THE POPULATION ECOLOGY OF THE LESION NEMATODE, *PRATYLENCHUS THORNEI* AND THE ROOT KNOT NEMATODE, *MELOIDOGYNE JAVANICA*

by

GORDON S. GRANDISON B.Sc.

Department of Plant Pathology
Waite Agricultural Research Institute
The University of Adelaide
South Australia

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STATEMENT

This thesis has not previously been submitted for an academic award at this or any other University, and is the original work of the author, except where due reference is made in the text.

Signed

G.S. Grandison

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SUMMARY

The purpose of this research was to study the influence of environmental factors on the numbers of *Pratylenchus thornei* and *Meloidogyne javanica* in soil and in roots of host plants.

In studies on the ecology of *Pratylenchus thornei* samples were taken from eight sites with different soil types in the Adelaide area of South Australia. The characteristics of each site were measured to determine which factors might most influence the nematode. Soil structure, soil texture, temperature, rainfall and irrigation, evapotranspiration, rate of drying, nutrient status, pH, soluble salts and other nematodes were measured.

Soil and root samples taken at monthly intervals were processed to extract the nematodes which were then counted. The measurements of environmental factors were correlated with nematode numbers to ascertain the key factors that determined differences in population density between sites and differences in density with time. The results indicated unequivocally that clay content was closely and positively correlated with numbers of *P. thornei*. Although there were highly significant differences in the numbers of *P. thornei* at different times within sites there were no significant

correlations with any of the weather factors. It was concluded that seasonal changes in nematode numbers were probably correlated with changes in root growth of the host plant, strawberry clover. Root growth was not measured as initially it was not considered to be an important environmental component determining seasonal population fluctuations.

Laboratory and glasshouse experiments were done to test hypotheses developed in the field. Invasion studies with P. thornei indicated that more nematodes invaded clover roots growing in clay soil than in sandy soil.

In studies on the ecological factors influencing Meloidogyne javanica experiments failed to show that volume of root system had any influence on percent invasion except at low root volumes. It was shown that the majority of nematodes invaded the root within the first 24 hours; that photoperiod had little influence on invasion provided there was sufficient light to maintain normal physiological functions in the plant; that numbers in the root declined 6 days after invasion started; that depth of inoculum in the soil had little influence on invasion rate; that ability to invade was lost in larvae 4 to 11 days old; that invasion decreased when the inoculum was introduced into the soil at a distance greater than 4 cm from the plant.

In both P. thornei and M. javanica mobility down columns of saturated soil increased with increase in temperature in sand but decreased in loam and clay. The optimum temperature for mobility of *P. thornei* is 15°C and maximum mobility occurs at a moisture content when most of the pores have drained of water.

The results are discussed under the following headings:

(a) The influence of environment on *P. thornei*

(b) Invasion of roots by *M. javanica*

(c) A comparison of the ecology of *P. thornei* and *M. javanica*

(d) Disease assessment

(e) The ecology of nematodes.

CHAPTER I

INTRODUCTION

The purpose of this research is to study the influence of environmental factors on the numbers of *Meloidogyne javanica* (Treub, 1885) and *Pratylenchus thornei* (Sher and Allen, 1953) in the soil and within the roots of host plants. Both species are known to cause serious losses in crops especially when their numbers reach high levels. This thesis is not concerned with the plant pathological aspects of these two nematodes but with their ecology and, in particular, it aims to devise a method whereby the data produced from systematic sequential sampling of areas can be used, after statistical analysis with the computer, to assess the relative importance of the various ecological components on nematode populations. Such information is, of course, relevant to the plant pathological problem because the greater the number of nematodes in the roots the greater the chance of their causing disease. Furthermore, the host plant is an important component of the nematodes' environment.

The root knot *M. javanica* and lesion *P. thornei* nematodes have quite different life-cycles and the way in which they cause disease in the host plant is also different.

The various aspects of these processes have also received different emphasis by nematologists. Thus, there are numerous papers on the relationship between host species and numbers of *Pratylenchus* in the soil, but less attention has been paid to their ecology and to the experimental study in the laboratory of environmental factors such as temperature and soil moisture on movement of and invasion by this nematode. Studies on the behaviour and physiology of *Meloidogyne* on the other hand have received more attention than those on the influence of various environmental factors on the nematode using whole plants growing in soil. Consequently, in this thesis, more emphasis will be placed on glasshouse experiments in the study of *Meloidogyne javanica* whereas with *Pratylenchus thornei* greater attention will be paid to field and laboratory experiments. In this way more convincing hypotheses can be put forward to explain the observed fluctuations in the numbers of these two species.

To emphasise these points and to set the stage for the descriptions of experiments that follow, a brief review of the published literature relevant to the ecology of root knot and lesion nematodes will be given.

I. *Pratylenchus thornei*

The numbers of nematodes in soil and host roots are influenced by a variety of interacting factors in the environment. In attempting to determine which of these factors are most important in the association of a particular nematode species with a particular host plant it is necessary to sample different sites with different soil types over a period of time. Having determined the trends in population numbers, hypotheses can be put forward to suggest which factors are influencing the nematode populations. Laboratory experiments then follow to test these hypotheses. This is the procedure adopted in this research project with P. thornei in clover.

There have been few critical studies given to the seasonal variation of Pratylenchus spp. in soil on plant hosts or other nematodes on specific crops. Most published reports have been on a single sample on a specific crop and hence are of little use for ecological studies. To give a true picture of the nematode-host plant relationships, the number of nematodes must be studied throughout the season. Graham (1951) observed in his studies on tobacco that a single population count made on only one sampling date may be very misleading. He found that numbers of Pratylenchus increased during spring and summer reaching a peak in early August and then decreased sharply, whereas with corn the peak numbers occurred in early September, but he gives no suggestions that explain these fluctuations.

Goheen and Williams (1955) studied the seasonal fluctuations of numbers of P. vulvus associated with brambles. They observed a peak of numbers in May and June followed by a rapid decline during the summer; the numbers remaining low throughout the winter. They suggested that there was a correlation between high numbers and increased root growth of this perennial crop. Similarly, Wehunt (1957) stated that there was a correlation between the maximum numbers of Pratylenchus and the greatest growth of white clover roots which occurs from January to June in Louisiana.

Mountain and Boyce's (1958) studies on the peach replant problem in Canada indicated that there was a seasonal fluctuation in numbers of P. penetrans, the peak occurring in spring, then as the summer progressed, numbers declined rapidly, supposedly due to high soil temperatures which were above the optimum for growth and development of P. penetrans. There followed a slight increase due to cooler autumn temperatures. They did not continue their sampling throughout winter to see if there were any further changes.

The influence of plant roots on numbers of Pratylenchus was indicated by Decker's (1960) studies on apple tree decline in Germany. Besides finding the highest nematode numbers in summer and the lowest in winter, which is presumably due to the influence of temperature, he found that there was a vertical distribution of numbers; there being higher numbers per gm of root at 16-24 cm depth which was the region of maximum root growth. The influence of the host on the numbers of *Pratylenchus* was also emphasized by Cichorius (1960) who showed that a favourable host such as carrots grown three years in succession caused a considerable increase in *P. penetrans* numbers. Notwithstanding this, there was still a seasonal fluctuation in numbers with a maximum in August-November apparently caused by higher soil temperature and higher moisture followed by a minimum number over the period of June-July, a time of little rainfall. In Di Edwardo's (1961) study on seasonal population variation of *P. penetrans* in strawberries, where moisture was not a limiting factor because of irrigation, the density of nematodes in roots increased from a minimum in February-March up to a peak in July, then declined suddenly. This was explained by the rapid increase in root development that reduced the numbers of nematodes per unit volume of root. As root growth slowed and the reproduction of *P. penetrans* increased, nematode density increased in the autumn. It is also possible that the soil temperature of 27°C may have exceeded the optimum developmental temperature for *P. penetrans*, which is 22.5°C (Patterson and Bergeson, 1967).

Winslow (1964) extended these ideas at Rothamsted in his studies on migratory soil nematodes over an experimental period of six years. He found a marked seasonal variation in numbers in the top 15 cm of soil; minimum numbers occurred during May-July, followed by a rise to a maximum in late summer or autumn which was, either maintained throughout the winter, or began to decline in the autumn. Factors such as the severe soil disturbance necessary for the husbandry of specific crops, and the relatively short growth period probably giving incomplete root penetration of the soil may explain the low numbers occurring after beet and potatoes compared to high numbers associated with cereals. Pratylenchus spp. showed clearly the influence of the crop in both heavy and light soils, being highest with cereals, but other tylench genera were more influenced by the soil type. Thus soil type appeared to be more important than the crop in determining presence or absence of migratory root nematodes, but within each soil type, the crop influenced the abundance of these nematodes, cereals generally supporting more than the root crops, beet and potatoes.

Dickerson et al. (1964) studied the influence of the combined effects of temperature and host on the seasonal variation in numbers of Pratylenchus. Numbers increased in the roots and soil to a peak in summer as the growing season progressed, followed by a decline in autumn and winter. They found that the optimum temperature for increase of *P. penetrans* varied with the host and was not correlated with the best host growth which occurred at 16°C. Highest numbers occurred at 16°C for potatoes and 24°C for corn. When potatoes followed corn, the population build-up shifted so that the largest population increases were at the cooler temperature. This may indicate that temperature has less influence on the nematode directly than indirectly by rendering the host more susceptible to infection.

Koen (1967) suggested that seasonal variation of numbers of *P. brachyurus* may be explained by the lack of host plants in the winter drought conditions and low temperatures of the veld regions in South Africa. The rapid increase in numbers during the spring and summer was correlated with the rapid growth phase of host roots, the increase in soil moisture and the higher soil temperatures. Although there were short periods of time (30 to 80 minutes) when temperatures exceeded 44°C, sufficient to kill nematodes, survival appeared to be enhanced by the insulating capacity of the organic matter content of the soil in which *Pratylenchus* occurred.

Similarly, Olthof (1967) suggested that there was a correlation between seasonal variation of numbers of *P. penetrans* on tobacco and soil moisture and temperature;

the numbers were directly proportional to moisture and inversely proportional to temperature.

Ferris and Bernard's (1967) study on populations of Pratylenchus over five years on soybeans could have provided some helpful information, but the sampling was done for only two months in each year.

In the more recent studies of Van Gundy et al. (1968) on Hemicycliophora arenaria, a peak of numbers was shown to occur in spring, followed by a decline in summer and autumn and an increase in winter. With the extra complicating factor of irrigation they found that soil temperature and acre-inches of water (from irrigation) showed an inverse relationship to numbers of nematodes. To explain their observed data, they postulated that environmental factors other than temperature are equally important in ecological studies. The increased frequency of irrigation reduced population numbers irrespective of temperature. This was probably an indirect effect through the reduced soil aeration which they determined. If the periods between irrigation were not long enough for the water to drain out of the larger pore spaces so that oxygen could diffuse in, then conditions in the soil were almost anaerobic and consequently reduced the numbers of nematodes in the summer and autumn. When water application was less frequent, aeration improved, and with decrease in temperature in the winter months there was an increase in Hemicycliophora numbers.

From his detailed studies Yeates (1968) correlated a considerable part of the annual variation in numbers of all nematodes with variation of environmental factors of moisture, temperature (though the single determinations at the time of sampling are of doubtful use), salinity, pH and organic matter. The overall picture gained from the data was that several factors influenced the seasonal variation in numbers.

The distribution of numbers of Pratylenchus in relation to root distribution, moisture and depth in the field have been only briefly considered by previous authors, each measuring a component to some degree and then speculating on the others. There are no published data on this aspect for P. thornei although temperature and moisture change with depth are likely to influence nematode mobility, penetration and survival in soil especially during hot drought conditions.

Seasonal changes in numbers of the plant feeding nematodes Helicotylenchus vulgaris and Rotylenchus pumilus in two non-cultivated sites in the United Kingdom were found by Yuen (1966) not to be related to soil moisture content. But from her figures, it is unlikely that moisture was ever limiting and it is more likely that the summer increase of numbers was related to growth of the host plants. Yuen concludes, from her studies on the vertical distribution of plant parasitic nematodes, that each maintains itself at a specific depth which may differ in different habitats. As Wallace (1963) suggested, the depth at which a species is most numerous is probably the zone in which conditions for reproduction are best, and depends on the interaction of several factors such as nutrition, moisture, temperature, aeration and soil structure.

Although the numbers of Pratylenchus spp. are usually greatest and damage is most severe where host plants are grown in sandy soils (Endo, 1959), P. thornei populations are highest in relatively heavy soils (Oostenbrink, 1954; Corbett, 1970). This suggests that the environmental factors which interact with soil type may influence the rate of population growth of Pratylenchus spp.

As Pratylenchus is an endoparasite with a migratory soil phase, its numbers will be influenced by soil temperature, structure, moisture, aeration, pH, and chemical properties. Soil moisture and aeration are interrelated with structure. Thus, in studying the ecology of Pratylenchus, the influence of the soil type on the survival, mobility and penetration of the root is an important aspect of the problem. Soil type can be expressed in terms of texture, i.e. sand, silt and clay and the factors associated with these: structure, moisture and suction.

Some work has been done on the relation of soil moisture to survival (Godfrey, 1929; Hastings, 1939; Lownsbery, 1956) and on the influence of soil moisture on population growth of *Pratylenchus* spp. as influenced by their ability to reach and penetrate host roots (Kable and Mai, 1968). Penetration of the root and hence the population growth of *P. penetrans* was probably influenced by the interaction of soil moisture with soil type, the higher populations occurring in sandy soil rather than in heavier soils; but the range of moisture stress favourable to population growth differed for different soil types. The higher the silt and clay content of a soil, the greater was the moisture tension necessary for drainage of excess moisture to give favourable conditions for satisfactory population growth. The length of time of the growing season, when such optimum conditions occur, is of shorter duration in clay soils than sandy soils (Kable and Mai, 1968). This hypothesis may explain observed field data for *P. penetrans* but, as *P. thornei* is generally found in heavier clay loam soils rather than sandy soils, a different explanation is required. However, the per cent penetration in Kable's experiments was very low (1-9%), hence it is difficult to assess the results of moisture tension on penetration. Furthermore, the highest percentage recovery was obtained from plants which had been infected over a period of 11 days during which time reproduction within the roots may have occurred. Further experiments are needed on this aspect of penetration. Thus penetration and migration are affected similarly by soil moisture, migration being best at about field capacity, declining in both wetter and drier soils (Wallace, 1961). The direction of nematode migration is influenced by orientation to the host roots and speed of movement is determined by the soil particle size, pore diameter and thickness of water films within the pores. Movement is quickest when the nematode can most efficiently use the surface tension forces of the water films in the soil pores (Wallace, 1958), and it is likely that these same physical factors in the soil and their interactions influence penetration in the same way as they affect migration.

This brief review raises the following questions that form the basis for the research described in this thesis:

1. Which of the numerous environmental components are chiefly responsible for variation in numbers of *P. thornei* with time (seasonal fluctuations), and for differences in population numbers between different localities?
2. How do the environmental factors influence the numbers of *P. thornei* and so enable it to survive the hot dry

conditions of the South Australian summer? How, for example, do soil moisture, temperature and soil structure influence the infection of clover roots by this nematode?

II. *Meloidogyne javanica*

There is an extensive literature on the detrimental influence of root knot nematodes on crop yield. This could be due to the fact that infected roots are grossly distorted and thus infection is very obvious. In fact root knot is one of the oldest known nematological disorders of plants; Berkeley (1855) described it from U.K. on cucumbers. Hence, more study has been assigned to this problem than to the more insidious debilitating effects on plant yield that some other plant parasitic nematodes cause. Due to the obvious need for immediate practical alleviation of the disease, an estimation of the degree of severity of infection and of susceptibility of a host was required.

The method of depicting the degree of root galling by an arbitrary numerical scale of 0 to 5 was at first used (Sasser, 1954), but more accurate methods were required to assess infection, as some hosts show very little external galling yet their yield is greatly reduced, e.g. lettuce, turnips, while with other crops yield increased at low numbers of *Meloidogyne* (Wallace, 1971). Hence critical studies are necessary on the factors that influence the infection of root knot larvae into host plants. Although Wallace's (1966, 1969, 1970) studies on the influence of environmental factors on M. javanica infection are very detailed, there are still phases which need investigation in order to explain in the ecological sense the complex interactions associated with nematode invasion of plants. For example more information is needed on the influence of the soil environment on the nematode so that we can determine the optimum conditions for the different phases of infection, migration through the soil and penetration of the host root, in order to determine any characteristics of the parasite that increase its chances of invading the host. The optimum temperature for migration is about 25-30°C and for invasion, 20-30°C. Thus temperatures fluctuating between 20-30°C probably give the maximum infection. These conditions could easily occur during diurnal temperature fluctuation in sub-tropical soils. Optimum soil moisture for movement and invasion is in the region of field capacity. Larvae of M. javanica migrate quickest through a medium when all the larger pore spaces have drained, but when there is still water in the smaller pore spaces and within the soil crumbs. The suction potential necessary to drain the soil of water varies according to the particle size, more suction being required for smaller particles than larger. Thus in the field it is easier to attain the lower suction necessary to give field capacity in sandy loam soils than in finer clay soils. The optimum particle size for migration is 250-500 μ and 150-250 μ for invasion. Soil moisture is the most important factor controlling the rates of diffusion of oxygen and carbon dioxide in the soil atmosphere. Thus migration and invasion by the infective second stage larvae of M. javanica are subjected to wide fluctuations of aeration with the changes in soil moisture content. We know that mobility of larvae is low when soil pore spaces are filled with water (Wallace, 1966). Also Van Gundy and Stolzy (1963) showed that there was a linear relationship between movement of larvae of M. javanica and the rate of oxygen diffusion in a porous medium. Relatively little water needs to drain from the soil to provide adequate aeration of pore spaces, thus the maximum effect of increased aeration following drainage of pores occurs at suctions that are lower than those required to drain all the larger pores (Wallace, 1968). Hence, low aeration in soil due to water-logging or to soil depth can, by inactivating larvae, prolong infectivity (Van Gundy et al., 1967) although absence of oxygen may reduce survival (Stolzy et al., 1960).

The pH of the medium appears to have little effect on invasion of larvae over the range tested, but the wide variation of the data and the short term of the experiment may not have revealed any influence (Wallace, 1966). Similarly, osmotic potential is probably of minor importance because it seldom exceeds two atmospheres in soil and mobility is only affected at values greater than this.

Although there is an increase in the numbers of M. javanica penetrating a root with increased inoculum, the rates of invasion are similar. There also appears to be a decrease in numbers infecting at about the sixth day after inoculation with M. javanica on tomato. This suggests that there may be a limitation of feeding sites and this is reflected in the decreased rate of development (Wallace, 1969). The main factors related to nematode density which appear to influence invasion are: multiple invasion at the same site and increased attraction to roots following initial invasion which increase in penetration; shortage of space at the root tip, destruction of root tissues and inhibition of root growth and thus loss of attraction to roots when growth stops; all of which decrease invasion (Wallace, 1966). The age and physiological state of the larvae greatly influences invasion. As the lipid content of the intestine (the main energy storage material) is used up, there is a decrease in mobility resulting in a decrease in infectivity. Although there is no significant loss in body contents during penetration, mobility in the soil prior to penetration of the root rapidly depletes the body reserves. Also, body contents are used up rapidly at high temperatures in dry soils and at high oxygen concentrations (Van Gundy *et al*., 1967).

These studies raise such questions as: Does the rate of invasion proceed at a steady rate until most of the population has entered the root? Is this rate linear or does it decrease with time? Does the distance a larva has to travel to reach a root and its age influence the rate of invasion? What is the influence of the host on invasion? Does the age of the host plant and hence the volume of roots influence invasion? Are the numbers of larvae of *M. javanica* that penetrate a host root influenced by host status, i.e. whether the plant is a susceptible or resistant variety. Experiments described later in this thesis attempt to answer these questions.

CHAPTER II

THE ENVIRONMENT OF PRATYLENCHUS THORNEI

This chapter is concerned with the description and measurement of those environmental factors that a preliminary survey indicated might most influence nematode numbers in the different sites.

To study the fluctuations in numbers of *Pratylenchus thornei* in the field, areas of strawberry clover (*Trifolium fragiferum* L.) at eight sites with a range of soil types were selected in the Adelaide region (Fig. 1).

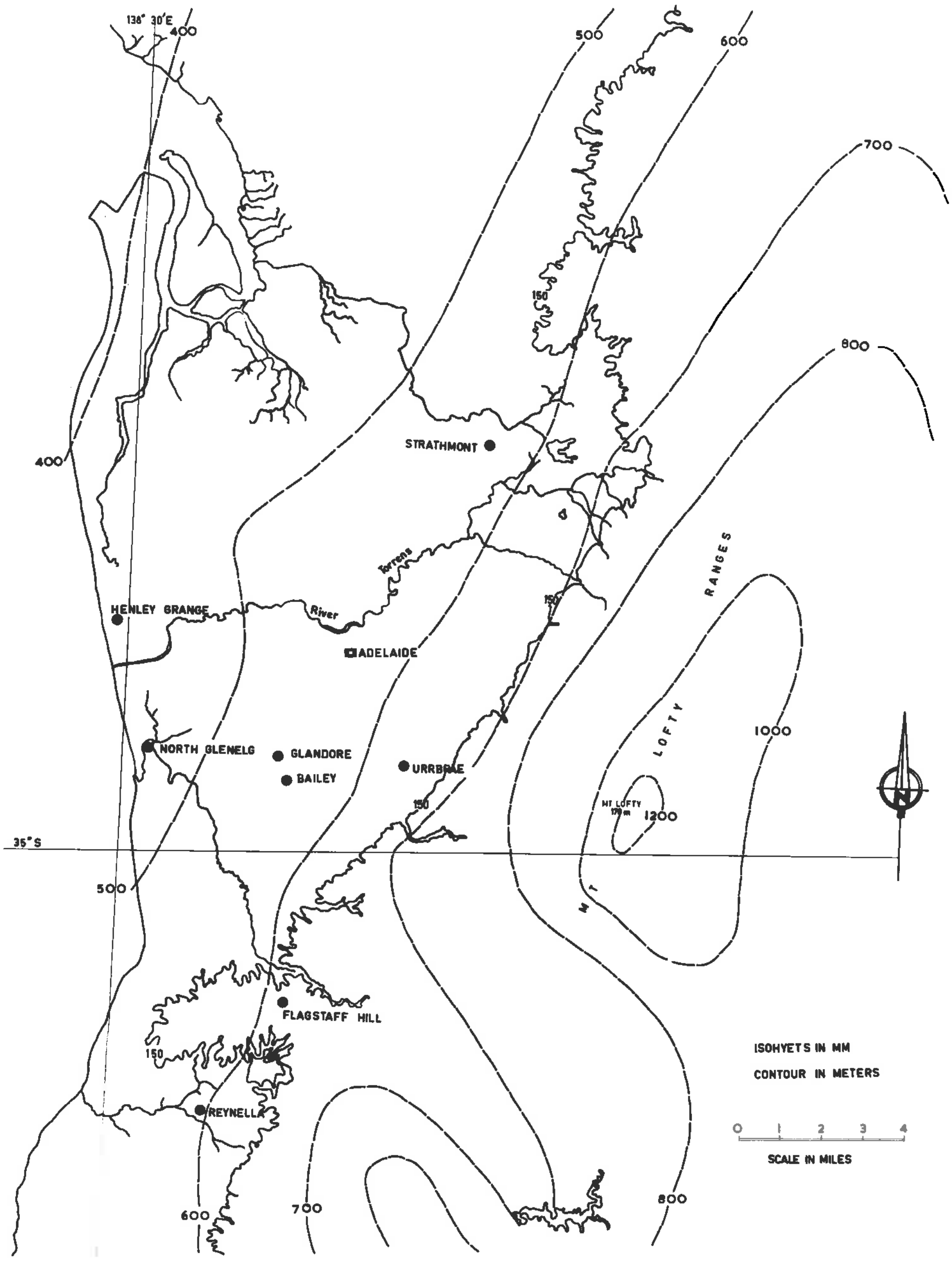
I. Site Characteristics

Site 1: North Glenelg - a level recreation ground where the dominant plant species is *T. fragiferum* with some *Poa pratensis* L. and *Cynodon dactylon* Pers. The site contained a sandy loam alluvial soil 400 metres from the coast at 6 m altitude (138° 30'E, 34° 58'S). The site was levelled in 1959 with no supplementary soil added. Drainage is natural, and no supplementary fertilizer is added.

Site 2: Bailey Grounds - a level recreation ground with the dominant plant species *T. fragiferum* with some *P. pratensis*, *C. dactylon* and *Festuca rubra* L. var. *commutata* Gaud.,

Figure 1. Map of Adelaide (South Australia) showing the eight sampling sites, the isohyets (discontinuous line) and 150 metre contour (continuous line).

FIG. 1


138° 30'E
400
500
600
700
800
150
STRATHMONT
Torrens
River
HENLEY GRANGE
ADELAIDE
RANGES
LOFTY
MT
1000
NORTH GLENELG
GLANDORE
BAILEY
URRBRAE
MT LOFTY
1200
35°S
FLAGSTAFF HILL
REYNELLA
ISOHYETS IN MM
CONTOUR IN METERS
0 1 2 3 4
SCALE IN MILES

growing in sandy loam red-brown earth 6150 metres from the coast at 30 m altitude (138° 34'E, 34° 58'S). The site was reformed in 1966 and supplementary sand and soil added. Drainage is natural and superphosphate is applied yearly in February at 1/8 ton/acre.

Site 3: Henley-Grange Oval - a level recreation ground where the dominant plant species is T. fragiferum with some P. pratensis and C. dactylon growing in a loam alluvial soil 1008 metres from the coast at 6 m altitude (138° 30'E, 34° 54'S). The site was formed in 1964 and supplementary sand was added. Drainage is natural and no fertilizer is added.

Site 4: Urrbrae College - a level recreation ground with the dominant plant species T. fragiferum with some P. pratensis, C. dactylon, F. rubra and Pennisetum claudestinum Hochst. growing in a loamy red-brown earth 11293 metres from the coast at 110 m altitude (138° 37'E, 34° 58'S). The site has been long established and no supplementary soil added. Drainage is natural and 'Pellagreen' (a commercial nitrogenous fertilizer) at 1-2 cwt/acre and superphosphate at 1 ton/acre, is added each spring.

Site 5: Flagstaff Hill Golf Club - an undulating fairway with the dominant plant species T. fragiferum with some P. pratensis, C. dactylon, F. rubra growing in a loamy sand podzolic soil 6857 metres from the coast at 186 m altitude (138° 35'E, 35° 03'S). The area was established in 1966 and no supplementary soil added. Drainage is natural and 'Nitrophoska' (commercial nitrogenous fertilizer) at 1 cwt/acre is added in autumn, and superphosphate at 1 ton/acre supplied in spring.

Site 6: Reynella Oval - a level recreation ground with dominant plant species T. fragiferum, with some P. pratensis, C. dactylon, F. rubra growing in a sandy loam red-brown earth 4840 metres from the coast at 114 m altitude (138° 33'E, 35° 05'S). The area was established in 1960 with supplementary sand added. Drainage is natural and superphosphate at 1 ton/acre is supplied in spring.

Site 7: Strathmont College - a flat site with a slight southerly slope with the dominant plant species T. fragiferum with some P. pratensis, C. dactylon and F. rubra growing in a heavy, dark, fine-textured, cracking clay 16130 metres from the coast at 100 m altitude (138° 39'E, 35° 52'S). The ground was formed in 1962 with no supplementary soil added. Drainage is natural. Superphosphate at 1 ton/acre is added each spring.

