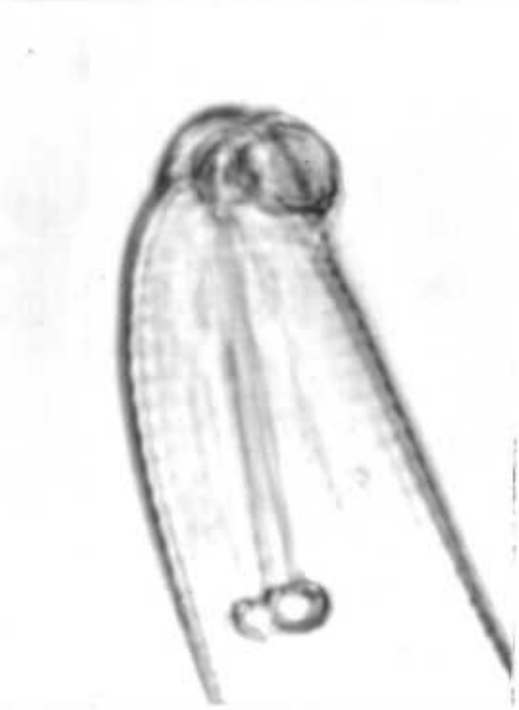


Plate 54.

Nematode in supernatant of macerated root. The saliva formed into a globule which was approximately 200 $\mu.^3$ (x3,000)

Plate 55.

Ventral gland secretion in H. dihystera. (x5,000)

Plate 54.

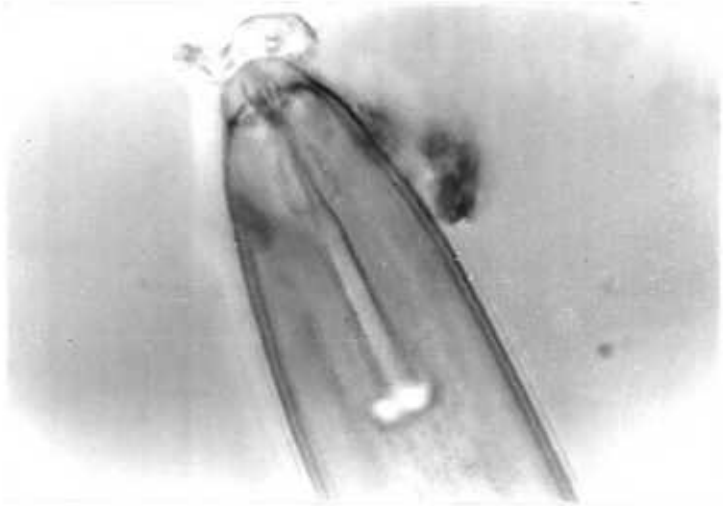
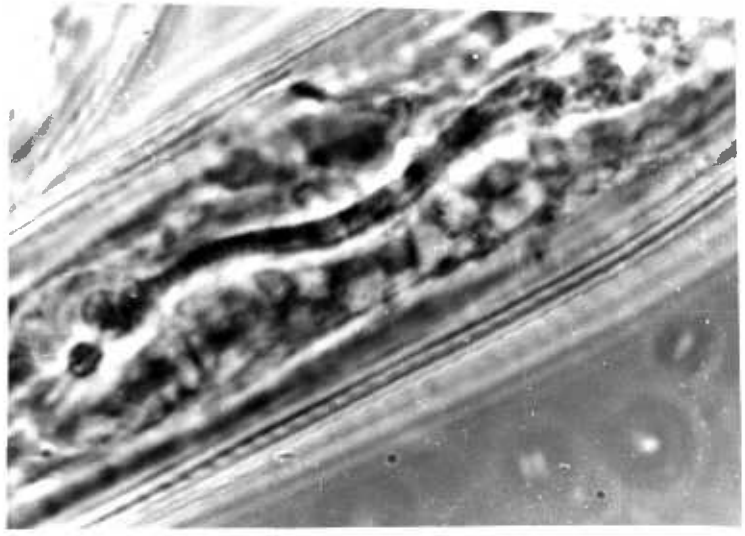


Plate 55.



5:4 Discussion.

Wyss (1973) divided the feeding behaviour of the ectoparasite T. dubius into four phases, exploration, penetration, salivation and ingestion. Doncaster and Seymour (1973) divided the behavioural activities of endoparasitic nematodes into:-

(A) behaviour leading to feeding comprising widespread exploration, local exploration and stylet thrusting, followed by

(B) feeding that comprised either a single or intermittent ingestion and salivation phases.

Successful feeding behaviour of H. diystera differed in that it involved firstly the penetration of the root and secondly prolonged feeding from a single cell. Widespread exploration of the root appeared to be a separate phase during which the nematodes orientate to roots but exhibit no real initiation of feeding activities. All the other sequences of feeding had a definite duration and all led to a predictable continuation of feeding if successful.

Root penetration consisted of firstly penetration site selection by localised exploration prior to stylet thrusting that led to cell wall rupture. Following entry to a cell, a nematode would immediately begin to select a new penetration site and this would continue at each successive cell wall encountered until root penetration activity was replaced by feeding.

Feeding comprised alternating salivation and ingestion but the stimulus needed to switch from root penetration to feeding is unknown. In endoparasites Doncaster and Seymour (1973) postulated that a chemical stimulus was the cause of the initiation of feeding.

Detailed observations of cell wall penetration by endoparasitic nematodes are uncommon, but Nacobbus and Heterodera spp. achieved entry by cutting a line of holes that fused into each other forming a slit (Doncaster and Seymour, 1973). This slit was then used as an entry hole,

and successful wall penetration by Heterodera spp. was achieved after 9 to 21 mins. Helicotylenchus spp. did puncture the cell wall more than once but penetration resulted from a combination of a weakened cell wall and the forward pressure of the body rupturing the cell wall. The ventral gland secretions of M. javanica pre-parasitic larvae are thought to aid root penetration (Bird, 1967a; 1967b). A similar secretion occurred in the sub-ventral glands of H. dihystera (Plate 55.) but no nematodes were seen to pass these secretions out to the penetration site.

The events of salivation and ingestion of the endoparasite H. dihystera were similar to those seen in ectoparasites and lasted for a comparable period (Wyss, 1973; Bridge and Hague, 1974). However, there is a degree of individuality in the feeding behaviour of different species, e.g. Merlinius icarus (Bridge and Hague, 1974) and Hemicycliophora arenaria (McElroy and Van Gundy, 1968) extend a feed by lengthening each of these phases. H. dihystera and H. varicaudatus extended a feed by alternating salivation and ingestion. Other endoparasites have been observed as having a rest period between ingestion phases, and the duration of these rest phases e.g. Hoplolaimus columbus 3 to 28 mins. (Fassuliotis, 1975), R. robustus 20 mins. (Klinkenberg, 1963) and Paratylenchus minutus 2 hours (Linford et al., 1949) suggests that feeding was also extended by alternating salivation and ingestion.

During feeding H. columbus was observed to periodically withdraw its stylet, re-insert it into the original holes and resume ingestion (Fassuliotis, 1975). At no time during a feed was H. dihystera seen to withdraw and re-insert its stylet. Stylet withdrawal only occurred at the end of a feed.

Irregular contraction at the onset of ingestion (Linford et al., 1949; Rhoades and Linford, 1971; Wyss, 1973) have been presumed to serve as a "warming up" phase prior to ingestion, but the contractions that occurred

were markedly different from normal bulb activity and thus a separate function may well be involved. The ventral glands have an outlet to the oesophageal lumen in the medium bulb (Plate 55.) and if these secretions have a digestive function, this spasmodic movement of the medium bulb may be involved in the release of such secretions prior to ingestion.

The lesion that developed around nematodes was of two types. Firstly a necrotic lesion caused by contaminating bacteria introduced to cells by the nematode, and seconly a feeding lesion that comprised a group of cells around and including the feeding cell.

The feeding lesion and necrotic lesion can be clearly separated in whole root pieces (Plates 7 and 24.) where the feeding cells are filled with a bead-like material. The central cell of the feeding lesion, the feeding cell was most commonly a pericyclic cell, yet root hairs cortical cells and endodermal cells were also fed from. Root hairs and epidermal cells were unfavourable feeding sites, and the close proximity and involvement of xylem cells in the feeding lesion seemed to be needed for sustained feeding. In this study no phloem cells became involved in the feeding lesion.

H. dihystera caused lignification of cortical cell walls of soybean (Orbin, 1973). Such lignification only occurred in the feeding cells of wheat and was mainly evident as a localised projection (Plate 36.). Similar projections occurred in roots infected by Pratylenchus fallax (Corbett, 1972) and Rotylenchus reniformis (Razak, 1975) and are also formed as a defence reaction to penetrating fungal hyphae (Nat. Acad. Sci., 1968).

Many organelles were present in the feeding cell cytoplasm yet for the nematode to be able to ingest material it has to be small enough to enter the 0.25 μ aperture of the stylet, and be present in the zone from which

ingestion occurs. Ingested material could be formed by the enzymic breakdown of cell cytoplasm such that the nematode is able to ingest the product. But this browsing habit seems unlikely to occur since the structural changes within the cell suggest that controlled changes of the cytoplasm have occurred. If the nematode controls these changes then the ribosomal-like particles of the dorsal gland exudate may function on specific sites to induce the changes. Alternatively, the feeding lesion may be formed by the reaction of plant cells to a loss of nutrients resulting in a uniform cell response.

Apart from the need for a control mechanism, the cell must form a product for the nematode. There is ample evidence that the feeding lesion is capable of achieving this, yet how this product is channelled to the nematode is unknown. It may be that local enzymic action around the salivary zone precludes the need for an organised channelling of nutrients, but the proximity of the lipid droplet and strands of granular E.R. (granular E.R. is thought to act as a protein transport system within a cell (Fawcett, 1966).) to the salivary zone suggests that a more organised system exists.

The feeding habits and lesion developed by R. reniformis on susceptible soybean roots (Rebois et al., 1975) resembles that formed by H. dihystera on wheat in that both nematodes invade the developed regions of roots, both have pericyclic endodermal and cortical cells involved in the feeding lesion, and within this lesion, the central vacuole of the cells is reduced and replaced by cytoplasm, containing increased numbers of organelles. A further similarity exists in the oesophageal structures of these nematodes (Seinhorst, 1966), and Seinhorst further postulated a phylogenetic relationship between these genera. The similarity in the feeding lesion gives additional support to this suggestion.

