THE DISTRIBUTION AND ABUNDANCE OF NEMATODES (especially the Plant Parasites) IN THE ARID REGION OF SOUTH AUSTRALIA.

by

J. M. Nobbs.

Department of Plant Pathology,

Waite Agricultural Research Institute,

The University of Adelaide,

South Australia.

Thesis submitted to the Universiy of Adelaide in fulfilment of the requirements for the Degree of Doctor of Philosophy.

September 1987.
Awarded 26/11/87

Acknowledgements.

I wish to thank Professor H. R. Wallace who supervised me during this work : his advice, encouragement and direction are gratefully acknowledged. I also wish to thank Dr. J. M. Fisher for his advice during glasshouse and laboratory experiments and Dr. Siddiqi and Mrs. F. Reay for taxonomic assistance.

Appreciation is given to Mr. G. Gloster and family for their aid in making the pastoral property, Plumbago Station, available for extensive field experiments and thanks are extended to all those managers and owners of pastoral properties who extended permission to sample on their property.

I wish to thank the Australian Bureau of Flora and Fauna for the travel scholarship which allowed the extensive field survey of the arid region to proceed. I would also like to thank the University of Adelaide for the award of a University Research Grant for the final 2 and a half years of work.

The contribution of the Biometry department and Word Processing Section is gratefully acknowledged. I express my appreciation for the assistance given by the students and staff of the Department of Plant Pathology, at the Waite Institute.

I would also like to express my deep appreciation of my parent's willingness to accompany me on my field excursions and their encouragement and support during the period of my study.

Declaration.

I hereby declare that this thesis contains no material which has been accepted for the award of any degree or diploma in any University. To the best of my knowledge and belief, no material described herein has been published or written by another person except when due reference is made in the text.

I give consent for this copy of my thesis, when deposited in the University library, to be available for loan and photocopying.

Candidate's Signature

8/9/07

Date

TABLE OF CONTENTS.

SUMMARY.

The composition of the plant parasitic nematode community in the arid region of South Australia was determined from a general field survey. Most species of plant parasitic nematodes identified had been described from other areas of Australia, but rarely outside Australia, indicating that they are probably endemic. Some new species, but no new genera, were found and the plant nematodes found were mainly migratory ectoparasites.

A system of classifying each site in relation to vegetation and landform characters was developed from existing systems, thereby enabling analysis to be made of relationships between particular site characteristics and the frequency of occurrence of particular plant parasitic nematodes. *Tylenchorhynchus tobari* was the most abundant and widely distributed plant nematode within the arid region of South Australia and an association was found between the distribution of *Atriplex vesicaria* and the nematode. *T.tobari* was successfully cultured on selected species of *Atriplex* (salt bush) and the biology and host/parasite relationships of the nematode were investigated. *T.tobari* had little or no effect on the growth of an ephemeral saltbush (*A.spongiosa*); *A.vesicaria* was found to be an unsuitable experimental plant. The duration of the lifecycle of *T.tobari*, its feeding behaviour and embryology were similar to other *Tylenchorhynchus* species. It is suggested that the lack of damage to the host plant may be associated with the feeding behaviour of *T.tobari*.

The association between nematode trophic groups and plant species was also investigated, with the sampling of particular areas of similar or different vegetation/landform formations. Particular plant parasitic nematodes (other than *T.tobari*) were found to be associated with specific vegetation/landform formations and host plants. The bacterial feeders were the most abundant trophic group in the arid region of S.A., with the other trophic groups occurring in differing proportions, depending on the site sampled. Survival of nematodes over time was also investigated. The method of survival of nematodes appeared to be associated with coiling. When soil was sampled during a 'dry' period, the total numbers of nematodes were low, as was the proportion of straight to coiled nematodes. When moist soil was sampled, the proportion of straight to coiled nematodes increased, with a corresponding increase in the total number of nematodes.

This study indicated that the structure of the nematode community in the arid region of S.A. is similar to that in other arid or desert regions. This study concentrated on determining associations and distribution patterns between plant nematodes, other trophic groups and host plants. A discussion of the influence of soil nematodes on the ecology of arid regions is presented in the concluding chapter, as well as some views on possible dispersal mechanisms and the evolution of particular plant parasitic nematodes.

CHAPTER 1. INTRODUCTION.

This study was aimed at investigating the composition, distribution and association of plant parasitic nematodes in soil from the arid region of South Australia. The arid regions of the world are characterised by the patchiness of precipitation and the relationship between adequacy of rainfall and a hypothetical evapotranspiration of natural vegetation (McGinnies et al., 1968). The nematode is fundamentally an aquatic animal (Jenkins and Taylor, 1967) whose main response to dehydration is to form a quiescent or anhydrobiotic state (Cooper and Van Gundy, 1971), the ability and form of which varies between species. The anhydrobiotic state (or coiling) can be induced in some species by using chambers in which the relative humidity can be altered (Demeure et al., 1979a;Ellenby, 1975; Huang and Huang, 1974; Roessner and Perry, 1975; Safed and Roessner, 1984; Townsend, 1984). One of the aims of this study was to investigate the changes in numbers of nematodes over time in the field, and to determine the ability (and form) of the nematodes which were able to survive desiccation. This subject has received little attention in Australia.

The composition of terrestrial nematode communities varies within natural and artificial ecosystems and between plants and plant communities. The distribution of nematodes in space is related to environmental influences, such as soil moisture and presence of food (Wallace, 1973; Whitford et al., 1983). There are four main trophic groups of nematodes (omnivore/predator, bacterial feeders, fungal feeders and plant parasites) which have been separated on their apparent food requirements. They are distinguished by the structure of the head and oesophageal regions which, for some species, have been shown to be specific for certain food sources. However, accurate knowledge of the precise food requirements of many soil inhabiting nematodes has not been determined. In this thesis, four trophic groups will be considered, although most emphasis will be placed on the plant parasitic Tylenchid nematodes. Classification of nematodes into trophic groups enables investigation of the association between the distribution of plant variables and nematodes.

Another factor which may influence the composition of the nematode fauna in Australia, is the isolation of the continent from invasion by animals and plants over a long time (most of the Tertiary) and the effect of a changing climate on those animals and plants. The Australian macro-fauna and the flora is largely endemic to Australia at the family, genus

and species level. Studies on the plant parasitic nematodes that occur in Australia, have shown that the majority of the pest species occur world-wide (Khair, 1986). However, most of the data came from surveys of agricultural crops and lands, hence many of these species may have been introduced to Australia in the last 200 years. Investigation of the native vegetation (Reay and Wallace, 1981a; Stirling and Vawdrey, 1985) has shown some association between climatic regions, host plants and certain plant parasitic nematodes. Few collections have been made in the arid region of South Australia, so little is known about the plant parasites occurring there. Consequently, it was decided that a significant amount of time would be spent on the identification and taxonomy of plant parasitic nematodes. It was considered likely that environmental stresses associated with the arid region (dehydration) and the presence of endemic flora, may have influenced the evolution of the Australian taxa at the genus and family level.

Within the soil ecosystem, microorganisms play an important role in the decomposition of organic matter (Daubenmire, 1974). These microorganisms also exhibit seasonal changes in activity and dormancy depending on environmental factors. Of the animals that occur in the soil (excluding Protozoa), nematodes constitute the greatest number of individuals and arthropods the greatest number of species (Dao, 1970). From studies on the role of soil organism (such as arthropods and nematodes) present in the Chihuahuan Desert of New Mexico, there is an apparent effect of rainfall and soil moisture on the abundance of these organisms (Wallwork et al., 1984; Whitford et al., 1981, 1983, 1985). Use of artificial rainfall and shading indicated that numbers of microarthropods increased with increased shade and rainfall (Mackay et al., 1986). There was, however, no apparent effect on the decomposition of leaf litter of creosote bush over a seven month period. With the use of biocides to eliminate target organisms within the soil, diagrams of the interaction between organisms and decomposition of nitrogen and carbon could be drawn (Parker et al., 1984). These diagrams indicated that bacterial feeding nematodes were the major consumers of bacteria in both the carbon and nitrogen decomposition cycles. The role of nematodes in the decomposition of surface litter was then found to be affected by rainfall, soil moisture and the activity of microarthropods (Whitford et al., 1985). Within the arid region of South Australia, resources did not permit the use of biocides to investigate this aspect of the role of

nematodes in the soil ecosystem, however, aspects of nematode abundance could be investigated.

Ferris (1982) postulated that the effect of nematodes on the soil ecology was not limited to numbers, size and weight (biomass), but also influenced by life-cycles, feeding behaviour, reproduction and survival strategies. The region adjacent to a plant root is a major source of nutrients for organisms (Dropkin, 1980). As with agricultural soils, the nematode community in the arid regions tend to occur close to the root systems of plants (Freckman and Mankau, 1977, and 1986). Any investigation of the distribution of nematodes involves the sampling of soil close to the plant root system and taking multiple samples from each site. To determine the effect of environment on abundance of nematodes, it is necessary to determine survival strategies.

Within the Chihuahuan desert, the presence of anhydrobiotic nematodes was observed to increase as leaf litter dried (Whitford et al., 1981). Before the application of artificial rainfall, the proportion of anhydrobiotic nematodes was between 78 and 97%. On application of rainfall, the proportion of anhydrobiotic nematodes decreased as the nematodes became active, but increased as the litter dried out. The study of active and anhydrobiotic nematodes was considered to be important in explaining changes in numbers of nematodes with time and the survival strategy of nematodes in a hostile environment.

Nematodes are seen as important in the soil ecosystem and appear to have an active and important part in the decomposition process in the arid or desert ecosystem. The Australian arid region is different from the American deserts, in being more diverse in vegetation (McGinnies et al, 1968), covering a wider area and in having been isolated from other land masses for a long time. An investigation of the composition and identity of plant nematodes in the arid region might, therefore, yield some information and ideas on their origin and evolution. In studying the nematode community in the arid region of South Australia the diversity of the vegetation needed to be accurately classified, and other environmental factors accounted for. Analysis and determination of any associations between these factors and nematode distribution could then be done.

Adequate sampling procedures are important in determining the factors that influence the distribution of any animal (Wallace, 1973). In this project, plant distribution

and abundance received particular attention. However, sampling needed to be accurate without being unwieldy (Goodell, 1982). Sample variation can be reduced by increasing the number of samples taken or sample size, but a point of diminishing returns can be reached in which effort to collect, extract, identify and count nematodes exceeds accuracy of sampling. The necessity of travelling long distances over poorly made roads, within a restricted period of time, reduced the number and amount of soil samples that could be transported back to the laboratory. However, the distribution of nematodes in the arid region of South Australia is considered at two levels ; the distribution of populations over a large area and the distribution of nematodes surrounding single plants and within small defined areas of vegetation .