Site 8: Glandore Oval - a level site with dominant plant species *T. fragiferum* with some *P. pratensis* and *C. dactylon* growing in a sandy loam red-brown earth, 5848 metres from the coast at 24 m altitude (138° 34'E, 34° 58'S). The ground has been established a long time with a considerable amount of supplementary sand added. Drainage is natural and superphosphate at 1 ton/acre is added each spring.

Soil water is probably the most important of the environmental factors controlling the behaviour of soil nematodes, and determines other aspects of the environment: soil atmosphere, soil temperature and plant growth. All these factors are controlled by three main soil physical properties: structure, texture and soil water suction.

II. Soil Structure at the Sampling Sites

The nematode moves through the soil pore spaces, the size and distribution of which depend on the arrangement and size of soil crumbs or aggregates, i.e. soil structure. Thus pore size, moisture, aeration, temperature and composition of the soil solution are likely to have an important influence on the movement and survival of the nematode.

By determining the moisture characteristic curve, pore size distribution, water holding capacity and available pore space were measured.

Using the method of Childs (1940) as described by

Wallace (1958), such curves were measured for each of the sites (Figs. 2 & 3), the soil first being passed through a 1 mm mesh sieve and a 20 cc sub-sample taken.

When the larger soil pores have drained of water under gravity, a state of equilibrium exists; the remaining water is held by surface tension and the soil is said to be at field capacity. The water retained at a suction of 100 cm of water, as measured by Childs' technique gives a fair indication of the water available for plant growth in the upper 6 cm where the bulk of strawberry clover roots occur. This volume of water remaining is expressed as a percentage of the volume of soil and is called the water holding capacity (WHC). The available pore space (APS) is another useful soil parameter; it refers to the volume of pore space through which the nematodes can move and is based on Wallace's 1958 studies which showed that *Heterodera* larvae which have a diameter of about 20 μ (the same as *Pratylenchus thornei*) moved best when soil pores between 30-60 μ diameter were just drained of water. Thus, available pore space is expressed as the per cent volume of water removed between suctions of 30 and 100 cm of water corresponding to pore diameters of 100 to 30 μ respectively. It gives a measure of the suitability of the soil for movement of *Pratylenchus*.

In natural soils there is a whole range of pore sizes

Figure 2. The moisture characteristic curve for four sampling sites (1-4).

APS = Available pore space

WHC = Water holding capacity

FIG. 2

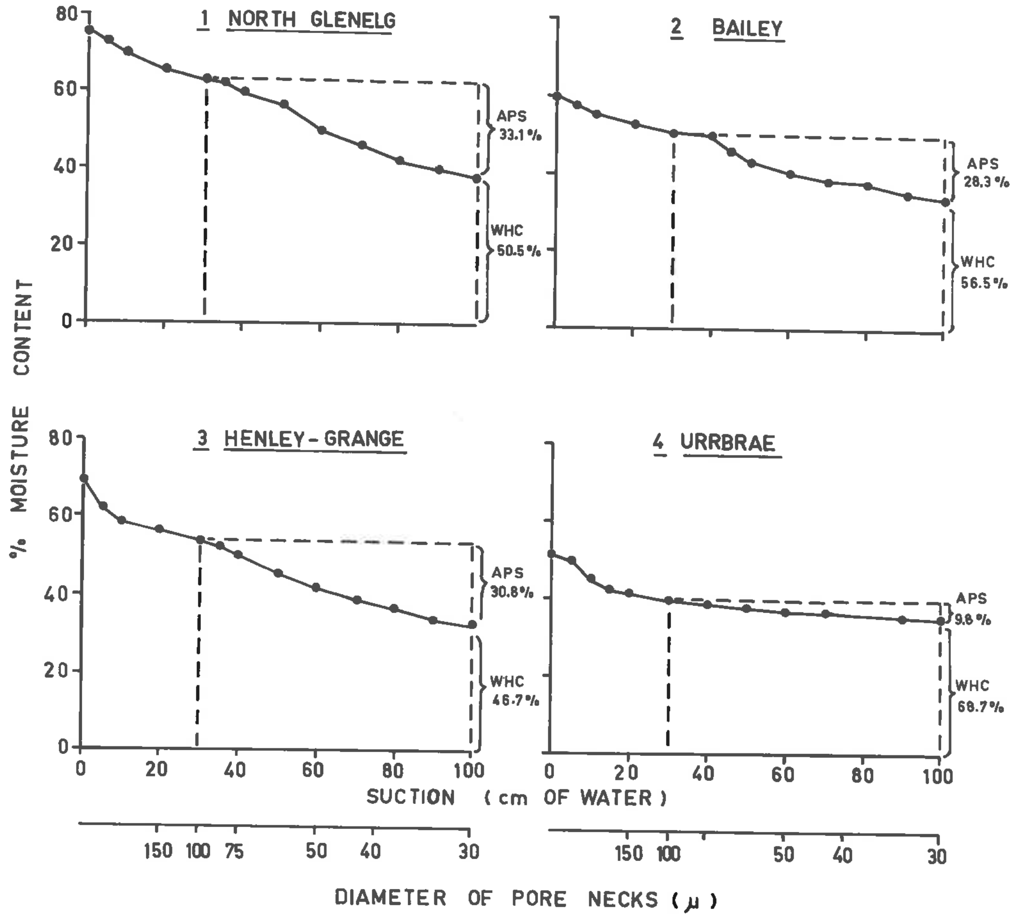
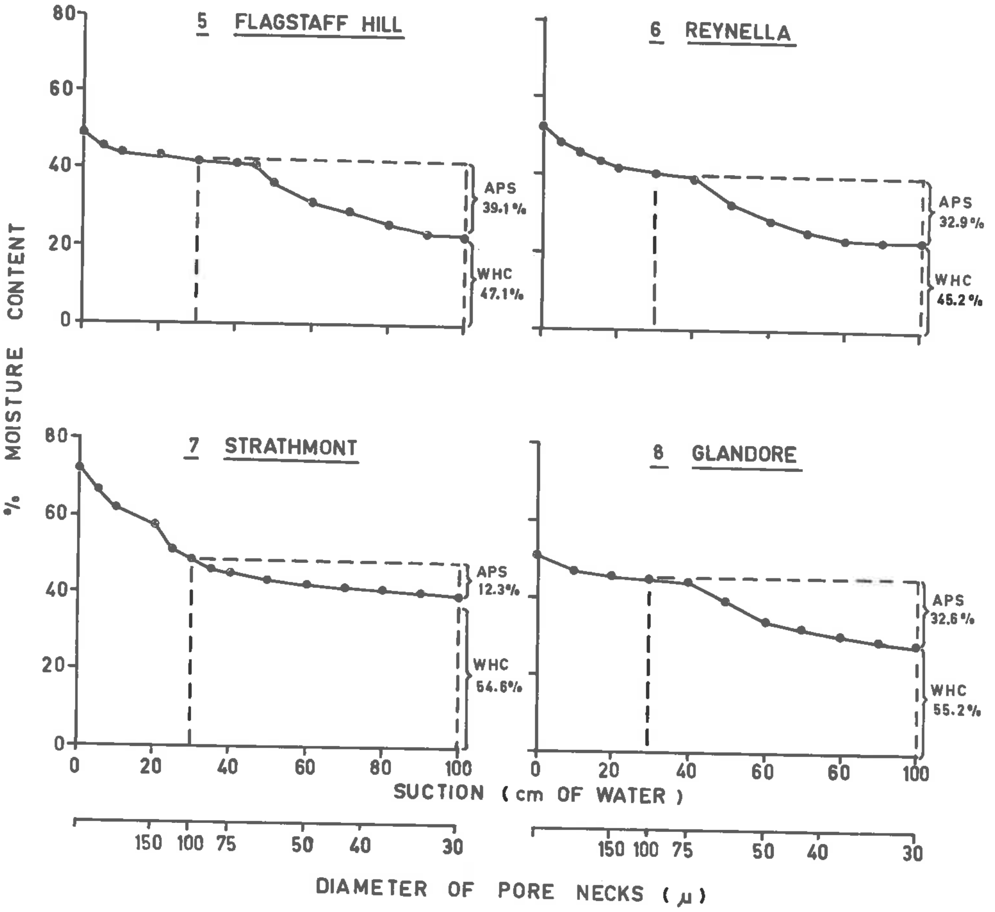

1 NORTH GLENELG
2 BAILEY
3 HENLEY-GRANGE
4 URRBRAE
APS 33.1 %
WHC 50.5 %
APS 28.3 %
WHC 56.5 %
APS 30.8%
WHC 46.7%
APS 9.8 %
WHC 68.7%
% MOISTURE CONTENT
SUCTION (cm OF WATER)
DIAMETER OF PORE NECKS (μ)
0
20
40
60
80
100
150
100
75
50
40
30

Figure 3. The moisture characteristic curves for four sampling sites (5-8).

APS = Available pore space

WHC = Water holding capacity

FIG. 3



5 FLAGSTAFF HILL
6 REYNELLA
7 STRATHMONT
8 GLANDORE
APS 39.1%
WHC 47.1%
APS 32.9%
WHC 45.2%
APS 12.3%
WHC 54.6%
APS 32.6%
WHC 55.2%
% MOISTURE CONTENT
SUCTION (cm OF WATER)
DIAMETER OF PORE NECKS (μ)

from which water is released in succession from pores of decreasing size as suction increases. Consequently, the form of the moisture characteristic curve is roughly linear, the familiar sigmoid curve is only obtained from soils with uniform crumb size or sands with uniform particle size.

The form of the moisture curves for sites 2, 3, 6, 8 is similar and hence their percentage available pore space of 28.3, 30.8, 32.9, 32.6 respectively are also similar. They generally have a lower moisture content at all suctions when compared to the clay site 7, due to the water being held within the clay crumbs themselves, as distinct from that contained in the pores between them. Site 4 (Urrbrae) has a moisture curve intermediate between that of a clay and a sand soil, i.e. there is an initial amount of water which is rapidly removed on increased suction. This indicates that the pores between the larger particles of sand are being drained, then the curve flattens out, possibly due to the high silt content, similar to the clay site. The packing of silt particles between the sand accounts for the low APS of 9.75%. The moisture curve for the sandy site 1 is possibly caused by the fairly high organic matter content in the soil which influences the moisture holding capacity at lower suctions, but this is overcome at higher suctions and the curve resembles that from a sandy site. The percentage APS figure of 33% substantiates this idea and indicates the general sandy nature of the soil.

Site 5 gives a moisture characteristic which would be generally expected from a sandy soil. There is a general lower moisture content at all suctions because sand grains unlike clay crumbs do not contain water. Furthermore, the soil drains at lower suctions than in the other sites; thus the sand particles must be relatively large. It is not surprising that this site has the highest APS of 39%.

III. Soil Texture of Sites

Soil texture gives an indication of the relative proportion of sand, silt and clay. The mechanical analysis of the soils from the eight sites was determined by the plummet balance method of Hutton (1955).

Table 1 indicates that the percentage of sand and clay are inversely related; percentage silt is low except at the Urrbrae College site. The sites tend to fall into five groups:

sand	North Glenelg and Flagstaff Hill Golf Club
sandy silt	Glandore Oval
silt	Urrbrae College
silty clay	Bailey Grounds, Henley-Grange Oval and Reynella Oval
clay	Strathmont College

TABLE 1

Mechanical analysis of soils from the eight sites at which samples of nematodes were taken

TABLE 1

Site	Coarse Sand >210μ	Fine Sand 210μ-50μ	Very Fine Sand 50μ-20μ	Total Sand	Silt 20μ-2μ	Clay <2μ
1. North Glenelg	23.7	63.5	5.4	92.6	1.9	6.6
2. Bailey Grounds	26.0	32.7	8.8	67.5	15.1	15.9
3. Henley-Grange Oval	41.3	30.8	7.4	79.5	8.3	10.5
4. Urrbrae College	11.6	24.2	18.8	54.6	24.3	15.7
5. Flagstaff Hill Golf Club	44.0	48.6	2.6	95.2	0.7	2.5
6. Reynella Oval	40.3	33.5	4.0	77.8	1.9	15.7
7. Strathmont College	8.4	16.9	11.1	36.4	8.2	51.7
8. Glandore Oval	44.9	36.8	5.0	86.7	5.3	5.6

The variation in the clay percentage between sites is correlated with the percentage APS - the higher the clay fraction the smaller the percentage APS, except that Urrbrae's 9.8% appears to be influenced by the high silt percentage; the pore spaces between the sand grains are probably packed with fine silt particles, thus markedly reducing the volume of pore space.

IV. Temperature, Rainfall and Irrigation

(a) Temperature

Soil temperatures at a depth of 6 cm were not taken because of lack of continuous recording instrumentation, no method of protection and the impracticability of leaving such stations unattended. It was considered that the taking of spot temperatures at the time of sampling would give no useful data due to the diurnal variation (Macfadyen, 1968). Thus air temperatures were recorded. Usually there is a close correspondence between changes in air and soil temperatures, although soil temperature having a lower variation is usually out of phase and lags behind that in air.

The micro-climate of bare soil may offer daily temperature changes at 5 cm depth of up to 20°C (Macfadyen, 1968), but the insulating effect of surface vegetation and organic material can protect the underlying soil from extremes of temperature change. Moist soils, because they have a higher thermal capacity, show less temperature fluctuation than dry soils.

The seasonal fluctuation of maximum and minimum air temperatures at the eight sites was similar, except that the two more southerly and higher sites of the Flagstaff Hill Golf Club and Reynella were about 1-2°C cooler all year than the other sites. Also there is a cooling coastal influence on the sites at Henley-Grange and North Glenelg. The highest mean monthly temperature of about 25°C is recorded for February; the lowest of 11°C being for July. Nevertheless, it is unlikely that temperature alone will be a major factor in influencing differences in numbers of nematodes between sites but, in conjunction with moisture, it could be important in influencing seasonal variations.

(b) Rainfall and Irrigation

From the monthly rainfall data, all sites showed a marked seasonal variation. February, the warmest month, had the least rainfall, in fact in most sites no rain fell at all. Then there was a sharp increase up to the yearly maximum in April which was 191 mm, followed by a gradual decline for the rest of the year.

The eight sites are broadly grouped into three areas according to their 50 year (1916-1966) average annual rainfall (Fig. 1); (i) the coastal sites of North Glenelg and Henley-Grange, 450 mm; (ii) the intermediate sites of Strathmont, Glandore, Bailey and Reynella, 550 mm; (iii) sites closest to the Mount Lofty Ranges of Urrbrae and Flagstaff Hill, 650 mm. All of the sites had supplementary water supplied by overhead irrigation sprinklers during the period of October, 1970 to March, 1971 but at different rates. The amount of water applied was only vaguely known by the groundsmen. Only Flagstaff Hill, Urrbrae and Strathmont tried to apply irrigation at a known rate of 140, 80 and 100 mm per month respectively. At the other sites, water was applied when the groundsmen 'considered it necessary to maintain vegetation'. Many methods and criteria are available for use in estimating the adequacy of the rainfall and the amount of irrigation required to sustain plant growth (Thornthwaite, 1948; Penman, 1948, 1949; Prescott, 1959; Butler and Prescott, 1955). However Slatyer's (1960) simplified technique, based on experience in a similar area in Australia, was applied. Slatyer made four assumptions. They were (i) the soil water reservoir in the root zone is 25 mm per foot of depth of soil and the root zone for most non-woody plants can be expected to extend to about 4 feet: thus 100 mm of soil water reservoir was used; (ii) that no deep drainage nor run-off occurs until this reservoir is recharged; (iii) that evapotranspiration of stored water takes place at an average yearly rate of 0.7 E_w (E_w = evaporation from a free water surface); (iv) excess rainfall over evapotranspiration was added to the next month's rainfall, and rainfall which replenished the soil water to more than 100 mm was disregarded.

The term 'evapotranspiration' or soil moisture regime, is meant to designate the process of movement of water from the land surface to the atmosphere in vapour form. Thus it includes evaporation from soil and plant surface, as well as transpiration of water by leaves and the flow of water vapour from the water film about the soil particles into the free pore space in the soil.

Estimation of irrigation required was determined by balancing the rainfall plus soil water storage against the evapotranspiration for the month. If evapotranspiration exceeds rainfall plus storage, the irrigation need was indicated by the difference. Table 2 indicates that Strathmont did not receive enough water during the hot weather of November to March, in fact in January the area only received half the amount of required water. Similarly, Urrbrae which received only 75 mm/month did not get sufficient water for the period of November to March; in January and February, the hottest months with the highest evapotranspiration rate, Urrbrae received only half the required amount of water. Observations indicated that all

TABLE 2

Estimation of the irrigation requirements at four sites at which samples of nematodes were taken

TABLE 2

Site	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June
			1970							1971		
STRATHMONT												
(100 mm/month irrigation water applied Sept. to April)												
Precipitation (mm)	69	93	52	7	37	30	7	12	32	125	106	78
0.7 E_W	42	47	66	114	150	173	195	179	142	103	52	29
Excess (mm)	56	53	34	-	-	-	-	-	-	22	78	127
Irrigation Requirement (mm)	-	-	-	74	111	143	187	167	111	-	-	-
URRBRAE												
(75 mm/month irrigation water)												
Precipitation	91	109	67	12	42	39	17	0	35	187	127	85
0.7 E_W	38	38	64	116	130	147	162	148	134	89	41	28
Excess	93	109	41	-	-	-	-	-	-	97	59	73
Irrigation Requirement	-	-	-	41	88	108	148	148	99	-	-	-
BAILEY and GLANDORE												
Precipitation	56	73	55	10	38	27	7	1	37	152	93	62
0.7 E_W	43	48	76	132	156	176	184	156	136	110	51	33
Excess	13	37	27	-	-	-	-	-	-	42	83	80
Irrigation Requirement	-	-	-	47	119	149	177	155	100	-	-	-

sites were under water stress during the hot season and consequently nematode multiplication was probably inhibited.

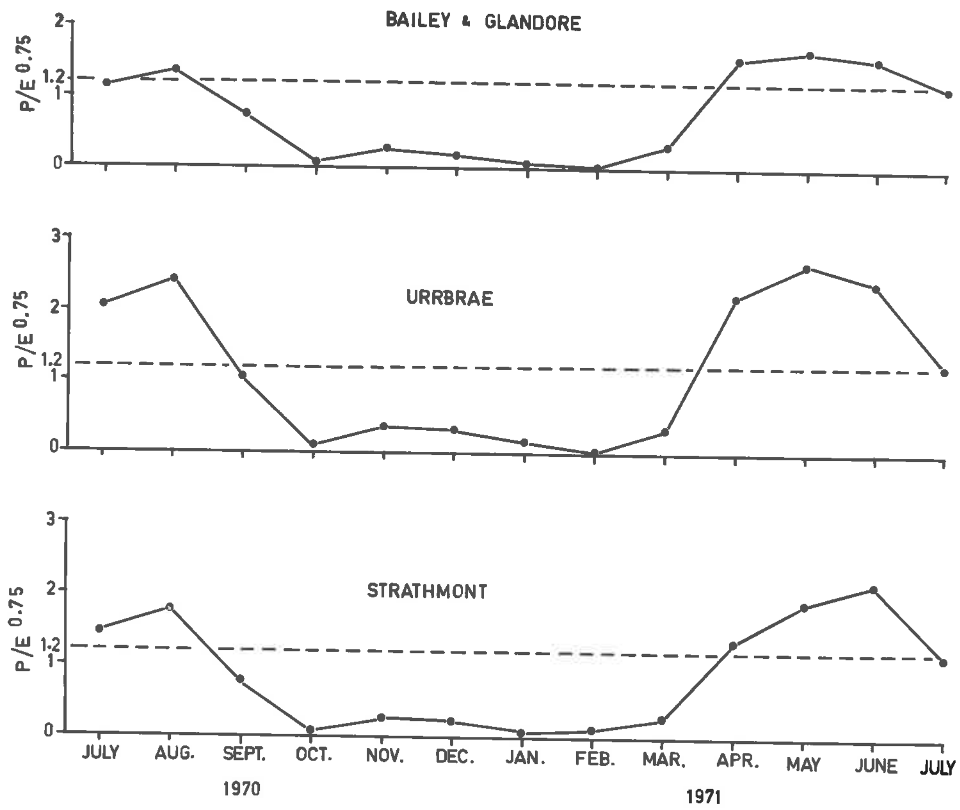
V. Precipitation and Evaporation

An important feature of turf management is to estimate the efficiency of any given rainfall, having in mind the water requirements of the plants as affected by the evaporation rate of the air. Strawberry clover needs a long growing season and this is satisfied by the plants' adaptability to soils such as clays which can retain moisture in the period of drought in Australia. Prescott (1956) was able to suggest a modification of the Transeau ratio of precipitation to evaporation (P/E) after studying the evapotranspiration from vegetation with the evaporation from a free water surface. This climatic index was $P/E^{0.75}$, and he gave values of this index which must be maintained or exceeded over most of the growing period for particular types of crop or vegetation cover. For average crops and pastures a value of 1.2 was considered necessary. Evaporation figures from an 'A' type Pan Evaporimeter were available from only four sites.

The main characteristics of the soil water regime at four of the sites (Fig. 4) indicate that the only period when soil water was continuously available for plant growth was from April to July, during the period of the observations (Nov. 1970 to July 1971).

Figure 4. The precipitation to evaporation ratio at three of the sampling sites over 13 months. The dotted line indicates the ratio above which water is continuously available for plant growth.

FIG. 4


BAILEY & GLANDORE
URRBRAE
STRATHMONT
P/E 0.75
2
1.2
1
0
3
JULY
AUG.
SEPT.
OCT.
NOV.
DEC.
JAN.
FEB.
MAR.
APR.
MAY
JUNE
JULY
1970
1971

Thus the important phases of the year in which plant growth can be expected are in the spring, utilizing stored soil moisture, and in the autumn. It is probable that the earlier the autumn rains occur the greater the pre-winter growth, and hence the greater the output of the turf for the year. Nematode numbers are probably influenced by this period of autumn growth.

VI. Rate of Drying of Soils at Three Sites

The importance and necessity of soil moisture to nematode survival has been discussed in the Introduction. Similarly soil water is necessary for survival and growth of the plant which is also the food resource of *Pratylenchus* and *Meloidogyne*.

Thus it is necessary to know if moisture is likely to be a limiting factor either for the movement of the nematode or the survival of the plant host, and if the moisture retention capacity varies with the different soil types. The most likely period of the year when water may be limiting is during February to March when rainfall is nil or only a few mm.

Thus soil moisture regime experiments were carried out on three sites, sand (North Glenelg), loam (Urrbrae) and clay (Strathmont) during February. The first 14 cm of the

North Glenelg profile (Fig. 5-1) corresponded roughly with the darker, high humus content region of this estuarine alluvial soil. The remaining 76 cm being sand showed no particular strata. This type of soil, because it is so juvenile, shows no true pedologic horizons. The levels that do show up are due to texture, colour and organic matter content.

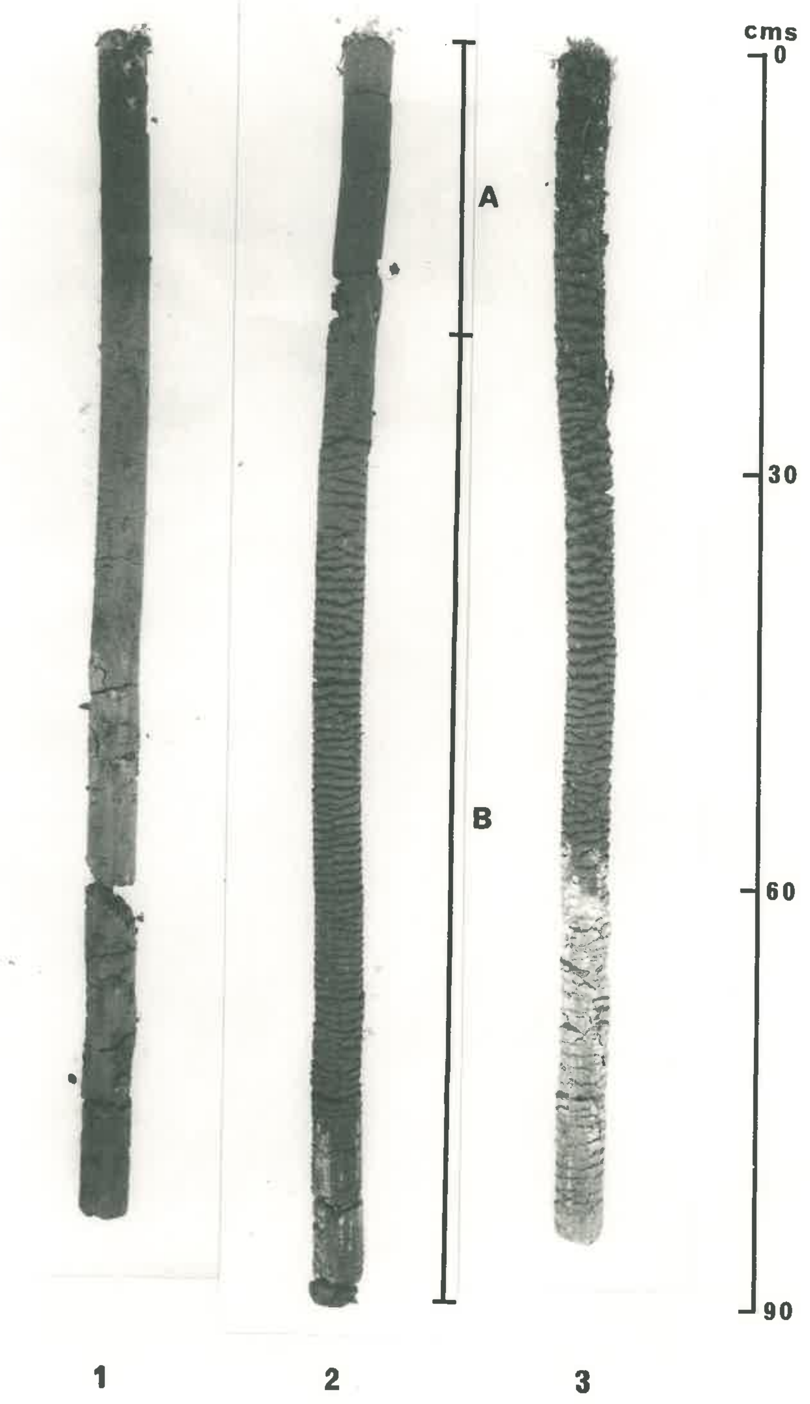
The Urrbrae site (Fig. 5-2) shows an A horizon of 20 cm of dark red-brown colour of fine silty texture. The B horizon is brighter and redder than the A horizon and becoming dark red-brown lower down, of finer clay texture showing marked transverse cracking and, at about 80 cm, containing slight amounts of lime in soft form. The C horizon was not reached at 90 cm.

The A horizon of Strathmont site is a black clay of granular structure down to 20 cm (Fig. 5-3). The B horizon is granular to coarsely structured clay changing from grey-brown to yellow with depth, and containing lime in the form of concretions. The C horizon was not reached at 90 cm.

The three sites were saturated with water for 24 hr, then sampling soil cores of 5 cm diameter were taken down to 90 cm every other day for ten days. The sites were protected from supplementary irrigation, though this was not avoided at Glenelg on the ninth day. Triplicate cores were cut into sections of 0-6, 6-14, 14-22, 22-30, 30-60,

Figure 5. Soil profiles from (1) North Glenelg, (2) Urrbrae and (3) Strathmont.