The cause of the termination of a feed is unknown, since no real indication of collapse of the feeding lesion was found. Doncaster and Seymour (1973) suggested that feeding stopped when the nematodes food requirements had been met. In this study no other explanation seems likely and the difference that exists between Helicotylenchus spp. and other vermiform nematodes studied is that nutrients derived from a single cell are sufficient to meet the needs of an adult egg laying nematode.

SECTION 6.

FINAL DISCUSSION

The expression of plant disease has been separated into:-

(A) The signs of disease, that are the visible and apparent changes in the growth of the plant, and

(B) The symptoms of disease that are the cellular and chemical changes that occur. (Nat. Acad. Sci., 1968).

Root feeding nematodes produce varied signs of disease but on the above ground tissues, disease is usually evident as stunted growth. H. pseudorobustus on winter wheat, H. digonicus on winter barley and populations of Helicotylenchus spp. in pot pathogenicity trials produced no signs of disease. The populations of the nematodes in these experiments were greater than populations that occurred in grassland soils in S.E. England (Bridge, 1971), and thus it is likely that cereal production is unaffected by populations of Helicotylenchus spp.

Field crops susceptible to Helicotylenchus spp. are usually perennial crops that allow large populations to build up and induce unthrifty growth (Minz et al., 1960; Tarjan et al., 1971; Wallace, 1971). Pasture soils are just such an environment, and thus when these pastures are ploughed and cereals planted, the nematodes are likely to invade the young cereal roots at a very susceptible growth stage. In this situation H. varicaudatus did invade the cereal roots, but in pathogenicity trials there was no evidence that the plants suffered diseases in either the early or subsequent stages of growth.

T. dubius was implicated in causing disease of cereals, where in winter wheat, populations that had developed on grass, were above the threshold for damage of cereals and induced stunted growth, whilst on winter barley these nematodes were also associated with a patchy early

yellowing of the host. T. dubius caused stunted growth of several hosts (Whitehead and Frazer, 1972; Kyrou, 1969; Gowen, 1971) and the inducement of disease by these root-tip feeding nematodes may be due to the sensitivity of their feeding site.

The effects of feeding of the individual genera of ectoparasitic nematodes are generally considered to be slight, but when taken as a group the host is regarded as being subject to a permanent insidious loss (Webster, 1969; Juska, 1972). This suspicion was not borne out in my pathogenicity field trials. In addition, the relative abundance of the separate genera in each of the plots were not related, and despite the similarity of feeding sites of Pratylenchus and Helicotylenchus spp., there was no evidence of competition for feeding sites.

The symptoms of disease are the cellular and chemical changes that are brought about by the invading organism. The symptoms caused by Helicotylenchus spp. are typically described as consisting of small necrotic lesions in the superficial cells of the cortex of their host roots (Minz et al., 1960; Taylor, 1961; Davis and Jenkins, 1966). The changes seen in the feeding lesion of H. dihystera using the light microscope were similar to the changes seen in feeding lesions of H. digonicus, H. pseudorobustus and H. varicaudatus. During penetration to a feeding site, cell walls are ruptured, and this exposes the contents of these cells to secondary organisms, be they other nematodes, bacteria or fungi, and it is these secondary organisms that cause the superfifical necrosis seen typically around these nematodes. Perry et al. (1959) stated that H. digonicus fed preferentially from within the central state of Poa pratensis and lima-bean roots. This was also true of Helicotylenchus spp. on cereal cells and the feeding lesion of these nematodes consisted of a small group of cells around the nematodes head. The changes occurring in

the feeding lesion indicate that the nematode is able to derive nutrients from a wider area than the specific cell from which it feeds because:-

1) The duration of a single feed from one cell precludes the possibility that a simple browsing feed occurs,

2) The formation of egg clusters during a single feed also indicates that the nematode must derive nutrients from many more cells than just the feeding cell,

3) The structural organisation of the feeding cell shows that browsing feeding does not coccur, and that the feeding cell has become a highly metabolically active cell.

4) The cytoplasmic alteration of a small group of cells around the feeding cell indicates that these cells are involved in supporting the nematodes feeding, and

5) Feeding lesions were preferentially formed adjacent to xylem tissues, suggesting that transport tissues were involved in a successful feeding lesion. The feeding cells that supported only short feeds were epidermal cells and root hairs, which are remote from the xylem tissues.

The pattern of feeding behaviour where cycles of ingestion were broken by short salivation phases, enabled the nematode to exploit the feeding sites. The events during these phases are still broadly similar to the events in other Tylenchida nematodes but do differ in that the dorsal gland secretions are retained in the pro-corporeal region during ingestion. The reason for this may be that the contents of the gland duct ampulla are likely to be drawn from the ampulla down the oesophageal lumen during ingestion. Thus the granules are retained in the pro-corporeal region until ingestion stops, when the granules can be safely released to the ampulla prior to passage out of the stylet into the feeding cell.

In the developmental stages of disease the success of the invading organism in creating the symptoms depends on whether the host tissues are susceptible or resistant. In the cereal roots, there was no evidence of any chemical resistance to infection but the cell wall knob that surrounded

the shaft of the nematode's stylet is similar to an evoked mechanical resistant mechanism that, in an attack on the plant by fungal disease is effective in limiting cell wall penetration by hyphae. The effectiveness of this barrier in precluding nematode feeding was obviously limited. A further mechanical resistance mechanism arose in seminal roots, so that, when they lost their cortical cells and the remaining cells became sclerotised they were no longer suitable for nematode feeding, but there was no indication that this resistance mechanism was induced by the nematode.

Following successful penetration to a feeding site, the reactions that lead to symptom expression are poorly understood. The morphological changes indicate that a well organised disease symptom is produced, but the biochemical basis of these changes may be controlled either enzymatically by the nematode, or by the plant responding to the nutrient draining of a cell, in which case only a limited enzymic action of the nematode's exudate might enable the ingestion of host cell cytoplasm.

The two genera, Helicotylenchus and Pratylenchus, have been thought to have similar feeding habits, browsing from cell contents (Yeates, 1971) with Pratylenchus spp. migrating within roots between feeds, and Helicotylenchus spp. typically migrating within the soil between feeds. But clearly Helicotylenchus spp. are not surface browsing feeders, and do differ from Pratylenchus spp. in that feeds from cells are very prolonged. Pratylenchus nematodes do, however, feed from similar cells in the root (Baxter and Blake, 1968; Townshend, 1963a; 1963b; Acedo and Rohde, 1971) but a general necrosis develops around feeding nematodes. In addition phenolic compounds (Acedo and Rohde, 1971) and cellulotytic enzymes (Krusberg, 1960) are found in the feeding site. Thus Pratylenchus nematodes feed by exploiting tissues already present in cells prior to the nematodes arrival in a truly browsing manner, whilst Helicotylenchus spp. cause no such general necrosis and in fact induce an ordered disease symptom.

Typically, adult female nematodes that feed as sedentary endoparasites are saccate and are able to lay large numbers of eggs while feeding. These nematodes produce a feeding lesion that comprises either the formation of a syncytium e.g. Rotylenchulus reniformis (Rebois et al., 1975; Razak, 1975) or else produce a group of giant cells e.g. Meloidogyne javanica (Bird, 1961)

The feeding lesion of H. dihystera comprised only a small group of cells around the nematode's head, but the cytoplasmic changes that occurred in these cells, the reduction of the central vacuole due to increased cytoplasm and cell organelles, is similar in kind to all the other sedentary endoparasites, and differs only in the degree of the alteration.

The syncytia of Heterodera spp. and the giant cells of Meloidogyne spp. (Jones and Northcote, 1972) passes the characteristics of cell wall in growths and protoplasmic changes of multinucleate transfer cells, whilst the syncytia of R. reniformis possess only the protoplasmic modifications of nuclear enlargement, reduction of the central vacuole and a dense cytoplasm containing prominent mitochondria and endoplasmic reticulum. The feeding lesion of H. dihystera differs in that no syncytia develops, no nuclear or cellular enlargement occurs, and the cytoplasm contains only limited E.R. yet the functions of the lesion are similar in that the nematode induces changes in the plant that are then used by the nematode as a means for providing nutrients for the production and laying of eggs.

Larval H. dihystera were occasionally found in roots both with unhatched eggs and whilst feeding (Plate 23.), but since the bulk of all endoparasitic H. dihystera were adult females, it seems probable that larval nematodes feed only for a limited period, and do not need to penetrate the root to the depth that adult egg laying females do. If this is so, then it provides a further link in the phylogenetic relationship between Helicotylenchus spp. and Rotylenchulus reniformis, since R. reniformis larvae are able to develop to adult nematodes without feeding (Razak, 1975).

Despite the ability of Helicotylenchus species to draw nutrients from

beyond the feeding cell, it appears that their feeding activities do not exploit the roots beyond their capacity to compensate for the stress conditions, and thus typical field populations of these nematodes do not affect the growth of cereals.

REFERENCES

Acedo, J.R. and Rohde, R.A. 1971 Histochemical root pathology of Brassica olercea capitata L. infected by Pratylenchus penetrans (Cobb) Filipjev and Schuurmans Stekhoven (Nematoda: Tylenchidae). J. Nematol. 3 62 - 68.

Anderson, R.V. 1964 Feeding of Ditylenchus destructor. Phytopathology 54 1121 - 1126.

Apt, W.J. and Hideo Koike. 1962 Pathogenicity of Helicotylenchus nannus and its relation with Pythium graminicola on sugarcane in Hawaii. Phytopathology 52 798 - 802.

Baker, A.D. 1953 Rapid method for mounting nematodes. Can. Ent. 85 77 - 78.

Banage, W.B. 1963 The ecological importance of free-living soil nematodes with special reference to those of moorland soil. J. Amin. Ecol. 32 133 - 140.

Baxter, R.I. and Blake, C.D. 1968 Pratylenchus thornei - a cause of root necrosis in wheat. Nematologica 14 351 - 361.

Benedict, W.G. and Mountain, W.B. 1956 Studies on the etiology of a root rot of winter wheat in Southwest Ontario. Can. J. Bot. 34 159 - 174.

Bird, A.F. 1961 The ultrastructure and histochemistry of a nematode induced giant cell. J. biophys. biochem. Cytol. 11 701 - 715.

Bird, A.F. 1967a Changes associated with parasitism in nematodes 1) Morphology and physiology of pre-parasitic and parasitic larvae of Meloidogyne javanica. J. Parasit. 53 768 - 776.

Bird, A.F. 1967b Changes associated with parasitism in nematodes II Histochemical and microspectrophotometric analysis of pre-parasitic and parasitic larvae of Meloidogyne javanica. J. Parasit. 53 1262 - 1269

Bird, A.F. 1969 Changes associated with parasitism in nematodes V Ultrastructure of the stylet exudate and dorsal gland oesophageal gland contents of female Meloidogyne javanica. J. Parasit. 55 337 - 345.