The effect of plant parasites on native plants was also considered. Cultures of certain plant parasites needed to be established and appropriate hosts and environmental conditions for nematode growth investigated. Tolerance / intolerance and resistance / susceptibility of native plants to a sedentary nematode (*Meloidogyne javanica*) has been investigated (Reay and Wallace, 1981b). There was a range of reactions to the nematode by the native hosts. However, the reaction of native plants to native nematodes was sought, as this may aid in understanding the ecology of the arid regions of South Australia and other areas. It was considered that a culture of plant parasitic nematode from the arid region could be used to investigate its biology and also host/parasite relationships. The effect of nematodes on native plants in the field could then be explained in terms of the numbers of nematodes found to be associated with their hosts. Similarly, observations on the feeding behaviour of the nematode *in vitro* could be related to the influence of the nematode on its host plant in the field.

Given the constraints of 3 1/2 years to complete the thesis (the vast areas to be covered for samples and the lack of laboratory assistance) the questions that could be asked had to be realistic. Such questions were : Are nematodes distributed randomly within an area or are specific plant nematodes associated with particular host plants? What effect do the nematode and plant have on each other? Are there any evolutionary trends within specific families of plant parasitic nematodes? In spite of constraints in sampling this region, it seemed that useful information could be obtained about an area of Australia few have investigated but many use.

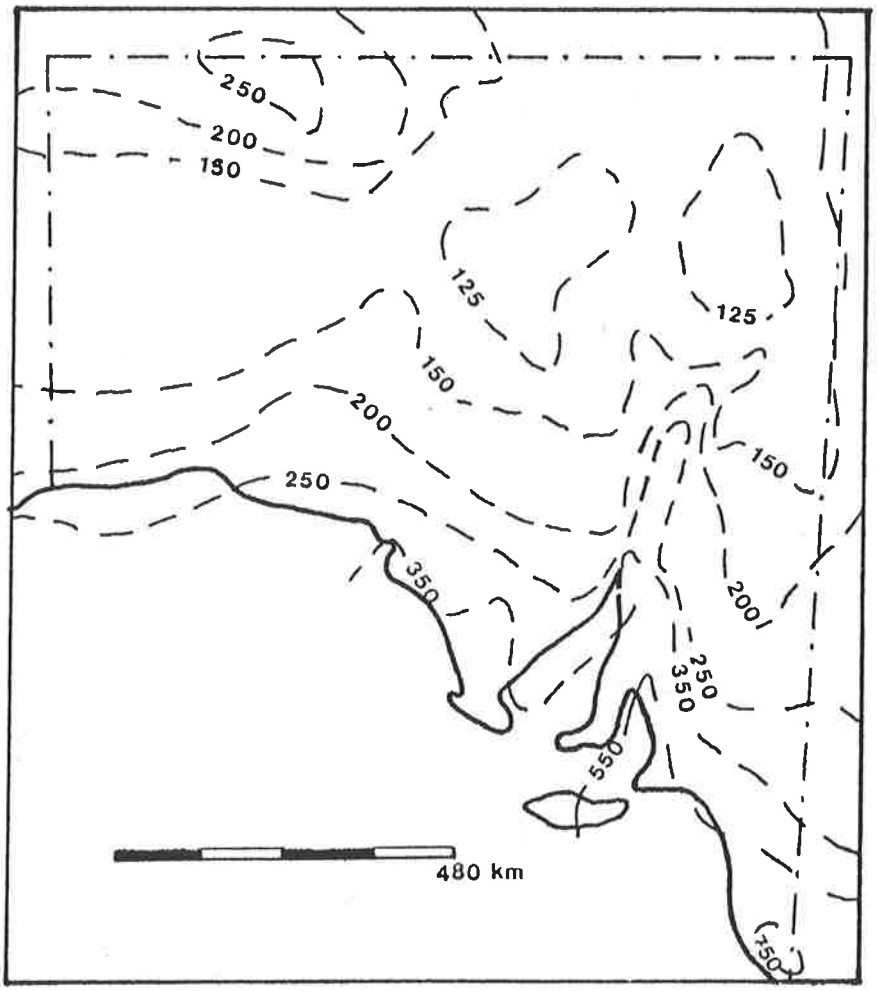


FIGURE 1. : MEAN ANNUAL RAINFALL ISOHYETS FOR SOUTH AUSTRALIA (after Specht, 1972).

——— = Coast line

—·— = State Boundary

— — = Isohyets (mm).

CHAPTER 2 : STUDY AREA.

2.1 INTRODUCTION.

A review of the climate and other environmental factors within the arid region of South Australia is presented because they were considered to be important in influencing the distribution of nematodes, particularly the plant parasites. The arid region of South Australia is characterised by an unpredictable and patchy rainfall, averaging less than 250mm per year (Figure 1). Arid regions comprise more than 83% of South Australia and 70% of Australia (Specht, 1972). Another characteristic of this region is its great age and low relief, a legacy of the general stability of the continent. The distribution of landform and vegetation, which has been influenced by past climatic events in conjunction with present climate, is also considered to play a large part in determining the distribution and numbers of nematodes. In determining the distribution of vegetation, the method of classifying types of vegetation should a) be adaptable for use in rapid reconnaisance as well as intensive study of small areas b) able to be operated by a single person at a reasonable efficiency level and c) able to use statistical analysis to measure variability and so confidence limits can be established (Shanks, 1954). A simple and accurate system of classifying the different vegetation and landform types is presented to allow analysis of any associations between nematode and plant distribution.

2.2 PAST CLIMATE.

Before 1980, studies suggested that the onset of aridity in Australia was fairly recent, starting about 1 million years ago (Laseron, 1971). However, with the development of accurate tests on fossil pollen and the opening up of the more inaccessible areas by mining companies, more detailed information about climatic changes within the last 30m years has been obtained. It was found that in the Miocene (25 million years B.P.), there were local pockets of aridity in an otherwise wet and warm climate (Truswell and Harris, 1982).

In the last 4-6 million years, the arid climate became more widespread as a general drying trend occurred. There were large fluctuations in climate, with periods of aridity followed by wetter periods. The last major period of aridity occurred 18,000 years

ago, with the climate of present day Australia becoming slightly wetter. Extinction, adaptation then radiation of both plant and animal species would have accompanied each successive change in climate. Truswell and Harris (1982) considered that a progression in vegetation type occurred in Australia, from rainforests in the early Tertiary through sclerophyll forests to present day arid vegetation. Almost all of the major components of the present day vegetation, excluding those imported by man, were present in the past vegetation. *Acacia* pollen has been found in sediments dating from the early Miocene; *Eucalyptus* seed pods have been found in early Tertiary fossils and elements of the family Chenopodiaceae have also been found as pollen in Tertiary sediments. Little vegetation would have survived the periods of great aridity, except in refuges (such as mountain ranges or permanent water) or on the outskirts of the arid region. It is likely that the distribution of the plant parasitic nematodes, and other nematode trophic groups, closely followed those of the plants and that they survived in places where plants survived and then re-invaded regions when conditions became more favourable. Some suggestions on how nematodes might have been dispersed are made at the end of the thesis.

Most of the geological activity which produced the mountain ranges and other landforms in S.A., occurred in the early Miocene with the uplift of the Flinders and Mount Lofty Complex, creating the central highlands. As a result, a large inland sea was formed when the outflow of a large river system to the ocean was blocked. At this time there was a warm and humid climate, indicated by the sediments, with a lush vegetation indicated by the plant fossils. With the onset of aridity, which appears to be related to the shift of the continent into its present latitude, the inland sea slowly dried out causing mineralisation of the soil and the formation of large salt lakes (a feature of the arid regions). Remnants of that large river system can be seen today as intermittent creeks and rivers that flow into Lake Eyre.

Other landform systems created during the periods of great aridity were the large sand dunes, which were caused by the denuding of the vegetation from the landscape which caused the soil to become unstable. This caused the formation of the large dune systems of the Simpson Desert and the Great Victorian Desert. With the onset of a wetter climate the dunes stabilised and were colonised by vegetation. This is a characterisic of the dune systems

PLATE 1 : STURT'S STONY DESERT.

Silcrete concretions present on the surface, with an Ephemeral Herbland vegetation. Present on the Plateaux area surrounding the Cooper Creek floodout.

PLATE 2 : THE BUBBLER.

Mound Spring ; a natural upwelling of the Great Artesian Basin. Reeds present at water's edge. Mound raised above ground level (approx. 5 metres).

of Australia which seldom occurs in other desert regions (McGinnies et al., 1968). The stony deserts of the Aroona Plateaux and Sturt's Stony Desert (Plate 1) were formed by a combination of wind erosion and chemical weathering of the Tertiary landscape. This landscape can be observed today, in the region surrounding Lake Eyre. Another feature of this region is the presence of natural upwellings of the Great Artesian Basin (the largest such basin in the world) which form the Mound Springs (Plate 2).

Present day vegetation and landforms are unique to Australia and, although similar plant genera and landforms can be found elsewhere, most plant species are endemic to Australia. There are 500 plant genera and 12,000 plant species endemic to the Australian continent, and of these 4,400 species are limited to the arid region (McCleary, 1968). This study aims to investigate associations between nematode, vegetation and landforms, with emphasis on establishing any relationship between plants and nematodes. Also to be considered will be the effect of plant distribution on nematodes of the different trophic groups. A classification system of vegetation and landforms is used to enable their distribution to be analysed, so that their influence on the distribution of nematodes can be assessed. Climate has an effect on the growth of the plant and so probably determines, to a large extent, the distribution of the nematodes. The dominant plant species present and the plant components of the understorey are also of interest, as these make up the vegetation formations.