FIG. 5



60-90 cm, placed into plastic bags and tied in the field.

The V_w (volume of water/cc of soil) was determined by drying a known volume of soil corrected for its density.

The percentage loss of available water is highest in the 0-6 cm section for the three sites for the first two days, being 26%, 32% and 18% for the sand, loam and clay sites respectively (Fig. 6). The deeper sections showed less loss of water, being virtually unaffected over the period of the experiment. The high water values for the 30-60 cm section at Urrbrae can be explained by the fact that this section was a heavy clay and corresponds to the Strathmont clay site at this same depth. Water was more easily lost from the sandy site, than the clay site where virtually no water was lost at all. This would be expected from the mechanical analyses (Table 1).

Different crops use moisture from different depths. Thus by comparing actual root distribution with depth, it was shown that soil moisture extraction was a good indication of the effective root depth (Nakayama and Van Bavel, 1963). As this correlation has not been made before with strawberry clover, root distribution was determined by a modification of Newman's (1966) method on five replicates.

From Fig. 7 we see that the largest root concentration (cm length of root/cc soil) is in the 0-5 cm zone.

Comparing the soil evapotranspiration graphs with the

Figure 6. The loss of available water from soil at six depths over 10 days. Graphs of water loss are shown for three sampling sites.

FIG. 6

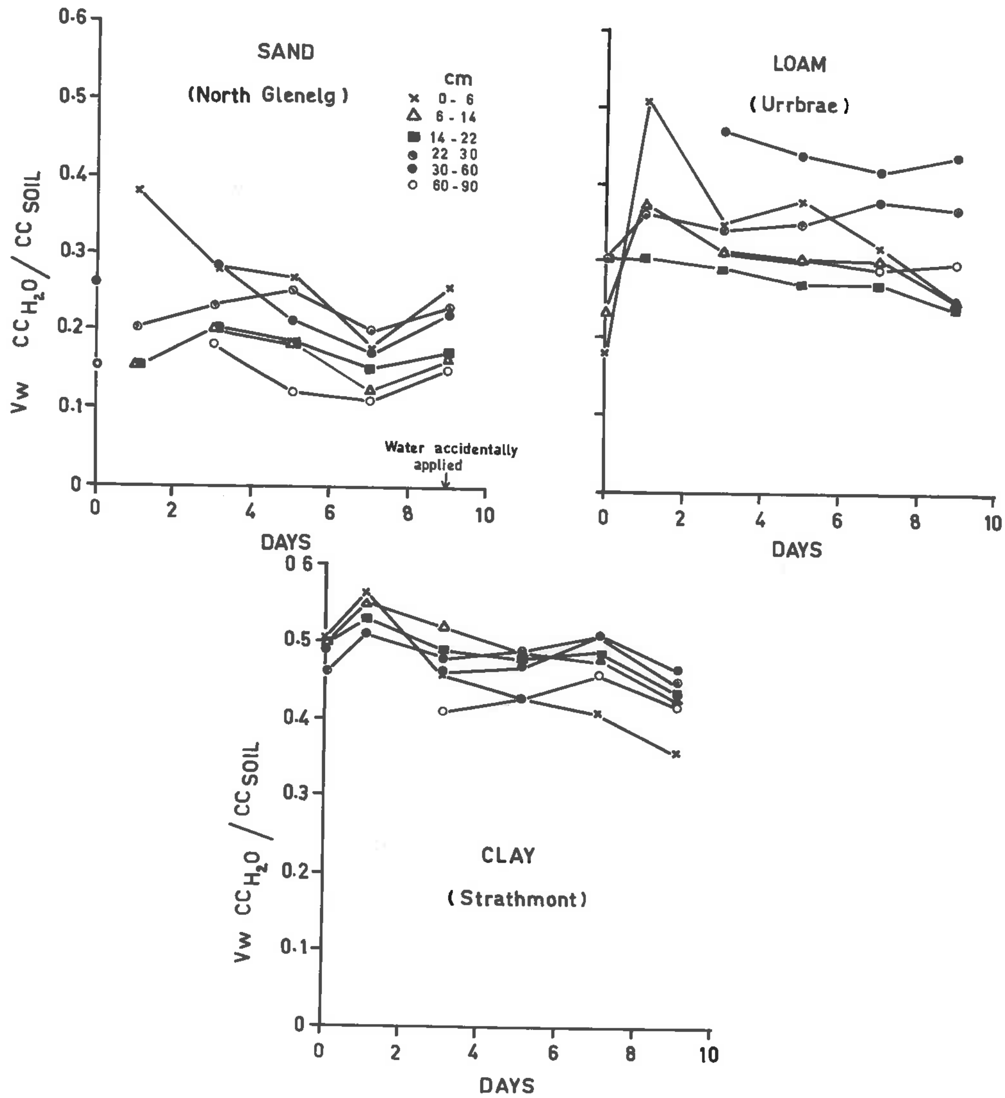
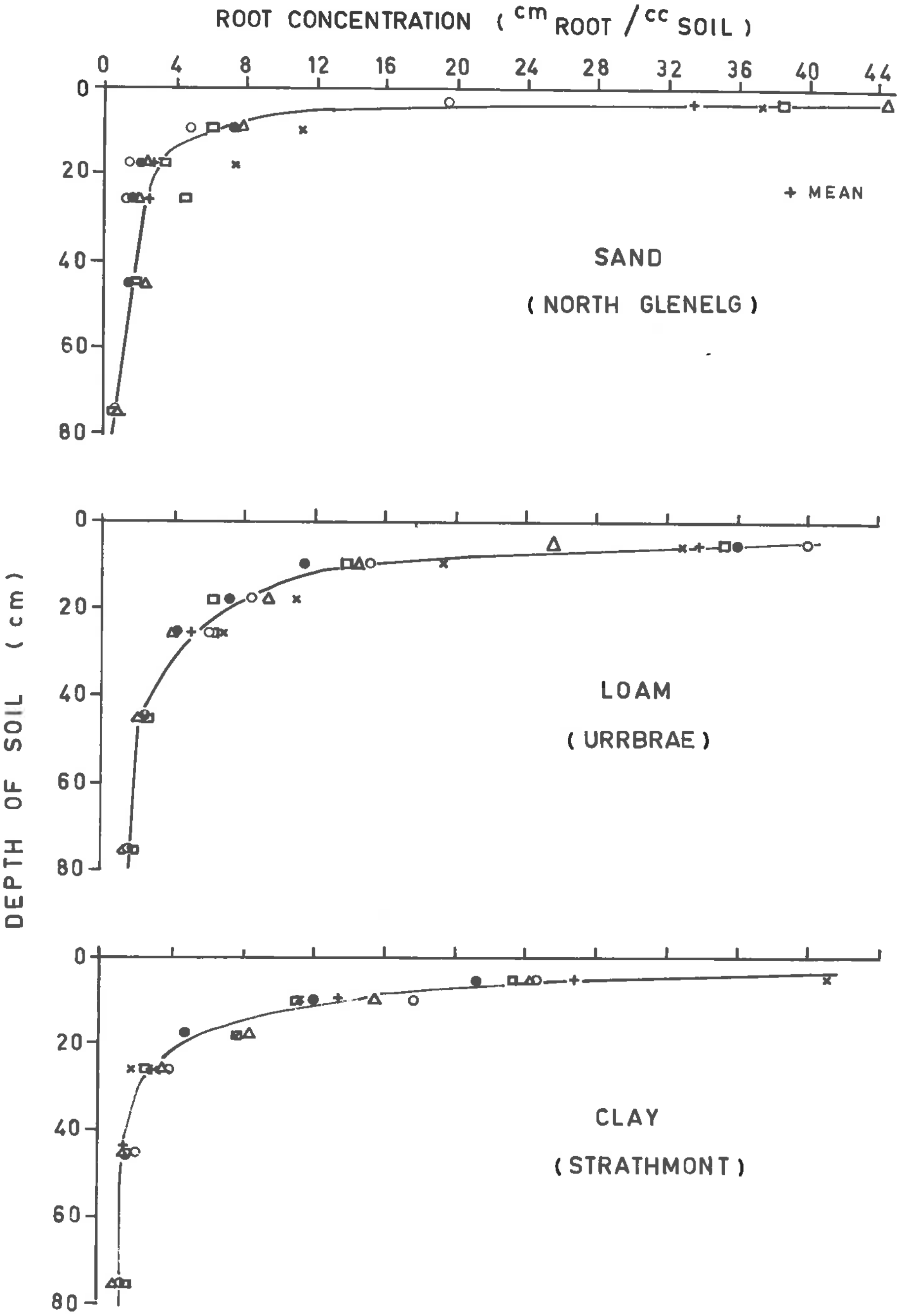

SAND
(North Glenelg)
cm
0 - 6
6 - 14
14 - 22
22 30
30 - 60
60 - 90
Water accidentally applied
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
10
DAYS
Vw CC H2O / CC SOIL
LOAM
(Urrbrae)
0
2
4
6
8
10
DAYS
CLAY
(Strathmont)
0 6
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
10
DAYS
Vw CC H2O / CC SOIL

Figure 7. The concentration of roots of strawberry clover in soil to a depth of 80 cm at three of the sampling sites.

FIG. 7



ROOT CONCENTRATION (cm ROOT / cc SOIL)
0
4
8
12
16
20
24
28
32
36
40
44
DEPTH OF SOIL (cm)
0
20
40
60
80
+ MEAN
SAND
(NORTH GLENELG)
LOAM
(URRBRAE)
CLAY
(STRATHMONT)

root distribution graphs, it seems likely that if the host plant is under any water stress that could influence the number of nematodes, it would be more likely to occur on the sandy sites than the clay.

VII. Nutrient Status of Sites

As no one figure representing fertility of the sites can be obtained, a sub-sample of six months' sample was submitted for nitrogen, phosphorus and potassium analysis (Table 3).

All of the determined elements were within the range of acceptable agricultural standards for plant growth.

VIII. pH and Percentage Soluble Salts (Table 4)

The pH range for all sites for all monthly samples was within the range which is acceptable for normal plant growth. Similarly the percentage soluble salts was within the tolerance range, except in the sandy Flagstaff Hill Golf Club site in February when 0.18% salts were recorded. This high value might be deleterious to plant growth; it occurred at a time when no rainfall was recorded, the rate of evaporation of the little irrigation applied was at its highest and there was little likelihood of salts leaching away from the root zone. From these observations it seems

TABLE 3

Nutrient status at the eight sites where samples of nematodes were taken

TABLE 3

Site	N ppm	P (total) ppm	K ppm
1. North Glenelg	500	59	550
2. Bailey Grounds	310	38	970
3. Henley-Grange Oval	300	41	690
4. Urrbrae College	260	42	1160
5. Flagstaff Hill Golf Club	145	22	200
6. Reynella Oval	185	28	460
7. Strathmont Oval	490	46	990
8. Glandore Oval	220	42	510

TABLE 4

pH and per cent soluble salts at the eight sites where samples of nematodes were taken

TABLE 4

Sites		pH	Percentage Soluble Salts
1. North Glenelg	Range	7.8-8.7	.02-.09
	Mean	8.2	.05
2. Bailey Grounds	Range	7.0-7.9	.01-.06
	Mean	7.5	.03
3. Henley-Grange Oval	Range	8.0-8.8	.02-.07
	Mean	8.5	.05
4. Urrbrae College	Range	6.7-7.7	.01-.03
	Mean	7.1	.02
5. Flagstaff Hill Golf Club	Range	7.1-8.3	.01-.18
	Mean	7.6	.07
6. Reynella Oval	Range	7.5-8.5	.01-.05
	Mean	8.2	.03
7. Strathmont College	Range	7.8-8.8	.04-.06
	Mean	8.5	.05
8. Glandore Oval	Range	7.3-8.5	.02-.09
	Mean	7.8	.05

likely that the levels of nutrient, pH and percentage soluble salts at the sites would only influence the population dynamics of nematodes indirectly via their influence on the growth of the host plant.

IX. Other Nematodes

A nematode's chance to survive and reproduce may be influenced by the presence and numbers of other nematodes, either of the same species or of other species. Fifty gm samples from 5 cm diameter soil core sections down to 30 cm were taken in triplicate from North Glenelg (sand), Urrbrae (loam), Strathmont (clay). The nematodes were extracted by the centrifugal flotation method. All roots were first removed (Table 5).

From similar cores, all strawberry clover roots were washed out weighed, stained in lacto-phenol cotton blue and the nematodes counted after squashing the roots between glass slides (see Table 6).

The results show that other nematodes as well as P. thornei occur in the soil and they show a gradual decrease in numbers with depth except at the clay site. In the sandy site M. javanica numbers are highest in the 0-6 cm zone. Saprophytes were more numerous in upper levels, presumably because of the higher humus content of the soil and the higher numbers of bacteria upon which they feed.

TABLE 5

The mean number of nematodes (3 replicate samples) per 50 g soil from three sites at 4 depths

TABLE 5

Depth (cm)	*P. thornei*	*M. javanica*	Other Tylenchids	Saprophytes
North Glenelg				
0-6	12	540	16	320
6-14	-	20	16	52
14-22	4	168	132	48
22-30	-	32	96	32
Urrbrae				
0-6	40	-	160	56
6-14	4	-	80	12
14-22	28	-	44	8
22-30	4	-	8	8
Strathmont				
0-6	220	-	184	48
6-14	400	-	-	24
14-22	385	-	20	48
22-30	224	-	12	12

TABLE 6

The numbers of nematodes in roots of strawberry clover at four depths in the soil

TABLE 6

Depth (cm)	Weight of Roots	*P. thornei*	*M. javanica*
North Glenelg			
0-6	0.055	1	408
6-14	0.151	-	885
14-22	0.160	-	12
22-30	0.042	-	16
Urrbrae			
0-6	0.285	24	-
6-14	0.280	12	-
14-22	0.085	12	-
22-30	0.079	-	-
Strathmont			
0-6	0.721	20	-
6-14	0.017	4	-
14-22	0.007	-	-
22-30	0.001	-	-

Only *P. thornei* and *M. javanica* were found within the roots, *M. javanica* being present only at North Glenelg. Numbers for both species decreased with depth of root material.

Despite the differences in soil type, the numbers of nematodes in the roots indicate that it is unlikely that other species are competing with *Pratylenchus*.

X. A Preliminary Appraisal of how the Environment might influence P. thornei

From the data on the abiotic features of the environment, a comparison of the main differences between sites shows that soil structure and texture, which determine soil pore size, might well be expected to directly or indirectly influence a number of other related factors, such as aeration and moisture, and thus influence nematode numbers. Soil texture in the sites ranges from sandy sites with 2.5% clay to clay sites with 51.7% clay. Since soil texture does have a marked influence on the general soil environment, it probably influences the upper limits of population numbers as well as particular phases of nematode activity (e.g. hatching of eggs, movement, penetration, etc.). Soil moisture, as well as being a necessary requirement for nematode movement, also influences the aeration of the soil and is determined by the size of soil pores. The soil moisture regime is indicated by the determinations of WHC and APS made from the moisture characteristic curves of the sites. There are two groups; those of low APS at the clay and silty sites of Strathmont and Urrbrae (12.3% and 9.8%) and the high APS of the rest of the sites (about 30%).

There are differences in the nutrient status of the sites that might possibly determine differences in nematode numbers between sites.

The other main group of features of the nematodes' environment are the factors that change with the season and these could greatly influence the monthly variation in nematode numbers. Temperature shows a seasonal fluctuation from a low of about 4°C in July to a maximum in February of 30°C. Similarly rainfall ranges from nil in February to a maximum in April of up to 190 mm.

Supplementary irrigation is applied at all sites in sufficient quantity to maintain plant growth throughout the period October to March. Thus, although the P/E ratio does not correspond exactly to so-called natural conditions without irrigation, it does give an idea of the water stress to which the plant and the nematode may be subjected during the hot drought season.

Irrigation may not cause an increase in nematode numbers if there is insufficient time between applications to allow the soil pores to drain and allow adequate aeration

(Van Gundy *et al.*, 1968), and irrigation may influence increases or decreases in soil micro-organisms which might be antagonistic to nematodes, as has been observed with fungi (Curl, 1961). pH and soluble salts could influence seasonal numbers also, but the range observed indicates that this is unlikely.

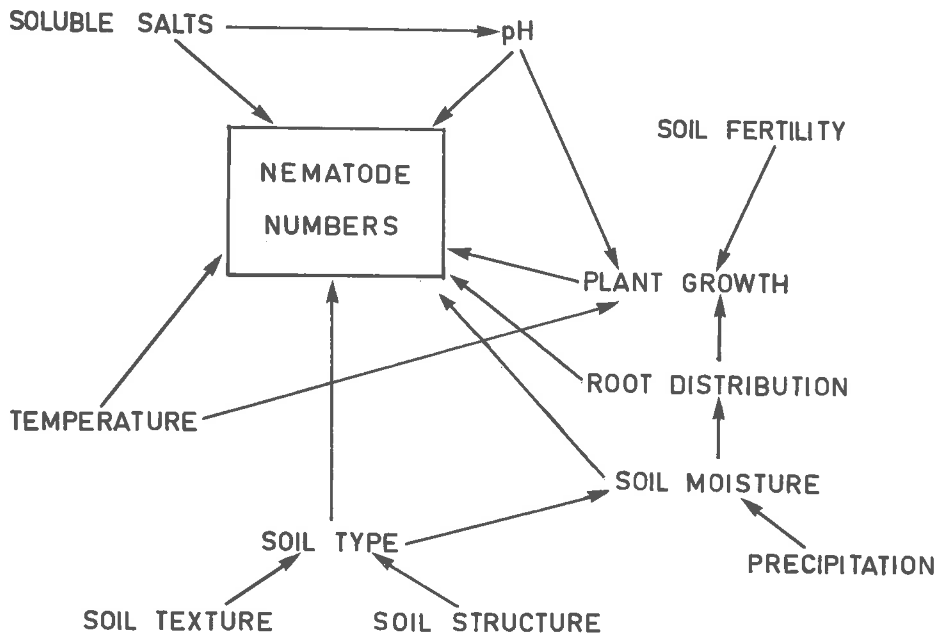
Variation in the density of the host plant (strawberry clover) could have an influence on nematode numbers, but from monthly observations, the percentage of overlapping cover provided by the host plant was approximately the same, the only variation being in the grasses which were not hosts to *P. thornei* nor *M. javanica*.

The study of the environment thus suggests that to explain the numbers of *P. thornei* in the roots of strawberry clover, correlations between nematode numbers and soil type, moisture, temperature, etc., should be obtained to ascertain which of these factors play an important part. Experiments should also be done to ascertain how these factors might influence numbers, e.g., by their effect on nematode mobility and infection.

The ecological situation can be expressed in the form of a flow diagram of immediate effects (Fig. 8). The system is obviously complex because of the number of interacting environmental factors that influence nematode numbers. The objective of this research project is to establish which

Figure 8. A flow diagram of immediate effects showing how environmental components might influence numbers of *P. thornei* in roots and soil.

FIG. 8


SOLUBLE SALTS
pH
SOIL FERTILITY
NEMATODE NUMBERS
PLANT GROWTH
ROOT DISTRIBUTION
TEMPERATURE
SOIL MOISTURE
SOIL TYPE
PRECIPITATION
SOIL TEXTURE
SOIL STRUCTURE

factors are most important so that a more simplified flow diagram can be proposed as a hypothesis to explain the differences in nematode numbers with time and between sites. In this chapter the chief environmental characteristics of the sites have been measured and their possible importance assessed; they form the basis for the next phase of the research - sampling of the various sites to determine nematode numbers.

CHAPTER III

NUMBERS OF PRATYLENCHUS THORNEI IN THE FIELD

I. Methods of Sampling and Extraction

From a pilot survey, a site 12 metre square was subdivided into a 4 x 4 grid; one sample from each of the twenty sectors was collected by using a 2.6 cm soil corer to a depth of 6 cm. Each sample included a strawberry clover plant from which nematodes were extracted. The variance between the numbers of nematodes per gm of root from each sample indicated that to obtain an acceptable standard error of the mean of 10%, twenty duplicate samples per site were required, giving a total bulked sample of not less than 1 Kg. This is considered to be a simple, but important and necessary step, in the initial stages of the investigation as the intensity and frequency of sampling adopted in the subsequent work should be sufficient to detect differences at a reasonable level of significance.

(a) Soil

From these duplicated bulked samples, two sub-samples of 200 gm of soil were treated to extract the nematodes. This was later modified to two, 50 gm soil samples after a comparison of results from 200 gm and 50 gm indicated that the accuracy was higher with 50 gm; it is likely that the accuracy increased because the resulting sample was clearer and easier to count. The nematodes were extracted from the soil by the centrifugal flotation technique (Jenkins, 1964) using a sucrose solution of specific gravity 1.18 (484.5 gm/litre water) (Goodey, 1963).

(b) Plant Material

The soil cores were carefully broken up and the strawberry clover plants and roots separated from soil and other plant roots. The roots were washed, blotted dry, carefully cut off from the stolons, weighed, finely cut up and placed over a funnel collector in a misting chamber, where the temperature of the water was controlled at 27°C. The nematodes that emerged from the roots were collected in a tube beneath the funnel and were counted in a Doncaster Counting Dish. From a preliminary experiment of 6 replicates, it was found that most of the nematodes emerged in 5 days, and all were out in 9 days; thus a standard time of 10 days for root extraction was adopted. Each month after the period of time in the mister, 0.5 gm of roots was taken and stained in 0.15% Lactophenol cotton blue at 55°C for 24 hrs. Samples were then washed, homogenized in a M.S.E. Homogenizer for 2 mins, sieved to remove coarse plant tissue, and the numbers of nematodes in an aliquot counted. Very few *Pratylenchus* were retained within the plant tissue.

II. Frequency of Sampling

The pattern of changes in nematode numbers becomes clearer as the sampling frequency increases; in this project the maximum frequency that could be usefully handled was monthly for all eight sites. Sampling was carried out from November 1970 to July 1971.

III. Factors Measured

The factors were broadly grouped into two categories - the site characteristics which would not be expected to change over the period of sampling, and those that were likely to show seasonal variation. Thus the nutrient status and the soil physical characters of structure and texture, which determine the water holding capacity and available pore space, were determined to see if there was a correlation between them and the numbers of *P. thornei* in the soil and in the roots and, more specifically, to determine whether the distribution of *P. thornei* in South Australia was similar to that in Europe where numbers tend to be higher in clay than in sandy soils (Oostenbrink, 1954, Corbett, 1970).

(a) Seasonal Characteristics

Those environmental features such as rainfall, evaporation, temperature, pH, and % soluble salts which show seasonal fluctuations were measured at sampling time so that correlations could be made which might explain the seasonal variation in the numbers of *Pratylenchus* in the soil and in clover roots.

The means of numbers of *P. thornei* recovered from *T. fragiferum* roots and from the soil for the eight sites over the nine month period from November 1970 to July 1971 are given in Tables 7 and 8.

As the variance of the nematode counts appeared to increase as the size of the count increased, the transformation $\log_{10}$ (1 + count) was applied.

The means of the transformed plant and soil counts are plotted in Figs. 9 to 12, and presented in Tables 9 and 10.

An analysis of variance was done on the transformed counts of nematodes in plants and on the transformed counts of nematodes in soil to determine the significance of the differences in nematode numbers between the eight different sites with time. Least significant differences (L.S.D.) were calculated to enable the appropriate comparisons to be made.

The significance of effects were determined by the F values which for the Transformed Nematode Plant counts were:

	F	
Sites	93.961	significant at $p < .001$
Months	4.915	" " $p < .05$
Interaction Sites x Months	4.765	" " $p < .001$

TABLE 7

Means of numbers of *Pratylenchus thornei* recovered from roots of strawberry clover at eight sites in the Adelaide area over 9 months

TABLE 7

Sites	Nov.	Dec.	Jan.	Feb.	March	April	May	June	July	Mean
N. Glenelg	85.5	16.0	1.5	1.5	16.0	0.0	0.0	18.0	0.5	15.4
Bailey	102.5	73.0	177.5	48.5	177.0	168.5	85.0	20.0	81.0	103.7
Henley-Grange	37.5	77.5	179.0	178.0	150.5	346.5	63.0	45.5	110.5	132.0
Urrbrae	123.5	10.5	37.5	63.5	12.0	254.0	101.0	34.0	117.0	83.7
Flagstaff Hill	5.5	4.0	2.5	4.0	11.5	6.5	2.0	9.5	76.5	13.6
Reynella	50.5	33.5	147.0	79.0	178.5	414.5	115.0	48.5	84.0	127.8
Strathmont	82.5	116.0	184.0	224.0	1134.0	606.0	246.5	90.0	179.5	318.1
Glandore	117.5	40.0	15.5	73.5	27.5	71.5	26.0	39.0	33.0	49.3
MEAN	75.6	46.3	93.1	84.0	213.4	233.4	79.8	38.1	85.3	105.4

TABLE 8

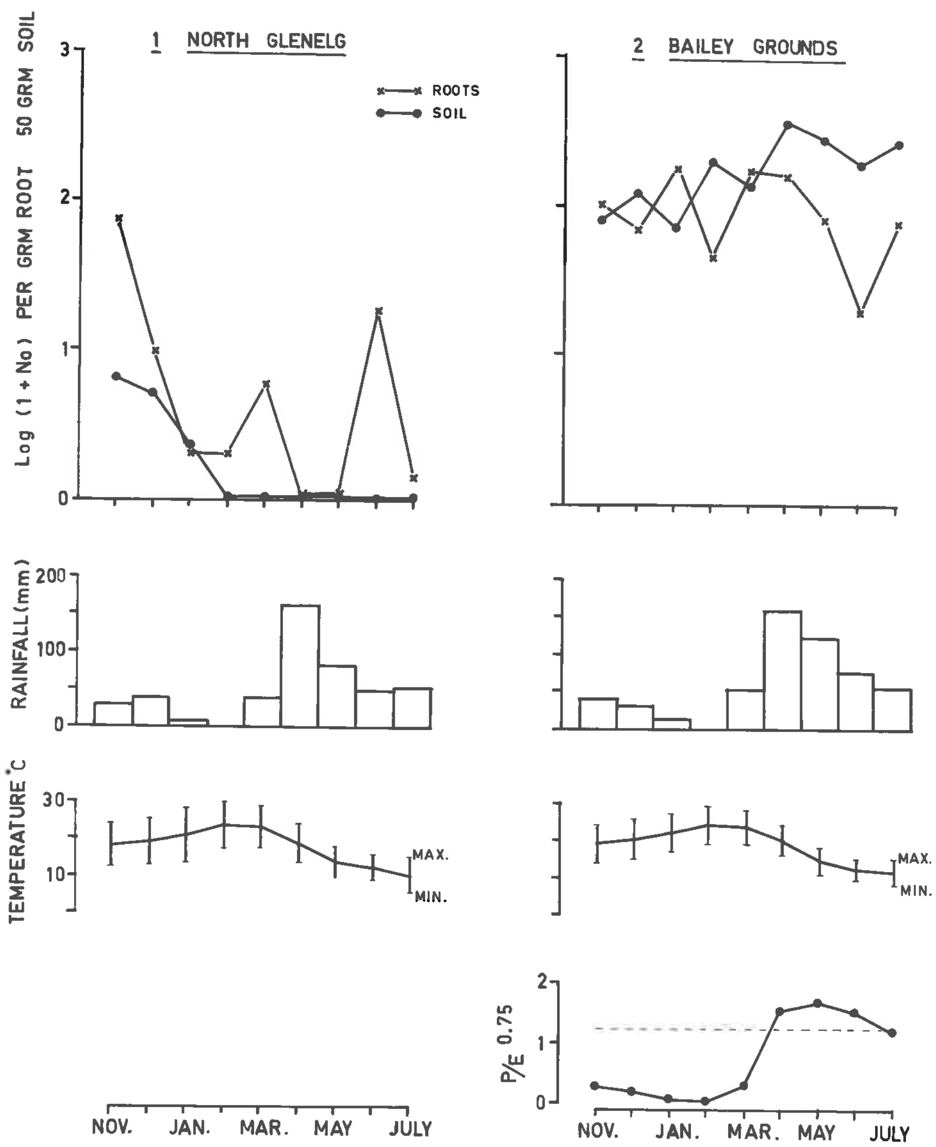
Mean numbers of *Pratylenchus thornei* recovered
from soil around roots of strawberry clover
at eight sites in the Adelaide area
over 9 months

TABLE 8

Sites	Nov.	Dec.	Jan.	Feb.	March	April	May	June	July	Mean
N. Glenelg	6.0	4.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3
Bailey	82.0	122.0	72.0	204.0	138.0	368.0	290.0	199.0	275.0	194.4
Henley-Grange	22.0	50.0	85.5	56.0	93.5	302.0	188.0	136.0	123.0	117.3
Urrbrae	28.0	48.0	18.0	74.0	42.0	104.0	140.0	70.0	164.0	76.4
Flagstaff Hill	12.0	14.0	2.0	4.0	6.0	8.0	2.0	2.0	7.0	6.3
Reynella	56.0	200.0	84.0	66.0	70.0	54.0	60.0	78.0	37.5	78.4
Strathmont	146.0	144.0	146.0	120.0	634.5	717.5	1162.0	717.5	934.0	524.6
Glandore	20.0	18.0	2.0	24.0	48.0	28.0	48.0	34.0	53.0	30.6
MEAN	46.5	75.0	51.4	68.5	129.0	197.7	236.3	154.6	199.2	128.7

Figure 9. The numbers of *Pratylenchus thornei* (transformed to logs) recovered from soil and roots of strawberry clover over 9 months at two sites. Rainfall and temperature data are shown and the precipitation - evaporation ratio for the Bailey Grounds.