Blake, C.D. 1966 The histological changes in banana roots caused by Radopholus similis and Helicotylenchus multicinctus. Nematologica 12 129 - 137

Bridge, J. 1971 Ecology and biology of nematodes associated with grass. Ph.D. Thesis London Univ. pp. 147.

Bridge, J. and Hague, N.G.M. 1974 The feeding behaviour of Tylenchorhynchus and Merlinius species and their effects on growth of perennial ryegrass. Nematologica 20 119 - 130.

Churchill, R.C. Jr. and Ruehle J.L. 1971 Occurrence, parasitism and pathogenicity of nematodes associated with Sycamore (Platanus occidentalis L.). J. Nematol. 3 189 - 96.

Collen, N.A. 1972 Plant parasitic nematodes in grass cereal rotations. Eur. Soc. Nematol. 11th Int. Sym. (Abst.) p.12.

Corbett, D.C.M. 1970 Cereal Pratylenchus spp. in England and Wales and their recognition. Pl. Path. 19 6 - 10.

Corbett, D.C.M. 1972 The effect of Pratylenchus fallax on wheat, barley and sugar beet roots. Nematologica 18 303 - 308.

Corbett, D.C.M. and Webb, R.M. 1970 Plant and soil nematode population changes in wheat grown continuously in ploughed and in unploughed soil. Ann. appl. Biol. 65 327 - 335.

Corbett, D.C.M., Winslow, R.D. and Webb, R.M. 1968 Nematode population studies on Broadbalk. Rothamsted Exp. Stn. Report 1968 157 - 174.

Cotton J. 1970 Field experiments with spring barley resistant to cereal cyst nematode. Ann. appl. Biol. 65 163 - 168.

Cotton, J. 1973 Feeding behaviour and reporduction of Xiphenema index on some herbaceous test plants. Nematologica 19 516 - 520.

Davis, R.A. and Jenkins, W.R. 1960 Nematodes associated with roses. Bull. A-106 Univ. of Maryland Agric. Exp. Stn. pp. 1 - 16.

Diab, K.A. and El-Eraki, S. 1968 Plant parasitic nematodes associated with olive decline in the United Arab Republic. Pl. Dis. Rept. 52 150 - 154

Dixon, G.M. 1969 The effect of cereal cyst eelworm on spring-sown cereals. Pl. Path. 18 109 - 112.

Doncaster, C.C. 1962 A counting dish for nematodes. Nematologica 7 334 - 336.

Doncaster, C.C. 1966 Nematode feeding mechanisms. 2. Observations on Ditylenchus destructor and D. myceliophagus feeding on Botryis cinerea. Nematologica 12 417 - 427

Doncaster, C.C. 1971 Feeding in plant parasitic nematodes. Mechanisms and behaviour. Plant Parasitic Nematodes, Vol. II pp. 137 - 157. Eds. B.M. Zuckerman, W.F. Mai and R.A. Rohde. Academic Press. (N.Y.). pp. 347.

Doncaster, C.C. and Seymour, M.K. 1973 Exploration and selection of penetration site by Tylenchida. Nematologica 19 137 - 145

Edwards, A. and Rogers, A. 1974 Agricultural resources. An Introduction to the farming industry of the United Kingdom. Faber and Faber (Lond.) pp. 304.

Farrar, L.L. 1957 Plant parasitic nematode genera found in the vicinity of small grain roots in Georgia. Pl. Dis. Rept. 41 703 - 704.

Fassuloitis, G. 1975 Feeding, egg laying, and embryology of the Columbria lance nematode, Hoplolaimus columbus. J. Nematol. 7 152 - 158.

Fawcett, D.W. 1966 An atlas of fine structure. The cell: Its organelles and inclusions. W.B. Saunders Co. (Philadelphia and Lond.). pp. 448

Feder, N. and O'Brien, T.R. 1968 Plant microtechnique: Some principles and new methods. Am. J. Bot. 55 123 - 142.

Ferris, V.R. and Bernard, R.L. 1971a Crop rotation effects on population densities of ectoparasitic nematodes. J. Nematol. 3 119 - 122

Ferris, V.R. and Bernard, R.L. 1971b Effect of soil type on population densities of nematodes in soybean rotation fields. J. Nematol. 3 123 - 128

Flegg, J.J. 1967 Extraction of Xiphenema and Longidorus species from soil by a modification of Cobb's decanting and sieving technique. Ann. appl. Biol. 60 429 - 437.

Gair, R., Mathias, P.L., and Harvey, P.N. 1969 Studies of cereal nematode populations and cereal yields under continuous or intensive culture. Ann. appl. Biol. 63 503 - 512.

Griffin, G.D. 1964 Association of nematodes with corn in Wisconsin. Pl. Dis. Rept. 48 458 - 459.

Gowen, S.R. 1971 Tylenchus emarginatus and Tylenchorhynchus dubius associated with Sitka Spruce (Picea sitchensis) seedlings. Pl. Path. 20 69 - 72.

Jacob, A., Nair, M.R.G.K. and Das, N.M. 1969 Helicotylenchus caribensis Roman, 1965 (Tylenchida Nematoda) associated with garden canna. Indian J. Ent. 31 179 - 180.

Johnson, P.W. and Bockhoven, L.W.D. 1969 Nematodes associated with tomato and cucumber greenhouse soils in Essex County, Ontario. Can. Pl. Dis. Surv. 49 132 - 134.

Jones, F.G.W. 1956 Soil populations of beet eelworm (Heterodera schachtii Schm.) in relation to cropping. II Microplot and field plot results. Ann. appl. Biol. 44 25 - 56.

Jones, F.G.W. 1965 The population dynamics and population genetics of the potato cyst nematode Heterodera rostochiensis Woll. on susceptible and resistant potatoes. Rothamsted Stn. Report 1965 pp. 301 - 316.

Jones, F.G.W. 1972 Nematodes and cereals. A.D.A.S. Quat. review 6 20 - 36.

Jones, M.G.K. and Dropkin, V.H. 1975 Cellular alterations induced in soybean roots by three endoparasitic nematodes. Physiol. Pl. Path. 5 119 - 124.

Jones, M.G.K. and Northcote, D.H. 1972 Nematode-induced syncytium - a multinucleate transfer cell. J. Cell Sci. 10 789 - 809.

Juniper, B.E., Cox, G.C., Gilchrist, A.J. and Williams, P.R. 1970 Techniques for Plant electron microscopy. Blackwell Sci. Pbl. (Oxford and Edinburgh) pp.108.

Juska, F.V. 1972 Nematodes, a contributing factor to Zoysiagrass decline in Maryland. Pl. Dis. Reptr. 56 568 - 572.

Klinkenberg, C.H. 1963 Observations on the feeding habits of Rotylenchus uniformis, Pratylenchus crenatus, P. penetrans, Tylenchorhynchus dubius and Hemicycliophora similis. Nematologica 9 502 - 506.

Krusberg, L.R. 1960 Hydrolytic and respiratory enzymes of species of Ditylenchus and Pratylenchus. Phytopathology 50 9 - 22.

Kyrou, N.C. 1969 Tylenchorhynchus dubius (Butschli, 1873) Filipjev 1936 on injurious root parasite of potatoes?
Eur. Potato J. 12 215 - 218.

Langdon, K.R., Struble, F.B. and Young,H.C. Jr. 1961 Stunt of small grains, a new disease caused by the nematode Tylenchorhynchus brevidens.
Pl. Dis. Reptr. 45 248 - 252.

Large, E.C. 1954 Growth stages in cereals. Illustration of the Feeke's scale.
Pl. Path. 3 128 - 129.

Lengvenyte, L. 1961 Some data on the rye, barley and maize nematode fauna in the Lithuanian S.S.R.
Acta. Parasitologica Lithuanica 3 Eng. Summ. pp.14 - 15.

Libman, G., Leach, J.G. and Adams, R.E. 1964 Role of certain plant parasitic nematodes in infection of tomatoes by Pseudomonas solanaceorum.
Phytopathology 54 151 - 153.

Linford, M.B. 1937 The feeding of the root-knob nematode in root tissue and nutrient solution.
Phytopathology 27 824 - 825.

Linford, M.B. 1939 Attractiveness of roots and excised shoot tissues to certain nematodes.
Proc. Helminth. Soc. Wash. 6 11 - 18.

Linford, M.B., Olivera, J.M. and Ishii, M. 1949 Paratylenchus minutus n. sp. a Nematode parasitic on roots.
Pacif. Sci. 3 111 - 119.

McElroy, F.D. and Van Gundy, S.D. 1968 Observations on the feeding processes of Hemicycliophora arenaria.
Phytopathology 58 1558 - 1565.

Minz, G., Zür, D. and Strich-Harari, D. 1960 Decline in banana plantations caused by spiral nematodes in the Jordan Valley, and its control by D.B.C.P.
Ktayim Quart. J. Nat. Univ. Inst. Agric. 10 147 - 157.

Nat. Acad. Sci. 1968 Principles of Plant and Plest control, Vol. 1 Plant disease development and control.
(Wash.) pp.205.

Norton, D.C. 1959 Relationship of nematodes to small grains and native grasses in North and Central Texas.
Pl. Dis. Reptr. 43 227 - 235

Orbin, D.P. 1973 Histophathology of Soybean roots infected with Helicotylenchus dihystera.
J. Nematol. 5 37 - 40.

Percival, J. 1921 The wheat plant. A monograph. Duckworth and Co. (Lond.) pp. 463.

Perez, J.B., Van Gundy, S.D., Stolzy, L.H., Thomason I.J. and Laird, R.J. 1960 Pratylenchus thornei a nematode pest of wheat in Sonora, Mexico. Phytopathology 60 1307.

Perry, V.G., Darling, H.M. and Thorne, G. 1959 Anatomy, taxonomy and control of certain spiral nematodes attracking blue grass in Wisconsin. Res. Bull. 207. Univ. Wisconsin Madison pp. 1 - 24.

Rasulov, Sh.A. 1970 The fauna of nematodes of the winter wheat and wild cereals of the foot hill zone of Dagestan. Zoologicheskii Zhurnal 49. Eng. Summ. p. 27

Razak, A.B.A. 1975 Host-parasite relationships of Rotylenchulus reniformis on cowpea and cotton. Ph.D. Thesis London Univ. pp.176.

Rebois, R.V., Madden, P.A. and Eldridge, B.J. 1975 Some ultrastructural changes induced in resistant and susceptible soybean roots following infection by Rotylenchulus reniformis. J. Nematol. 7 122 - 139.