2.3 PRESENT CLIMATE.

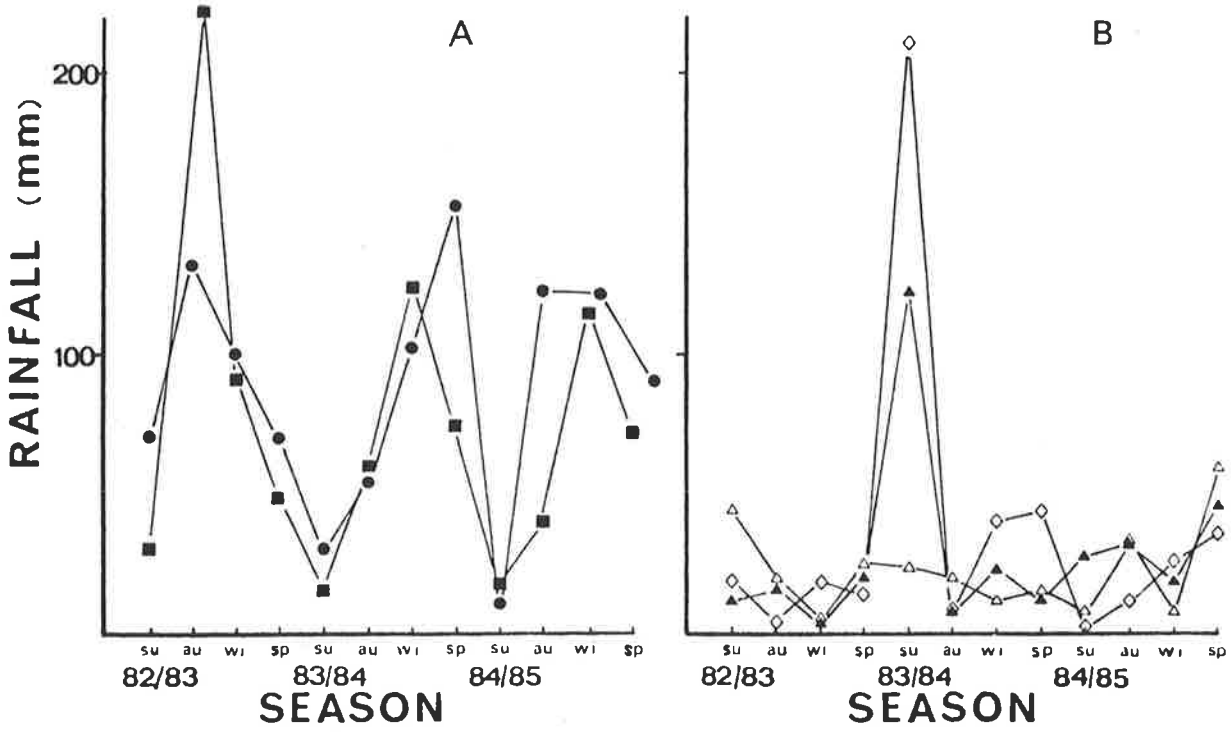
Precipitation has a major influence on the growth and development of plants and on the spatial distribution of plant species. After a period of drought, rain of less than 6mm is insufficient for plant growth, as the water does not penetrate to the root system before evaporating (Specht, 1972). Rainfall that promotes plant growth usually falls in January and Febuary (as part of the monsoonal trough from the north) or in June and July (from southerly, anticyclonic depressions), but the distribution is very patchy.

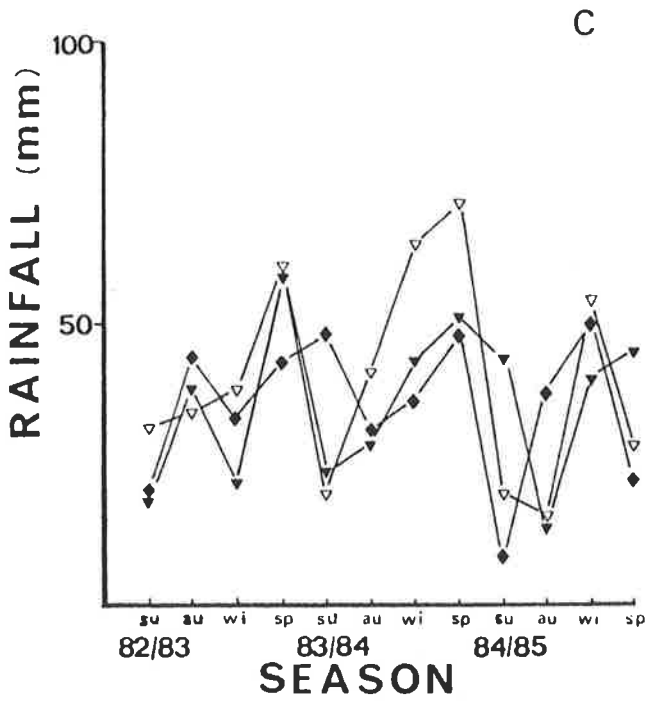
The region has long, hot summers and short, cool winters. The fluctuations in daily temperature can be high and reflects continentality. In most areas, the mean temperature for January (the hottest month) is 32 ^{o}C, and the minimum is 15 ^{o}C.

FIGURE 2 : RAINFALL FIGURES FOR NINE OF THE SELECTED SITES FROM WITHIN THE ARID AND MARGINAL REGIONS OF SOUTH AUSTRALIA.

2A)	Marginal	: -	Ceduna	- ■
			Port Pirie	- ●
2B)	North	: -	Oodnadatta	- △
			Coober Pedy	- ▲
			Marree	- ◇
2C)	South	: -	Kokatha	- ▽
			Tarcoola	- ▼
			Woomera	- ◆

(Season :- su = summer , au = autumn , wi = winter , sp = spring).
(year changes represented by :- 1982 to 1983 = 82/83, 1983 to 1984 = 83/84, 1984 to 1985 = 84/85).


A
B
RAINFALL (mm)
200
100
su au wi sp su au wi sp su au wi sp
82/83
83/84
84/85
SEASON


C
RAINFALL (mm)
100
50
su au wi sp su au wi sp su au wi sp
82/83
83/84
84/85
SEASON

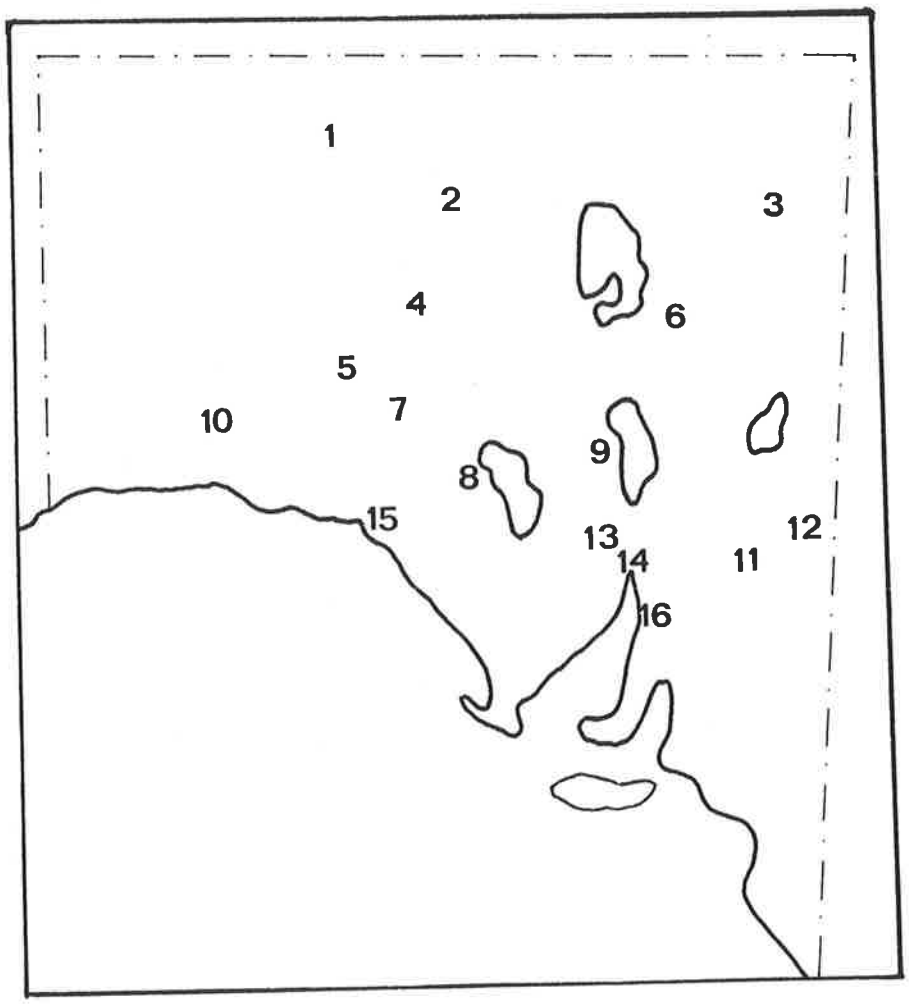


FIGURE 3 : SELECTED RAINFALL COLLECTION STATIONS IN THE ARID REGION AND MARGINAL LANDS OF SOUTH AUSTRALIA.

(numbers correspond to those in Table 1)

——— - Coast-line and inland lakes

— · — · — - State Boundary

TABLE 1 : AVERAGE RAINFALL FIGURES FOR SIXTEEN STATIONS AND TOWNS IN FIVE DIFFERENT ZONES IN THE ARID AND MARGINAL REGIONS IN SOUTH AUSTRALIA (Data collected from 1982 to 1985 inclusive).

	Average Rainfall (mm) Monthly												
Zone/Station	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
North													
(1) Granite Dns	115	7	55	6	3	1	3	2	16	14	2	12	236
(2) Oodnadatta	75	13	24	6	2	3	3	2	7	17	9	23	184
(3) Moomba	51	9	6	1	1	5	10	4	6	13	4	7	117
Centre North													
(4) Coober Pedy	26	12	21	5	7	8	8	12	7	12	5	15	138
(5) Comm. Hill	5	6	16	5	7	8	8	12	7	17	3	15	109
(6) Marree	50	5	4	2	3	5	8	9	9	12	12	8	127
Centre South													
(7) Tarcoola	4	19	16	4	10	10	9	13	15	20	7	6	133
(8) Kokatha	4	7	17	8	8	14	14	17	14	21	6	14	144
(9) Woomera	6	10	13	9	12	10	6	17	11	10	8	11	123
(10) Cook	9	10	46	25	5	3	10	14	13	17	4	25	181
South													
(11) Yunta	15	8	8	20	13	10	18	22	15	26	10	20	185
(12) Olary	28	5	8	17	13	6	14	22	15	14	11	20	173
(13) Nonning	7	2	41	26	15	17	28	35	48	24	13	10	266
(14) Pt Augusta	10	9	21	18	14	14	21	34	23	26	11	13	214
Marginal													
(15) Ceduna	4	3	37	40	20	18	42	37	23	25	5	16	270
(16) Port Pirie	6	2	27	39	36	20	29	43	36	28	18	11	295

(numbers in brackets correspond to numbers in Figure 3)
(Comm.Hill = Commonwealth Hill, Pt Augusta = Port Augusta and Granite Dns = Granite Downs

Temperatures as high as 50 ^{o}C, in the shade, have been recorded in the far north. The mean maximum for July is 15 ^{o}C and the minimum 3 ^{o}C. The evaporation rate during the summer months is very high, reaching 90% in January. During the winter months, evaporation is low and the relative humidity is high, especially at night, due to low temperatures and the considerable diurnal change in temperature.