FIG. 9



1 NORTH GLENELG
2 BAILEY GROUNDS
ROOTS
SOIL
Log (1 + No) PER GRM ROOT 50 GRM SOIL
3
2
1
0
RAINFALL (mm)
200
100
0
TEMPERATURE °C
30
10
MAX.
MIN.
P/E 0.75
2
1
0
NOV.
JAN.
MAR.
MAY
JULY

<u>Figure 10</u>. The numbers of *Pratylenchus thornei* (transformed to logs) recovered from soil and roots of strawberry clover over 9 months at two sites. Rainfall and temperature data are shown and the precipitation - evaporation ratio for Urrbrae College.

FIG. 10

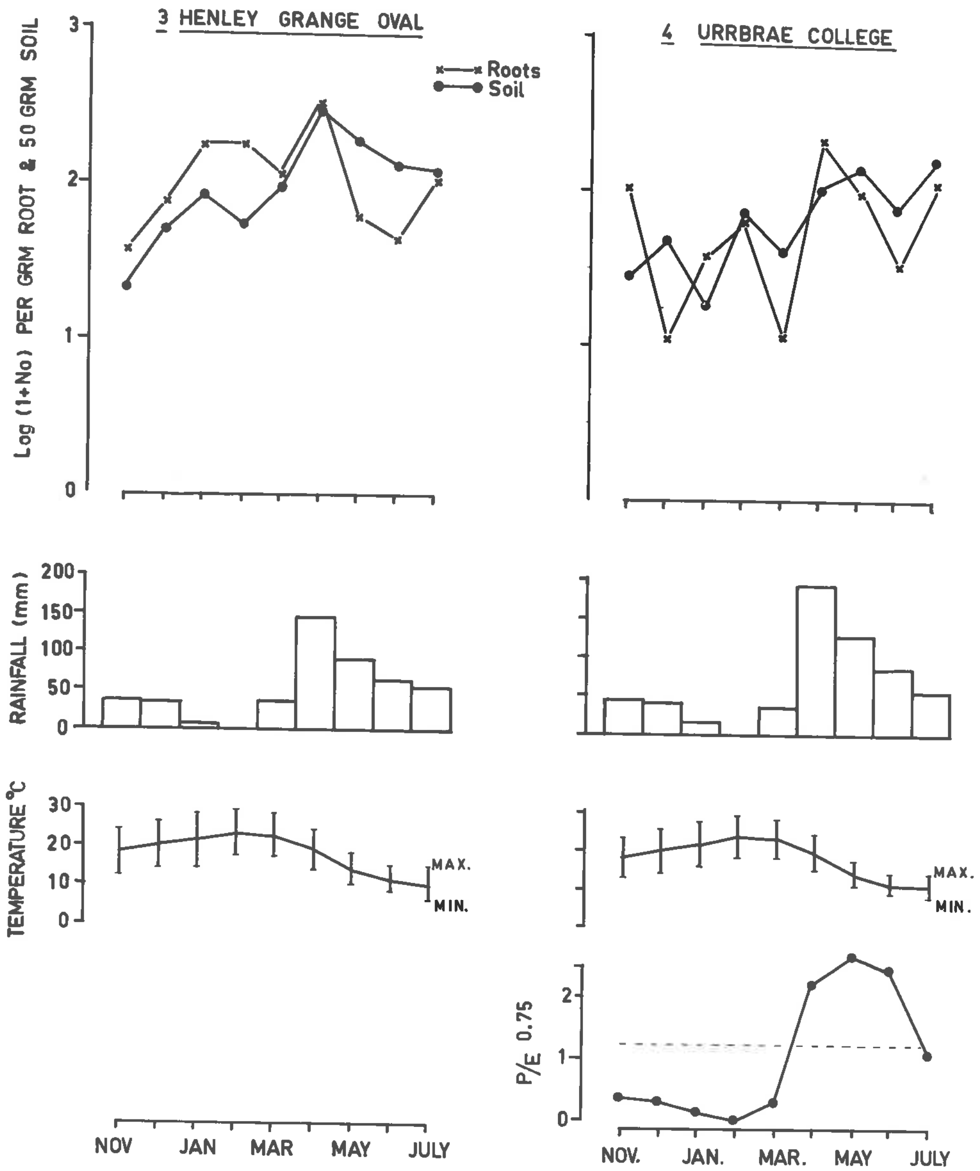

3 HENLEY GRANGE OVAL
4 URRBRAE COLLEGE
Roots
Soil
Log (1+No) PER GRM ROOT & 50 GRM SOIL
RAINFALL (mm)
TEMPERATURE °C
MAX.
MIN.
P/E 0.75
NOV
JAN
MAR
MAY
JULY
NOV.
JAN.
MAR.
MAY
JULY

Figure 11. The numbers of *Pratylenchus thornei* (transformed to logs) recovered from soil and roots of strawberry clover over 9 months at two sites.

Rainfall and temperature data are shown.

FIG. 11

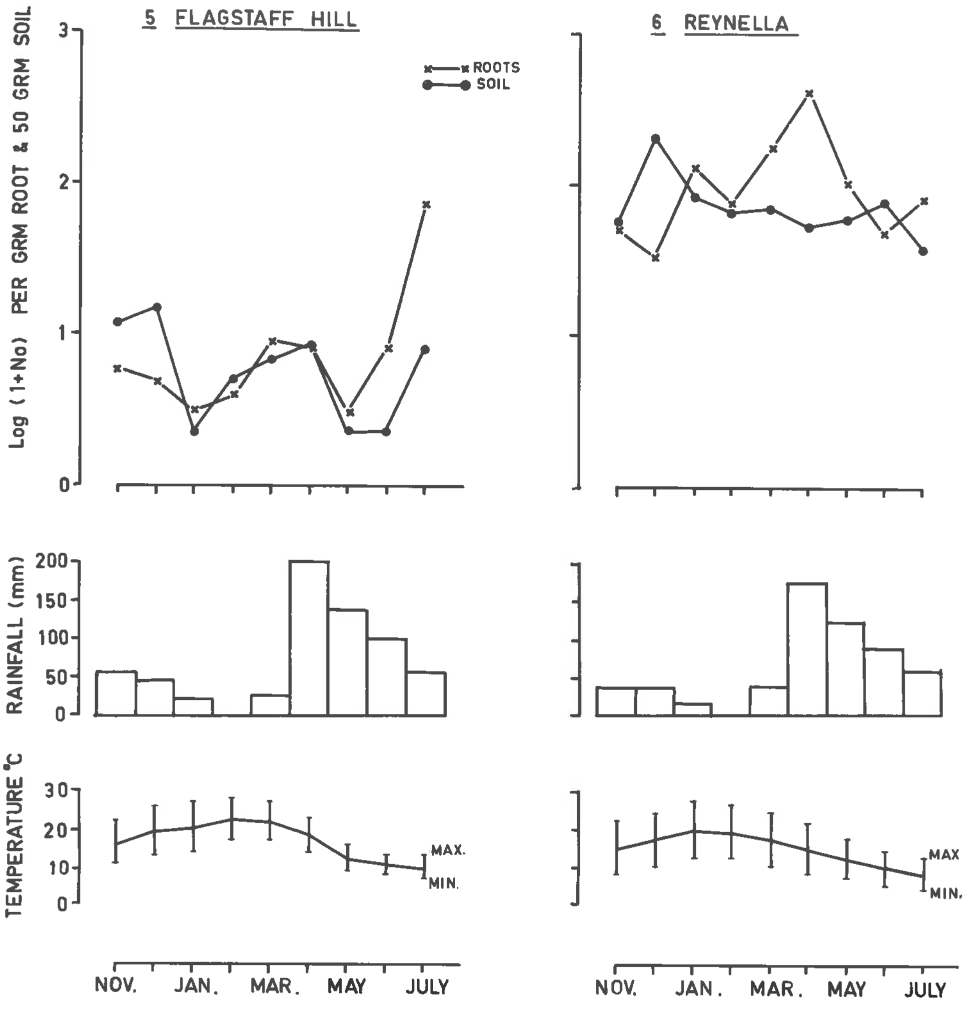
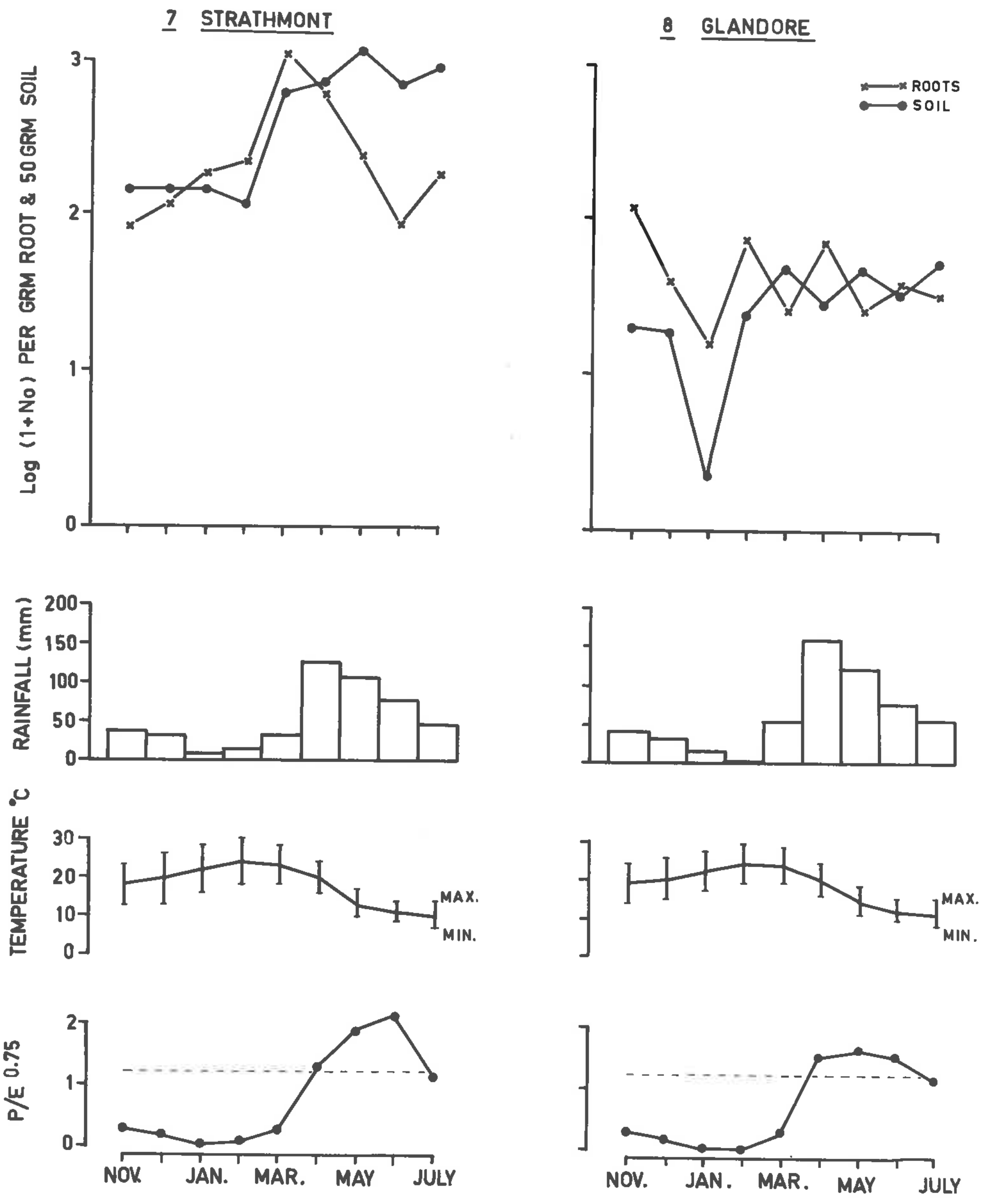

5 FLAGSTAFF HILL
6 REYNELLA
ROOTS
SOIL
Log (1+No) PER GRM ROOT & 50 GRM SOIL
0
1
2
3
RAINFALL (mm)
0
50
100
150
200
TEMPERATURE °C
0
10
20
30
MAX.
MIN.
MAX
MIN.
NOV.
JAN.
MAR.
MAY
JULY
NOV.
JAN.
MAR.
MAY
JULY

Figure 12. The numbers of *Pratylenchus thornei* (transformed to logs) recovered from soil and roots of strawberry clover over 9 months at two sites. Rainfall and temperature data are shown and the precipitation - evaporation ratio for Strathmont and Glandore.

FIG. 12



7 STRATHMONT
8 GLANDORE
ROOTS
SOIL
Log (1+No) PER GRM ROOT & 50 GRM SOIL
RAINFALL (mm)
TEMPERATURE °C
MAX.
MIN.
P/E 0.75
NOV.
JAN.
MAR.
MAY
JULY

TABLE 9

Means of transformed numbers of *Pratylenchus thornei* recovered from roots of strawberry clover at eight sites in the Adelaide area over 9 months

TABLE 9

Sites	Nov.	Dec.	Jan.	Feb.	March	April	May	June	July	Mean
N. Glenelg	1.8703	0.9842	0.3010	0.3010	0.7593	0.0000	0.0000	1.2782	0.1505	0.6272
Bailey	2.0071	1.8682	2.2516	1.6537	2.2442	2.2106	1.9287	1.3202	1.9064	1.9323
Henley-Grange	1.5570	1.8779	2.2546	2.2529	2.0674	2.5336	1.7997	1.6654	2.0346	2.0048
Urrbrae	2.0949	1.0246	1.5851	1.8085	1.1127	2.3412	2.0076	1.5324	2.0481	1.7283
Flagstaff Hill	0.7782	0.6990	0.5000	0.6021	0.9621	0.8495	0.4771	0.9163	1.8766	0.8512
Reynella	1.6702	1.5378	2.1686	1.8981	2.2297	2.6096	2.0290	1.6941	1.9283	1.9739
Strathmont	1.8906	2.0563	2.2638	2.3521	3.0445	2.7814	2.3925	1.9588	2.2332	2.3304
Glandore	2.0556	1.6116	1.2075	1.8514	1.3997	1.8573	1.4287	1.5880	1.5221	1.6135
MEAN	1.7405	1.4575	1.5665	1.5900	1.7275	1.8979	1.5079	1.4942	1.7125	1.6327

TABLE 10

Means of transformed numbers of *Pratylenchus thornei* recovered from soil around the roots of strawberry clover at eight sites in the Adelaide area over 9 months

TABLE 10

Sites	Nov.	Dec.	Jan.	Feb.	March	April	May	June	July	Mean
N. Glenelg	0.8266	0.6990	0.3495	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2083
Bailey	1;9086	2.0898	1.8526	2.3050	2.1426	2.5669	2.4524	2.2995	2.4391	2.2285
Henley-Grange	1.3162	1.7072	1.9082	1.7548	1.9669	2.4798	2.2716	2.1300	2.0871	1.9580
Urrbrae	1.4624	1.6843	1.2559	1.8555	1.6292	2.0161	2.1346	1.8368	2.2111	1.7873
Flagstaff Hill	1.0923	1.1722	0.3495	0.6990	0.8266	0.9065	0.3495	0.3495	0.8997	0.7383
Reynella	1.7546	2.3029	1.9114	1.8164	1.8469	1.7401	1.7606	1.8907	1.5845	1.8454
Strathmont	2.1670	2.1442	2.1488	2.0577	2.8012	2.8533	3.0629	2.8554	2.9688	2.5621
Glandore	1.3222	1.2763	0.3495	1.3979	1.6768	1.4452	1.6768	1.5256	1.7323	1.3781
MEAN	1.4813	1.6345	1.2657	1.4858	1.6113	1.7510	1.7136	1.6109	1.7403	1.5883

and for the Transformed Nematode Soil Counts:

	F		
Sites	358.688	significant at	$p < .001$
Months	12.703	" "	$p < .001$
Interaction Sites x Months	6.415	" "	$p < .001$

Thus there are highly significant differences in the numbers of nematodes between sites and at different times within sites.

<u>Least Significant Differences</u> (L.S.D.)

For the variates log (1 + plant count) and log (1 + soil count) L.S.D.'s at the 5% and 10% levels were calculated to enable appropriate comparisons to be made.

<u>Plant Nematode Count</u> (Table 9)

	5%	10%
Sites for a given month	0.510	0.426
Months for a given site	0.510	0.426

<u>Soil Nematode Count</u> (Table 10)

	5%	10%
Sites for a given month	0.349	0.292
Months for a given site	0.349	0.292

Since there is a highly significant ($p < .001$) interaction of sites with monthly readings, we cannot make any generalizations about the way numbers of nematodes change with time because variations of numbers with time differed considerably between sites or groups of sites. For example the numbers of nematodes at the sandy sites of North Glenelg and Flagstaff Hill Golf Club were lower in the summer period and tended to increase with the onset of moist cooler winter conditions. In the clay sites of Strathmont, numbers significantly increased throughout the summer until March, then afterwards there was a significant decrease as winter conditions prevailed. The silty clay sites of Bailey, Henley-Grange and Reynella also tended to follow this trend of the clay site though not so markedly. Similarly, the sandy silt site of Glandore had higher numbers overall than the sand sites, but numbers decreased throughout the summer followed by a slight increase during winter. The silty site of Urrbrae also showed a gradual decrease in numbers throughout the summer reaching its lowest value in March just before the rains started and, except for a significant increase in April, it continued at this low level until the winter. This might be explained by the fact that moisture was now available and the soil had not cooled down. After that, the numbers showed no clear trend.

Similar trends are seen in the soil counts with time and with the different groups of sites. However, the numbers of nematodes in the clay and silty clay sites increased during summer and they continued to do so throughout the winter. In the sandy sites, numbers remained low even in the winter; however it is possible that if measurements had continued, they would have increased at these sites. With silty and sandy silt sites, soil numbers tended to fluctuate considerably, although they follow the same trend as the numbers of nematodes in the plants.

This raises the question: what factors caused these different variations in numbers between sites with time? Once again the answer to this question will not come from a consideration of site characteristics alone, their variation with time must be considered too.

The reason for the highly significant site by time interaction on population change is the dominant feature of soil type influencing numbers of nematodes in plant roots and soil. It seems likely that in the hot dry weather, in spite of irrigation, nematodes in sandy sites survived less well than in clay soils. For example, in the sandy soil of North Glenelg, numbers gradually declined throughout summer whereas, in the clay soils of Strathmont, numbers gradually increased. This may be due to the fact that what little water the soils did receive via irrigation, drained off too quickly in the sandy soils to have much influence on the survival of nematodes, whereas by the colloidal nature of the clay site, water was retained and together with the favourable warmer conditions produced optimum conditions for nematode survival and even reproduction. It is also possible that nematode mortality was higher in the loose, sandy soils whereas ability to migrate from one plant to another was inhibited. In general, therefore, survival of nematodes is less in sites where the soil has a high percentage of sand or, conversely, a low percentage of clay. The different moisture relations of the sites caused by their different soil types and the influence of these characteristics on nematode survival is therefore probably the chief cause of the interactions in the field experiment. To examine these aspects, glasshouse and laboratory experiments were carried out and are described in the next chapter.

It was thought at the outset that the factors pH, % soluble salts, maximum and minimum temperature, rainfall and the precipitation-evaporation index which varied with time, might play a large part in determining population changes with time. It was also decided that the amount and quality of plant root material might influence nematode numbers but to a lesser degree; as the sites were regularly mown, no estimation of top growth could be obtained.

An analysis of covariance was done to see which, if any, of these factors influenced nematode numbers and to ascertain whether any of the covariates (factors) varied as the number of nematodes varied. Thus, if only an analysis of variance was done on the nematode populations, the value of the information so derived would be hard to assess because what may have been measured was the variation of the covariate. If any of the covariates varied significantly with the nematode counts, then the original data would have to be corrected for that covariate. The original analysis of variance was used to determine the L.S.D.'s.

Using the transformation $\log_{10}$ (1 + count), an analysis of covariance was done for each factor (pH, % soluble salts, maximum temperature, minimum temperature, precipitation/evaporation ratio) separately and for the three factors maximum temperature, minimum temperature, and rainfall combined. Thus, by this analysis, the effects due to sites and due to months were eliminated from both the soil and plant nematode counts and the factors being considered, leaving a residual set of readings which could be examined for structure.

A simple linear regression was done for each factor against the nematode counts, and the fitted linear regression lines were plotted for visual inspection. A graph could not be done for the three variables maximum and minimum temperature and rainfall as this is a multiple regression.

The regressions indicated that for each of the six factors and for combinations of factors, the regressions were not significant as tested by the F-ratio at a given level of significance.

It is worthwhile noting that the effects of irrigation were not considered, and this, with rainfall could have influenced the quantity of water available at the sites. Furthermore a temperature effect may have interacted with a rainfall effect. For example, if the nematodes preferred high temperatures and high moisture, the moisture requirements would be satisfied in winter and thier temperature requirements satisfied in summer; but neither would be satisfied at the same time except, perhaps, in March to April with the commencement of the wet season when temperatures were still relatively high. The data reveal a definite peak in March-April in the heavier soil sites of clay, clay silts and silt, but such a peak is less noticeable in the sandy silt and sandy sites. This could be related to the different thermal capacity properties of clay and sandy soils as the heavier soils might remain warmer for longer with the onset of the rainy season. These effects would not be revealed by statistical analysis of the field data; the only way of demonstrating them would be by laboratory controlled experiments.

The available field data failed to establish that any of the factors measured (pH, % soluble salts, maximum and minimum temperature, P/E) influenced the numbers of nematodes in either plant or soil. It now seems probable that in fact, the amount of plant material (roots, new root tips) or some chemical quality of the roots had a large influence on nematode numbers. These factors were not measured because it was thought at the outset that factors other than the plants would exert more influence on variation of nematode numbers with time; but this is not so and hence it is clear that the plant material factor should be included in future studies.

(b) Site Characteristics

Since none of the factors that fluctuated with time significantly affected nematode numbers, other factors must have an influence.

The site characteristics of nutrient status (N,P,K,), soil texture (% sand, silt and clay), water holding capacity, available pore space (for nematode movement) and mean annual rainfall were investigated to see which factor or combination of factors was responsible, and to what degree, for the differences in population numbers between the eight sites sampled.

To ascertain the influence of these factors, simple linear regression analyses were carried out in an attempt to explain the differences between sites. Linear regression for numbers of nematodes in the plant and in the soil against each of the nine untransformed and transformed measured site factors were determined for each month.

Tables 11 and 12 show the significance of the F-ratios of the regressions.

The results of both untransformed and transformed data indicate that the factors % clay and % sand have significant effects in many places and that, while there are few significant F-ratios for the others, the factors nitrogen and potassium, % silt and available pore space appear to have some influence. Phosphorus, water holding capacity and mean annual rainfall do not warrant further consideration. As both untransformed and transformed data were used, it must be decided which sets of regressions to use. A comparison of the F-ratios show that:

a. The majority of the F-ratios for the % sand, % clay, nitrogen and available pore space are higher in the untransformed data.

b. The majority of the F-ratios for the potassium and % silt are higher in the transformed data.

c. Phosphorus, water holding capacity and mean annual rainfall are not very significant anywhere.

As % sand and % clay contain most of the significant results and the untransformed data give regressions of a better fit, untransformed data are used.