Reynolds, E.S. 1963 The use of lead citrate at high pH. as an electron-opaque stain in electron microscopy. J. Cell Biol. 17 208 - 212.

Rhoades, H.L. and Linford, M.B. 1961 A study of the parasitic habit of Paratylenchus projectus and P. dianthus. Proc. Helminth. Soc. Wash. 28 185 - 190.

Ruehle, J.L. 1975 Response of short leaf pine to parasitism by plant-parasitic nematodes. Pl. Dis. Reptr. 59 290 - 292.

Russell, C.C. and Perry, V.G. 1966 Parasitic habit of Trichodorus christiei on wheat. Phytopathology 56 357.

Saxena, P.K., Chhabra, H.K. and Sneh Lata 1973 Biology of Helicotylenchus elegans Roman (Rotylenchoidinae : Nematoda) Zool. Anz. Leipzig 190 142 - 148

Saynor, M. 1972 The influence of host plants and soil cultivation on Pratylenchus, Tylenchorhynchus and Helicotylenchus species in natural habitat. Eur. Soc. Nematol. 11th Int. Sym. (Abst.). pp. 60 - 61.

Saynor, M. 1972a Observations on the biology and economic importance of certain free-living nematodes associated with cereals in East Anglia. Ph.D. Thesis London Univ. pp. 194.

Sechler, D.T., Tappan, W.B. and Luke, H.H. 1967 Effect of variety and fumigation on nematode populations in oats, wheat and tye.
Pl. Dis. Reptr. 51 915 - 919.

Seinhorst, J.W. 1966 The relationships between population increase and population density in plant parasitic nematodes. I Introduction andmigratory nematodes.
Nematologica 12 157 - 169.

Seinhorst, J.W. 1967 The relationships between population increase and population density in plant parasitic nematodes. III Definition of the term host, host status and resistance. IV The influence of external conditions on the regulation of population density.
Nematologica 13 429 - 442.

Seinhorst, J.W. 1971 The structure of the glandular part of the oesophagous of Tylenchida.
Nematologica 17 431 - 443.

Semkina, A.I. 1971 The influence of the nematode Longidorus elongatus and the fungus Helminthosporium sativum on the yield of spring wheat in Kustanaj district of U.S.S.R.
Byull. Vses. Inst. Gel'mintologii im. K.I. Skryabina 6 79 - 83.
Eng. Summ. p. 83.

Skarbilovich, T.S. 1972 Susceptibility of different plants to parasitic nematodes of clover.
Trudy Vses. Soveshchaniya Fitogel'mintologov. (5th) Samarkand 1962.
Eng. Summ. p. 268.

Sharma, R.D. 1971 Studies on the plant parasitic nematode Tylenchorhynchus dubius.
Meded. Landb. Hoogesch. Wageningen 1 pp. 154.

Skuodyte, Z. 1962 The distribution of nematodes in some cereal species and localisation of these parasites in various plant organs in the Lithuanian S.S.R.
Acta. Parasitologica Lithuanica 4 Eng. Summ. p.135.

Sledge, E.B. 1956 Pathogenicity of the spiral nematode, Helicotylenchus nannus Steiner 1945, in relation ot selected varieties of corn.
Alabama Acad. Sci. 28 123.

Sledge, E.B. 1959 The extrusion of saliva from the stylet of the spiral nematode, Helicotylenchus nannus.
Nematologica 4 356.

Spurr, A.R. 1969 A low-viscosity epoxy resin embedding medium for electron microscopy.
J. Ultrastruct. Res. 26 31 - 43.

Stewart, R.N. and Schindler, A.F. 1956 The effect of some ectoparasitic and endoparasitic nematodes on the expression of bacterial wilt in carnations.
Phytopathology 46 219 - 222.

Strover, R.H. 1972 Banana, Plantain and Abaca diseases.
Commw. Agric. Bur. pp. 316.

Tarjan, A.C., Jiménez, M.F. and Soria, J. 1971 Reaction of nematised cacao to chemical treatment.
Nematropica 1 16.

Tarjan, A.C. and Jiménez, M.F. 1973 Debilitation of cacao in Costa Rica by plant nematodes.
Nematropica 3 25 - 28.

Taylor, D.P. 1960 Host range study of the spiral nematode, Helicotylenchus microlobus.
Pl. Dis. Reptr. 44 747 - 750.

Taylor, D.P. 1961 Biology and host-parasite relationships of the spiral nematode, Helicotylenchus microlobus.
Proc. Helminth. Soc. Wash. 28 60 - 66

Taylor, D.P. and Schleder, E.G. 1959 Nematodes associated with Minnesota crops. II Nematodes associated with corn, barley, oats, rye and wheat.
Pl. Dis. Reptr. 43 329 - 333.

Troughton, A. 1962 The roots of temperate cereals (wheat, barley, oats and rye).
Mimeographed Pub. No.2 Commw. Bur. Past. Fld. Crops pp. 91.

Townshend, J.L. 1963a The pathogenicity of Pratylenchus penetrans to celery.
Can. J. Pl. Sci. 43 70 - 74.

Townshend, J.L. 1963b The pathogenicity of Pratylenchus penetrans to strawberry.
Can. J. Pl. Sci. 43 75 - 78.

Wallace, H.R. 1971 The influence of the density of nematode populations on plants.
Nematologica 17 154 - 166.

Wallace, H.R. and Greet, D.N. 1964 Observations on the taxonomy and biology of Tylenchorhynchus macrurus (Goodey, 1932) Filipjev, 1936 and Tylenchorhynchus icarus sp. nov.
Parasitology 54 129 - 144.

Webster, J.M. 1969 The host-parasite relationships of plant-parasitic nematodes.
Advances in Parasitology 7 1 - 40.

Whitehead, A.G. and Hooper, D.J. 1970 Needle nematodes (Longidorus spp.) and stubby-root nematodes (Trichodorus spp.) harmful to sugar beet and other field crops in England.
Ann. appl. Biol. 65 339 - 350.

Whitehead, A.G. and Hemming, J.R. 1965 A comparison of some quantitative methods of extracting small vermiform nematodes from soil.
Ann. appl. Biol. 55 25 - 38.

Whitehead, A.G. and Frazer, J.E. 1972 Injury to field beans (Vicia faba L.) by Tylenchorhynchus dubius.
Pl. Path. 21 112 - 113.

Whitehead, A.G., Frazer, J.E. and Greet, D.N. 1970 The effects of D-D, chloropicrin and previous crops on numbers of migratory root-parasitic nematodes and on the growth of sugar beet and barley.
Ann. appl. Biol. 65 351 - 359.

Winslow, R.D. 1964 Soil nematode population studies. 1) The migratory root Tylenchida and other nematodes of the Rothamsted and Woburn six-course rotations.
Pedobiologia 4 65 - 76.

Wyss, U. 1973 Feeding of Tylenchorhynchus dubius.
Nematologica 19 125 - 136.

Yeates, G.W. 1971 Feeding types and feeding groups in plant and soil nematodes.
Pedobiologia 11 173 - 179.

Yuen Pick-Hoong 1966 The nematode fauna of the regenerated woodland and grassland of Broadbalk Wilderness.
Nematologica 12 195 - 214.

Zuckerman, B.M. and Strick-Harari, D. 1963 The life stages of Helicotylenchus multicinctus (Cobb) in banana roots.
Nematologica 9 347 - 353.

Gupta, J.C. and Atwal, A.S. 1971 Biology and ecology of Hoplolaimus indicus (Hoplolaiminae : Nematoda) I The Life stages and the feeding behaviour.
Nematologica 17 69 - 74.

Winslow, R.D. 1956 Nematode ecology pp. 341 - 415.
Nematology Eds. Sasser, J.N. and Jenkins, W.R.

APPENDIX A

Trial to determine effects of ploughing on nematode numbers.

Table 1. Nos. of Nematodes in 200 c.c. soil. 0-3 inches. Ley Pasture Field.

Sampling Date Weeks	Tylenchus	Para-tylenchus	Tylencho-rhynchus	Praty-lenchus	Helico-tylenchus	Dory-laims	Non Plant Para-sites	Total
0	336	168	1,350	392	168	0	1,850	4,264
3	900	1,100	700	700	200	100	2,600	6,300
6	400	1,000	800	600	200	100	3,300	6,400
9	300	500	500	300	100	50	4,600	6,350
12	400	700	1,400	200	100	200	8,900	11,900

Table 2. Nos. of Nematodes in 200 c.c. of soil. 0 - 3 inches. Permanent Pasture Field.

Sampling Date Weeks	Tylenchus	Para-tylenchus	Tylencho-rhynchus	Praty-lenchus	Helico-tylenchus	Dory-laims	Non Plant Para-sites	Total
0	496	302	372	62	124	0	1,736	3,092
3	300	1,000	300	500	100	200	3,300	5,700
6	700	1,400	300	400	400	200	14,600	18,000
9	700	700	200	30	50	200	7,000	8,880
12	600	300	600	100	0	200	14,400	16,200

APPENDIX A

Table 3. Nos. of Nematodes in 200 c.c. of soil. 3 - 6 inches. Ley Pasture Field.

Sampling Date Weeks	Tylenchus	Para-tylenchus	Tylencho-rhynchus	Praty-lenchus	Helico-tylenchus	Dory-laims	Non Plant Para-sites	Total
0	518	518	740	700	441	0	1,330	4,247
3	200	2,300	1,000	1,100	600	300	5,600	11,100
6	200	1,600	1,000	800	300	200	5,200	9,300
9	300	1,800	800	500	100	100	3,800	7,400
12	700	800	600	300	300	100	4,500	7,300

Table 4. Nos. of Nematodes in 200 c.c. of soil. 3 - 6 inches. Permanent Pasture Field.

Sampling Date Weeks	Tylenchus	Para-tylenchus	Tylencho-rhynchus	Praty-lenchus	Helico-tylenchus	Dory-laims	Non Plant Para-sites	Total
0	324	162	270	270	162	0	1,944	3,132
3	300	700	600	600	300	300	4,300	7,100
6	1,500	1,000	300	700	200	300	12,200	16,200
9	200	900	300	300	100	200	9,500	11,500
12	200	300	200	100	100	100	6,600	7,600

APPENDIX A

Table 5. Nos. of Nematodes in 200 c.c. of soil. 6 - 9 inches. Ley Pasture Field.

Sampling Date Weeks	Tylenchus	Para-tylenchus	Tylencho-rhynchus	Praty-lenchus	Helico-tylenchus	Dory-laims	Non Plant Para-sites	Total
0	196	862	764	576	96	0	2,350	4,844
3	400	1,600	700	500	500	0	5,200	8,900
6	600	1,700	600	700	100	200	5,900	9,800
9	300	900	300	700	200	0	3,100	5,500
12	400	800	600	400	100	0	3,000	5,300

Table 6. Nos. of Nematodes in 200 c.c. of soil. 6 - 9 inches. Permanent Pasture Field.