There is a trend towards predominantly summer rainfall in the northern part of the state and for winter rainfall in the south (Mabbutt, 1971), but the whole region is characterised by the unpredictability of rainfall. A drought can occur over a five year period and then, in the sixth year, over three times the yearly average may fall, giving an exaggerated value to the rainfall figure. In line with the aims of this thesis, vegetation was sampled during the months Febuary to December inclusive, from 1983 to 1985. Rainfall figures for selected sites were obtained from the Bureau of Meteorology in Adelaide. Figure 2A shows the cyclical nature of the rainfall pattern over the sample period in areas with rainfall above 225mm per annum (marginal and southerly zones). Considering the seasonal rainfall figures from stations within the arid zone (Figure 2B and 2C), it is clear that there was less rainfall than in the marginal lands and the rainfall pattern was very erratic. The average monthly rainfall figures from fourteen sites, in five different zones in the arid region and the marginal agricultural lands (Figure 3) are presented in Table 1. These data show that in the north, heavy rainfall occurs mainly in the summer months. There was a gradual change to more winter rainfall in the south, with the central zones having an even distribution of low rainfall over the year.

The mean values for the year before sampling and the three years of sampling were then compared to mean values collected over the last 30 years (Table 2). They show that from 1982 to 1985 had thirteen out of sixteen sites lower than average rainfall, even within the agricultural zones. Therefore, it can be considered that in the years when sampling was done conditions were drier than average. This may have affected the number of individuals and species of plant nematodes collected during this time as rainfall probably affected the growth of the plant species. Conversely, it seems likely that in wetter years the numbers and diversity of nematodes of all trophic groups would increase in the soil samples.

TABLE 2 : AVERAGE RAINFALL FROM SIXTEEN STATIONS WITHIN ZONES IN THE ARID AND MARGINAL REGIONS IN SOUTH AUSTRALIA (data collected from 1982 to 1985 inclusive, and the average presented from the last 30 years).

Zone/Station	Average Annual Rainfall (mm) 1982-85	Last 30years
North		
1) Granite Downs	236	*
2) Oodnadatta	184	176
3) Moomba	117	*
Centre North		
4) Coober Pedy	138	157
5) Commonwealth Hill	109	185
6) Marree	127	160
Centre South		
7) Tarcoola	133	173
8) Kokatha	144	188
9) Woomera	123	294
10) Cook	181	172
South		
11) Yunta	185	231
12) Olary	173	205
13) Nonning	266	241
14) Port Augusta	214	243
Marginal		
15) Ceduna	270	315
16) Port Pirie	295	346

(numbers in brackets correspond to numbers in Figure 3)

FIGURE 4 : THE DISTRIBUTION OF THE MAJOR VEGETATION FORMATIONS IN SOUTH AUSTRALIA (after Specht, 1972).

1 - Open Woodland

2 - Low Woodland A) *Acacia aneura* dominant
B) *Casuarina cristata* dominant

3 - Tall Shrubland

4 - Mallee Scrub

5 - Low Shrubland A) *Atriplex vesicaria*/ *Maireana sedifolia* dominant
B) *Atriplex rhagodiodes* dominant

6 - Grassland

7 - Ranges A) Musgrave
B) Flinders
C) Gawler

▦ - Agricultural Lands

▩ - Salt Lakes

— - Coast-line


7A
7A
6
5B
6
6
1
5B
6
1
6
1
4
1
5B
3
6
5B
1
6
3
5A
6
2A
7B
2A
5A
5A
5A
3
4
4
7B
5A
7C
4
2B
4

2.4. PRESENT DISTRIBUTION OF VEGETATION.

The arid region of South Australia is vegetated rather than desertified, with a diverse and unique flora, influenced by landforms, soil type and previous and present climatic changes. The system of classifying the composition of the native flora in this project was based largely on that of Specht (1972), while considering the system presented in the CSIRO province codes (Laut et al., 1977a, b, c, d). The system uses the height and growth habit of the dominant plant species to classify the different types of vegetation. Due to the dry climate, there are constraints on the growth of plant species, so they seldom reach the optimum height that plants of the same species reach in more favourable climates.

A 'tree' is defined as a woody plant with a major axis, with the greater portion of the branches and foliage in the upper half of the plant. A 'shrub' is defined as a woody plant with no major axis and the branches and foliage are not confined to the upper half of the plant. The 'mallee' form of *Eucalyptus* is a woody plant with multiple stems, arising from a swollen, woody base from which the stems and roots can regenerate after fire or felling. The vegetation is classified on the criteria of growth habit (as above) of the most common or dominant plant species and the height to which it grows (in the field). The composition of the understorey is also recorded, as plant species or groups that comprise the main component under the dominant host plant. The plants of the understorey are probably a major source of food for the plant parasitic nematodes and other trophic groups that rely on decomposition of leaf litter as their food source.

The major formations used in classifying the vegetation are given below and the general distribution of each formation given in Figure 4.

1. OPEN WOODLAND : The main dominant plant species of this formation are *Eucalyptus camaldulensis* , *E.microtheca* and *Acacia cambadgeii* . These species can grow to a height of between 10-30 metres, although in some very arid areas they are smaller. The foliage cover is usually less than 30% and they are closely associated with large water-courses and surrounding flood-plains (Plate 3). The understorey varies greatly, but often consists of ephemerals.

2. LOW WOODLAND : The dominant species of this formation are the tree form of *Acacia aneura* and *A.papyrocarpa* (Plate 4A), and *Casuarina cristata* (Plate 4B). The height of the

PLATE 3 : COOPER'S CREEK FLOODPLAIN.

View of Creek and floodplain from distant hill. Open Woodland vegetation, with varied and sparse understorey. Can observe creek line in the background, against far hills.

PLATE 4A : LOW WOODLAND

This vegetation type is dominated by *Acacia papyrocarpa*, with an understorey based on *Atriplex vesicaria*. Typical shape of *A.papyrocarpa*, with umbrella like canopy cover.

dominant species is between 5-10 metres, with less than 30% foliage cover. The *C.cristata* based formation mainly occurs in the southern zone of the region and often has an understorey of Chenopodiaceae. The understorey of the other dominant species is varied but often of the Chenopodiaceae.

3. TALL SHRUBLAND : The dominant species tend to be the shrub form of *Acacia aneura* , *A.kempeana* , *A.papyrocarpa* and other shrubby species. The height of the plants is from 2-8 metres, with less than 50% foliage cover. It is found as a central strip in the region, small pockets on the eastern border and in areas in which the climate is less favourable for the plant species to develop into trees. The understorey is variable but is mainly consists of Chenopodiaceae.

4. MALLEE SCRUB : The most common dominant species are *Eucalyptus socialis* and *E.gracilis* , which can reach a height of between 2-5 metres with a varied foliage cover (can be 100%). This formation is mainly confined to the southern zones of the region on limestone based soils and with relatively high rainfall (Plate 5). The understorey usually consists of *Spinifex* spp. (in wetter areas) or Chenopodiaceae species.

5. LOW SHRUBLAND : The height of the shrubs is usually less than 1 metre, with the dominant species *Atriplex vesicaria* and *Maireana sedifolia* on the alkaline soils and *A.rhagodiodes* on saline soils (Plate 6). *Eremophila* , *Cassia* and *Acacia* spp. may be present as taller shrubs but usually occur sparsely or in small stands of local distribution. *Salicornia* and *Nitraria* spp. are common on saline soils surrounding salt lakes and pans. The understorey usually consists of ephemeral herbs and grasses. This formation has a varied foliage cover.

6. GRASSLAND : There are two forms of grassland :-

Hummock Grassland ; *Zygocloa paradoxa* and/or *Trioda* spp. are the dominant species. The height of the clumps of grasses is less than 2 metres and has a varied foliage cover.

Tussock Grassland ; Consists of discrete, compact tussocks between 30 and 40 centimetres tall of perennial sedges, grasses, rushes or iron grasses (*Lomandra* spp.). The main dominant species are *Astreleba pectinales*, *Eragrostis australis* and *Spinifex* spp..

Both Hummock and Tussock Grasslands are mainly confined to the northern zones of the state, but are present as the understorey component of a range of other vegetation formations.

PLATE 4B : LOW WOODLAND.

This vegetation is dominated by *Casuarina cristata* and has a mixed Chenopod understorey.

PLATE 5 : MALLEE SCRUB.

This vegetation was present near Port Germain, in the marginal lands, therefore, plant species present are taller than in the arid or semi-arid regions. *Eucalyptus socialis* is the dominant species present, with a mixed chenopod understorey.

7. RANGES : Consist of many different vegetation formations. They are an important source of runoff and a refuge for plants and animals during drought. In Figure 4, three ranges are shown.

A) The Musgrave Ranges occur in the north-west of the state with a vegetation consisting mainly of Sparse (less than 10% foliage cover) Low Woodland with a grass understorey. To travel in this region it is necessary to obtain permission from the aboriginal owners. Unfortunately, at the intended time of sampling, permission was refused as tribal initiatation ceremonies were taking place.

B) The Flinders Ranges comprise a central highland area of the state and have highly varied vegetation formations. Previous work has been done in this area, so few samples were taken.

C) The Gawler Ranges have a Low Woodland, Tall Shrubland and Low Shrubland as the most common vegetation formations. These ranges have a higher rainfall than the surrounding plains. The understorey is very diverse.

An eighth formation is included in the general classification, although it's distribution is erratic and not included in Figure 4.

8. EPHEMERAL HERBLAND : Ephemeral herbs and grasses emerge after heavy rain and can grow up to 40 cm. They occur as understorey components and as a discrete formation in such areas as swales of sand dune, overgrazed pastoral lands, clay pans and in between clumps of trees and shrubs. Particular species of ephemerals (e.g. grasses) have special requirements for germination, such as temperature and moisture to break dormancy. This means that at any particular time of the year, the composition of Ephemeral Herblands is determined by temperature and soil moisture. Such influences probably affect the composition of the nematode species.

The vegetation formations that occur in the arid region can be placed into one of the above categories. Within each major formation, there are local distributions of different vegetations which are caused by local changes in landform and soil types. Such classification enables the description of local vegetation formation and so any association between vegetation and plant parasitic nematodes to be assessed. It seems likely that different landforms may also affect the distribution of plant species and so the nematode community. A description of the classification of landform systems follows.

PLATE 6 : LOW SHRUBLAND.

This vegetation is present surrounding a salt lake (Lake Gilles). *Atriplex vesicaria* (light green) and *Salicornia* spp. (dark green or purple) are present. In the distance, *Acacia papyrocarpa* is present on the first dune, before the shore of the salt lake.

PLATE 7 SAND DUNES AND HUMMOCK GRASSLAND.

This is a view of the Simpson Desert from the crest of a dune. *Zygocloa paradoxa* is present in the fore-ground, stony swale between the dunes and in the distance, small *Acacia* species.

2.5 PRESENT DISTRIBUTION OF LANDFORMS.

The relief of the region is low and shows the effect of erosion on a landscape of great age and stability. Figure 5 shows the general distribution of the major landforms within the arid region of S.A.. The classification criteria used to distinguish these landforms is presented.

1. SAND DUNE SYSTEMS : The direction of the dune crests varies between each area. There are dunes (1A) that run predominantly in an east-west direction and others (1B) that run in a predominantly north-south direction. The sand dunes have a Low Shrubland or Grassland on their crests and may have a Tall Shrubland or Ephemeral Herbland in the swales (Plate 7). The dunes consist of silaceous sands on the crest and clays in the swales.