From the linear regressions of untransformed data, the % sand and % clay regressions are significant for many of

TABLE 11

The influence of nine environmental factors on the F-ratios of the numbers of *Pratylenchus thornei* recovered from roots of strawberry clover and from soil:-

*	significant at 5%
**	significant at 1%
***	significant at 0.1%

In the left hand column P = plant
S = soil

<u>TABLE 11</u>

Variates	N	P	K	Sand	Silt	Clay	Water Holding Capacity	Available Pore Space	Mean Annual Rainfall
P Nov.	0.62	3.07	5.07	1.28	3.88	0.23	10.98*	2.90	0.81
S Nov.	1.17	0.01	2.27	13.17*	0.20	41.63***	0.10	2.70	0.11
P Dec.	1.64	0.28	1.59	4.11	0.06	8.46	0.03	0.94	0.44
S Dec.	0.00	0.55	0.44	2.38	0.01	3.34	0.13	0.41	0.26
P Jan.	0.22	0.04	1.36	2.82	0.19	3.53	0.20	0.47	0.19
S Jan.	0.79	0.00	1.29	5.99*	0.03	13.55*	0.17	1.26	0.24
P Feb.	0.67	0.12	1.34	5.05	0.12	7.97*	0.01	2.09	0.52
S Feb.	0.16	0.01	5.44	4.43	2.57	2.31	0.72	1.42	0.58
P Mar.	2.47	0.18	1.10	8.84*	0.00	84.32***	0.00	2.53	0.09
S Mar.	2.42	0.22	1.64	11.37*	0.04	93.42***	0.05	3.24	0.03
P Apr.	0.36	0.00	1.83	10.08*	0.25	14.59**	0.00	3.39	0.03
S Apr.	2.18	0.22	3.42	11.33*	0.45	22.14**	0.09	2.99	0.12
P May	0.85	0.02	3.69	37.12***	0.64	76.31***	0.33	7.36*	0.08
S May	2.71	0.31	2.41	14.79**	0.18	95.09***	0.17	4.29	0.02
P June	0.97	0.20	1.15	7.55*	0.02	18.98**	0.01	3.18	0.09
S June	2.50	0.23	2.17	13.66*	0.12	101.18***	0.08	3.64	0.03
P July	0.05	0.27	2.64	14.62**	1.20	10.34*	0.30	5.49	0.11
S July	2.59	0.31	3.08	18.72**	0.35	91.94***	0.36	5.29	0.00
P TOTAL	1.30	0.07	2:26	15.52**	0.17	63.40***	0.02	3.85	0.05
S TOTAL	2.22	0.18	2.78	16.56**	0.25	91.90***	0.13	3.92	0.01

TABLE 12

The influence of nine environmental factors on the F-ratios of the transformed numbers of *Pratylenchus thornei* recovered from roots of strawberry clover and from soil:-

*	significant at 5%
**	significant at 1%
***	significant at 0.1%

In the left hand column P = plant
S = soil

TABLE 12

Variates	N	P	K	Sand	Silt	Clay	Water Holding Capacity	Available Pore Space	Mean Annual Rainfall
P Nov.	2.80	8.51*	12.61*	1.26	6.53*	3.05	3.81	1.80	0.03
S Nov.	0.15	0.03	2.52	9.62*	1.96	14.26**	0.24	1.77	0.87
P Dec.	2.06	1.25	3.27	1.88	2.12	6.12*	0.01	0.14	0.43
S Dec.	0.02	0.38	1.54	3.66	1.42	7.83*	0.02	0.92	0.75
P Jan.	0.10	0.00	3.59	3.22	3.60	9.34*	0.02	0.84	0.04
S Jan.	0.40	0.00	3.05	4.33	1.98	13.79**	0.01	1.04	0.03
P Feb.	0.02	0.03	3.05	3.75	4.32	5.83	0.22	1.60	0.12
S Feb.	0.01	0.06	3.75	4.13	6.17*	5.97	0.71	1.73	1.23
P Mar.	1.07	0.07	1.33	4.04	0.54	10.48*	0.25	0.29	0.16
S Mar.	0.02	0.02	2.46	6.67*	3.29	7.60*	0.24	1.65	0.52
P Apr.	0.04	0.07	2.57	4.07	3.76	6.18*	0.26	1.88	0.58
S Apr.	0.05	0.01	3.62	6.09*	5.63	6.68*	0.41	1.99	0.28
P May	0.00	0.00	4.25	6.52*	5.23	10.50*	0.62	2.88	0.77
S May	0.21	0.07	6.15*	9.12*	8.12*	11.47*	0.84	3.15	0.35
P June	0.87	0.85	3.05	5.17	1.75	10.67*	0.09	2.23	0.06
S June	0.14	0.02	4.75	7.72*	5.68	11.90*	0.44	2.30	0.31
P July	1.00	1.93	0.29	2.42	1.08	1.29	0.13	1.18	1.65
S July	0.06	0.00	4.54	9.70*	7.39*	7.48*	1.20	3.59	0.78
P TOTAL	0.47	0.16	5.48	8.69*	4.25	24.39**	0.20	2.52	0.03
S TOTAL	0.06	0.00	3.92	7.77*	5.01	10.22*	0.43	2.31	0.46

the months for numbers of nematodes in both plant and soil. The graphs show that in all cases the slope of the graph for sand is negative and for % clay it is positive. As this happens for so many months, it seems that the nematode counts in plant and soil are related to soil composition (i.e. as measured by % sand and % clay). In particular, the nematode count increases, as the clay content increases; as the sand content increases, the nematode count decreases. Furthermore, the F-ratios for % sand and % clay indicate that these effects are less marked in the summer than in winter months.

The results of the linear regressions indicated that it might be useful to do Selective Regressions of monthly nematode counts against the most likely site factors of nitrogen, potassium, % sand, % silt, % clay and available pore space for all sites. This procedure initially selects the factor which has the highest F-ratio or best fit, and this factor is then combined with each of the other factors in turn and a multiple regression of nematode numbers against each pair is done. The pair which has the highest F-ratio for regression is chosen, i.e. those factors which best explain the variation in nematode numbers. Then these two factors are combined with each of the remaining factors in a similar fashion to give the best fitting triplet. The process was continued up to four factors.

At each stage a test is made to determine whether the additional factor gives a significantly better explanation of how the nematode count varies.

The selective regressions, Table 13, show that % clay and % sand are the most important factors from the nine considered. Furthermore:

a. If % clay or % sand has 'appeared' in the selective regression, the other one generally does not appear, or appears a lot later in the list. This indicates that the variation in the data can be explained by either the clay content or sand content, but that we do not really need both in any particular instance. Thus % clay and % sand are highly correlated so that only one is needed. The real factor that is significant in these regressions is soil composition.

b. Although nitrogen, potassium, % silt and available pore space appear in various positions, they are not significant in any case and do not occur in any regular pattern.

Although available pore space was not a significant influence in either the linear regression or selective regressions, the F-ratios in the regressions are higher than those for the other factors (except sand and clay). Also in the selection regression for soil counts, available

TABLE 13

Results of selective regression analysis on numbers of *Pratylenchus thornei* recovered from the roots of strawberry clover and from soil over 9 months

The factors are listed in order of appearance in the selective procedure and the significance of the F-ratio for the addition of the factor is indicated by the stars:-

*	significant at 5%
**	significant at 1%
***	significant at 0.1%
N.S.	not significant
Space =	available pore space

TABLE 13

MONTH	PLANT		SOIL	
NOVEMBER	K	N.S.	Clay	***
	Sand	N.S.	Space	N.S.
	Space	N.S.	Sand	*
	Silt	N.S.	Silt	N.S.
DECEMBER	Clay	*	Clay	N.S.
	Space	N.S.	N	N.S.
	K	N.S.	Space	N.S.
	N	N.S.	Sand	N.S.
JANUARY	Clay	N.S.	Clay	*
	Space	N.S.	Space	N.S.
	Sand	N.S.	Sand	N.S.
	K	N.S.	K	N.S.
FEBRUARY	Clay	*	K	N.S.
	N	N.S.	Space	N.S.
	Space	N.S.	Sand	*
	K	N.S.	Silt	N.S.
MARCH	Clay	***	Clay	***
	Sand	*	Space	N.S.
	Space	N.S.	Sand	N.S.
	Silt	N.S.	Silt	N.S.
APRIL	Clay	**	Clay	**
	N	N.S.	K	N.S.
	K	N.S.	Space	N.S.
	Silt	N.S.	Silt	N.S.
MAY	Clay	***	Clay	***
	N	N.S.	Space	N.S.
	K	N.S.	Silt	N.S.
	Silt	N.S.	Sand	N.S.
JUNE	Clay	**	Clay	***
	Silt	N.S.	Space	N.S.
	Sand	N.S.	Silt	N.S.
	K	N.S.	Sand	N.S.
JULY	Sand	**	Clay	***
	K	N.S.	N	N.S.
	Silt	N.S.	Silt	N.S.
	Space	N.S.	Sand	N.S.
T O T A L	Clay	***	Clay	***
	N	N.S.	Space	N.S.
	Space	N.S.	Silt	N.S.
	K	N.S.	Sand	N.S.

pore space appears second in the list in seven of the nine cases, so that although not significant in these data, it does occur frequently. Hence available pore space is a factor worth considering when dealing with nematode counts in the soil, although this experiment failed to demonstrate this in a statistically significant way.

IV. Root-Knot Nematode (M. javanica) and other Nematodes in the Field Sites

The root-knot nematode was observed in the clover host plant at only two sandy soil sites of North Glenelg and Flagstaff Hill Golf Club. The numbers were low in spring being 40 per gm of root, increasing to a summer peak in February of 12,000 per gm of root and then declining to a winter drop of 1,500 per gm of root. The only other site in which root-knot nematodes appeared was the silty site of Urrbrae and then it was found only in summer.

Other nematodes found in the soil extractions were Helicotylenchus dihystera (Cobb, 1893) Sher, 1961 and Trichodorus lobatus Colbran. H. dihystera was recovered from all soil types except the heavy clay and was more numerous on the sandy silt, silt and silty clay sites than on the sand sites, while T. lobatus was only found on the two sandy sites.

The results of the measurements in the field suggest that soil type and moisture are the dominant factors influencing numbers of *Pratylenchus*. To test this hypothesis, experimental evidence is required to ascertain how these environmental components affect the nematode's ability to move, invade and emerge from the host root, for these processes will play a large part in determining the population density of *Pratylenchus* in the root.

The same is likely to be true for *Meloidogyne*, but in a quite different way because the root-knot nematode is generally more prevalent on sandy soils (Kincaid, 1946; O'Bannon and Reynolds, 1961) and this is certainly true in the Adelaide area, at least in those areas which receive constant and adequate watering during the hot dry season.

In the next chapter, experiments are described that test hypotheses derived from these field data.

CHAPTER IV

THE INFLUENCE OF ENVIRONMENT ON THE NUMBERS OF PRATYLENCHUS THORNEI AND MELOIDOGYNE JAVANICA

How environmental factors might influence numbers of *Meloidogyne* and *Pratylenchus* was studied in a series of laboratory and pot experiments. The approach was different for the two species because, as explained previously in the Introduction, the various aspects of the ecology of these two species have received unequal attention in the past. Thus for *Pratylenchus*, laboratory experiments were done to ascertain the influence on mobility and invasion of temperature and soil moisture. Experiments with *Meloidogyne* involved the influence of various environmental components on invasion of tomatoes as this is a convenient experimental plant.

Field data from the previous chapter indicated that soil type was an important aspect in influencing numbers of nematodes, e.g. the numbers in the sandy sites were much lower than in the clay sites. To test this hypothesis and to determine possible causes glasshouse pot experiments were carried out.

I. A Comparative Study of the Influence of Soil Type and Nematode Numbers on the Invasion of Clover Roots by *Meloidogyne javanica* and *Pratylenchus thornei*

(a) Methods: O'Connor strain of strawberry clover seedlings was grown in 75 mm clay pots with three soil types - sand (ex Flagstaff Hill), loam (Urrbrae) and clay (Strathmont) all being previously sieved to a crumb size of 1 to 0.25 mm. The pots were plunged in damp vermiculite and maintained at field capacity by weighing to constant moisture. Three levels of inoculum of nematodes (0, 1000, 5000 for *M. javanica* and 0, 300, 1500 for *P. thornei*) were used with fourfold replication. The pots were randomly distributed in the vermiculite container. 15 ml of full strength Hoagland's solution were added to each pot, weekly. After 42 days the roots were carefully washed free from soil, blotted dry and weighed. Top weights were also recorded. The roots were stained in 0.15% lactophenol-cotton-blue at 55°C for 24 hrs, washed and homogenized for 2 min, sieved to remove the larger plant debris and the extracted nematodes counted in a Doncaster counting dish.

The aim of these experiments was to investigate the influence of soil type and inoculum level on (i) root weight, (ii) percentage invasion, and (iii) the ratio of percentage invasion to root weight.

By comparing the means and the range of the sets of data it was found that the following transformations were needed -

(i) root weight - no transformation

(ii) % invasion - $\log_{10}$ transformation

(iii) % invasion/root weight - $\log_{10}$ transformation

An analysis of variance was done on the data for M. javanica and P. thornei and for each of the above three variates. Least significant differences were calculated.

(b) Results: A summary of the significance of effects follows. The one, two and three stars refer to probability levels of .05, .01 and .001 respectively. N.S. is not significant.

M. javanica

	Inoculation Level (0, 1000, 5000)	Soil Type (Sand, Loam, Clay)	Interaction (Inoculation Level x Soil Type)
Root Weight	N.S.	N.S.	N.S.
% Invasion	*	N.S.	N.S.
% Invasion/ Root Weight	N.S.	N.S.	N.S.

P. thornei

	Inoculation Level (0, 300, 1500)	Soil Type (Sand, Loam, Clay)	Interaction (Inoculation Level x Soil Type)
Root Weight	N.S.	**	N.S.
% Invasion	***	***	N.S.
% Invasion/ Root Weight	**	N.S.	N.S.

As none of the interactions for either species was significant comparisons can be made between inoculation levels and soil types independently. The calculated L.S.D.'s and estimated means are given in Tables 14 and 15.

As reproduction occurred during the experiment

(42 days), the number of nematodes recovered is the result of the number invading, establishing and their rate of reproduction. It was assumed that the establishment and rate of reproduction was the same in roots grown in the three soil types. Hence the numbers recovered are directly related to the numbers invading. There is, of course, the possibility that roots grown in the three different soils differed biochemically thereby influencing the nematodes' physiology. But in this experiment no such variations were expected as the nutrient status and pH of the soils used were all within the tolerance range for plant growth. Further studies are required on this aspect; they were not investigated in this thesis.

There is very little of significance in the *M. javanica* data. The percentage invasion is influenced ($P < .05$) only by the inoculation level - the higher percent invasion occurring with the lowei inoculation level which confirms the result obtained in (c) below, that the population density in the roots may be regulated by lack of feeding sites. There was a slight tendency towards a higher

TABLE 14

The influence of soil type and inoculum level on the invasion of clover roots by *Meloidogyne javanica*

TABLE 14

Estimated Means

Meloidogyne javanica

Root Weight

Inoculation level	0	1000	5000	LSD 5%	LSD 10%
Mean	2.17	2.46	2.11	0.55	0.46

Soil type	Sand	Loam	Clay	LSD 5%	LSD 10%
Mean	1.97	2.30	2.47	0.55	0.46

Percentage Invasion

Inoculation level	0	1000	5000	LSD 5%	LSD 10%
Mean	-	2.83	2.58	0.22	0.18

Soil type	Sand	Loam	Clay	LSD 5%	LSD 10%
Mean	2.56	2.80	2.76	0.27	0.23

Percentage Invasion/Root Weight

Inoculation level	0	1000	5000	LSD 5%	LSD 10%
Mean	-	2.46	2.29	0.23	0.19

Soil type	Sand	Loam	Clay	LSD 5%	LSD 10%
Mean	2.33	2.41	2.40	0.29	0.24

TABLE 15

The influence of soil type and inoculum level on the invasion of clover roots by *Pratylenchus thornei*

TABLE 15

Estimated Means

Pratylenchus thornei

Root Weight

Inoculation level	0	300	1500	LSD 5%	LSD 10%
Mean	1.67	1.66	1.58	0.41	0.34
Soil type	**Sand**	**Loam**	**Clay**		
Mean	1.16	1.93	1.82	0.41	0.34

Percentage Invasion

Inoculation level	0	300	1500	LSD 5%	LSD 10%
Mean	-	2.29	2.05	0.12	0.10
Soil type	**Sand**	**Loam**	**Clay**		
Mean	1.98	2.19	2.34	0.15	0.12

(1% = 0.20; 0.1% = 0.27)

Percentage Invasion/Root Weight

Inoculation level	0	300	1500	LSD 5%	LSD 10%
Mean	-	2.10	1.89	0.15	0.12
Soil type	**Sand**	**Loam**	**Clay**		
Mean	1.99	1.91	2.08	0.18	0.15

percentage invasion in clay than in sandy soils, which is contrary to previous observations (Sasser, 1954) but this was significant only at the 10% level.

The P. thornei data show more interesting results because it is apparent that root weight is influenced by the soil type, being heavier in loam and clay than in sand ($P < .01$), but the inoculation level had no significant influence on the root weight.

Assuming that the quantity of roots does not influence the number of nematodes that invade, it is appropriate to consider the analysis of % invasion. The % invasion by P. thornei is highly significantly ($P < .001$) influenced by the inoculation level, being greater at the lower 300 level than 1500 and also by the soil type, being highly significantly ($P < .001$) greater in clay than in sand, in loam than in sand ($P < .01$) and in clay than in loam ($P < .05$). This again confirms the observed field data. In drawing conclusions from the analysis of % invasion/root weight, it must be assumed that the amount of root present influences the number of nematodes that invade. With an inoculation level of 300, significantly more ($P < .01$) invaded per unit weight of root than the 1500 level, whereas soil type had no effect. This result is not unexpected as high invasion rates and large root weight are associated with the heavier soils whereas low invasion rates and small root weight are associated with the sandy soils. The ratios would thus be similar. The results do not indicate whether differences in root weights in the different soils wholly account for the differences in invasion rates; there may be other factors such as differences in soil structure that influence the mobility of the nematodes through the soil. This aspect will be the subject of later experiments.

II. Some Ecological Factors influencing *Meloidogyne javanica*

Several factors determine the numbers in a population that invade the host plant roots. The questions that arise are - does the rate of invasion proceed at a steady rate until most of the population has entered the root? - does the population density influence the percentage invasion? - how do environmental factors influence invasion? Pot experiments were set up to investigate these points.

(a) Influence of population density on invasion

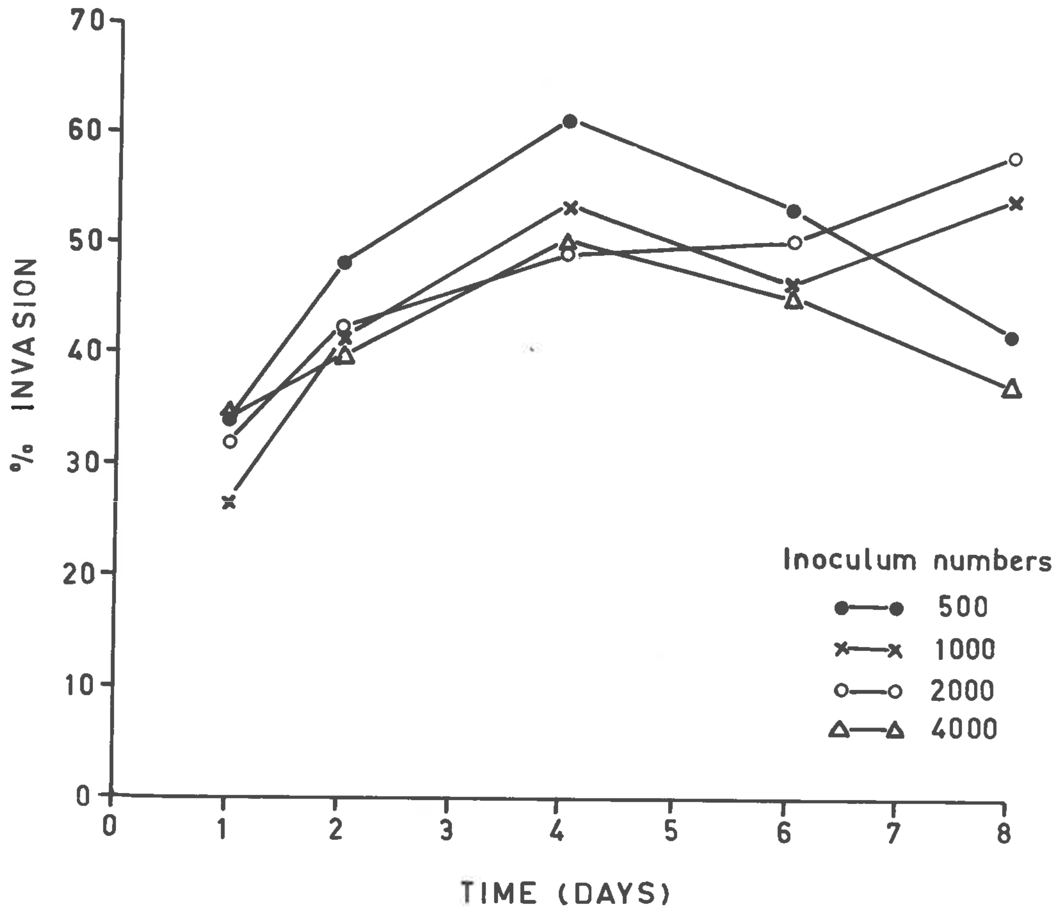
Tomato plants (var. Tatura dwarf) 2 weeks old, were grown in plastic cylinders 3.5 cm diam. by 7 cm, in U.C. soil mixture. They were grown in a glasshouse without artificial lighting and were watered daily. Temperatures were controlled to give an approximate daily fluctuation of 20 to 26°C. Fourfold replication was used and four levels (500, 1000, 2000, 4000) of 0 to 2 day old *M. javanica* larvae were introduced on to the surface of the soil. Four plants were removed 1, 2, 4, 6 and 8 days after inoculation, washed, blotted dry, weighed, stained in boiling cotton-blue-lactophenol, macerated in an M.S.E. homogenizer and the extracted larvae counted in a Doncaster counting dish.

There was a steady increase in percentage invasion with all rates of inoculum up to about the 4th day, the lowest inoculum of 500 larvae giving the highest percentage. Thereafter there was a gradual decline in % invasion for the 500 and 4000 inocula, but an increase in the 2000 (Fig. 13).

The results indicate that the rate of invasion is not linear, but decreases progressively with time, so that after about 4 to 5 days, the numbers in the root do not increase (except for the 2000 inoculum which cannot be explained). An analysis of variance of the data in this experiment did not indicate any significant differences in invasion rates between levels of inoculum, i.e. there was no evidence of competition for suitable feeding or invasion sites. There was an indication that numbers invading decreased after 6 days but the duration of the experiment was too short to give an unequivocal result. Thus to determine this point, a further experiment was done [see (c)].

Figure 13. The influence of inoculum density on percent invasion of tomato roots by *Meloidogyne javanica* over 8 days.

FIG. 13



70
60
50
40
30
20
10
0
% INVASION
0
1
2
3
4
5
6
7
8
TIME (DAYS)
Inoculum numbers
500
1000
2000
4000

(b) <u>The influence of population numbers and root volume on invasion</u>

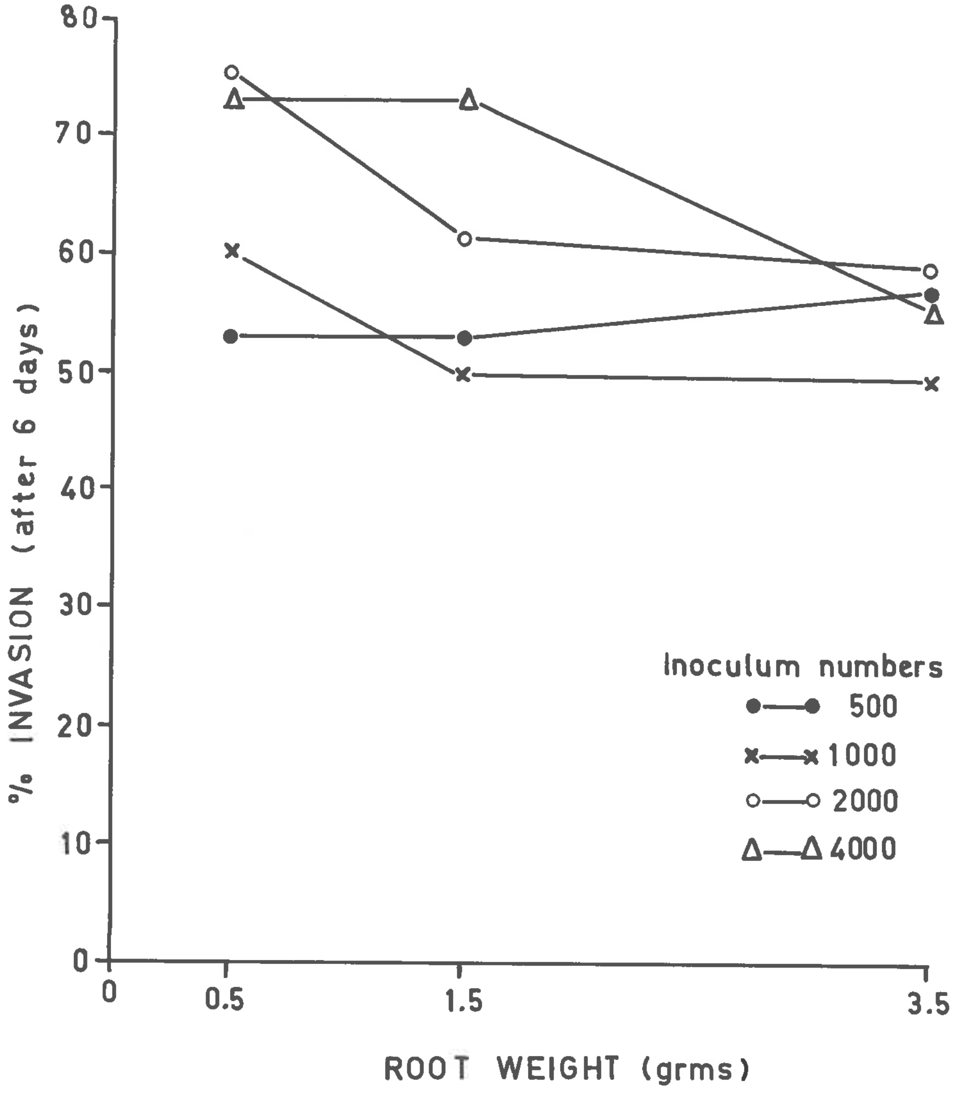
It is possible that as the amount of roots in a given volume of soil increases the number of nematodes invading the root would also increase because there was less distance for the nematode to travel and more invasion sites.

Tomato plants, 2 weeks old, were grown in a plastic pot containing 350 cc of U.C. soil mixture. Cultural conditions were the same as the previous experiment, there being five replicates. Larvae at four levels of inocula (500, 1000, 2000, 4000) were introduced at 2 cm from the base of the plant, there being three times of inoculation, one week apart. Plants were harvested and the number of nematodes invading the roots were counted, after six days, because the previous experiment indicated that the bulk of larvae had invaded by this time.

An analysis of variance of the results (Fig. 14) failed to show that root volume had any significant influence on the % invasion except with low root volumes where a greater percentage of the high population (4000) invaded than the smaller population (500). Thus it appeared that location of the host root did not greatly restrict invasion and furthermore there was no indication of competition caused by population density, in fact the contrary appeared to occur because the high population had a greater

Figure 14. The influence of inoculum density and root weight on percent invasion by *Meloidogyne javanica* after 6 days.

FIG. 14


80
70
60
50
40
30
20
10
0
% INVASION (after 6 days)
0
0.5
1.5
3.5
ROOT WEIGHT (grms)
Inoculum numbers
500
1000
2000
4000

percentage invasion, possibly due to the attraction of nematodes to previously invaded sites.

(c) <u>The influence of time and photoperiod on invasion</u>

To elaborate further on experiment (a), the question arose, Did the second stage larvae that invaded the plant roots over the initial six days remain in the plant?

Tomato plants in 3.5 cm by 7 cm plastic tubes of U.C. soil mixture were inoculated with 1000 two-day old *M. javanica* larvae and grown in the glasshouse as previously described. Each day for fifteen days after inoculation, five plants were removed and the nematodes that had invaded were counted as previously described. Other host plants of Mung bean (*Phaseolus aureus* Roxb.) and French bean (*P. vulgaris* L.) were similarly used. The results are shown in Fig. 15.

Also, to determine the percentage invasion for the first 24 hours, tomato plants were harvested every two hours. The plants were grown in a controlled environment cabinet on a 12 hr photoperiod of 1600 lumens/sq. ft at a constant temperature of 25°C. A duplicate set of plants was set up in another cabinet with the photoperiod reversed. The purpose was to see if invasion was influenced by light.

In the 24 hr experiment (Fig. 16) numbers started invading after about 10 hrs, after which there was a high rate of invasion up to 24 hrs. The experiments confirmed

__Figure 15.__ The peroent invasion of the roots of three host plants over 15 days. The inoculum contained 1,000 larvae of __Meloidogyne javanica__ per plant.