Sampling Date Weeks	Tylenchus	Para-tylenchus	Tylencho-rhynchus	Praty-lenchus	Helico-tylenchus	Dory-laims	Non Plant Para-sites	Total
0	112	168	504	168	280	0	1,624	2,856
3	300	700	400	700	300	0	4,700	7,100
6	800	1,400	400	600	300	400	10,200	14,100
9	200	1,000	300	200	200	50	7,500	9,450
12	700	100	200	300	100	100	9,700	11,200

APPENDIX A

Table 7. Nos. of Nematodes per 0.1 grm. of fresh roots. Ley Pasture.

Sample No.		Sampling Date Weeks			
		3	6	9	12
Pratylenchus	1	4	1	0	1
	2	2	2	10	5
	3	1	1	1	3
	4	1	10	5	1
	5	2	0	2	5
Total		10	14	19	15
Helicotylenchus	1	2	5	3	2
	2	4	14	2	1
	3	3	5	1	2
	4	1	7	1	2
	5	2	3	3	2
Total		12	34	10	9

Table 7. (contd.) Nos. of Nematodes per 0.1 grm. of fresh roots. Permanent Pasture.

Sample No.		Sampling Date Weeks			
		3	6	9	12
Pratylenchus	1	1	1	4	5
	2	2	5	1	2
	3	0	20	8	3
	4	4	2	8	4
	5	2	3	6	6
Total		9	31	27	20
Helicotylenchus	1	0	0	3	1
	2	0	0	2	3
	3	0	0	4	3
	4	0	0	0	2
	5	0	0	5	3
Total		0	0	14	12

APPENDIX B

Relationship between nematode numbers and yield of Winter Wheat.

Table 1. Nos. of Nematodes per 200 c.c. of soil. Mean of two extractions. Sampling date 11th June, 1973.

Plot No.	Tylenchus	Paratylenchus	Tylenchorhynchus	Pratylenchus	Helicotylenchus	Aphelenchoides	Criconemoides	Paratrichodorus	Non Plant Parasites	Total
1	2,736	4,251	105	105	585	0	0	0	8,114	15,896
2	1,985	104	0	172	134	0	239	0	11,179	13,813
3	547	0	0	132	340	0	0	0	5,927	6,946
4	917	1,030	810	0	35	0	226	0	10,394	13,412
5	346	172	346	1,242	103	0	35	0	7,239	9,483
6	517	400	83	858	33	0	0	0	5,337	7,228
7	240	344	0	1,142	0	0	0	0	9,482	11,208
8	580	65	65	104	101	0	0	0	7,264	8,179
9	1,034	654	42	308	731	0	115	0	9,101	11,985
10	714	178	316	0	751	0	0	0	9,141	11,100
11	449	344	0	207	686	0	0	0	4,979	6,665
12	928	121	67	229	40	0	0	0	7,771	9,156

Table 2. Nos. of Nematodes per 200 c.c. of soil. Mean of two extractions. Sampling date 23rd September, 1973.

Plot No.	Tylenchus	Paratylenchus	Tylenchorhynchus	Pratylenchus	Helicotylenchus	Aphelenchoides	Criconemoides	Paratrichodorus	Non Plant Parasites	Total
1	1,476	2,457	1,032	192	643	0	0	0	5,815	11,615
2	1,424	0	43	211	125	0	0	0	5,812	7,615
3	305	0	0	275	232	0	37	0	4,682	5,531
4	444	444	277	248	315	0	0	0	3,699	5,427
5	366	83	286	649	163	0	0	0	3,386	4,933
6	661	80	165	436	42	0	0	0	4,870	6,254
7	352	175	0	795	88	174	219	0	4,336	6,139
8	770	170	727	0	43	0	171	0	6,544	8,425
9	1,202	542	507	77	1,587	190	0	0	8,270	12,375
10	720	40	160	280	720	80	0	0	4,720	6,720
11	566	217	477	561	220	40	32	0	4,599	6,712
12	267	46	89	271	42	0	0	0	4,989	5,704

Tables 3 and 4

APPENDIX B

Table 3. Nos. of Nematodes per 200 c.c. of soil. Mean of two extractions. Sampling date 12th March, 1974.

Plot No.	Ty-lenchus	Paraty-lenchus	Tylencho-rhynchus	Praty-lenchus	Helico-ty-lenchus	Aphe-lench-oides	Crico-nemoides	Para-tricho-dorus	Non Plant Para-sites	Total
1	350	6,150	400	50	400	100	0	0	5,450	12,900
2	200	200	0	50	200	0	0	0	3,800	4,450
3	200	50	0	100	50	100	0	0	4,050	4,550
4	300	600	100	350	600	0	0	0	5,050	7,000
5	100	0	100	300	50	0	0	0	4,550	5,100
6	200	50	100	0	0	50	0	0	3,500	3,900
7	100	0	0	250	100	0	200	0	2,400	3,050
8	50	50	50	0	0	100	0	0	3,050	3,300
9	350	300	50	100	350	0	0	0	5,700	6,850
10	600	100	0	50	300	100	0	0	4,550	5,600
11	250	50	100	100	0	200	0	0	3,250	3,950
12	50	50	0	50	0	300	0	0	2,700	3,150

Table 4. Nos. of Nematodes per 200 c.c. of soil. Mean of four extractions. Sampling date 23rd July, 1974.

Plot No.	Ty-lenchus	Paraty-lenchus	Tylencho-rhynchus	Praty-lenchus	Helico-ty-lenchus	Aphe-lench-oides	Crico-nemoides	Para-tricho-dorus	Non Plant Para-sites	Total
1	422	305	127	19	80	149	0	27	2,484	3,613
2	897	224	222	29	57	324	19	66	3,687	5,525
3	536	20	87	164	66	211	10	17	3,925	5,036
4	410	20	180	60	160	120	0	40	2,510	3,500
5	180	17	269	213	65	122	0	71	3,351	4,288
6	282	39	205	161	9	159	49	27	3,155	4,086
7	220	37	9	89	9	286	35	10	2,785	3,480
8	590	70	100	0	50	497	95	19	5,716	7,107
9	691	516	44	218	481	87	17	140	5,189	7,383
10	330	65	209	109	276	135	9	36	3,381	4,550
11	456	62	604	91	134	144	0	94	6,124	7,709
12	122	37	134	99	45	131	9	35	7,234	7,836

APPENDIX B

Table 5. Nos. of Nematodes per 200 c.c. of soil. Mean of two counts from one extraction. Sampling date 23rd July, 1974.

Plot No.		Ty-lenchus	Paraty-lenchus	Tylencho-rhynchus	Praty-lenchus	Helico-ty-lenchus	Aphe-lench-oides	Crico-nemoides	Para-tricho-dorus	Non Plant Para-sites	Total
1	1	360	520	160	40	120	0	0	40	2,200	3,440
	2	210	105	245	0	175	0	0	0	1,085	1,820
	3	840	245	35	0	140	70	0	35	3,290	4,655
	4	280	350	70	35	140	525	0	35	3,360	4,795
2	1	400	560	240	0	40	520	40	40	3,880	5,720
	2	520	0	160	0	80	280	0	0	2,200	3,240
	3	920	160	280	80	40	320	0	120	4,400	6,320
	4	1,750	175	210	35	70	175	35	105	4,270	6,825
3	1	320	0	40	40	40	240	40	0	5,360	6,080
	2	360	80	120	320	80	120	0	0	1,800	2,880
	3	480	0	120	120	40	240	0	0	4,760	5,760
	4	665	0	70	175	105	245	0	70	3,780	5,110
4	1	160	40	160	80	240	160	0	80	2,560	3,480
	2	240	40	0	0	80	160	0	0	1,920	2,440
	3	680	0	160	80	160	160	0	0	3,120	4,360
	4	560	0	400	80	160	0	0	80	2,440	3,720
5	1	105	0	315	105	70	105	0	35	1,575	2,310
	2	105	70	245	245	0	175	0	105	2,660	3,605
	3	160	0	200	120	120	120	0	40	5,040	5,800
	4	140	0	315	385	70	105	0	105	4,130	5,250
6	1	240	0	0	160	0	20	0	40	2,480	2,940
	2	315	35	245	35	0	70	35	0	1,890	2,625
	3	175	0	455	210	35	105	0	70	4,410	5,460
	4	400	120	120	240	0	440	160	0	3,840	5,320

APPENDIX B

Table 5. (contd.)

Plot No.		Ty-lenchus	Paraty-lenchus	Tylencho-rhynchus	Praty-lenchus	Helico-ty-lenchus	Aphe-lench-oides	Crico-nemoides	Para-tricho-dorus	Non Plant Para-sites	Total
7	1	80	0	0	0	0	280	0	0	1,720	2,080
	2	245	35	0	280	35	420	105	0	3,290	4,410
	3	240	80	0	40	0	200	0	40	2,840	3,440
	4	315	35	35	35	0	245	35	0	3,290	3,990
8	1	560	80	0	0	80	480	0	40	3,760	5,000
	2	760	40	280	0	40	320	120	0	4,880	6,440
	3	480	80	80	0	80	280	120	0	7,400	8,520
	4	560	80	40	0	0	910	140	35	6,825	8,590
9	1	735	210	70	0	175	0	35	0	4,410	5,670
	2	525	70	70	280	630	140	0	35	4,620	6,370
	3	630	1,225	0	315	525	140	35	350	6,790	10,010
	4	875	560	35	280	595	70	0	175	4,935	7,525
10	1	320	80	80	200	200	240	0	40	2,880	4,040
	2	665	140	175	105	490	70	35	70	2,730	4,480
	3	175	0	420	70	175	70	0	35	3,675	4,620
	4	160	40	160	160	240	160	0	0	4,240	5,160
11	1	320	40	560	120	80	120	0	200	5,560	7,000
	2	595	70	735	140	105	175	0	0	5,775	7,595
	3	490	0	630	105	140	105	0	140	5,775	7,385
	4	420	140	490	0	210	175	0	35	7,385	8,855
12	1	350	0	245	35	0	210	35	70	8,260	9,205
	2	0	40	80	0	0	40	0	0	6,400	6,560
	3	140	70	210	140	140	35	0	70	7,035	7,840
	4	0	40	0	200	40	240	0	0	7,240	7,760

APPENDIX B

Table 6. Nos. of Nematodes per 200 c.c. of soil. Mean of two extractions. Sampling date 22nd September, 1974.