2. RANGES : As for vegetation, these are divided into three areas;

2A) The Musgrave Ranges consist of highly metamorphosed rocks of igneous origin. The soils are highly variable but usually shallow.

2B) The Flinders Ranges consist of highly metamorphosed rocks of sedementary origin with a thin soil cover.

2C) The Gawler Ranges consist of granitic outcrops which have undergone much weathering. The soils between the outcrops are fairly deep, red earths, or hard, red duplex soils.

Throughout the whole region there are occasional hills and small outcrops, these are classified as rises or hills.

3. MURRAY PLAINS SYSTEM : These plains overlie a predominantly limestone bedrock with soils of calcareous earths on undulating plains. They surround the present day Murray River and have a mainly Chenopodiaceae based Low Shrubland which are present as the understorey component of Low Woodlands dominated by *Acacia* spp., *Myporum* spp. and *Casuarina cristata* . *Atriplex vesicaria* is found to be mainly associated with shallow red duplex soils and *Maireana sedifolia* with deeper soils. Mixtures of these two species of the Chenopodiaceae can occur.

4. PLAINS AND PLATEAUX SYSTEMS : These occur mainly around the salt lakes. The plateaux areas are remnants of the Tertiary landscape and the plains are the product of the weathering of this surface (Plate 8). The soils in the plains are siliceous sands or hard, red duplex

PLATE 8 : STONY PLAIN.

Undulating plain, near Marree, with an occasional low shrub and ephemeral Herbland as the main vegetation.

PLATE 9 : SALT LAKE AND SURROUNDING SALINE SOILS.

View of Lake Everard in the Gawler Ranges.

Maireana spp. present in a Shrubland Vegetation.

soils. The plateaux soils consist of shallow, red soils. The plains support a variety of vegetation formations, and the plateaux usually have a Low Shrubland or Ephemeral Herbland formation.

5. STONY DESERT SYSTEMS : These have evolved over a long period of intense weathering of Tertiary soils, with the formation of silcrete concretions on the surface of the soil. Occasional depressions occur into which soil is blown and small trees and shrubs can colonise, but most of the vegetation consists of Ephemeral Herbland, Low Shrubland (*Atriplex* spp.) or Hummoch Grassland (*Zygocloa paradoxa*). The soils are mainly red duplex soils with large concretions of silcrete.

6. NULLARBOR PLAIN SYSTEM : The Nullarbor Plain was formed as the sea retreated from the land as Australia rose. It is composed of thin Tertiary, marine sediments (sandy limestones which lie close to the surface) that give rise to shallow calcareous loams. The plain is characterised by an almost featureless Low Shrubland with no tall shrubs or trees growing on an almost totally flat landscape. The dominant plant species present are *Atriplex vesicaria* and *Maireana* spp..

Also shown on Figure 5, is the distribution of large river systems that flow into Lake Eyre and the River Murray that flows into the sea. These mainly have an Open Woodland vegetation and have an associated flood out or flood plain. Other topographic features that occur are of local distribution such as water-courses, large intermittent creeks, small salt lakes and saline surrounds (Plate 9), clay pans, slightly undulating hills and steep gorges with associated microclimates and large hills. The main types of landforms used to classify sites were plain, plateaux, dune, water-course, flood-plain, swamp (salt lake surrounds and areas of accumulation after rain) and hills or rises.

2.6 CONCLUSION AND DISCUSSION.

The whole region has a diverse vegetation and landforms which reflect the great age and stability of the region. Most of the region is vegetated, rather than desertified, and with the use of artesian water extensive livestock grazing occurs. The host plants have adapted to great changes in climate, from periods of long droughts to abundant rainfall, that still occur at the present time. The present day vegetation has evolved from ancient plants

FIGURE 5 : THE DISTRIBUTION OF THE MAJOR LANDFORM SYSTEMS WITHIN THE ARID REGION OF SOUTH AUSTRALIA. (after Boomsma and Lewis, 1981).

1 - Sand Dunes A) Crests run East/West
B) Crests run North/South

2 - Ranges A) Musgrave
B) Flinders
C) Gawler

3 - Murray Plains

4 - Plains and Plateaux

5 - Stony Deserts

6 - Nullabor Plain

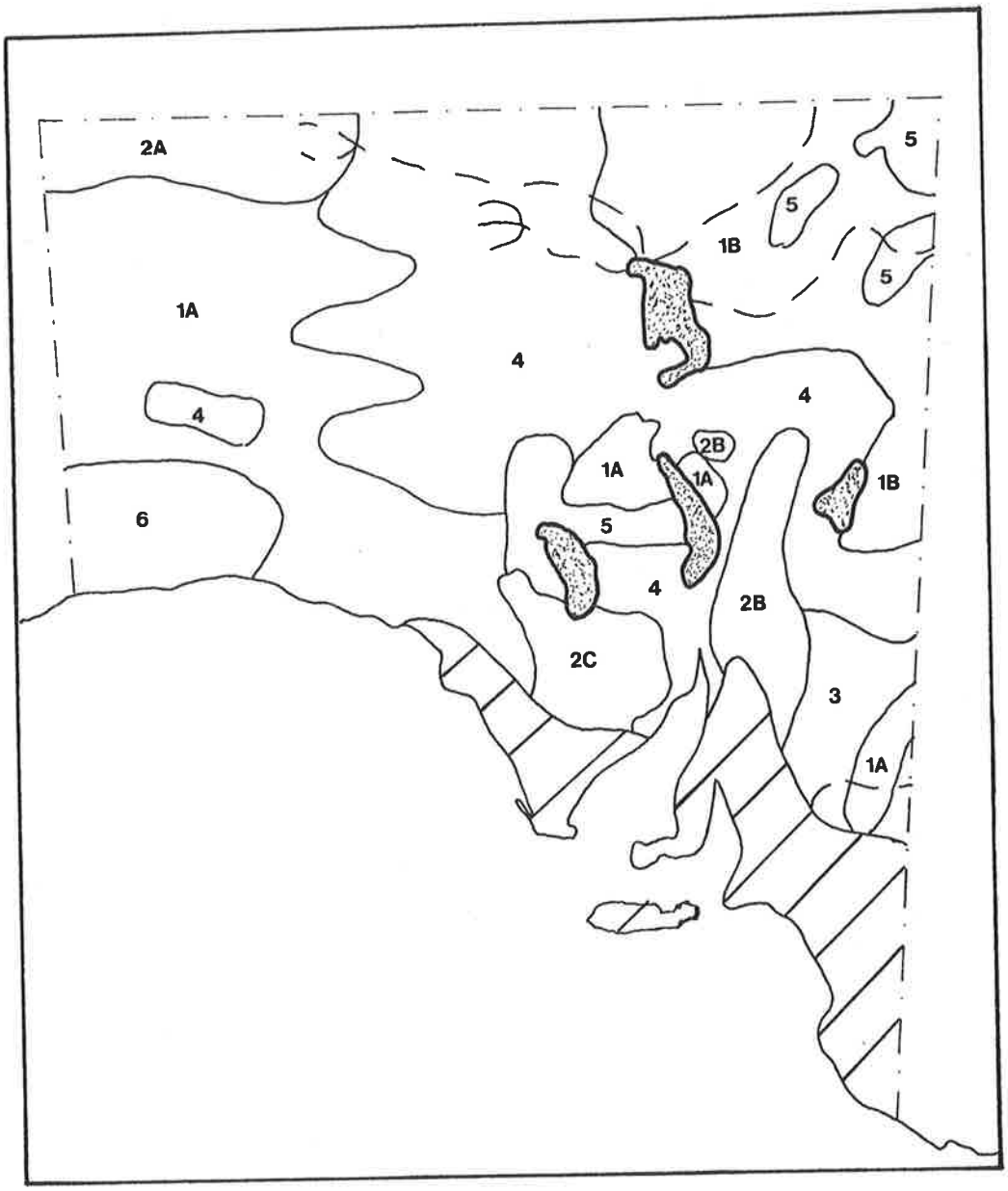
 - Agricultural Lands

 - Salt Lakes

— · — - State Boundary

— — - Major River Systems

——— - Coast Line


2A
5
5
1B
5
1A
4
4
4
2B
1A
1A
1B
6
5
4
2B
2C
3
1A

which had characters that enabled them to adapt to these changes in climate. This adaptation would be expected to have occurred with the nematodes occurring in the region

In this study, a survey was made to determine the relationship between different vegetation associations, landforms, dominant host plants, understorey components, host plants and the occurrence of plant parasitic nematodes. Consequently, this chapter has described a classification system devised for this particular project to define the sites from which the soil samples were taken. As the influence of rainfall on growth of host plants and, therefore, on nematode populations is considered to be of importance, rainfall figures for the last four years have been presented. Previous records indicate that during the years of sampling, and the year before sampling commenced, there was lower than average rainfall. Such conditions may have reduced the chance of collecting nematodes that occur in low numbers or those nematodes associated closely with ephemeral plants. These plants (and possibly the nematodes) have particular requirements for germination and growth, and so are restricted to growing at certain times of the year. Only further work in wetter years will establish whether the conclusions reached in this thesis require modification.

CHAPTER 3 : THE IDENTIFICATION AND GENERAL DISTRIBUTION OF THE TYLENCHID PLANT PARASITES IN THE ARID REGION OF SOUTH AUSTRALIA.

3.1. INTRODUCTION.

There is considerable disagreement between taxonomists on the 'correct' classification of the higher and lower groups of nematodes (Hooper, 1978). Most classification systems are based on attempts to develop the possible evolutionary relationship between species and within families (Dropkin, 1980). The classification system used in this thesis is the one developed by Siddiqi (1986) which provides a comprehensive series of keys and figures upon which classification can be based.

To determine the distribution of particular plant parasitic nematodes, it is necessary to identify them accurately to, at least, genus level. Any ecological study depends on accurate identification of nematodes, so that associations between nematodes and other factors can be determined. Previous work by Reay and Wallace (1981a) showed that there were several genera of plant parasites associated with native vegetation occurring in the northern section of the Mt. Lofty and Flinders Ranges complex. It was considered that these genera might occur in the arid areas of the state.

Sauer and Annells (1981) identified two new species of *Tylenchorhynchus* and a *Morulaimus* spp., from an area in N.S.W. with a similar climate to the arid region of S.A.. Of those plant parasitic nematode genera identified from soil collected around Australia, none appear to be endemic to Australia, although only one species of *Morulaimus* has been identified outside of Australia (Yeates, 1967). It was felt that environmental conditions in the arid region of Australia might exert selection pressure on nematodes that would influence their evolution at the family and genus level. Identification and classification of the plant parasitic nematodes was attempted to species level so that broad associations between the distribution of plants and their nematode parasites could be analysed using the classification systems described in the previous chapter.