__Figure 16.__ The influence of photoperiod on the invasion of tomato roots by larvae of __M. javanica__ over 24 hours.

FIG. 15

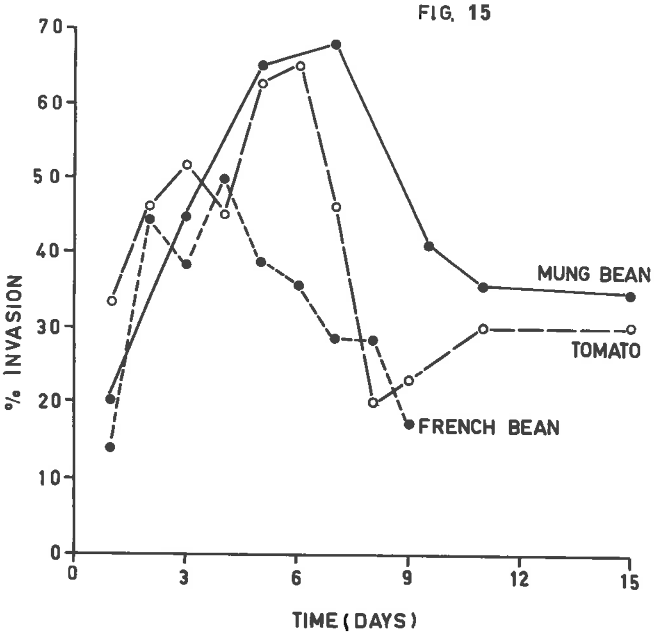
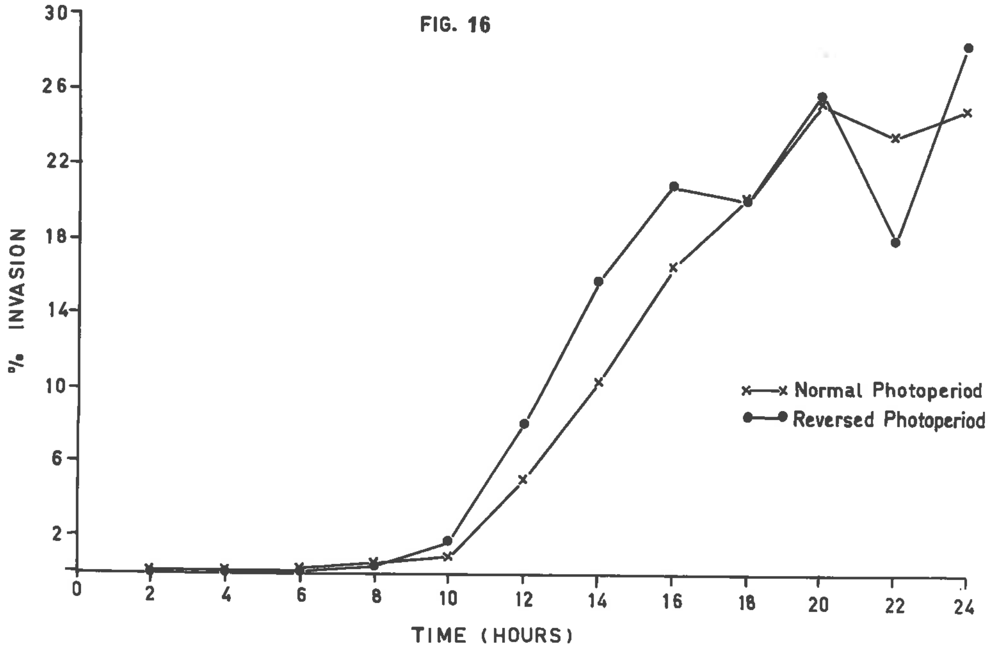

70
60
50
40
30
20
10
0
% INVASION
0
3
6
9
12
15
TIME (DAYS)
MUNG BEAN
TOMATO
FRENCH BEAN

FIG. 16


30
26
22
18
14
10
6
2
% INVASION
0
2
4
6
8
10
12
14
16
18
20
22
24
TIME (HOURS)
Normal Photoperiod
Reversed Photoperiod

each other in that the percentage invasion for the first day for both was about 30%.

An analysis of variance between the values obtained for the normal and reversed photoperiod at 12, 14 and 16 hrs showed no significant difference. Thus it appeared from this experiment that invasion can occur just as well when the plants are receiving light as when they are not, as long as the plant receives sufficient light to maintain normal physiological functions.

The results (Fig. 15) confirmed the previous (a) investigation that there was an initial increase in numbers invading up to about the sixth day; then they declined considerably depending on the host species.

This decline in numbers after 6 days may have been caused by death due to lack of feeding sites, or the larvae may have left the root which was more likely. It thus appeared that whereas numbers of nematodes in the inoculum and numbers of invasion sites may not have greatly restricted invasion, the population density in the roots may have been influenced by lack of feeding sites.

(d) <u>The influence of distance from the host plant on invasion</u>

To confirm that nematodes find and invade roots efficiently over distances up to 4 cm, an experiment was carried out using tomato seedlings in 10 cm pots of U.C. soil mixture, grown under glasshouse conditions as previously described. Two-day old larvae at four levels of inocula (500, 1000, 2000, 4000) were introduced at the base of the plant, around the plant in 2 cm and 4 cm concentric circles, and as an overall flooding of the soil surface. Four replicate plants were harvested after six days and the numbers of nematodes within the roots were counted.

The results (Fig. 17) indicated that distance of inoculation from the plant had no influence on the percentage invading. M. javanica larvae appeared to be able to locate roots at a distance of at least 4 cm from the plant. As this experiment did not extend to a limiting condition, a further test was necessary using greater distances and nematodes of different physiological age [see (f)].

(e) The influence of depth of inoculum on invasion

The vertical location of the nematode to the root system might also influence its ability to infect. Tomato seedlings were grown as in (d) until the plants' roots had grown throughout the soil. The plants were then carefully tipped out of their pots and inoculated with 2000 two-day old larvae by a Luerlock continuous syringe at four equidistant positions around the soil cone, at either a quarter, a half or three quarters the way down the root system. An overall surface inoculum was given as well. After six days, five replicates of each of the four treatments were harvested. The roots were carefully washed, and

<u>Figure 17</u>. The influence of distance from the host plant (tomato) on invasion by *Meloidogyne javanica* at four inoculum levels. (OA = overall)

<u>Figure 18</u>. The influence of depth of inoculum in the soil on the invasion of tomato roots by *M. javanica*.

FIG. 17

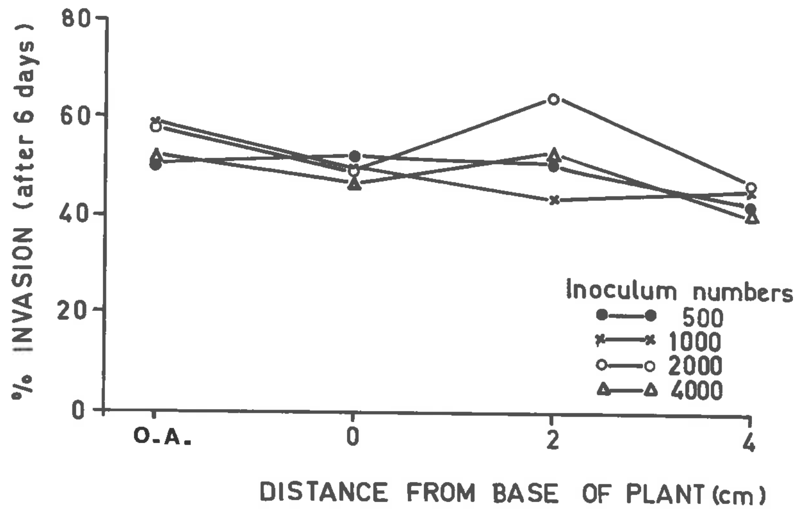
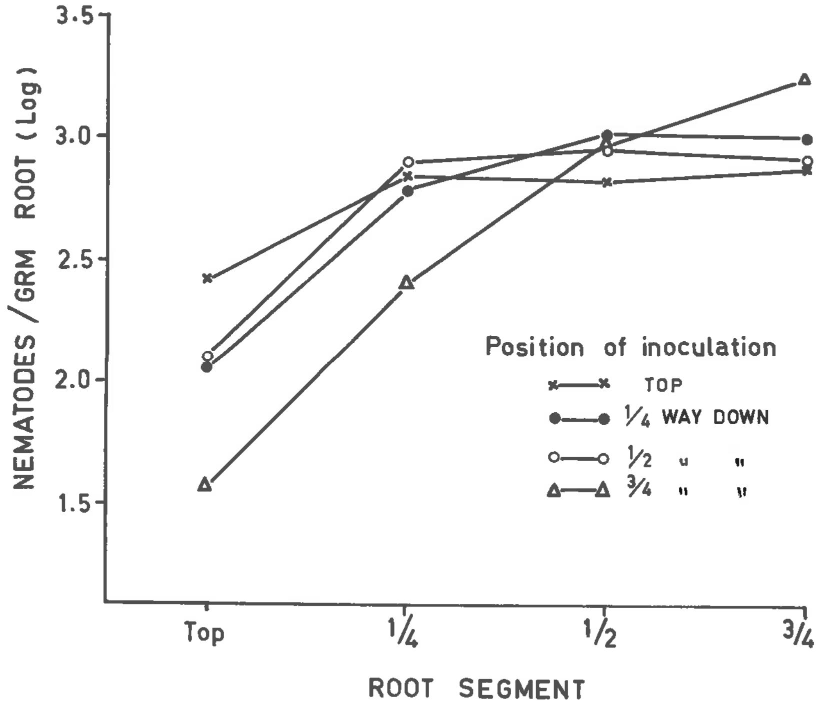

80
60
40
20
0
% INVASION (after 6 days)
O.A.
0
2
4
DISTANCE FROM BASE OF PLANT (cm)
Inoculum numbers
500
1000
2000
4000

FIG. 18


3.5
3.0
2.5
2.0
1.5
NEMATODES /GRM ROOT (Log)
Top
1/4
1/2
3/4
ROOT SEGMENT
Position of inoculation
TOP
1/4 WAY DOWN
1/2 " "
3/4 " "

out into quarters with respect to depth. The invading nematodes were counted for each of these regions.

Results (Fig. 18) showed that the greatest invasion occurred in the vicinity of the original inoculation, but was greater below than above the inoculum point. This could have been caused by water percolation carrying the nematodes deeper, or due to a positive geotaxis. But the total invasion was uninfluenced by position of inoculum. Thus nematodes appeared to invade at the same rate wherever their location relative to the plant, although invasion at lower depths may have put greater stress on the plant because that was where the majority of new roots and root tips occurred.

(f) <u>The influence of age of nematode and position of inoculum on invasion</u>

To test a previous suggestion, experiment (d) was repeated using larvae of different ages. The larvae for ageing, after being collected from infested roots held in a misting chamber, were kept in shallow water in a Petri dish and stored at 27°C in a polyethylene bag to prevent drying over the period of storage, which was 0, 2 and 4 days. The results (Fig. 19) showed that larvae from 0 to 4 day old invaded equally well over distances from 0 to 4 cm from the plant.

To ascertain the limiting time for infectivity, larvae

Figure 19. The influence of age of larvae of *Meloidogyne javanica* on invasion of tomato roots.

Figure 20. The influence of age of larvae of *M. javanica* and distance from tomato plant on invasion.

FIG. 19

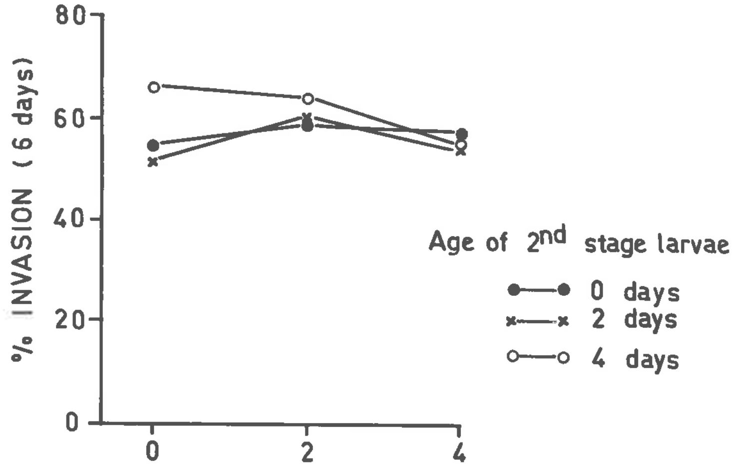
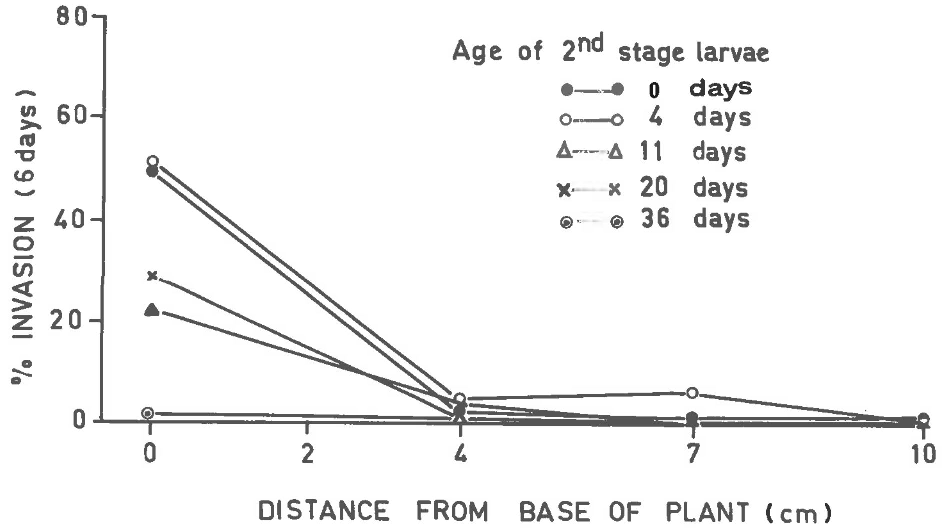

80
60
40
20
0
% INVASION (6 days)
0
2
4
Age of 2nd stage larvae
0 days
2 days
4 days

FIG. 20


80
60
40
20
0
% INVASION (6 days)
Age of 2nd stage larvae
0 days
4 days
11 days
20 days
36 days
0
2
4
7
10
DISTANCE FROM BASE OF PLANT (cm)

were aged for C, 4, 11, 20 and 36 days and the distances of inoculation from the plant were increased to 0, 4, 7 and 10 cm.

Increasing the age range of larvae and increasing the distance showed unequivocally (Fig. 20) that larvae older than 4 days were less infective and that 4 cm is about the distance when ability to reach the root begins to influence percentage invasion. Thus it can be concluded that maximum infection is achieved by newly hatched larvae that occur within a short range of the host plant roots.

(g) <u>Influence of age of plant and volume of soil on invasion</u>

The previous experiment indicated that larval age influenced percentage invasion; it is also possible that the age of the plant in terms of increased root volume or change in physiology may also influence invasion.

Tomato seedlings were grown in 3.5, 9.5 and 14.5 cm pots of U.C. soil mixture in a glasshouse. When the two first true leaves had expanded the plants were inoculated with 2000 one-day old larvae in equivalent volumes of water to cover the surface of the soil of all pot sizes.

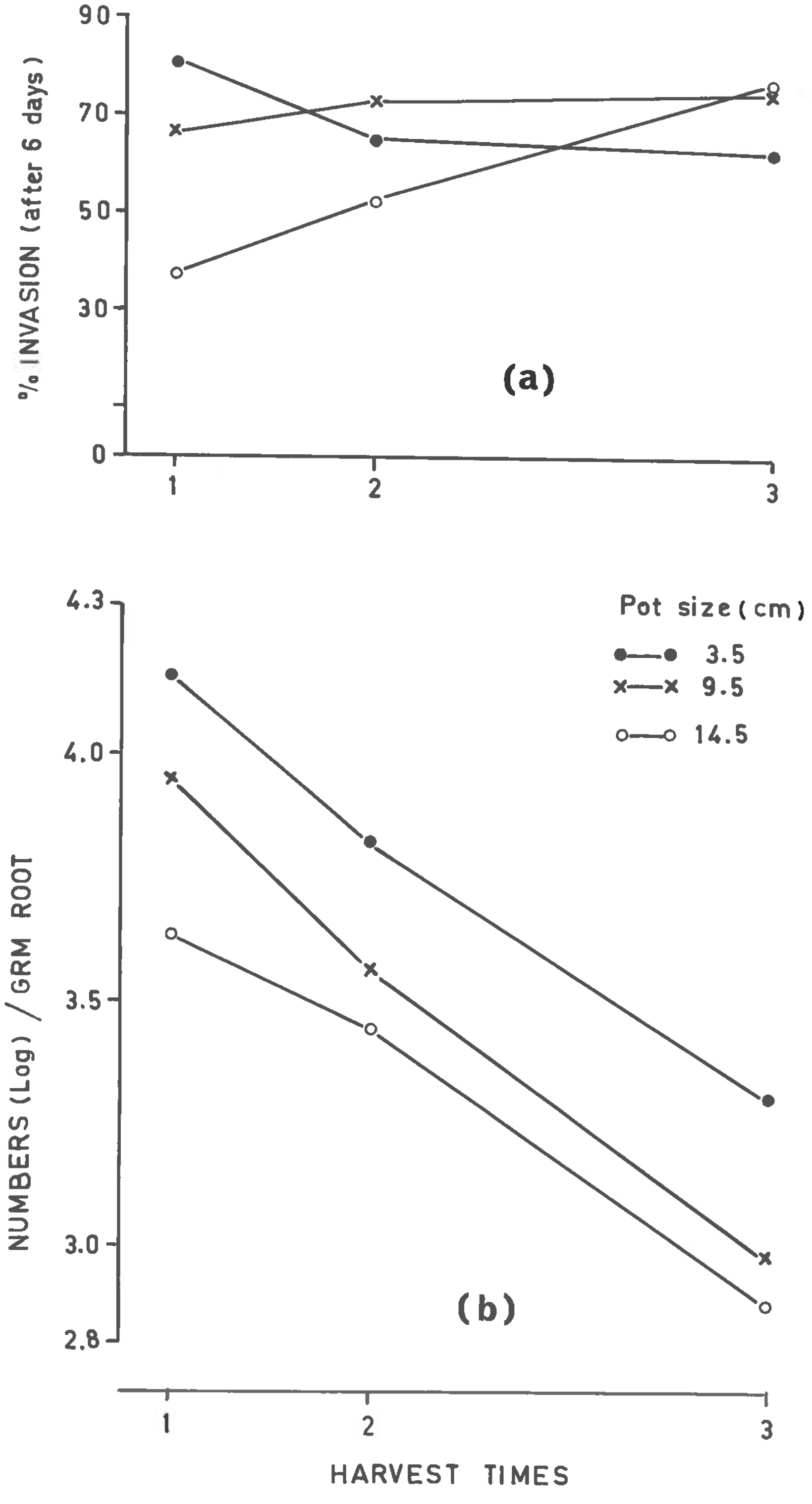
Two further inoculations, four and twelve days later, using one-day old larvae, were given to other sets of plants. This gave the range of increased root volumes. Five replicated plants were harvested six days after inoculation and

Figure 21. The influence of age of plant and volume of soil on invasion of tomato roots by *M. javanica*. The harvest times 1, 2 and 3 correspond to (1) the time at which the two first true leaves have expanded, (2) 4 days later, (3) 12 days later.

(a) percent invasion

(b) same data as (a) expressed as numbers of nematodes/gm of root.

FIG. 21



% INVASION (after 6 days)
90
70
50
30
0
1
2
3
(a)
NUMBERS (Log) / GRM ROOT
4.3
4.0
3.5
3.0
2.8
Pot size (cm)
3.5
9.5
14.5
(b)
1
2
3
HARVEST TIMES

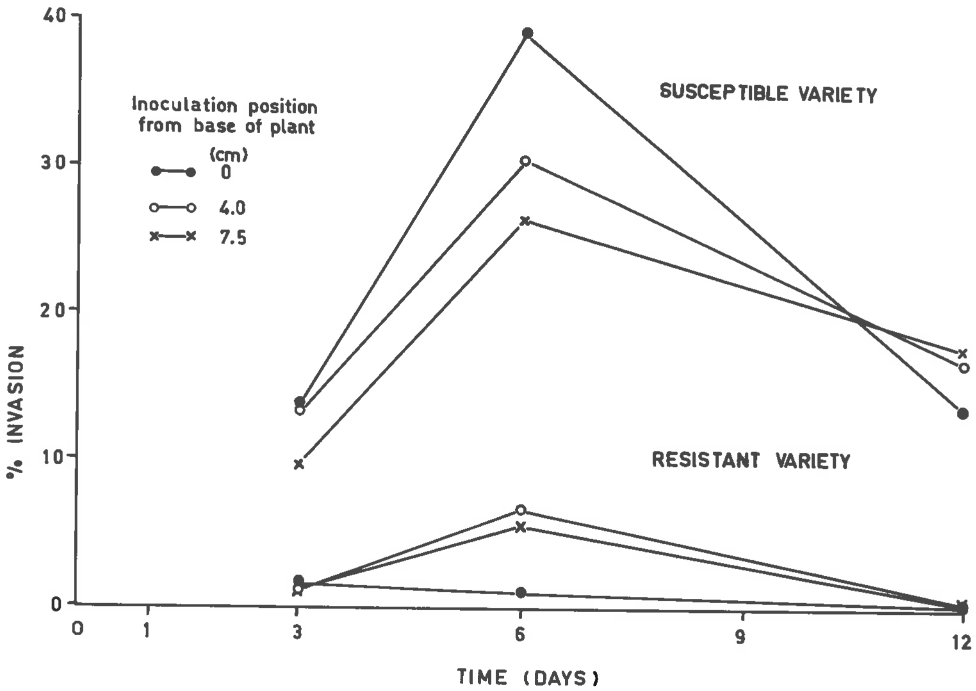
the invading nematodes counted. Results showed (Fig. 21 b) that although the numbers of larvae per gram of root decreased with age of plant, this was due to the increased amount of root as the plant aged. Percentage invasion was unaffected by the amount of root (Fig. 21 a). Thus there was no indication of increasing resistance in the plant with age.

(h) Influence of host resistance, position of inoculum and time on invasion

Although there was no apparent physiological change in the host plant with ageing that influenced invasion, this aspect was studied further by using a resistant strain of a host plant. Tomato seedlings of the susceptible strain, Tatura dwarf, and resistant strain HE4242 were grown in U.C. soil mixture in the glasshouse. Plants were inoculated with one-day old larvae at 0, 4 and 7.5 cm from the base of the plant. Four replicates were harvested 3, 6 and 12 days later and the invading nematodes counted. Results (Fig. 22) showed that nematodes invaded susceptible plants in greater numbers than resistant plants, and the decrease in populations in the roots after 6 days was again confirmed, as was the fact that the distance of placement of nematodes from the roots does influence numbers invading. Resistance in the tomato variety HE4242 is thus expressed early in the nematode's life cycle unlike other situations where

Figure 22. The influence of host resistance, position of inoculum and time on invasion of tomato roots by *M. javanica*.

FIG. 22



Inoculation position from base of plant
(cm)
0
4.0
7.5
SUSCEPTIBLE VARIETY
RESISTANT VARIETY
% INVASION
40
30
20
10
0
0
1
3
6
9
12
TIME (DAYS)

resistance appears after invasion.

(i) Summary

It seems likely from these data that the number of *M. javanica* in the roots of a host plant will be influenced by the age of the nematodes, by the distance it has to travel to the roots and by the density of nematodes that a root will support - possibly due to a limitation of feeding sites. Consequently the results support the previous hypothesis of Wallace (1966) and Van Gundy *et al*. (1967), that after hatching the nematode has only a limited time to invade the roots before it loses its infectivity. The results indicate the important fact that in relating nematode numbers to possible plant growth, it is the number of nematodes in the roots that should be measured, numbers in the soil are likely to be an unreliable indication of the potential damage *M. javanica* might cause.

The influence of soil type, previously considered to be important in determining the distribution of the nematode, was not significant in these experiments, although this factor requires further investigation as all previous field observations indicated that *M. javanica* was more prevalent in coarser textured soils. It is possible that some other factor associated with soil type other than nematode mobility may be important under field conditions. Two such possibilities are the influence of soil type on root growth and on desiccation of the nematode.

It is evident from the few factors that were studied in this research project that the abundance of *M. javanica* in the root is determined by rate of invasion which is in turn influenced by a complex, interacting system of factors.

The experiments just described help to indicate what is happening in the field situation and they define the more important environmental and physical factors that influence plant growth and nematode distribution. Conclusions based on the combined information from the field, laboratory and glasshouse will be discussed in the final chapter.

III. Some Ecological Factors Influencing *Pratylenchus thornei*

(a) The influence of temperature on mobility

The field observations indicate that numbers of *P. thornei* increase after the hot summer season, thus it is possible that temperature might contribute to this increase, possibly by influencing nematode mobility.

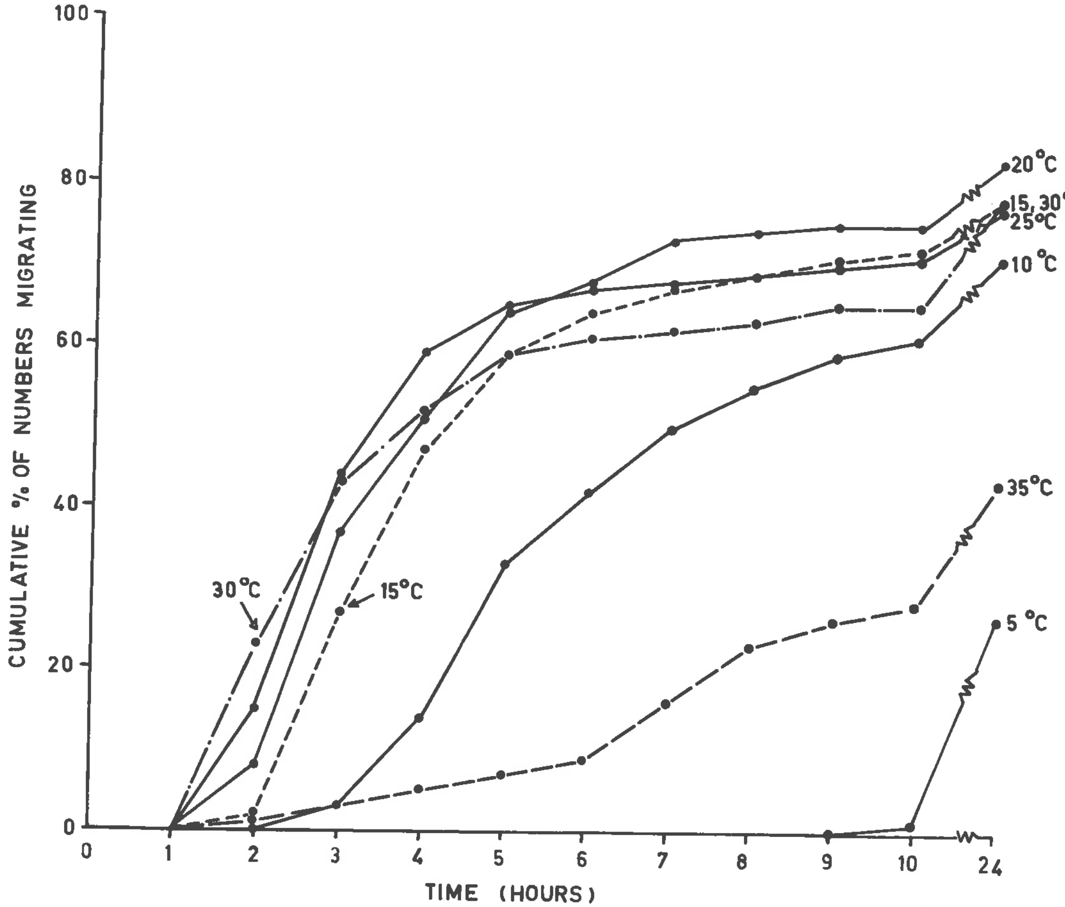
To test the effect of temperature on the migration of *P. thornei* a modification of Wallace's (1958) vertical migration technique was used. Plastic tubing 0.5 cm diam. x 2 cm long was covered at one end with a piece of fine cloth. The covered end was immersed in water in a solid watch glass block after being filled with 150-250 μ particle size sand and tapped to ensure uniform compaction. About 100 nematodes were then pipetted onto the surface of the sand. Each hour the number of nematodes which had migrated down through the sand and through the cloth were counted in the water in which the end of the tube was immersed. After 24 hours those larvae that had moved through the sand, together with those still in the sand were counted and the percentage migrating through the column of sand was calculated.