Plot No.	Ty-lenchus	Paraty-lenchus	Tylencho-rhynchus	Praty-lenchus	Helico-ty-lenchus	Aphe-lench-oides	Crico-nemoides	Para-tricho-dorus	Non Plant Para-sites	Total
1	540	765	180	90	405	270	0	45	8,640	10,935
2	1,035	45	315	90	45	855	0	45	6,075	8,505
3	400	0	120	120	40	480	80	40	6,560	7,840
4	280	200	280	700	40	1,595	0	40	10,760	13,895
5	120	40	320	640	80	600	40	120	10,360	12,320
6	315	0	225	315	90	765	90	90	5,895	7,785
7	450	45	450	495	40	495	0	0	5,760	7,735
8	440	80	120	0	240	1,480	0	200	11,520	14,080
9	765	450	45	315	765	225	0	315	7,785	10,665
10	637	150	75	225	137	300	0	112	4,762	6,398
11	760	120	480	360	80	760	0	40	9,040	11,640
12	240	0	480	80	80	240	0	160	8,160	9,440

APPENDIX B

Table 7. Straw weight and Leaf surface area of sub-plots and totals for main plots.

Plot No.	Straw Weight grms.	Leaf Area sq. cm.	Plot No.	Straw Weight grms.	Leaf Area sq. cm.
1 1	689	57.7	7 1	1,208	59.0
2	977	60.4	2	1,417	75.2
3	939	51.7	3	1,332	76.2
4	786	44.5	4	1,472	70.9
Total	3,391	214.3	Total	5,429	281.3
2 1	1,061	66.9	8 1	1,012	49.7
2	1,316	65.3	2	1,127	51.0
3	1,220	60.7	3	1,238	50.8
4	1,378	66.4	4	1,216	69.0
Total	4,975	259.3	Total	4,593	220.5
3 1	692	59.5	9 1	1,212	45.8
2	911	90.1	2	1,296	53.7
3	546	48.5	3	1,033	46.0
4	1,038	109.1	4	1,282	41.0
Total	3,187	307.2	Total	4,823	186.5
4 1	940	73.4	10 1	1,290	67.4
2	968	71.2	2	1,299	60.0
3	706	64.5	3	1,189	84.5
4	820	66.0	4	1,222	62.6
Total	3,434	275.1	Total	5,000	274.5
5 1	876	54.7	11 1	1,235	66.0
2	1,161	65.9	2	1,182	53.1
3	1,039	86.1	3	1,230	74.8
4	1,098	68.7	4	1,237	56.3
Total	4,174	275.4	Total	4,884	250.3
6 1	1,187	57.9	12 1	1,180	71.1
2	1,554	82.5	2	1,124	53.2
3	1,236	74.4	3	1,007	54.4
4	1,273	80.0	4	1,429	54.3
Total	5,250	294.8	Total	4,740	233.0

APPENDIX C

Nematicidal Trials

Sampling Dates: 1 = 27th Nov., 1973 2 = 1st April, 1974
3 = 31st July, 1974 4 = 18th Nov., 1974
5 = 17th March, 1975 6 = 29th July, 1975

Table 1. Tylenchus spp.
Nos. of Nematodes per 200 c.c. of soil. Mean of two extractions. Extraction Method:- Whitehead Tray.

Sampling Region	Sampling Depth cm.	CONTROL Sampling Dates			TREATED Sampling Dates		
		1	2	3	1	2	3
Even Growth Region	0 - 10	402	200	485	0	0	265
	10 - 20	312	200	455	0	200	245
Patchy Growth Region	0 - 10	120	200	730	0	100	1,120
	10 - 20	243	100	485	0	100	150
		4	5	6	4	5	6
Even Growth Region	0 - 13	265	340	460	200	0	360
	13 - 26	560	1,000	340	480	330	200
	26 - 39	240	400	240	210	300	105
Patchy Growth Region	0 - 13	90	90	322	290	0	100
	13 - 26	205	70	240	80	40	82
	26 - 39	90	45	65	85	0	40

Table 2. Paratylenchus spp.
Nos. of Nematodes per 200 c.c. of soil. Mean of two extractions. Extraction Method:- Whitehead Tray.

Sampling Region	Sampling Depth cm.	CONTROL Sampling Dates			TREATED Sampling Dates		
		1	2	3	1	2	3
Even Growth Region	0 - 10	134	200	0	0	0	40
	10 - 20	104	300	35	0	100	0
Patchy Growth Region	0 - 10	240	0	360	0	0	105
	10 - 20	0	200	120	0	100	70
		4	5	6	4	5	6
Even Growth Region	0 - 13	0	0	40	0	0	0
	13 - 26	0	0	0	0	0	0
	26 - 39	40	80	40	0	0	0
Patchy Growth Region	0 - 13	175	45	207	85	0	20
	13 - 26	130	0	0	80	0	0
	26 - 39	45	50	0	0	0	20

APPENDIX C

Table 3. T. dubius.
Nos. of Nematodes per 200 c.c. of soil. Mean of two extractions. Extraction Method:- Whitehead Tray.

Sampling Region	Sampling Depth cm.	CONTROL Sampling Dates			TREATED Sampling Dates		
		1	2	3	1	2	3
Even Growth Region	0 - 10	469	100	880	0	0	195
	10 - 20	312	100	140	0	100	35
Patchy Growth Region	0 - 10	840	400	1,225	0	200	210
	10 - 20	770	300	675	0	0	110
		4	5	6	4	5	6
Even Growth Region	0 - 13	130	130	140	160	45	180
	13 - 26	85	160	100	110	210	120
	26 - 39	120	200	180	215	170	107
Patchy Growth Region	0 - 13	125	540	360	340	300	300
	13 - 26	770	320	220	360	320	42
	26 - 39	305	230	165	335	165	80

Table 4. Pratylenchus spp.
Nos. of Nematodes per 200 c.c. of soil. Mean of two extractions. Extraction Method:- Whitehead Tray.

Sampling Region	Sampling Depth cm.	CONTROL Sampling Dates			TREATED Sampling Dates		
		1	2	3	1	2	3
Even Growth Region	0 - 10	3,685	1,000	1,990	0	200	280
	10 - 20	2,808	900	1,120	0	500	175
Patchy Growth Region	0 - 10	1,080	300	495	0	100	140
	10 - 20	2,808	700	780	0	500	375
		4	5	6	4	5	6
Even Growth Region	0 - 13	600	695	560	600	720	280
	13 - 26	265	1,160	400	800	510	220
	26 - 39	760	1,160	200	270	555	280
Patchy Growth Region	0 - 13	565	540	187	540	470	180
	13 - 26	935	605	320	330	360	172
	26 - 39	635	730	87	415	425	60

APPENDIX C

Table 5. H. digonicus
Nos. of Nematodes per 200 c.c. of soil. Mean of two extractions. Extraction Method:- Whitehead Tray.

Sampling Region	Sampling Depth cm.	CONTROL Sampling Dates			TREATED Sampling Dates		
		1	2	3	1	2	3
Even Growth Region	0 - 10	268	0	140	0	100	35
	10 - 20	104	100	280	0	100	70
Patchy Growth Region	0 - 10	480	400	75	0	100	70
	10 - 20	616	200	730	0	200	75
		4	5	6	4	5	6
Even Growth Region	0 - 13	35	90	0	0	45	0
	13 - 26	130	120	80	160	0	20
	26 - 39	0	40	40	120	45	0
Patchy Growth Region	0 - 13	40	135	42	0	125	40
	13 - 26	255	630	80	125	160	22
	26 - 29	80	465	180	130	205	0

Table 6. Longidorus spp.
Nos. of Nematodes per 200 c.c. of soil. Mean of two extractions. Extraction Method:- Flegg's.

Sampling Region	Sampling Depth cm.	CONTROL Sampling Dates			TREATED Sampling Dates		
		1	2	3	1	2	3
Even Growth Region	0 - 10	0	0	0	0	0	0
	10 - 20	0	0	0	0	0	22
Patchy Growth Region	0 - 10	30	0	0	0	0	0
	10 - 20	90	150	22	0	40	0
		4	5	6	4	5	6
Even Growth Region	0 - 13	0	0	0	0	0	0
	13 - 26	10	0	0	5	0	0
	26 - 39	0	0	0	0	0	0
Patchy Growth Region	0 - 13	10	0	20	15	0	10
	13 - 26	20	0	0	40	0	20
	26 - 39	80	40	30	25	35	60

APPENDIX C

Table 7. Total Tylenchid spp.
Nos. of Nematodes per 200 c.c. of soil. Mean of two extractions. Extraction Method:- Whitehead Tray and Flegg's.

Sampling Region	Sampling Depth cm.	CONTROL Sampling Dates			TREATED Sampling Dates		
		1	2	3	1	2	3
Even Growth Region	0 - 10	4,958	1,400	3,495	0	300	815
	10 - 20	3,640	1,600	2,030	0	1,000	1,125
Patchy Growth Region	0 - 10	2,760	1,300	2,885	0	500	1,645
	10 - 20	4,437	1,500	2,790	0	900	780
		4	5	6	4	5	6
Even Growth Region	0 - 13	1,030	1,255	1,200	960	810	820
	13 - 26	1,040	2,600	920	1,550	1,050	560
	26 - 39	1,160	1,880	700	1,570	1,070	492
Patchy Growth Region	0 - 13	995	1,350	1,118	1,255	895	640
	13 - 26	2,295	1,625	860	975	880	318
	26 - 39	1,155	1,600	497	975	795	200

Table 8. Non Plant Parasitic.
Nos. of Nematodes per 200 c.c. of soil. Mean of two extractions. Extraction Method:- Whitehead Tray.

Sampling Region	Sampling Depth cm.	CONTROL Sampling Dates			TREATED Sampling Dates		
		1	2	3	1	2	3
Even Growth Region	0 - 10	3,720	2,600	3,840	0	1,400	2,685
	10 - 20	2,584	2,100	1,866	0	800	1,435
Patchy Growth Region	0 - 10	4,087	2,700	4,270	0	1,700	3,185
	10 - 20	2,080	1,500	1,865	0	1,800	1,350
		4	5	6	4	5	6
Even Growth Region	0 - 13	1,495	1,525	1,040	1,640	1,620	660
	13 - 26	660	2,240	620	640	825	300
	26 - 39	1,000	760	140	775	410	185
Patchy Growth Region	0 - 13	2,460	3,645	2,000	4,395	2,585	2,240
	13 - 26	1,480	1,985	860	1,460	1,760	807
	26 - 39	1,260	800	385	715	925	500

Table 9. Nematicidal trial season 1974/75. Plot Yields.