3.2. MATERIALS AND METHODS.

Soil was collected from native, undisturbed vegetation which occurred on the side of the main tracks within the region. Soil was taken to a depth of 25 cm (depending on the depth and hardness of the soil), placed in plastic bags, sealed with tape and placed in a card-board box. The site number and host plant were noted on the bag and at least 3 samples from each dominant plant species and understorey components were taken. For all soil samples taken in this and subsequent field work, the soil was thouroughly mixed within each plastic bag before sub-samples were taken. This was to ensure that the nematodes were evenly distributed within the soil sample. The nematodes were extracted using a modified Baermann's Funnel technique (Schindler, 1961).

Baermann's Funnel Technique (modified) : A 50 ml sample of soil was placed on a single ply tissue, which had been placed on a wire mesh standing in a 22 cm diameter by 3 cm deep glass petri-dish containing water. The soil was left for at least three days before counting the nematodes. The nematode suspension was then decanted through a 25µm aperture sieve (Yeates, 1968), washed into a measuring cylinder and the nematodes identified to at least family level under the dissecting microscope. The extraction efficiency of this method was found to be about 65% (tested by sampling the soil every day removing the soil, washing out and counting any nematodes still present). Depending on the dryness of the soil, a high proportion of juveniles was obtained due to hatching of eggs over the three days, but this was not considered deleterious to the work done in this chapter. The soil was left for three days, which was the time taken for most of the plant parasitic nematodes to move from the soil into the base of the petri-dish.

After extraction, the nematodes to be identified were removed by hand to a small, glass vial containing 2 ml of water. The nematodes were heat killed (60 ^{o}C) and 2 ml of 3% formalin solution added to make a concentration of 1.5%. The fixed nematodes were then kept for at least 7 days before mounting on a permanent slide. Immediate identification was possible using lactophenol (Tarjan, 1973) and the wax method of mounting (Appendix 1). Care was taken when placing the nematode specimens into the lactophenol to ensure that

the nematode specimens did not shrink and were complete when mounted. These specimens could be maintained in their original form for up to 3 years (possibly more).

The specimens required for permanent mounting were transferred through an alcohol series to pure glycerol (Southey, 1986) and then mounted in glycerol surrounded by pure wax. For identification to species level, the nematodes were measured under a high powered light microscope and the important morphological criteria for the different species assessed. Important measurements used in taxonomy are :- total body length, body width, length of stylet, tail length and width, length of oesophagus from lip to junction with intestine and base of oesophageal gland, length of spicules and gubernaculum and length from lip region to vulva.

The ratios used in taxonomy are :-

a = total body length / mid-body width

b = total body length / length from head to junction of oesophagus with intestine.

b' = total body length / length from head to posterior end of esophageal gland (used for those genera with an overlapping oesophageal gland).

c = total body length / tail length (anus to tail terminus)

c' = tail length / body width at anus.

v = total body length / length from head to vulva x 100 (%)

From these measurements and ratios, comparisons with and relationship to described species could be established. The general morphology of the nematodes is important in establishing whether the specimens belong to a new species, genus or family.

The classification system for landform, vegetation, dominant plant species and understorey components presented in the previous chapter, were used to classify the sites from which soil was collected. Chi-square analysis was used to test the Null Hypothesis that : The frequency of the occurrence of particular plant nematodes at each type of vegetation landform, dominant plant species, understorey components and plants sampled is equal to the total number of each type sampled. The expected values (E) were estimated from the % of

total sites sampled within each type and calculated for the number of sites from which the particular plant nematode was collected.

3.3. CLASSIFICATION.

3.3.1. The Genera of Plant Parasitic Nematodes found in the arid region.

Thirteen plant parasitic genera were identified from soil from the arid region of South Australia. These are *Tylenchorhynchus, Hemicycliophora, Pratylenchus, Paratylenchus, Radopholus, Hoplolaimus, Scutellonema, Rotylenchus, Helicotylenchus, Dolichorhynchus, Morulaimus, Telotylenchus, Heterodera/Globodera* and *Paralongidorus*. The last genus is a dorylaimid and was not identified further. These Tylenchid genera belong to five families (Siddiqi, 1986):-

Dolichodoridae : *Tylenchorhynchus, Dolichorhynchus, Morulaimus* and *Telotylenchus*.

Hoplolaimidae : *Hoplolaimus, Pratylenchus, Scutellonema, Radopholus, Scutellonema* and *Rotylenchus*.

Hemicycliophoridae : *Hemicycliophora*.

Heterodoridae : *Heterodera./Globodera*

Paratylenchidae : *Paratylenchus*

Most of the specimens of the genus *Heterodera/Globodera* were males or juveniles and so, were not identified further (this genus is not included in the key below). A key is presented for the identification of the plant parasitic genera of the Tylenchida collected from the arid region of South Australia.

3.3.2. Key to the genera of Plant Parasitic Nematodes in the arid region.

(after Siddiqi, 1986).

(based on female characteristics).

1. Body length less than 0.5mm ..... *Paratylenchus*

   Body length greater than 0.5mm 2

2. Post-corpus massive and amalgamated with pre-corpus, phasmid absent ..... *Hemicycliophora*

2. Cont.

Post-corpus not massive or amalgamated with pre-corpus, phasmid or phasmid-like structure present 3

3. Subventral oesophageal glands enlarged, usually extending past the dorsal gland 4

Subventral glands not enlarged, not extending past the dorsal oesophagealglands 9

4. Juveniles and females with low arched cephalic framework 5

Juveniles and females with high arched cephalic framework

5. Oesophageal glands overlapping intestine mostly ventrally, no sexual dimorphism in the cephalic region *Pratylenchus*

Oesophageal glands overlapping intestine mostly dorsally, sexual dimorphism in cephalic region *Radopholus*

6. Phasmid Scutella-like (large) 7

Phasmid pore-like 8

7. Stylet knobs tulip shaped, each with 1-3 anteriorly directed tooth-like projections *Hoplolaimus*

Sytlet knobs not tulip-shaped, without tooth-like projections *Scutellonema*

8. Cephalic region large, offset, with indented basal annule *Rotylenchus*

Cephalic region small, generally continuous, with smooth basal annule *Helicotylenchus*

9. Longitudinal ridges or fields or lamellae outside lateral fields present *Dolichorhynchus*

No longitudinal ridges or fields or lamellae outside lateral fields 10

10. Oesophageal gland lobe-like extending over intestine *Morulaimus*

Oesophagealglands in a bulb, abutting intestine *Tylenchorhynchus*

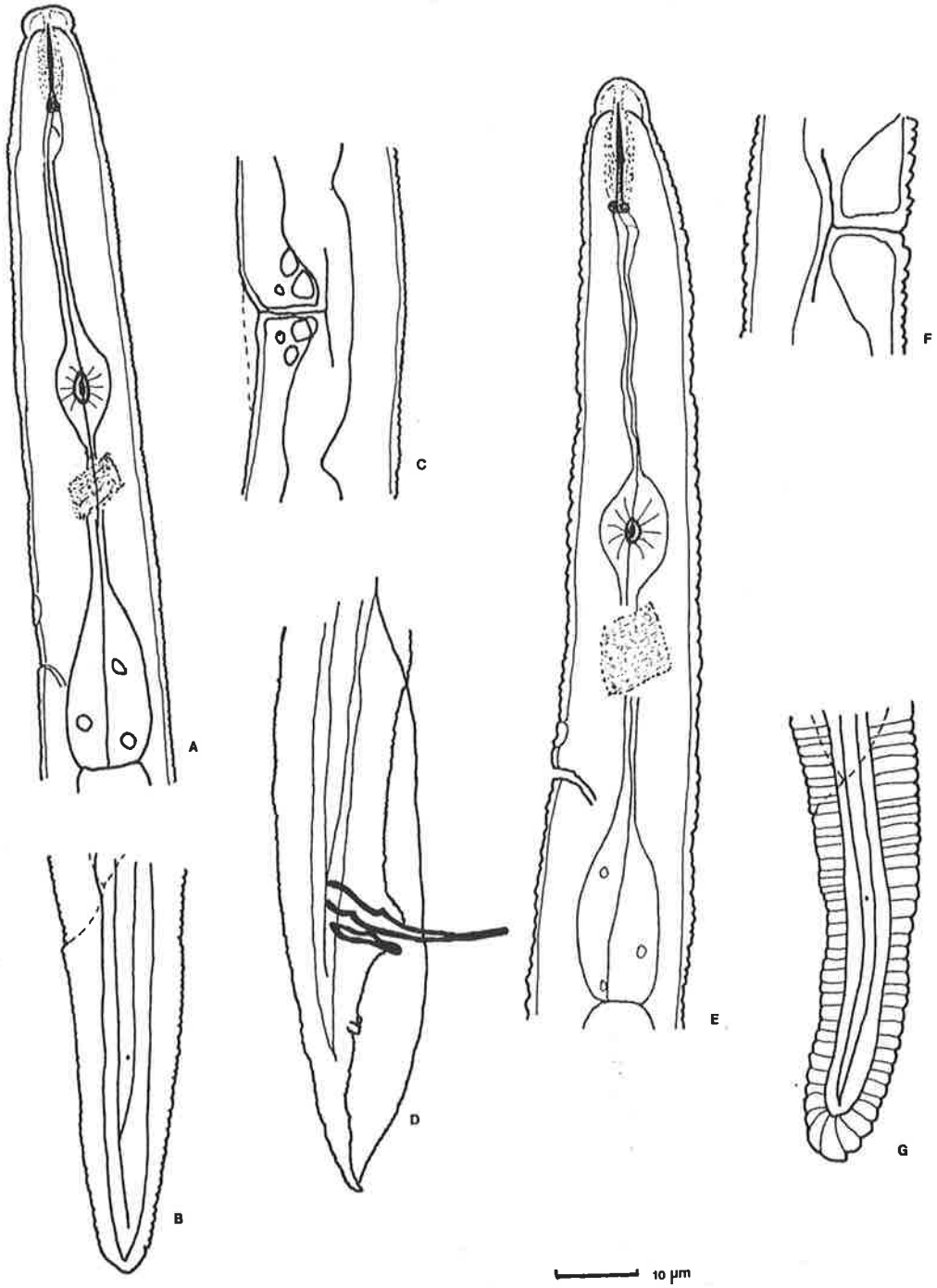


FIGURE 6 : Tylenchorhynchus tobari (A-D) ; oesophageal region (A), female tail (B), vulval region (C), male tail (D) T.annulatus (E-G) ; oesophageal region (E), vulval region (F) female tail (G).

Description and diagnosis of the different species of those genera which have sufficient numbers of females has been attempted. There was only one specimen of *Hemicycliophora* collected which was subsequently lost. *Pratylenchus* specimens were often collected and identified from juveniles and so identification to species level was not attempted. The distribution of the nematodes not included in the description of species below are discussed later. The species which were identified have been separated into their two families Dolichodoridae and Hoplolaimidae and are presented below.