Seven temperatures of 5°, 10°, 15°, 20°, 25°, 30° and 35°C were investigated with four replications for each temperature. The results are shown in Fig. 23. About 75% of the nematodes migrated at the temperatures of 10° to 30°, but only 45% and 25% at 35°C and 5°C respectively. An analysis of variance of the data transformed to angles of equal information indicated that mobility was not significantly different between 15°, 20°, 25° and 30°C for any time for the duration of the experiment but migration at 35° was significantly slower than at 10°C and both were slower than the 15°, 20°, 25°, 30°C group after 4 hrs duration until the termination. Migration at 5°C had only just commenced at 10 hrs.

These results support the field observations that the higher temperature of 35°C which is commonly prevalent in the surface layers of soil in Adelaide during the summer

Figure 23. The influence of temperature on the mobility of *Pratylenchus thornei*.

FIG. 23


100
80
60
40
20
0
CUMULATIVE % OF NUMBERS MIGRATING
0
1
2
3
4
5
6
7
8
9
10
24
TIME (HOURS)
20°C
15, 30°C
25°C
10 °C
35°C
5 °C
30°C
15°C

and the low 10°C, a common winter temperature, could have a marked influence on the migration of the nematodes and thus influence the numbers invading the host plant. The cooler temperatures after mid-summer are within the optimum range for migration and hence might explain the autumn increase in numbers found in the host plant.

(b) Influence of temperature and soil type on mobility of *P. thornei*

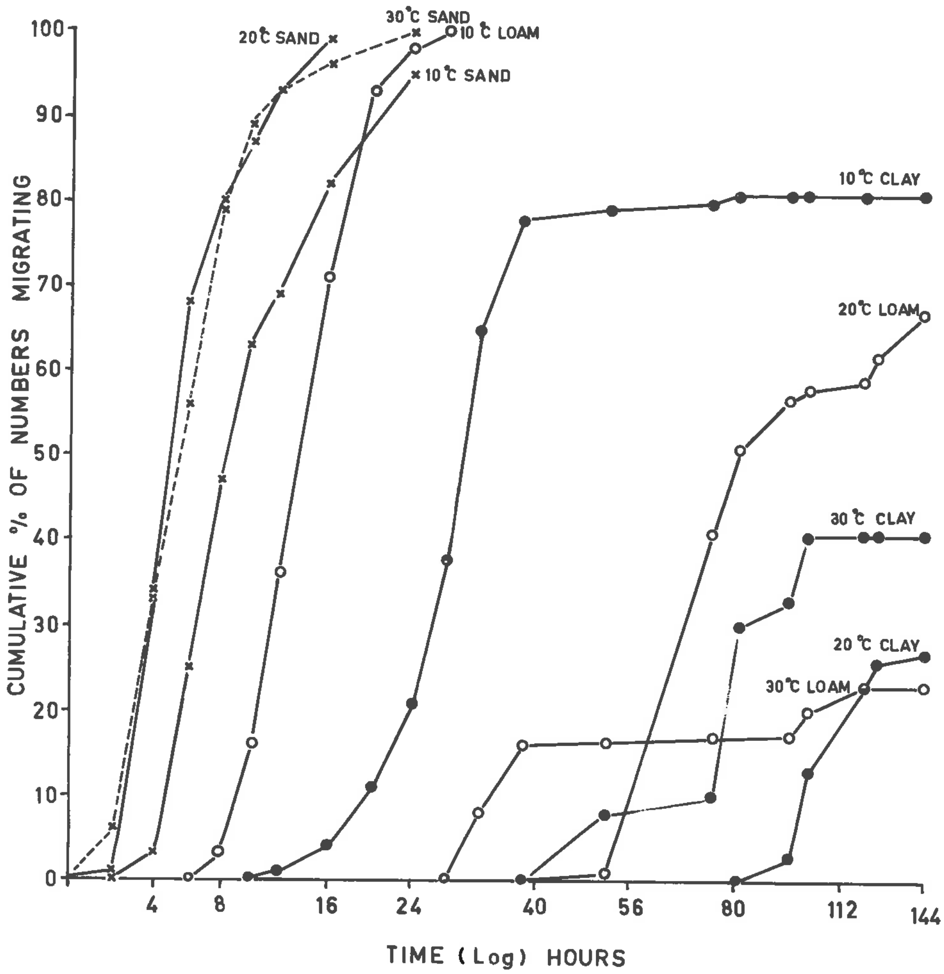
From the results of the field observations in the previous chapter, numbers tend to be lower in the coarser textured soils. Thus a combination of soil texture and temperature as seen from (a) above, might influence mobility and thereby partly explain the association of lower numbers with sandy soils.

The problem was investigated by using the method described in (a) except that 1.5 cm length of tube was used. Five replicates of sand (Flagstaff Hill), loam (Urrbrae) and clay (Strathmont), sieved to 150-250 μ particle size, were studied at 10°, 20° and 30°C.

From the results obtained (Fig. 24) it is evident that *P. thornei* migrated through the sand at the three temperatures within 24 hrs, whereas in the loam and clay migration took up to 150 hrs when the experiment was ended, depending on the temperature. Migration was slower in clay than loam. Considering the influence of temperature, migration

Figure 24. The influence of temperature and soil type on the mobility of *P. thornei*.

FIG. 24



CUMULATIVE % OF NUMBERS MIGRATING
100
90
80
70
60
50
40
30
20
10
0
20°C SAND
30°C SAND
10°C LOAM
10°C SAND
10°C CLAY
20°C LOAM
30°C CLAY
20°C CLAY
30°C LOAM
4
8
16
24
40
56
80
112
144
TIME (Log) HOURS

through the column was quicker at 20° and 30°C in sand reaching a maximum rate in about 4 hrs, whereas it took 8 hrs at 10°C. The lowest temperature of 10°C gave the quickest migration rates for both loam and clay, with maximum rates at 16 hrs and 32 hrs respectively after commencement of the experiment. At the higher temperature of 20°C migration did not commence until 50 hrs and 95 hrs in the loam and clay respectively; and at 30° until 32 hrs and 50 hrs for loam and clay respectively. As it is difficult to obtain a clear picture from these results, the data were statistically analysed at a reference point of time taken for 20% migration, in order to test the differences in times for different soils, and the differences in times for different temperatures. Due to the fact that there were some missing values of the replications non-orthogonal two-way analysis of variance was done on the logarithmic transformed ($\log_{10}$) data. The means of the log transformed data were as follows:

Means of log time (hours) taken for 20% migration of *P. thornei*

	10°C	20°C	30°C
Sand	0.75	0.53	0.50
Loam	1.02	1.82	1.99
Clay	1.38	2.05	1.89

Because the analysis is non-orthogonal, we must determine the F value for each factor 'corrected' for the other.

The results are summarized as follows:-

Temperature (adjusted for soil type)

highly significant $P < .001$

Soil Type (adjusted for temperature)

highly significant $P < .001$

Interaction (Temperature x Soil Type)

highly significant $P < .001$

As the interaction (Temperature x Soil Type) is significant, the only appropriate comparisons to make are soil types for a given temperature and temperatures for a given soil type.

As the analysis is non-orthogonal it is not appropriate to calculate L.S.D.'s as the L.S.D. would be different for almost all comparisons. The t distribution is used for testing because the variance is estimated by the Error Mean Square (0.0115).

From the results of significance in the comparisons of soil types for a given temperature, and temperatures for a given soil type (Tables 16 and 17), we see that sand is significantly ($P < 0.001$) a better medium for migration than loam or clay at 10°, 20° and 30°C, while loam is highly significantly ($P < 0.001$) better than clay at 10°C, but only significantly ($P < 0.05$) better at 20°C and not significant

TABLE 16

Comparisons of the influence of soil type on the migration of *Pratylenchus thornei* at different temperatures

* significant at 5%

** significant at 1%

*** significant at 0.1%

N.S. not significant

TABLE 16

Comparison of Soil Types for a Given Temperature:

Temperature	Comparison	Values of t_{29}	Significance
10°C	Sand, Loam	4.025	***
	Sand, Clay	9.307	***
	Loam, Clay	5.281	***
20°C	Sand, Loam	17.886	***
	Sand, Clay	19.020	***
	Loam, Clay	2.477	*
30°C	Sand, Loam	13.679	***
	Sand, Clay	18.755	***
	Loam, Clay	1.313	N.S.

TABLE 17

Comparisons of the influence of temperature on the migration of *Pratylenchus thornei* in different soil types

*	significant at 5%
**	significant at 1%
***	significant at 0.1%
N.S.	not significant

TABLE 17

Comparison of Temperatures for a Given Soil Type:

Soil Type	Comparison	Value of t_{29}	Significance
Sand	10°, 20°C	3.228	**
	10°, 30°C	3.640	**
	20°, 30°C	0.413	N.S.
Loam	10°, 20°C	11.048	***
	10°, 30°C	7.884	***
	20°, 30°C	0.941	N.S.
Clay	10°, 20°C	8.166	***
	10°, 30°C	6.549	***
	20°, 30°C	2.056	*

at $30^{o}C$. For both loam and clay migration tended to be quicker at $10^{o}C$ than at $20^{o}C$ or $30^{o}C$, whereas the reverse is true for sand.

From the results of these two experiments (a) and (b), it appears that if the factor of migration was the determinant of numbers of nematodes, then the experiments do not support the hypothesis based on field observations that the highest numbers of nematodes are found in clay rather than in sandy soils. Possibly the laboratory experiments do not sufficiently simulate the physical conditions found in the field, but more likely migration is an unimportant factor influencing number in roots. The temperature factor does confirm the field observation of reduced migration rate at the high temperature of $30^{o}C$. Thus it appears likely that the plant host through its response to soil type is influencing the numbers of nematodes.

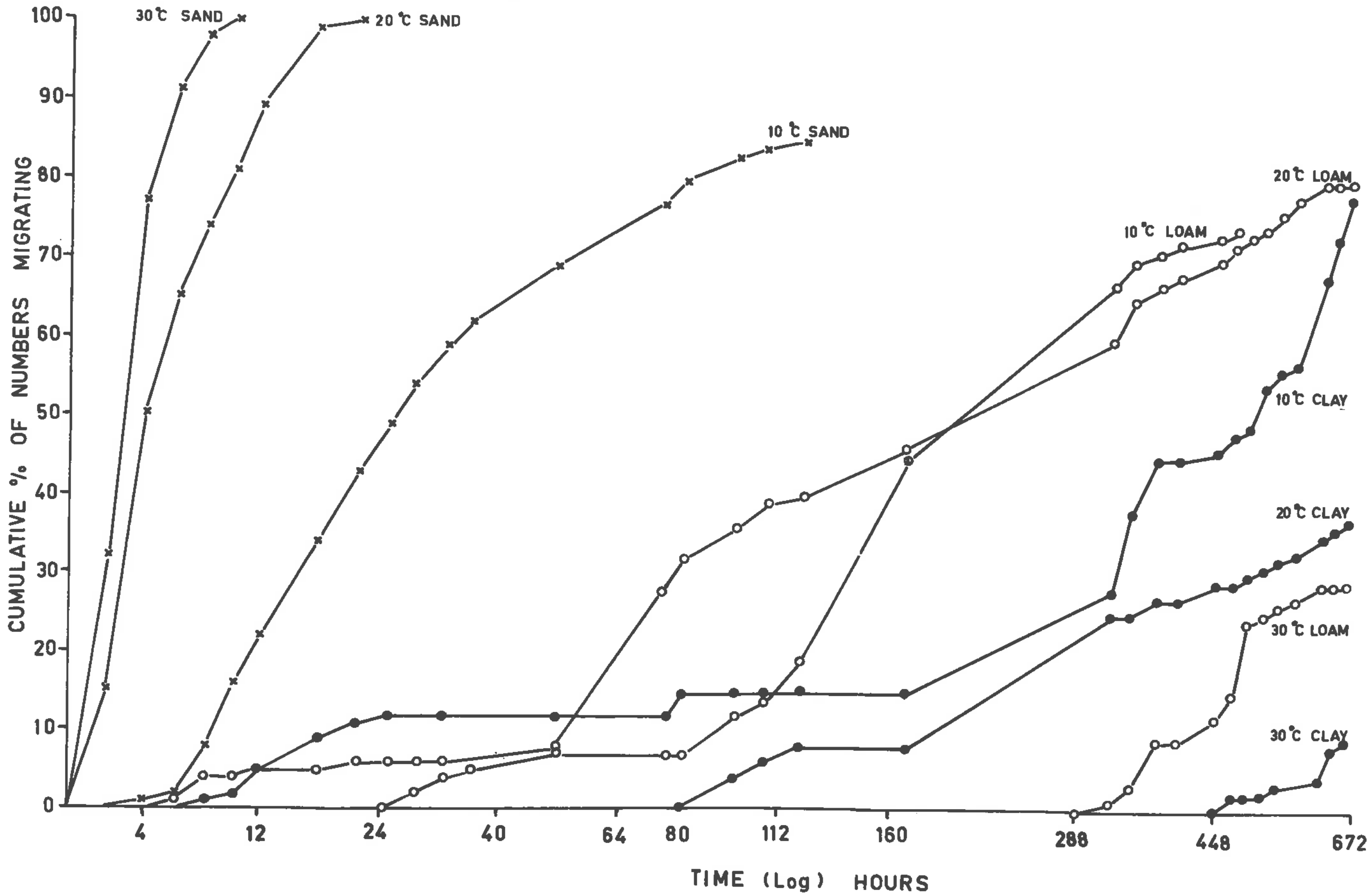
(c) Influence of temperature and soil type on mobility of *M. javanica*

A similar experiment to that just described was carried out to investigate the influence of soil type and temperature on the migration of *M. javanica*. The experiment is included here to facilitate comparison with the previous data for *P. thornei*.

The results (Fig. 25) confirm the previous studies of Wallace (1966) that the rate of migration increases with

Figure 25. The influence of temperature and soil type on the mobility of *M. javanica*.

FIG. 25


30°C SAND
20°C SAND
10°C SAND
20°C LOAM
10°C LOAM
10°C CLAY
20°C CLAY
30°C LOAM
30°C CLAY
CUMULATIVE % OF NUMBERS MIGRATING
100
90
80
70
60
50
40
30
20
10
0
4
12
24
40
64
80
112
160
288
448
672
TIME (Log) HOURS

temperature, the fastest rate being in sandy soil at 4 hrs after commencement of the experiment at 30°C. Migration was completed after 120 hrs, 21 hrs, 10 hrs for 10°, 20° and 30°C respectively. The optimum range for maximum mobility in sand of 150-250 μ particle size appears to be between 20° and 30°C.

With the loam and clay migration was a lot slower, a higher percentage coming through by the termination of the experiment at 652 hrs at 10° and 20° than at 30°C. The commencement of migration was later in the clay than in the loam at 20° and 30°C, but earlier (8 hrs compared to 28 hrs) at 10°C. Both loam and clay had comparable percentages of nematodes migrating by the termination of the experiment at 652 hrs at the three temperatures. A higher percentage was observed at 10° and 20° than at 30°C.

Percentage Migration at 27 Days

	Loam	Clay
10°C	74	78
20°C	80	37
30°C	29	9

The conclusions drawn from those results suggest that the optimum conditions for migration of *M. javanica* are coarse soil texture and a temperature range of 20-30°C.

Comparing the influence of soil type and temperature on migration of P. thornei and M. javanica, a coarse textured soil type is best for both species, the higher temperatures favouring M. javanica more than P. thornei. In the finer textured soils of loam and clay M. javanica, like P. thornei, migrated quicker at the lower temperature of 10°C than at 20° or 30°C. As far as is known this interaction between temperature and soil type has not been reported before.

These results support the hypothesis that root knot nematode occurs most abundantly in the sandy regions of Adelaide and that maximum numbers occur in the spring and summer.

With P. thornei, the experimental data do not directly confirm the hypothesis that this nematode is found in higher numbers in the clay soils than in the sands, but they do support the idea that numbers increase in the cooler periods of the year.

(d) The influence of temperature on the emergence of P. thornei from roots

The number of P. thornei in the roots is determined by the relative rates of invasion and subsequent emergence as in Meloidogyne. To evaluate how temperature influences such emergence 4 replicates of one gm aliquots of a thoroughly mixed sample of clover roots extracted from the field were set up in distilled water at 5°, 15°, 25°, 35°C, and

P. thornei were allowed to emerge. From Fig. 26, it is seen that the emergence was higher at 15°C than at any other temperature, thus confirming that maximum emergence occurs within the somewhat low temperature range corresponding to spring and autumn temperatures. It is also within the optimum temperature range of 15-25°C for mobility as shown in (a).

(e) Influence of soil moisture on invasion

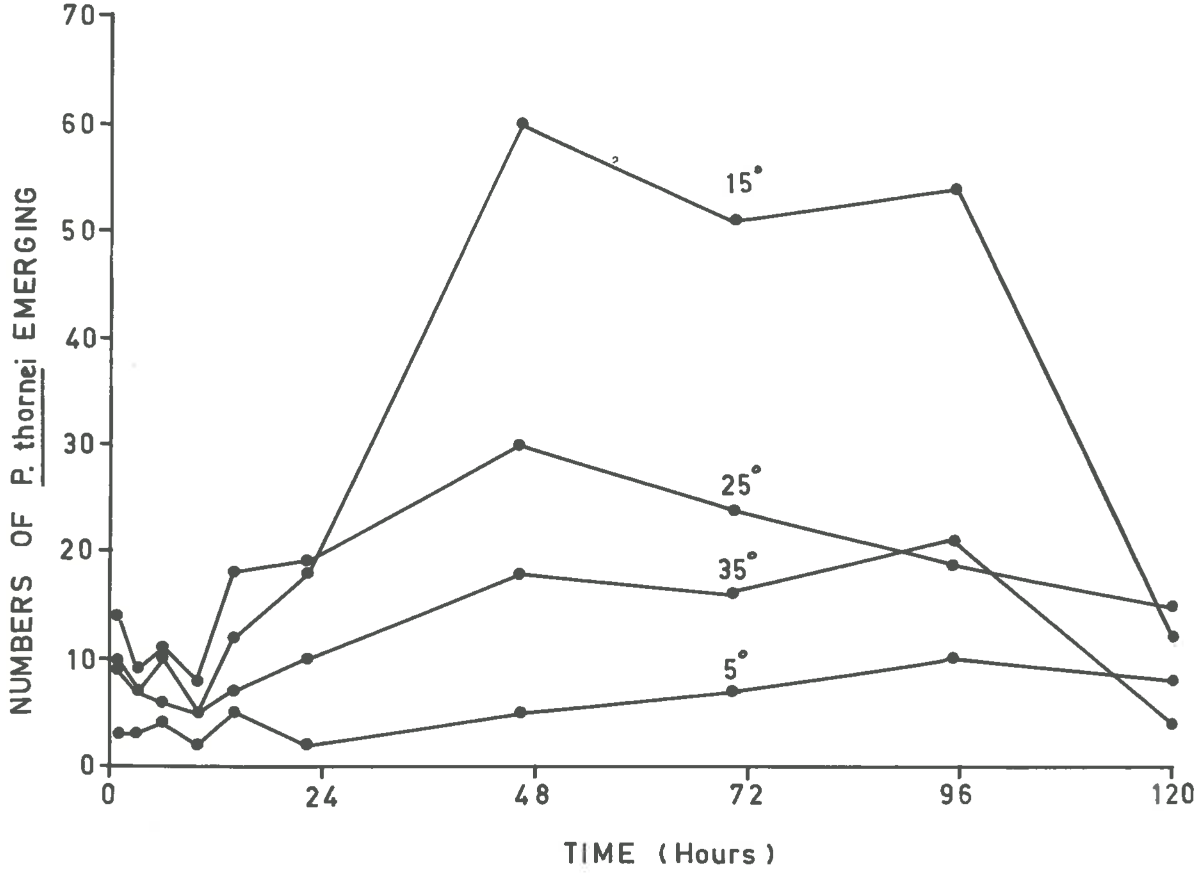
Soil moisture may also have an important effect on invasion. Field observations indicate that numbers of P. thornei were low during the dry summer period, and in the wet winter season, but high during autumn with the commencement of the rainy period. Thus to ascertain the influence of soil moisture on invasion of clover plants, the sintered funnel method was used to obtain the required suction potentials in sand (Wallace, 1966).

O'Connor strain of strawberry clover seedlings germinated from surface sterilized seed, were potted into 4 cm diam. by 10 cm plastic propagation tubes in 150-250 μ particle size sterilized sand and given full strength Hoagland's solution.

After the plants became established (10 days) the tubes were placed vertically on the sintered plate of a large 9 cm funnel. The space between the tubes was filled with sterile sand of smaller particle size to keep the tubes

Figure 26. The influence of temperature on the emergence of *P. thornei* from clover roots in water.

FIG. 26



70
60
50
40
30
20
10
0
NUMBERS OF P. thornei EMERGING
15°
25°
35°
5°
0
24
48
72
96
120
TIME (Hours)

upright.

A layer of "Perlite" which remains moist because of its porous crumbs was placed on the surface of the sand in the tubes to prevent the rapid surface drying of the sand. The tops of the funnels were covered with transparent polythene to maintain moist growing conditions. The funnel and side arm of the apparatus were filled with sterile distilled water and the suction potentials were adjusted and maintained at the required potentials of 30, 40, 50, 60 and 90 cm of water (Fig. 27). Four replications were used in each treatment. After seven days one ml aliquots of an aqueous solution of 1000 P. thornei nematodes were pipetted into the sand at the base of the plant.

The funnels were kept in a glasshouse where temperatures were controlled to fluctuate between 30°C during the day and 20°C during the night. No supplementary lighting was given. At the end of the experiment, seven days later, the seedlings were washed from the sand, blotted dry and top and root weights recorded. Roots were stained and all the nematodes counted by crushing the roots in clear lactophenol between two pieces of flat glass.

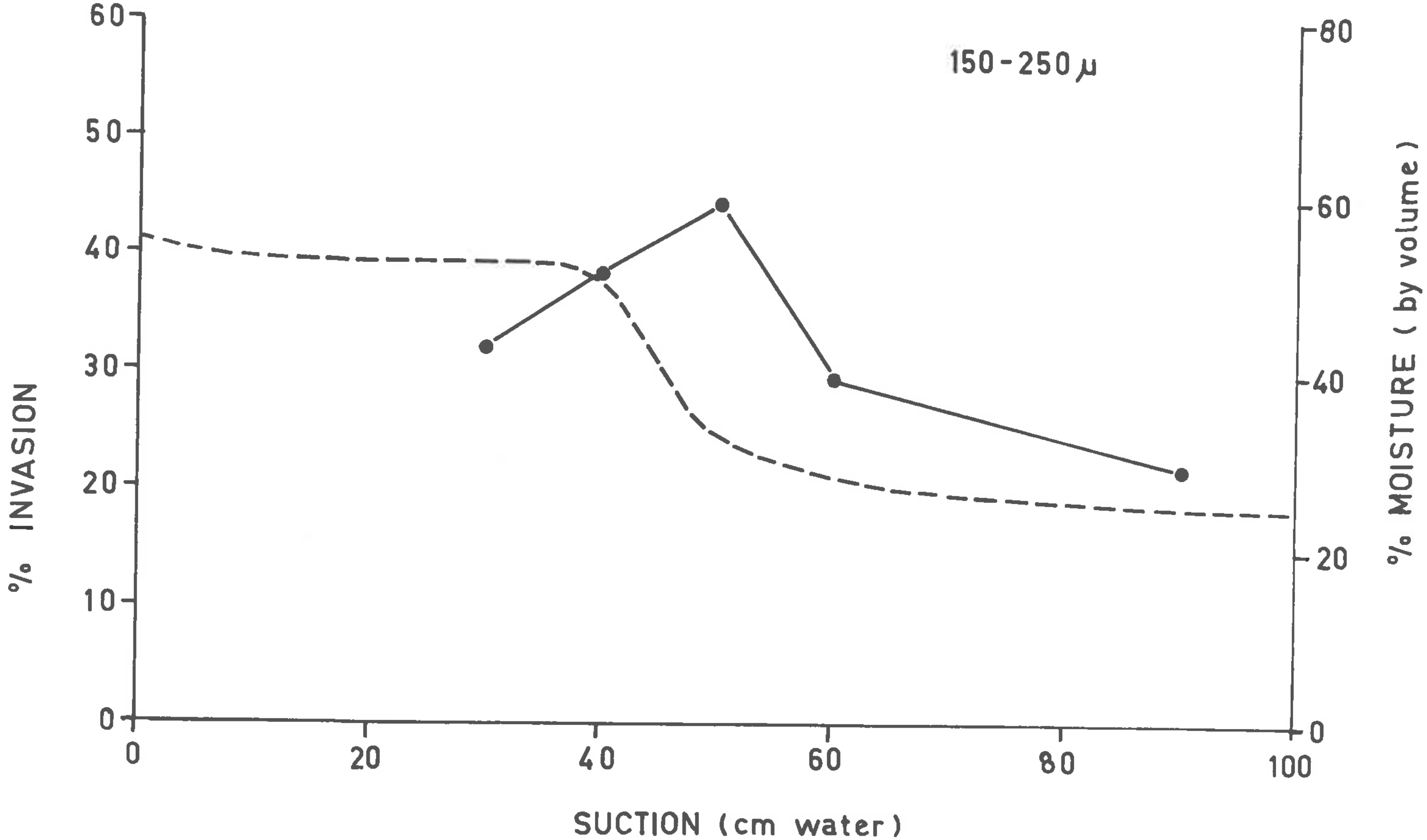
The results are shown in Fig. 28. The mean numbers of nematodes invading the roots transformed to arc sines are:

Figure 27. Sintered funnel apparatus to maintain suction in soil around plant roots.

FIG. 27

Figure 28. The influence of soil suction on the invasion of strawberry clover roots by *P. thornei*. The dotted line indicates the moisture characteristic of the sand (150-250 μ particle size) in which the plants were grown.

FIG. 28



150-250 μ
60
50
40
30
20
10
0
% INVASION
80
60
40
20
0
% MOISTURE (by volume)
0
20
40
60
80
100
SUCTION (cm water)

Suction (cm)	Nos.	
30	34.20	L.S.D.$_{.05}$ = 3.04
40	38.08	L.S.D.$_{.01}$ = 4.26
50	41.60	L.S.D.$_{.001}$ = 6.02
60	33.09	
90	27.82	

Numbers invading are relatively low at 30 cm suction potential, increasing at 40 cm, and reaching a peak at 50 cm followed by a drop at 60 and 90 cm.

From the results above and the L.S.D.'s it can be seen that all adjacent points are significantly ($P < .05$) different from each other - in fact between 50 cm and 60 cm it is highly significant ($P < .001$). By comparing the graph of invasion with the moisture characteristic of the medium (Fig. 28) it is evident that maximum penetration occurs at 50 cm suction at which point most of the pores have drained although there is still water in the smaller pores, i.e. it is in the region of field capacity. Such conditions occur after rainfall. At suction potentials lower than 50 cm, correspondingly more pores are filled resulting in conditions which are unfavourable for movement (Wallace, 1968).