Sample Plots	Total Grain Weight grms.	1,000 grain Weight grms.	Sample Plots	Total Grain Weight grms.	1,000 grain Weight grms.
Even Region			Even Region		
Control 1	5,610	55.06	Treated 1	5,088	56.80
2	5,364	53.16	2	5,440	54.47
3	6,513	54.23	3	5,148	56.30
4	6,261	56.29	4	4,505	55.95
5	4,902	55.75	5	5,914	54.76
Mean	5,730	54.90	Mean	5,219	55.7
Patchy Region			Patchy Region		
Control 1	4,781	54.10	Treated 1	5,869	51.40
2	5,188	53.15	2	5,701	51.30
3	4,674	49.80	3	6,290	51.18
4	5,933	50.69	4	5,576	53.05
5	5,931	51.54	5	6,529	53.93
Mean	5,301	51.90	Mean	5,993	52.2

Total Grain Weight. N.S.

1,000 Grain Weight. 1% significance. L.S.D. at 5% = 1.80.

APPENDIX D

Table 1

Host Preference Experiments.

Table 1. Original soil. Nos. per 200 c.c. of soil. Two Extracts, each counted twice.

Sample Origin		Tylenchus sp.	Tylenchorhynchus	Paratylenchus	Pratylenchus	Helicotylenchus	Rotylenchus	Non Plant Parasites
Royston *H. digonicus*								
1	1	280	65	1,365	-	1,625	-	7,800
	2	195	560	650	-	1,755	-	7,995
2	1	305	0	1,260	-	2,457	-	6,993
	2	189	63	1,071	-	2,331	-	6,993
Mean		242	172	1,086	-	2,042	-	7,445
Ascot *H. pseudorobustus*								
1	1	250	550	2,900	50	1,750	-	3,350
	2	150	350	3,600	200	1,850	-	3,500
2	1	360	400	4,230	90	1,440	-	2,610
	2	180	360	2,790	90	1,170	-	2,880
Mean		235	415	3,380	107	1,552	-	3,085
Broomhill *H. varicaudatus*								
1	1	864	72	792	-	576	-	4,536
	2	792	72	432	-	720	-	4,176
2	1	715	65	585	-	485	-	4,290
	2	650	65	390	-	325	-	4,030
Mean		755	68	550	-	526	-	4,258
Broadbalk *H. vulgaris*								
1	1	100	50	700	0	450	300	1,000
	2	50	100	500	50	450	200	850
2	1	80	40	160	40	560	200	1,680
	2	40	40	120	40	480	120	1,040
Mean		67	57	370	32	485	205	1,142

APPENDIX D

Table 2. Tested soils. Nos. per 100 c.c. following dilution.

Sample Origin	Ty-lenchus	Tylencho-rhynchus	Paraty-lenchus	Praty-lenchus	Helico-tylenchus	Non Plant Parasites
Royston	240	80	240	-	80	560
	80	0	120	-	160	640
	90	45	135	-	270	1,350
	90	0	180	-	360	1,215
Mean	125	31	169	-	217	941
Ascot	175	70	665	0	385	720
	175	245	770	0	350	975
	200	200	920	60	200	760
	280	280	520	0	320	920
Mean	207	199	719	15	314	844
Broomhill Farm	80	40	80	-	40	600
	40	40	80	-	40	640
	210	70	175	-	70	735
	175	70	35	-	105	385
Mean	126	55	92	-	64	590

APPENDIX D

Table 3. Nos. of H. vulgaris per 100 c.c. of soil.

Sample		Nos. per 100 c.c.	Sample		Nos. per 100 c.c.
Fallow	1	0	Winter Wheat	1	15
	2	0		2	0
	3	0		3	0
	4	30		4	15
	5	0		5	0
Mean		6	Mean		6
Poa	1	20	Spring Barley	1	10
	2	0		2	0
	3	20		3	20
	4	0		4	10
	5	0		5	0
Mean		8	Mean		8
Ryegrass	1	0	Winter Barley	1	15
	2	0		2	0
	3	0		3	25
	4	0		4	20
	5	0		5	20
Mean		0	Mean		16
Festuca	1	30	Spring Oats	1	0
	2	20		2	10
	3	50		3	40
	4	30		4	50
	5	10		5	20
Mean		28	Mean		24
Spring Wheat	1	0	Winter Oats	1	0
	2	0		2	0
	3	0		3	0
	4	0		4	0
	5	0		5	0
Mean		0	Mean		0

APPENDIX D

Table 4. Nos. of H. digonicus per 100 c.c. of soil.

Sample		Numbers per 100 c.c.			Sample		Numbers per 100 c.c.		
		3 Months	6 Months	9 Months			3 Months	6 Months	9 Months
Fallow	1	40	0	0	Winter	1	0	125	40
	2	0	30	80	Wheat	2	280	0	80
	3	60	30	0		3	200	150	80
	4	50	25	40		4	80	40	360
	5	120	40	40		5	80	275	660
Mean		54	25	32	Mean		128	118	244
Poa	1	50	665	280	Spring	1	60	60	550
	2	60	125	1,000	Barley	2	40	0	0
	3	0	300	650		3	150	125	0
	4	120	245	200		4	360	30	90
	5	90	60	1,640		5	180	50	120
Mean		64	279	752	Mean		154	53	152
Ryegrass	1	0	280	60	Winter	1	90	100	320
	2	40	245	100	Barley	2	0	70	240
	3	30	25	270		3	240	330	160
	4	0	30	120		4	80	150	80
	5	80	175	520		5	30	360	320
Mean		30	191	214	Mean		80	202	224
Festuca	1	120	120	360	Spring	1	40	25	80
	2	120	240	1,020	Oats	2	120	0	80
	3	30	330	210		3	50	60	160
	4	50	330	80		4	120	0	280
	5	90	135	200		5	120	30	50
Mean		80	231	374	Mean		90	23	130
Spring	1	0	0	40	Winter	1	180	300	1,100
Wheat	2	0	150	120	Oats	2	280	460	90
	3	200	0	350		3	120	300	300
	4	60	0	40		4	80	180	720
	5	100	0	80		5	30	120	1,200
Mean		72	30	126	Mean		138	272	686

APPENDIX D

Table 5. Nos. of H. pseudorobustus per 100 c.c. of soil.

Sample		Numbers per 100 c.c. Months			Sample		Numbers per 100 c.c. Months		
		3	6	9			3	6	9
Fallow	1	50	50	60	Winter	1	520	320	1,170
	2	0	40	120	Wheat	2	120	300	400
	3	0	100	40		3	720	200	1,880
	4	80	0	240		4	0	850	720
	5	40	50	80		5	200	240	560
Mean		34	48	108	Mean		312	382	946
Poa	1	120	1,000	760	Spring	1	360	200	2,000
	2	280	560	240	Barley	2	160	100	1,620
	3	120	50	440		3	200	350	2,000
	4	100	400	480		4	120	100	2,440
	5	200	40	600		5	100	120	1,400
Mean		164	412	504	Mean		188	174	1,892
Ryegrass	1	0	700	780	Winter	1	200	450	240
	2	120	160	1,180	Barley	2	120	320	1,120
	3	0	350	720		3	0	200	690
	4	40	1,250	360		4	120	100	330
	5	50	200	440		5	80	300	560
Mean		42	532	696	Mean		104	274	588
Festuca	1	90	1,080	1,040	Spring	1	160	30	532
	2	80	480	270	Oats	2	40	80	400
	3	200	100	640		3	150	160	240
	4	120	400	960		4	120	100	600
	5	80	720	1,160		5	80	50	480
Mean		114	556	814	Mean		110	84	450
Spring	1	250	500	850	Winter	1	30	0	920
Wheat	2	210	300	400	Oats	2	120	450	2,720
	3	180	1,000	640		3	120	150	800
	4	360	200	1,450		4	80	360	420
	5	500	0	400		5	360	1,200	540
Mean		300	400	748	Mean		142	432	1,080

APPENDIX D

Table 6. Nos. of H. varicaudatus per 100 c.c. of soil.

Sample		Numbers per 100 c.c. Months			Sample		Numbers per 100 c.c. Months		
		3	6	9			3	6	9
Fallow	1	40	30	0	Winter	1	10	140	0
	2	40	40	0	Wheat	2	40	30	0
	3	40	20	0		3	60	100	0
	4	20	0	80		4	0	50	0
Mean		35	22	20	Mean		27	80	0
Poa	1	10	60	250	Spring	1	0	0	40
	2	20	0	160	Barley	2	50	60	150
	3	40	120	80		3	180	20	110
	4	10	50	80		4	180	0	80
Mean		20	57	142	Mean		102	20	95
Ryegrass	1	60	40	80	Winter	1	100	0	120
	2	30	0	40	Barley	2	20	100	90
	3	30	40	40		3	40	80	70
	4	0	120	0		4	20	175	40
Mean		30	50	40	Mean		45	89	80
Festuca	1	20	0	160	Spring	1	30	60	0
	2	30	0	80	Oats	2	10	25	0
	3	40	0	80		3	20	0	30
	4	30	0	160		4	60	20	170
Mean		30	0	120	Mean		30	26	50
Spring	1	70	20	0	Winter	1	20	0	30
	2	10	100	25	Oats	2	50	0	0
	3	40	25	25		3	30	90	90
	4	50	75	0		4	0	20	0
Mean		42	55	12	Mean		25	27	30

APPENDIX D

Table 7. Toyston site. H. digonicus.
Nos. per 100 c.c. of soil.
Mean of 5 replicates.

Sampling Date	3 Months				6 Months				9 Months			
Treatment	Tylenchus	Paratylenchus	Helicotylenchus	Non Plant Parasites	Tylenchus	Paratylenchus	Helicotylenchus	Non Plant Parasites	Tylenchus	Paratylenchus	Helicotylenchus	Non Plant Parasites
Fallow	16	62	54	1,080	22	0	25	206	0	0	32	268
Poa trivalis	8	46	64	1,158	54	68	279	308	68	272	752	94
Festuca Pratensis	0	6	80	830	69	31	231	150	28	24	374	338
Lolium perenne	10	54	30	940	42	35	191	243	54	144	214	174
Spring Wheat	0	26	72	768	18	0	30	173	28	0	126	120
Winter Wheat	0	16	128	860	0	0	118	46	0	0	244	226
Spring Barley	15	26	154	525	0	0	53	188	0	0	152	298
Winter Barley	8	16	88	1,098	13	30	202	42	8	8	224	48
Spring Oats	0	46	90	746	0	0	23	92	72	0	130	334
Winter Oats	0	22	138	1,400	59	194	272	212	16	152	686	687
Grass hosts	6	36	58	976	55	45	234	234	50	147	447	202
Cereal hosts	4	25	112	899	15	37	116	125	19	27	260	285

APPENDIX D

Table 8. Silwood, Ascot site. H. Pseudorobustus.
Nos. per 100 c.c. of soil.
Mean of 5 replicates.