3.3.3. The Family Dolichodoridae.

Tylenchorhynchus tobari Sauer and Annells, 1981.

Measurements

Females (n=825) : L=0.56-0.92mm(0.72) ;V=49-60%(54) ; a=24-37(30); b=4.3-6.6(5.2); c=10.0-20.8(13.8); c'=2.1-3.9(3.1); stylet=14-23µm(19).

Males (n=14) : L=0.59-0.71mm(0.67); a=28-36(31); b=4.0-5.6(5.5); c=9.5-12.2(10.3); c'=3.4-4.7(4.1); Gubernaculum=10-14µm(11); Spicules=24-29µm(26); stylet=15-19µm(17).

Description (amended from Sauer and Annells, 1981).

Body cuticle with fine annulations (<1µm). Lateral field of four lines, areolation of outer bands indistinct along length of body. Lip region set off, stylet slender, knobs sloping anteriorly. Oesophagus typical of the genus, dorsal oesophageal gland opening < 1µm behind stylet knobs (Figure 6).

Female :- Reproductive tract didelphic, spermathecae occasionally full of sperm, vulva a transverse slit, with irregular depressions occurring posterior to slit. Rectum short, anus pore-like, no extension of intestine into tail cavity. Tail tapering to bluntly rounded, non-annulated terminus, slightly thickened. Phasmid pore-like, in anterior section of tail, tail annules about 50.

Male :- Slightly smaller than female, similar. Rare. Testis single, outstretched, T=62%. Spicules slender, gubernaculum sclerotised and protrusible from cloaca. Phasmid in

FIGURE 7 : THE DISTRIBUTION OF SITES FROM WHICH Tylenchorhynchus tobari WAS COLLECTED (dots indicate sites from which T.tobari was found).

A = Sites from which males were extracted.

anterior section of tail. Caudal alae just encompasses tip. Distribution of males restricted to only a few sites (Figure 7).

Discussion.

The taxonomy of the genus *Tylenchorhynchus* is complex. In 1986, Siddiqi re-erected the genus *Bitylenchus* Filipjev, 1936, to include those species that had areolated outer bands of the lateral fields, a post anal sac filling half or more of the tail cavity, the cuticle on the tail terminus thickened and the gubernaculum not protrusible. Siddiqi included the species *T.tobari* in this re-erected genus. From specimens collected by myself, and from the original description and paratypes provided by Sauer and Annells, it was observed that this species had indistinct areolation of the outer lateral fields, no post anal sac filling half of the tail terminus and the tail with only a slightly thickened tail terminus. The males had not previously been described and were found to have a protrusible gubernaculum. Due to *T.tobari* having some characters of the genus *Tylenchorhynchus* (no post-anal sac and a protrusible gubernaculum) and some of *Bitylenchus* (areolated lateral fields and cuticle of tail terminus slightly thickened), it is felt that more study is required to determine if the re-erection of *Bitylenchus* is advisable. Therefore, in this study, the genus *Tylenchorhynchus* will be used to describe those species that belong in this group.

This species was very widespread and occurred at some sites in high numbers. The physical distribution of this nematode is presented in Figure 7. Due to *T.tobari* being the most widespread plant parasite, further analysis using observed and expected frequency of occurrence was undertaken in the last section of this chapter.

Tylenchorhynchus annulatus Cassidy, 1930.

syn. *T.martini* Fielding, 1956.

Measurements.

Females (n=18) : L=0.63-0.76mm(0.68); V=53-62(57); a=23-30(27); b=4.2-7.8(5.2); c=13.3-19.5(14.8); c'=1.9-3.4(2.7); stylet=15-24µm(19).

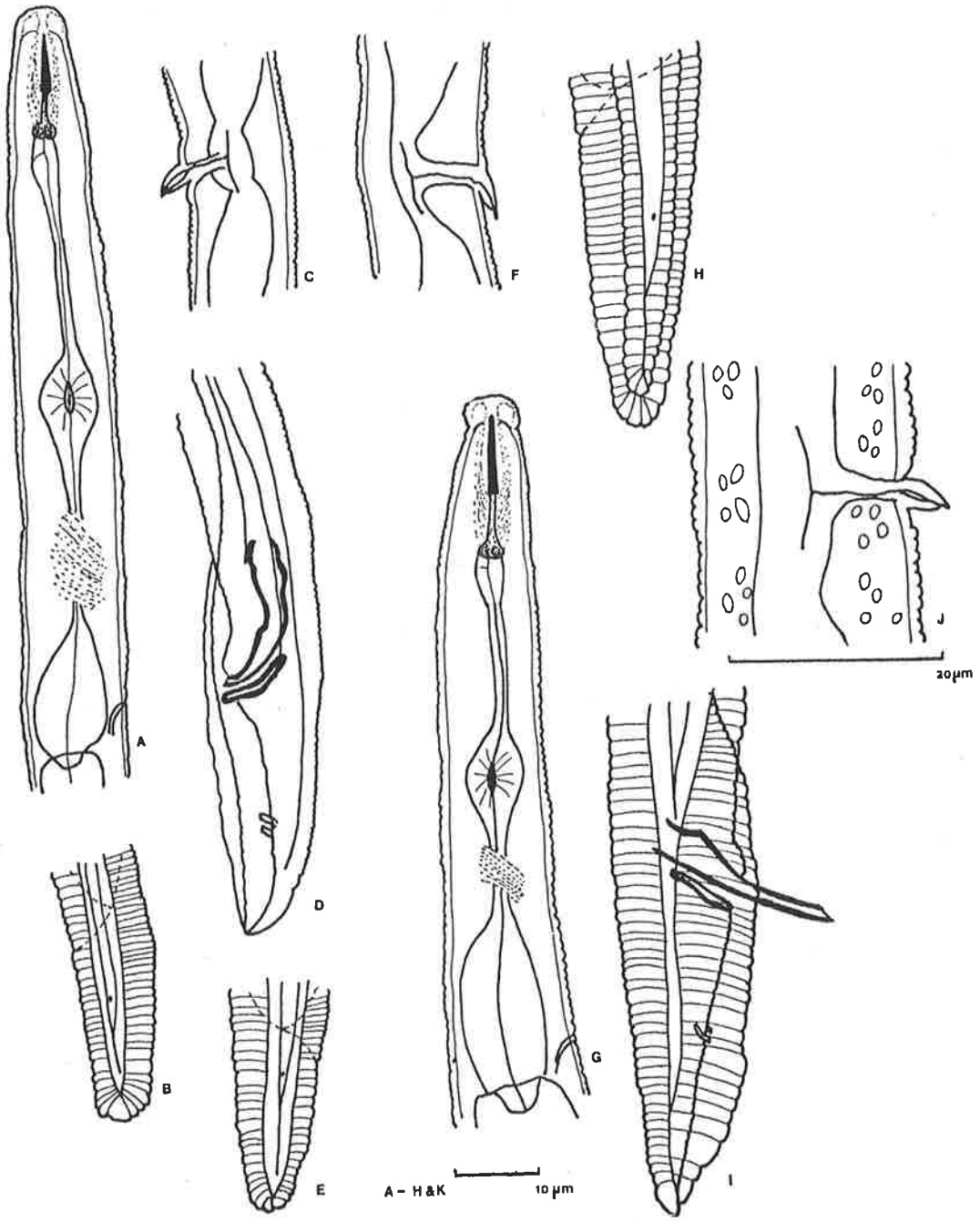


FIGURE 8 : Tylenchorhynchus velatus (A-D) ; paratypes from Sauer, oesophageal region (A), female tail (B), vulval region (C), male tail (D).
T.velatus (E and F) ; specimens from the arid region of S.A. female tail (E), vulval region (F).
T.siccus (G-J) ; oesophageal region (G), female tail (H), male tail (I), vulval region (J).

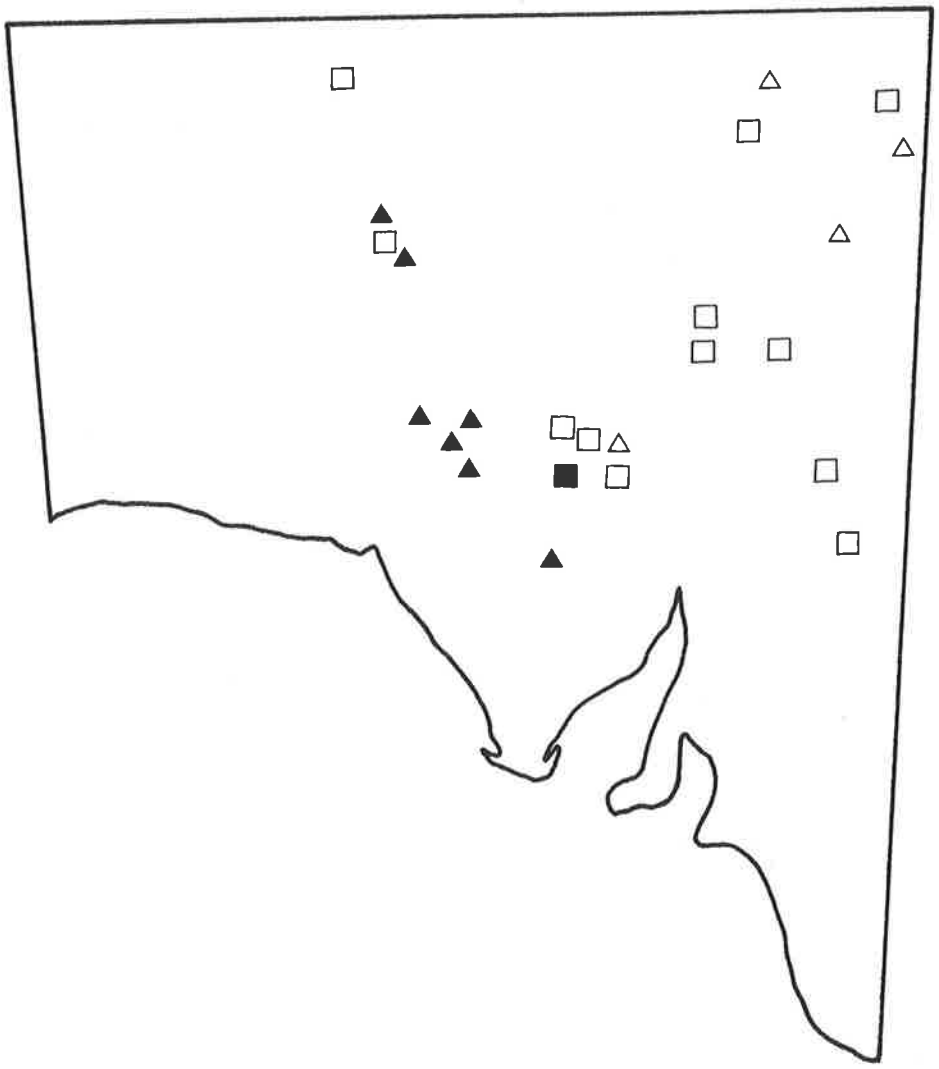


FIGURE 9 : THE DISTRIBUTION OF Tylenchorhynchus velatus, T.annulatus, T.siccus AND Dolichorhynchus sedecimstriatus WITHIN THE ARID REGION OF SOUTH AUSTRALIA.