Lack of aeration is unlikely to be a significant factor here, as Van Gundy and Stolzy (1963) showed that a suction of 30 millibars drained a sufficient number of pores to allow maximum oxygen diffusion. This means that the maximum effect of increased aeration will occur at suctions that are lower than those necessary to drain all the larger pores. With suctions above 50 cm, there is probably insufficient moisture to allow nematode movement. Thus suction potential or soil moisture is probably one of the most important physical factors influencing nematode activity in the soil. The present study confirms Wallace's (1966) studies on *M. javanica*.

(f) Summary

The results suggest that soil physical factors may be of primary importance in determining migration and invasion. Other factors such as oxygen deficiency and biological factors, however, cannot be overlooked. The increased survival and hence higher numbers in clay loam soils than in sandy soils during the dry summer season when there is a higher moisture tension, may be due to the fact that clay soils, because they contain fine capillaries, retain water more efficiently than sandy soils at high moisture tension; in the sands, however, there are relatively few fine capillaries (Willis, 1960). Of the biological factors, Johnston (1957, 1958) and Rodriguez-Kabana *et al*. (1965) have shown that toxins produced by anaerobic bacteria can cause death of nematodes in saturated soils.

Notwithstanding these factors, the physiological status of the host plant can influence the population rate of P. penetrans (Dolliver, 1961). Thus it is possible that the soil physical factors influence the numbers of nematodes indirectly through their influence on the physiological processes of the host plant. Morphological, anatomical and physiological changes in the roots can result from very wet or very dry conditions (Crafts, Currier and Stocking, 1949), and so influence nematode numbers especially those feeding on particular root tissues. Also, root growth in certain soils has been observed frequently to be hindered or entirely prevented by unfavourable soil structure. Aubertin and Kardos (1965) found that under experimental conditions roots did not grow into rigid porous systems which had pore diameters smaller than approximately the root diameter. If the pore space was large enough then the root became deformed to some extent as it grew around the immovable particles. But under a non-rigid system, roots will penetrate regardless of pore diameter by means of particle displacement. It is possible therefore that physiological changes in the roots caused by mechanical impedence could influence the attractiveness of the roots and their suitability for invasion by nematodes. The possibility of biochemical processes may be involved. It has been shown that polyphenols and related compounds in plant tissues often increase disease resistance (Farkas and Király, 1962) and that there is a much higher phenol content in a poor host than in a good host of P. penetrans (Macdonald, 1966).

CHAPTER V

DISCUSSION

It was stated in the Introduction that the purpose of this research was to study the influence of environmental factors on the numbers of *Meloidogyne javanica* and *Pratylenchus thornei* in the soil and within the roots of host plants. In this chapter some generalisations relevant to the ecology and plant pathological aspects of these two nematodes will be made and the questions that the work has raised will be discussed.

I. The Influence of Environment on *Pratylenchus thornei*

Environment is defined here as the total of all the factors that influences the nematode's survival, and one of the purposes of the research on *P. thornei* was to determine those factors that most influenced the population density of the nematode. The flow diagram of immediate effects (Fig. 8) indicates how environment might affect the nematode; the results of the work just described indicate that water and, to a lesser extent, available pore space were important. The other key factor influencing the nematode was probably the plant itself. However, as explained previously, measurements on plant growth were not done for practical reasons;

it was thought initially that because the plant grew little during the winter it would not play an important part in determining the differences in numbers between sites or with time. The results indicated that between sites, differences could be attributed mostly to soil type. In accounting for variations in population density as time progressed it became clear that components such as temperature, rainfall and precipitation-evaporation ratio were unimportant compared with some other unmeasured factor. It seems likely that this factor was the plant. Presumably, the volume and metabolism of the roots of strawberry clover show marked changes with season that influences them as a substrate for nematode nutrition. It is also possible that the nematode counts from roots and soil were so variable that a significant correlation was not obtained; this is unlikely however in view of the highly significant correlations between numbers and the percent clay in the soil from the different sites.

The higher numbers of *P. thornei* in clay soils was one of the most clearcut results of this research. The black clay of granular structure at Strathmont with its low evapotranspiration rate contained the most nematodes probably for the following reasons: (1) Clays, because of their smaller pore spaces within and between crumbs, retain water more firmly than the sandy soils and thereby alleviate death by desiccation. (2) Strawberry clover grows better on the clay soils possibly because of increased nutrients but more importantly because the clay soils offer the root system protection from desiccation as well. The largest root concentration was at 0-5 cm depth (Fig. 7) and in the hot South Australian summers sandy soils soon lose water to this depth even when irrigated. Thus the key factor is water.

Differences in population density between the sampling sites were closely correlated with soil type but the real cause was probably difference in soil water which was also associated with soil type. On the other hand the key factor determining changes in nematode numbers with time at each site was probably plant growth as mentioned previously, but what were the factors most influencing plant growth?

There is little information on the nutritive value of roots in general, and in particular to nematodes. Plant analyses usually refer to entire root systems including old parts which may have accumulated metabolites or excretions. Thus they do not show the composition of the young fibrous roots where invasion by nematodes is more likely to occur. The extent and chemical properties of the roots are liable to be influenced by variations of the soil conditions. For the efficient function of the plant there is an important, fairly constant ratio of equilibrium between the root system relative to shoot (Rogers and Booth, 1960). The environmental factors mainly responsible for determining this ratio are water conditions, light and nitrogen supply. The water factor is most important as it determines the oxygen and mineral nutrient supply to the roots. Root morphology is influenced by moisture; in a dry soil a root rapidly extends over a large distance but branching is reduced whereas in soil of high water content, roots are short and profusely branched. Drought conditions give a reduced top growth and a high root/shoot ratio, whereas excess water gives excess top growth and decreased root/shoot ratio until shortage of oxygen eventually limits the development of the plant. The factors of light and nitrogen supply determine the balance of carbohydrate to nitrogen ratio which in turn regulates the growth of the roots and hence the root/shoot ratio. Also, high nitrogen supply causes changes in the chemical and morphological properties of the roots which may affect the ability of the plant to withstand and inhibit nematode infection, and the value of the root as a food source. Similarly, light intensity, by influencing photosynthesis, affects the carbohydrate supply. Reduced carbohydrate means less is available for cell wall synthesis. The cell walls become weaker and their mechanical strength is reduced (Burström, 1958). Also, it has been observed that root exudates from plants under water stress contain less kinetin activity (Itai and Vaadia, 1965). All these factors conceivably could influence the attractiveness of roots to nematodes. Thus moisture together with temperature play an important part; perhaps their influence on the nematode was too indirect to give a significant correlation, or more likely the data were too variable. There is a good chance that weather factors influenced seasonal changes of nematode numbers to a marked degree although the indirect nature of the influence may make it more difficult to establish a correlation. Further research is required on these aspects.

II. Invasion of Roots by *Meloidogyne javanica*

The present work indicated two important features of infection by *Meloidogyne javanica*: (1) Most nematodes entered the root during the first 24 hours under the experimental conditions. (2) Numbers invading continued to increase for 6 days, thereafter numbers declined. The invasion process appears to be rapid and efficient so that more nematodes enter the host root than there are feeding sites. If, as seems possible, the nematodes that fail to establish themselves vacate the root, then they may have a further chance of re-invading another root. Such an interpretation of the results indicates that it would be worthwhile to establish if the concept of a feeding site is a useful one; are there in fact only a limited number of cells capable of becoming giant cells and providing the developing nematode with nutrient? Equally plausible is the hypothesis that the plant responds to infection by some change in its physiology that acts as a signal to nematodes that have not established themselves in the root to vacate the root. Further study is required to ascertain whether such a defence mechanism exists.

The other results on infection by *Meloidogyne javanica* show that the nematode has only a limited time (about 4-11 days) after which it is incapable of invading the plant. Thus nematodes that enter and subsequently leave probably have less chance of re-invading another root unless they feed while in the root and make up for body reserves lost during previous activities in reaching and penetrating the root. Further work is needed to elucidate this point.

The fact that fewer *Meloidogyne javanica* invaded tomato roots of the resistant variety HES 4242 than the susceptible Tatura Dwarf indicates that resistance may be due to a lack of attraction to the root, or to a repellant or that the nematodes vacated the root soon after invasion. Whichever mechanism operates it is evident that the generalisation that resistant and susceptible plants are invaded by nematodes to the same extent does not apply to tomato var. HES 4242 and *Meloidogyne javanica*. The experiments on the influence of nematode numbers on invasion also indicate that low inoculum densities and increased root size are not always associated with high percent infection as suggested by Wallace (1966).

III. A Comparison of the Ecology of *Pratylenchus thornei* and *Meloidogyne javanica*

One of the major differences between these two species is their response to temperature. *Pratylenchus thornei* has an optimum temperature in the region of 15°C or higher. This probably explains to a large extent why populations of *Pratylenchus thornei* increase during the winter in South Australia, whereas *Meloidogyne javanica* is most noticeable in the summer.

The other major difference between the two species is their relation to soil type. *Pratylenchus thornei* occurs more abundantly in clay soils as this research project has shown, whereas *Meloidogyne javanica* occurs most frequently in sandy soils. This difference cannot be explained by their mobility characteristics in the different soil types because results from the experiment relating invasion and soil moisture for *Pratylenchus thornei* closely resemble those for *Meloidogyne javanica* as do the temperature-soil type interaction results. The explanation may lie once again in their temperature requirements. It is possible that *Meloidogyne javanica* is able to tolerate the higher temperatures of the South Australian summer and hence is more prevalent in irrigated sandy soils. *Pratylenchus thornei* has a much lower temperature requirement and in summer its chief hazard is desiccation. Hence clay soils offer a more secure environment. Thus it seems possible that desiccation is less a hazard for *Meloidogyne javanica* because of its higher temperature requirements and because its eggs are enclosed in an egg sac, consequently it can thrive in sandy soils. Such an environment is far more of a hazard to *Pratylenchus thornei* which is therefore confined to heavier soils. However, further experiments are needed before such a hypothesis can be accepted.

IV. Disease Assessment

Although it was stated in the Introduction that this research was more concerned with ecology than with plant pathology, the results provide some useful pointers in the latter field. The scheme adopted in this project was to measure the nematode's environmental components and by regressional and covariance analyses determine what factors have most influence on nematode numbers. The same procedures could be used to assess the influence of the plant's environment on crop yield. The nematode would be part of that environment with the plant at the centre of the interacting web of components. By this means variations in crop growth in different localities and within a crop during its growing season could be correlated with the factors that inhibited it. Thus it could be established whether nematodes were an important factor inhibiting growth and yield. There was no obvious indication from observations in the eight sites in the Adelaide area that *Pratylenchus thornei* was ever the cause of reduced growth of strawberry clover. The reasons are not known, but it is possible that in the South Australian climate the clay soil environment favours the plant more than the nematode; clover flourishes in summer under irrigation when temperatures are too high for the nematode, whereas in winter the clover becomes dormant, and although the nematode increases in numbers the roots may not provide the sort of nutrient that enables the nematode to increase to damaging levels. With *Meloidogyne javanica* on the other hand, the summer temperatures, together with irrigation, provide optimum conditions and consequently there is a great deal of damage in a wide variety of crops in South Australia. Thus once it becomes known just why one or another species of nematode is to be found in a particular niche at a particular time, the possibility arises for disturbing in some way the environment of an undesirable nematode to the benefit of crop yield, thereby achieving some measure of control.

V. The Ecology of Nematodes

The study of the ecology of *Pratylenchus thornei* and *Meloidogyne javanica* involved measurements of nematode numbers, their rates of movement and their infectivity; it also included measurements of the plant and of the environment. These three parts of the ecological system are only a small fraction of a very complex web of interacting factors, and one of the main problems of ecology is to reduce this complexity to some kind of simple picture containing a few major key factors. The key factors that influence *Pratylenchus thornei* in soil and in roots of strawberry clover have been described on the basis of regression analyses. The correlations between two factors do not indicate a causal relationship however. From the data available, no one regulating factor can be applied to populations of *Pratylenchus thornei*. This reflects a lack of information about some factors that influence population processes, but it also seems likely that a few particular factors, for example soil type and survival under dry conditions, exert their influences more strongly than other factors such as speed of movement. Consequently, Wallace's (1968) hypothesis that nematodes will increase in numbers when conditions for movement are optimal, overlooked the possibility that some other characteristic of the nematode such as survival from desiccation might be more important in some environments. It would be informative to study the behavioural pattern of the nematode under these extreme conditions of the hot dry South Australian summer. Do the nematodes migrate downwards in the hot dry season? Or do they undergo a reduction of metabolic activity induced by this environmental stress and thereby survive this stage either in the soil or within dried host tissue in a state of quiescence or even cryptobiosis? Baxter and Blake (1968) found that only 4% of Pratylenchus thornei survived 20 weeks of drying to 5% moisture by weight. But it is possible that this number is sufficient to re-establish a population when environmental factors become favourable. For non-feeding nematodes, survival under favourable conditions is dependent on the conservation of energy which is mainly in the form of stored lipids, but prolonged survival under unfavourable conditions is not understood (Cooper and Van Gundy, 1971). It may even be conceivable that Pratylenchus thornei may be able to feed on soil fungi, as some soil fungi can grow quite favourably at moisture tensions of pF5.5 (Griffin, 1969).

In an ecological system where several factors are measured, there are sometimes interactions between factors; two factors acting together may have a different effect from what might be expected if we considered them separately. The influence of temperature and soil type on the downward migration of *Meloidogyne javanica* and *Pratylenchus thornei* is an example. In these experiments (Figs. 24 and 25) mobility increased in sand as the temperature increased from 10 to 30°C. In clay however the reverse was true, the nematodes were most mobile at 10° and least mobile at 30°C. It seems likely that there is a third factor operating here that is the cause of this result, microorganisms. In the mobility experiments saturated columns of soil were used hence bacteria, for example, might be expected to deplete the oxygen in the soil at a greater rate at 30 than at 10°C, hence the better aeration in the clay at the lower temperature might more than offset the reduced nematode mobility at that temperature. In the sand, where there are usually fewer microorganisms, the reduction in oxygen concentrations is probably less and so the increased mobility response to increased temperature occurred. In dealing with the ecology of nematodes in soils that tend to be very wet, it would therefore be necessary to consider microorganisms and soil aeration as two important environmental components. The microorganism aspect also provides a further possible explanation why *Pratylenchus thornei* is more abundant on clay soils. For a nematode to live in clay soils it is possible that it should have a low temperature tolerance range otherwise at higher temperatures the reduced aeration caused by activity of microorganisms would inactivate the nematode.

It is also possible of course that *Pratylenchus thornei* has a greater tolerance to low oxygen concentration than *Meloidogyne javanica*. These questions require further research before they can be answered.

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BIBLIOGRAPHY

AUBERTIN, G.M. & KARDOSS, L.T. (1965). Root growth through porous media under controlled conditions: I. Effect of pore size and rigidity.
Proc. Soil Sci. Soc. America 29, 290-293.

BAXTER, R.I. & BLAKE, C.D. (1968). Pratylenchus thornei - a cause of root necrosis in wheat.
Nematologica 14, 351-361.

BERKELEY, M.J. (1855). [Vibrio forming cysts on cucumber.]
Gardener's Chronicle 14, 220.

BURSTRÖM, H. (1958). The influence of growth regulators on composition of the cell wall.
Fysiogr. Sällsk. (Lund) Förh. 28, 53-64.

BUTLER, P.F. & PRESCOTT, J.A. (1955). Evapotranspiration from wheat and pasture in relation to available moisture.
Aust. j. agric. Res. 6, 52-61.

CHILDS, E.C. (1940). The use of soil moisture characteristics in soil studies.
Soil Sci. 50, 239-52.

CHICHORIUS H.-D. (1960). Über das auftreten Freilebender Wurzelnematoden in Rheinschen Böden unter besonderer berücksichtigung der Standortverhältnisse.
Nematologica 5, 231-252.

COOPER, A.F. (Jr.) & VAN GUNDY, S.D. (1971). Senescence, Quiescence and Cryptobiosis. In "Plant Parasitic Nematodes" (B.M. Zuckerman, W.F. Mai and R.A. Rohde, eds.) pp 297-318.
Academic Press N.Y.

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

CRAFTS, A.S., CURRIER, H.B. & STOCKING, C.R. (1949). Water in the physiology of plants.
Chronica Botanica Co., Waltham, Mass., 240 pp.

CURL, E.A. (1961). Influence of sprinkler irrigation and four forage crops on populations of soil microorganisms including those antagonistic to *Sclerotium rolfsii*.
Pl. Dis. Reptr 45, 517-519.

DECKER, H. (1960). *Pratylenchus penetrans* als Ursache von 'Müdigkeitserscheinungen' in Baumschulen der DDR.
Nematologica (Suppl. II), 68-75.

DICKERSON, O.J., DARLING, H.M. & GRIFFIN, G.D. (1964). Pathogenicity and population trends of *Pratylenchus penetrans* on potato and corn.
Phytopathology 54, 317-322.

DI EDWARDO, A.A. (1961). Seasonal population variations of *Pratylenchus penetrans* in and about strawberry roots.
Pl. Dis. Reptr 45, 67-71.

DOLLIVER, J.S. (1961). Population levels of *Pratylenchus penetrans* as influenced by treatments affecting dry weight of Wando pea plants.
Phytopathology 51, 364-367.

ENDO, B.Y. (1959). Responses of root-lesion nematodes, *Pratylenchus brachyurus* and *P. zeae*, to various plants and soil types.
Phytopathology 49, 417-421.

FARKAS, G.L. & KIRALY, Z. (1962). Role of phenolic compounds in the physiology of plant diseases and disease resistance.
Phytopath. Z. 44, 105-150.

FERRIS, V.R. & BERNARD, R.L. (1967). Population dynamics of nematodes in fields planted to soybeans and crops grown in rotation with soybeans. I. The genus *Pratylenchus* (Nemata:Tylenchida).
J. econ. Ent. 60, 405-410.

GODFREY, G.H. (1929). A destructive root disease of pineapples and other plants due to Tylenchus brachyurus n.sp.
Phytopathology 19, 611-629.

GOHEEN, A.C. & WILLIAMS, C.F. (1955). Seasonal fluctuations in the population of meadow nematodes in the roots of cultivated brambles in North Carolina.
Pl. Dis. Reptr 39, 904-905.

GOODEY, J.B. (1963). Laboratory methods for work with plant soil nematodes.
Tech. Bull. No.2, Ministry Agriculture (4th edition) Lond. 72 pp.

GRAHAM, T.W. (1951). Nematode root-rot of tobacco and other plants.
Sth. Carolina Agr. Exp. Sta. Bull. 390: 25 pp.

GRIFFIN, D.M. (1969). Soil water in the ecology of fungi.
A. Rev. Phytopath. 7, 289-310.

HASTINGS, R.J. (1939). The biology of the meadow nematode Pratylenchus pratensis (De Man) Filipjev 1936.
Can. J. Res. Ser. D. 17, 39-44.

HUTTON, J.T. (1955). A method of particle size analysis of soils.
C.S.I.R.O. Soils Divisional Report No. 11/55 Adelaide.

ITAI, C. & VAADIA, Y. (1965). Kinetin-like activity in root exudate of water stressed sunflower plants.
Physiologia Pl. 18, 941-944.

JENKINS, W.R. (1964). A rapid centrifugal-flotation technique for separating nematodes from soil.
Pl. Dis. Reptr 48, 692.

JOHNSTON, T. (1957). Further studies on microbiological reduction of nematode population in water-saturated soils.
Phytopathology 47, 525-526. (Abstr.)

JOHNSTON, T. (1958). The effect of soil moisture on *Tylenchorhynchus martini* and other nematodes. Proc. Louisiana Acad. Sci. 20, 52-55.

KABLE, P.F. & MAI, W.F. (1968). The influence of soil moisture on *Pratylenchus penetrans*. Nematologica 14, 101-122.

KINCAID, R.R. (1946). Soil factors affecting incidence of root knot. Soil Sci. 61, 101-109.

KOEN, H. (1967). Notes on the host range, ecology and population dynamics of *Pratylenchus brachyurus*. Nematologica 13, 118-124.

LOWNSBERY, B.F. (1956). *Pratylenchus vulnus*, primary cause of the root-lesion disease of walnuts. Phytopathology 46, 376-379.

MACDONALD, D.H. (1966). Some relationships between chemical composition of plant roots and the population levels of *Pratylenchus penetrans*. Nematologica 12, 95 (Abstr.).

MACFADYEN, A. (1968). 'The measurement of climate in studies of soil and litter animals' in Wadsworth, R.M. (Edt.) The Measurement of Environmental Factors in Terrestrial Ecology 59-67.

MOUNTAIN, W.B. & BOYCE, H.R. (1958). The peach replant problem in Ontario. V. The relation of parasitic nematodes to regional differences in severity of peach replant failure. Can. J. Bot. 36, 125-134.

NAKAYAMA, F.S. & VAN BAVEL, C.H.M. (1963). Root activity distribution patterns of sorghum and soil moisture conditions. Agron. J. 55, 271-274.

NEWMAN, E.I. (1966). A method of estimating the total length of root in a sample. J. appl. Ecol. 3, 139-145

O'BANNON, J.H. & REYNOLDS, H.W. (1961). Root knot nematode damage and cotton yields in relation to certain soil properties.
Soil Sci. 92, 384-386.

OLTHOF, TH. H.A. (1967). Seasonal fluctuations in soil populations of Pratylenchus penetrans under tobacco in Ontario.
Nematologica 13, 148 (Abstr.).

OOSTENBRINK, M. (1954). Over de betekenis van vrijlevende wortelaaltjes in land- en tuinbouw.
Versl. Meded. Pl. ziekt. Dienst Wageningen 124, 196-233.

PATTERSON, M.T. & BERGESON, G.B. (1967). Influence of temperature, photoperiod and nutrition on reproduction male-female-juvenile ratio and root to soil migration of Pratylenchus penetrans.
Pl. Dis. Reptr 51, 78-82.

PENMAN, H.L. (1948). Natural evaporation from open water, bare soil and grass.
Proc. Roy. Soc. A 193, 120-146.

PENMAN, H.L. (1949). The dependence of transpiration on weather and soil conditions.
J. Soil Sci. 1, 79-89.

PRESCOTT, J.A. (1949). Climatic expressions and generalized climatic zones in relation to soils and vegetation.
Proc. Specialist Confr. Agriculture, Australia, 1949.

PRESCOTT, J.A. (1956). Macro- and micro-climatic factors in relation to pastures.
7th Intr. Grasslands Congress; p24-32.

RODRIQUEZ-KABANA, R., JORDAN, J.W. & HOLLIS, J.P. (1965). Nematodes: biological control in rice fields: role of hydrogen sulfide.
Science, N.Y. 148, 524-526.

ROGERS, W.S. & BOOTH, G.A. (1960). The roots of fruit trees.
Sci. Hort. 14, 27-34.

SASSER, J.N. (1954). Identification and host-parasite relationships of certain root-knot nematodes (*Meloidogyne* spp.).
Uni. Maryland Agr. Exp. Stat. Bull. A-77 (Tech). 30pp.

SLAYTER, R.P. (1960). Agricultural climatology of the Yass Valley.
C.S.I.R.O. Div. of Land Research and Regional Survey, Tech. Paper No.6.

STOLZY, L.H., VAN GUNDY, S.D. & LETEY, J. (1960). Oxygen tolerances of four plant-parasitic nematodes.
Phytopathology 50, 656.

THORNTHWAITE, C.W. (1948). An approach towards a rational classification of climate.
Geogrl Rev. 38, 55-94.

VAN GUNDY, S.D. & STOLZY, L.H. (1963). Oxygen diffusion rates and nematode movement in cellulose sponges.
Nature Lond. 200, 1187-1189.

VAN GUNDY, S.D., BIRD, A.F. & WALLACE, H.R. (1967). Ageing and starvation in larvae of *Meloidogyne javanica* and *Tylenchulus semipenetrans*.
Phytopathology 57, 559-571.

VAN GUNDY, S.D., McELROY, F.D., COOPER, A.F. & STOLZY, L.H. (1968). Influence of soil temperature, irrigation and aeration on *Hemicycliophora arenaria*.
Soil Sci. 106, 270-274.

WALLACE, H.R. (1958). Movement of eelworms. I. The influence of pore size and moisture content of soil on the migration of larvae of the beet eelworm, *Heterodera schachtii* Schmidt.
Ann. appl. Biol. 46, 74-85.

WALLACE, H.R. (1961). The bionomics of the free-living stages of zoo-parasitic and phyto-parasitic nematodes - a critical survey.
Helminthological Abstracts 30, 1-22.

WALLACE, H.R. (1963). The biology of plant parasitic nematodes.
Arnold, London, 280 pp.

WALLACE, H.R. (1966). Factors influencing the infectivity of plant parasitic nematodes.
Proc. Roy. Soc. (London), Ser. B., 164, 592-614.

WALLACE, H.R. (1968). The dynamics of nematode movement.
A. Rev. Phytopath. 6, 91-114.

WALLACE, H.R. (1969). The influence of nematode numbers and of soil particle size, nutrients and temperature on the reproduction of *Meloidogyne javanica*.
Nematologica 15, 55-64.

WALLACE, H.R. (1970). Some factors influencing nematode reproduction and the growth of tomatoes infected with *Meloidogyne javanica*.
Nematologica 16, 387-397.

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

WEHUNT, E.J. (1957). Population trends of nematodes associated with white clover in Louisiana. (Abst.)
Phytopathology 47, 36.

WILLIS, W.O. (1960). Evaporation from layered soils in the presence of a water table.
Proc. Soil Sci. Soc. America 24, 239-242.

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

YEATES, G.W. (1968). An analysis of annual variation of the nematode fauna in dune sand, at Himatangi Beach, New Zealand.
Pedobiologia 8, 173-207.

YUEN, P.-H. (1966). The nematode fauna of the regenerated woodland and grassland of Broadbalk wilderness.
Nematologica 12, 195-214.

Grandison, G. S. (1963). The clover cyst nematode (Heterodera trifolii Goffart) in New Zealand: (a note). *New Zealand Journal of Agricultural Research, 6*(5), 460-462.

Grandison, G. S. (1964). A new species of Doryllium (Nematoda Dorylaimida) from Auckland and Campbell Islands. *New Zealand Journal of Science, 7*(2), 169-173.

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Grandison, G. S. (1965). The stem nematode (Ditylenchus dipsaci) in clovers in New Zealand: (a note). *New Zealand Journal of Agricultural Research, 8*(4), 1090-1091.

Gibbins, L. N. & Grandison, G. S. (1966). An improved centrifugal-flotation technique for the isolation of Ditylenchus dipsaci. *Nematologica*, *12*(4), 642–643.

Gibbins, L. N. & Grandison, G. S. (1968). An assessment of serological procedures for the differentiation of biological races of Ditylenchus Dipsaci. *Nematologica*, *14*(2), 184–188.

Brewerton, H. V., McGrath, H. J. W. & Grandison, G. S. (1969). Thionazin residues on Lucerne and red clover (experiments for control of Ditylenchus dipsaci). *New Zealand Journal of Agricultural Research, 12*(1), 171-176.

Taylor, J. B. & Canter-Visscher, T. W. (1970). Prolonged control of Verticillium wilt and root-lesion nematodes by soil fumigation. *New Zealand Journal of Science*, *13*(4), 591-601.