Sampling Date	3 Months					6 Months					9 Months				
Treatment	Tylenchus	Paratylenchus	Tylenchorhynchus	Helicotylenchus	Non Plant Parasites	Tylenchus	Paratylenchus	Tylenchorhynchus	Helicotylenchus	Non Plant Parasites	Tylenchus	Paratylenchus	Tylenchorhynchus	Helicotylenchus	Non Plant Parasites
Fallow	118	10	28	34	442	260	20	80	48	1,360	40	0	46	108	454
Poa trivalis	382	40	188	164	938	372	36	322	412	1,204	384	32	864	504	648
Festuca pratensis	344	24	48	114	616	412	34	256	556	448	448	14	478	814	268
Lolium perenne	330	0	84	42	686	488	30	156	532	694	666	34	246	680	598
Spring Wheat	544	16	246	300	1,556	132	0	158	380	534	306	10	248	748	426
Winter Wheat	352	152	292	312	1,592	290	0	96	382	1,116	248	6	594	946	578
Spring Barley	392	8	130	188	1,208	202	0	108	174	822	218	0	216	1,892	764
Winter Barley	266	16	78	104	1,270	246	0	100	274	884	392	0	232	588	658
Spring Oats	310	30	140	110	1,258	182	10	118	84	418	513	14	541	450	802
Winter Oats	262	30	108	142	1,878	304	0	194	432	1,064	486	0	664	1,080	928
Grass hosts	352	21	107	107	747	424	33	245	500	782	499	27	529	666	505
Cereal hosts	354	42	165	192	1,460	226	2	129	288	806	360	5	401	951	693

APPENDIX D

Table 9. Broomhill site. H. varicaudatus.
Nos. per 100 c.c. of soil.
Mean of 4 replicates.

Sampling Date	3 Months					6 Months					9 Months				
Treatment	Tylenchus	Paratylenchus	Tylenchorhynchus	Helicotylenchus	Non Plant Parasites	Tylenchus	Paratylenchus	Tylenchorhynchus	Helicotylenchus	Non Plant Parasites	Tylenchus	Paratylenchus	Tylenchorhynchus	Helicotylenchus	Non Plant Parasites
Fallow	127	92	98	35	2,712	115	10	0	22	495	10	60	0	20	1,290
Poa trivalis	112	102	0	20	1,247	131	135	0	57	837	150	0	0	142	217
Festuca pratensis	190	680	20	30	1,207	47	88	6	0	197	30	840	0	120	160
Lolium perenne	27	682	40	30	1,457	40	270	10	50	282	10	432	0	40	510
Spring Wheat	75	300	7	42	1,622	55	61	0	55	367	12	35	0	12	224
Winter Wheat	27	232	0	27	2,347	64	222	28	80	260	10	47	0	0	165
Spring Barley	222	1,185	7	102	1,717	45	0	0	20	522	0	112	0	95	232
Winter Barley	27	400	0	45	1,375	119	216	12	89	302	40	640	0	80	200
Spring Oats	90	110	14	30	1,190	74	45	6	26	221	10	20	0	50	122
Winter Oats	12	565	50	25	1,012	42	99	0	27	195	10	820	0	30	600
Grass hosts	110	488	20	27	1,304	73	164	5	36	439	63	424	0	101	296
Cereal hosts	75	465	13	45	1,544	66	107	8	49	311	14	279	0	44	257

APPENDIX D

Table 10

Table 10. Braodbalk site. H. vulgaris.
Nos. per 100 c.c. of soil.
Mean of 5 replicates.

Treatment	Tylenchus	Paraty-lenchus	Helico-tylenchus	Rotylenchus	Non Plant Parasites
Fallow	0	0	6	6	18
Poa trivalis	16	18	8	0	104
Festuca pratensis	0	0	28	0	90
Lolium perenne	16	0	0	20	156
Spring Wheat	6	6	0	12	24
Winter Wheat	6	6	6	24	32
Spring Barley	0	8	8	32	16
Winter Barley	8	0	16	8	72
Spring Oats	0	0	24	8	80
Winter Oats	8	0	0	16	116

APPENDIX E

Pathogenicity Trials.

Table 1. Pathogenicity of H. dihystera on wheat. Inoculated Nematodes.

Treatment	Replicate	Final Nematode Nos./Pot	Number of Tillers	Shoot Dry Weight grms.	Root Dry Weight grms.
Control	1	0	9	4.60	1.177
	2	0	8	5.93	1.487
	3	0	8	6.25	1.012
	4	0	8	5.31	3.031
Mean	-	0	8.25	5.52	1.83
1,000 Nematodes	1	660	10	9.79	1.496
	2	310	9	5.65	1.152
	3	620	8	4.60	1.258
	4	570	8	6.47	2.781
Mean	-	490	8.75	6.63	1.67
2,000 Nematodes	1	0	10	8 .06	2.517
	2	265	8	3.97	1.063
	3	570	7	4.68	0.931
	4	600	8	7.47	2.687
Mean	-	359	8.25	6.04	1.80
5,000 Nematodes	1	1,120	9	4.28	1.278
	2	610	6	7.49	2.540
	3	1,095	7	3.54	0.950
	4	365	8	4.69	1.557
Mean	-	795	7.50	5.00	1.58

APPENDIX E

Table 2. Pathogenicity of H. pseudorobustus on wheat. Inoculated Nematodes.

Treatment	Replicate	Final Numbers of Nematode/ Pot	Number of Tillers	Shoot Dry Weight (grms.)	Root Dry Weight (grms.)
Control	1	0	8	5.36	1.594
	2	0	9	4.45	1.111
	3	0	8	5.27	0.998
	4	0	7	7.48	2.312
Mean		0	8.00	5.64	2.00
1,000 Nematodes	1	0	9	4.05	1.764
	2	0	10	4.39	1.254
	3	335	7	4.28	0.690
	4	330	7	5.71	2.211
Mean		166	8.25	4.61	1.48
2,000 Nematodes	1	315	9	5.22	1.655
	2	330	7	5.95	1.255
	3	0	7	4.61	3.132
	4	0	8	5.01	1.890
Mean		161	7.75	5.20	1.97
5,000 Nematodes	1	2,800	8	6.12	1.930
	2	990	7	6.49	1.805
	3	1,890	7	3.84	1.068
	4	610	7	5.85	2.867
Mean		1,572	7.25	5.57	1.92

APPENDIX E

Table 3. Rate of increase of H. dihystera.
Nos. per 250 c.c. of soil plus cut roots.

Treatment	Replicate	Nos. of H. dihystera	Treatment	Replicate	Nos. of H. dihystera
1 week	1	114	16 weeks	1	300
	2	90		2	1,922
	3	75		3	100
Mean		93	Mean		774
5% Confidence Limit		34	5% Confidence Limit		1,113
4 weeks	1	84	32 weeks	1	1,962
	2	80		2	1,980
	3	90		3	1,800
Mean		85	Mean		1,914
5% Confidence Limit		1.4	5% Confidence Limit		108
8 weeks	1	210	48 weeks	1	4,547
	2	187		2	3,840
	3	300		3	4,187
5% Confidence Limit	Mean	232	5% Confidence Limit	Mean	4,191
		72			363

Table 4. Pathogenicity of H. dihystera. Infested soil.
Initial and Final Populations.

Treatment Soil	Extract	Number of H. dihystera per 200 c.c. soil.	
		Initial	Final
Control	1 1)	0	0
	2)	0	0
	2 1)	0	0
	2)	0	0
Mean		0	0
Infested	1 1)	975	480
	2)	925	620
	2 1)	750	675
	2)	700	575
Mean		837	587

APPENDIX E

Table 5. Pathogenicity of H. dihystera on wheat. Infested soil.

Uninfested Soil				Infested Soil			
Pot No.	Growth Stage (1)	Shoot Dry Wt. grms.	Root Dry Wt. grms.	Pot No.	Growth Stage (1)	Shoot Dry Wt. grms.	Root Dry Wt. grms.
1	10	1.35	0.30	1	10	0.54	0.19
2	10	0.49	0.20	2	10.1	0.74	0.19
3	9	0.70	0.17	3	10.1	0.67	0.14
4	10.1	1.03	0.20	4	10	0.35	0.09
5	10.1	1.05	0.23	5	10.1	0.90	0.28
6	10.1	1.34	0.38	6	10	1.44	0.47
7	10	1.88	0.35	7	10.3	1.61	0.14
8	10.4	1.22	0.45	8	10	0.90	0.28
9	10	1.10	0.33	9	10.2	1.29	0.34
10	10.2	1.67	0.32	10	10.1	1.57	0.56
11	10.3	1.36	0.41	11	10.3	1.03	0.37
12	10	0.82	0.46	12	10	1.76	0.54
13	10.5	1.57	0.44	13	10	1.44	0.37
14	10	0.89	0.31	14	10.2	1.61	0.35
15	10	1.51	0.36	15	10	1.51	0.42
16	10.5	0.93	0.34	16	10.5	0.58	0.19
17	10	0.92	0.27	17	10.4	1.67	0.41
18	10.5	1.29	0.45	18	10	1.15	0.47
$\bar{Z}_x$		21.12	5.97			20.76	5.80
$\bar{x}$		1.18	0.33			1.15	0.32

(1) Growth stage based on Feekes scale, Large (1954).

APPENDIX E

Table 6. Root Invasion by H. dihystera.

Treatment		Main		Secondary		Total
		Endoparasitic	Semi-Endoparasitic	Endoparasitic	Semi-Endoparasitic	
5 days	1	0	0	0	0	0
	2	1	0	0	0	1
	3	0	0	0	0	0
	4	0	0	0	0	0
Total		1	0	0	0	1
10 days	1	2	0	0	0	2
	2	4	0	1	0	5
	3	4	0	1	0	5
	4	14	0	3	0	17
Total		24	0	5	0	29
15 days	1	14	0	12	0	26
	2	16	0	7	1	24
	3	12	0	3	0	15
	4	23	2	7	0	32
Total		65	2	29	1	97
25 days	1	27	1	31	0	59
	2	10	1	18	0	29
	3	10	0	5	1	16
	4	20	2	9	0	31
Total		67	4	63	1	135
40 days	1	10	0	28	0	38
	2	11	0	15	0	26
	3	8	0	18	0	26
	4	17	0	11	0	28
Total		46	0	72	0	118