T.velatus - □
T.annulatus - △
T.siccus - ■
D.sedecimstriatus - ▲

Description (amended from Fielding, 1956).

Fixed body cylindroid, cuticle with coarse annulations (>1µm). Lateral field with four lines, outer bands distinctly areolated. Lip region continuous, with indistinct annulations. Stylet slender. Oesophagus typical of the genus (Figure 6).

Females :- Reproductive system didelphic, outstretched. Vulva a transverse slit, slightly sunken. Rectum short, anus pore-like. Tail long, tapering to hemispherical, annulated terminus. Tail annules 30, ventrally. Phasmid in anterior section of tail. No males were collected.

Discussion

T.annulatus was mostly collected from sites confined to the northeastern corner of the region (Figure 9). It is possible that this nematode was transported, via the intermittant creeks and rivers that flow into Lake Eyre, from the southwestern part of Queensland, as it has been identified from native vegetation in that area (Khair et al. 1986). The presence of the nematode at one site north of Whyalla in the south west part of the state could be a remnant population or wind dispersed population. A discussion on the modes of dispersal and migration is presented in the last chapter.

Tylenchorhynchus velatus Sauer and Annells, 1981.

Measurements.

Females (n=22) : L=0.63-0.85mm(0.70); V=53-61%(56); b=4.5-6.4(5.5); c=10.1-23.4(18.3); c'=1.8-3.3(2.3); stylet=20-23µm(22).

Description (amended from Sauer and Annells).

Body cylindroid, annules distinct, lateral field of four lines, indistinctly areolated. Lip region continuous, stylet well developed. Oesophagus typical of the genus (Figure 8).

Females :- Reproductive system didelphic, gonads outstretched, spermathecae lacking sperm in populations from the arid region of S.A.. Vulva with double epitygma, sloping anteriorly. Short rectum, pore-like anus, tail tapering to bluntly rounded terminus with no distinct thickening. Phasmid pore-like, in anterior section of tail. Tail annules about 25. No males were collected and identified from soil from the arid region of S.A..

Discussion.

T.velatus did not occur in as many sites as *T.tobari* but, the sites from which they were extracted were widely spread throughout the area (Figure 9).

Tylenchorhynchus siccus n.sp.

Measurements.

Holotype Female : L=0.75mm; V=56%; a=27; b=5.7; c=20.9; c'=2.1; stylet=26µm.

Paratype Females (n=24) : L=0.68-0.94mm(0.77); V=54-59%(56); a=24-37(25); b=4.6-7.1(5.8); c=14.1-22.1(19.4); c'=1.7-2.6(2.1); stylet=24-30µm(27).

Allotype Male : L=0.74mm; a=35; b=6.0; c=12.6; c'=3.9; Spicules=31µm; Gubernaculum=16µm; stylet=26µm; T=62%.

Paratype Males (n=16) : L=0.66-0.82mm(0.74); a=27-31; b=4.6-6.3(5.7); c=10.4-13.5(12.0); c'=3.1-4.3(3.4); Spicules=27-33(31)µm; Gubernaculum=12-16µm; Stylet=25-31µm(26); T=52-78%(67).

Description.

Body slightly curved, cylindroid. Cuticular annulation coarse (1.9µm). Lateral fields with four incisures, outer bands indistinctly areolated at mid-body, distinctly on tail. Lip region offset, rounded, with six or seven indistinct annules, cheilorhabdions lightly sclerotised. Stylet slender, knobs rounded, sloping anteriorly. Dorsal oesophageal gland opening 1 to 2 µm behind knobs. The outer lateral lines begin just behind stylet knobs and the inner lines begin between the posterior end of the median bulb and the base of the terminal bulb. Oesophagus typical of the genus (Figure 8), with the excretory pore between 3 to 11 annules anterior to base of terminal bulb. Hemizonid present, 3 to 8 annules anterior to pore, 2 to 4 annules long. Junction of intestine and oesophagus with small overlap of terminal bulb over intestine.

Female : Reproductive tract didelphic, outstretched. Spermathecae present, full of sperm. Vulva with epiptygma, about 5 µm long, sloping anteriorly. Rectum short, anus pore-like, post anal sac absent. Tail with sixteen to thirty annules, tapering to an annulated rounded terminus, not obviously thickened.

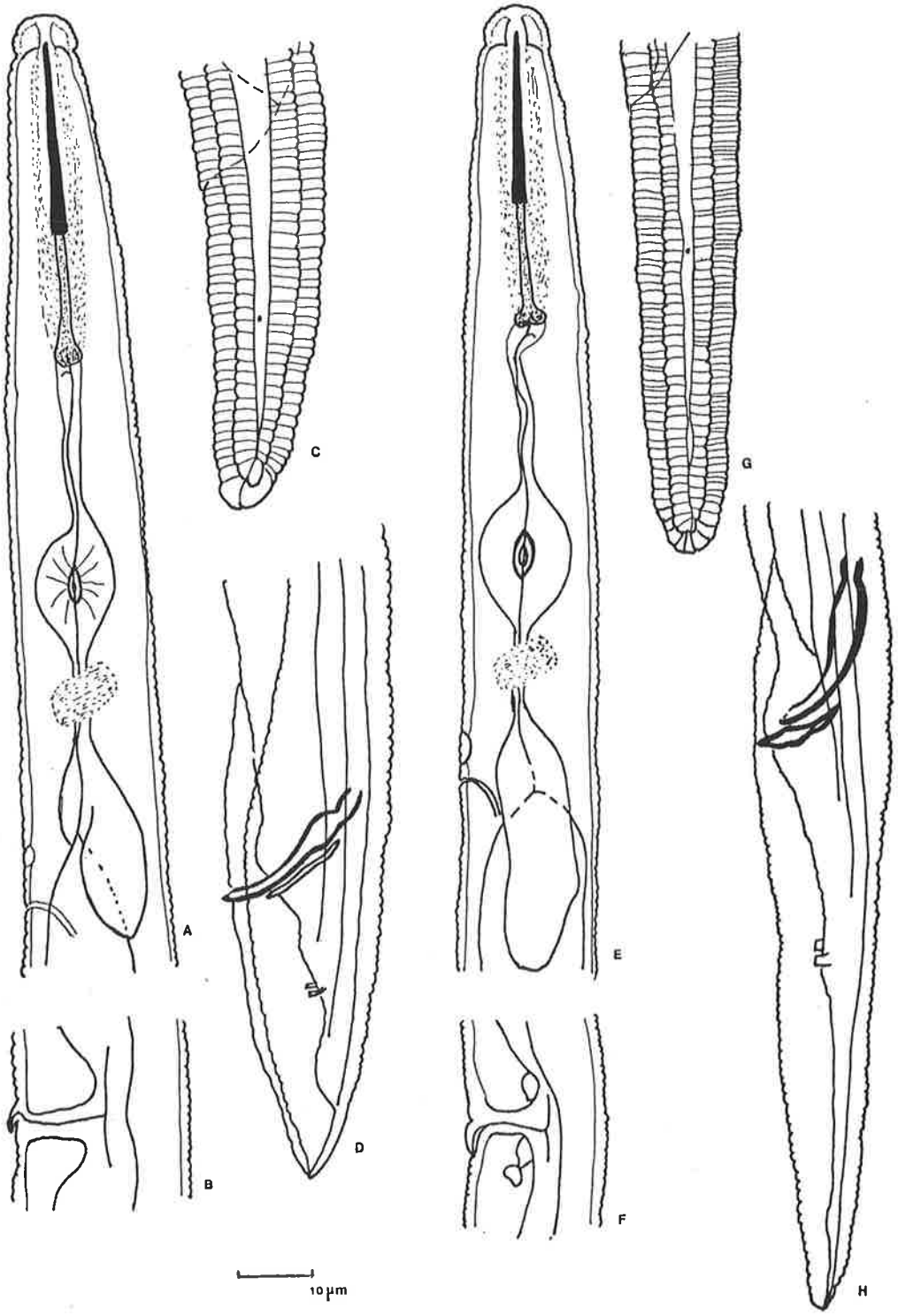


FIGURE 10 : Morulaimus geniculatus (A-D) ; oesophageal region (A), vulval region (B), female tail (C), male tail (D).

M. simpsonii (E-F) ; oesophageal region (E), vulval region (F) female tail (G) , male tail (H).

Male : Similar to female, except smaller. Testis single, outstretched. Spicules stout, gubernaculum sclerotised, protruding from cloaca, distally flanged. Caudal alae extending to tail terminus.

Juveniles found, similar to females except for reproductive system.

Discussion.

This new species is distinguished by having a large stylet, strongly annulated cuticle, offset lip region, gubernaculum protruding from the cloaca, outer lateral field areolated, no post anal sac, non-thichened tail terminus (females) and an epiptygma. The only other *Tylenchorhynchus* species with an epitygma is *T.velatus* which has a continuous lip region, and other characters different. At present it has only been found at two sites close to each other. The location from which *T.siccus* was collected is shown in Figure 9. It is closely associated with a Low Woodland vegetation with *Acacia papyrocarpa* dominant and with a mixed chenopod understorey. A more intensive sampling was carried out within a defined area and is discussed in the next chapter.

Morulaimus geniculatus Sauer, 1965.

Measurements.

Females (n=13) : L=1.14-1.36mm(1.23); V=49-55%(52); a=33-54(42); b=5.1-8.3(6.5); c=19.6-34.5(22.5); c'=1.9-3.0(2.3); stylet=39-65µm(59).

Males (n=13) : L=0.83-1.19mm(1.12); a=36-49(39); b=6.0-8.3(6.7); c=11.5-21.9(12.1); c'=2.7-5.4(4.8); Gubernaculum=12-18µm(14); Spicules=27-36µm(30); stylet=36-59µm(54).

Description (amended from Sauer, 1965).

Large nematode (>1mm), body cylindroid, lip region broad and set-off. Four lateral lines, outer bands areolated the length of the body. Oesophagus typical of the genus (Figure 10).

Female : Reproductive tract didelphic, outstretched. Vulva with double epitygma, sloping anteriorly. Tail conoid, tapering to bluntly rounded, annulated terminus. Rectum short, anus pore-like, phasmid in posterior section of the tail. Tail with 32 annules, terminus annules larger than rest, paired.

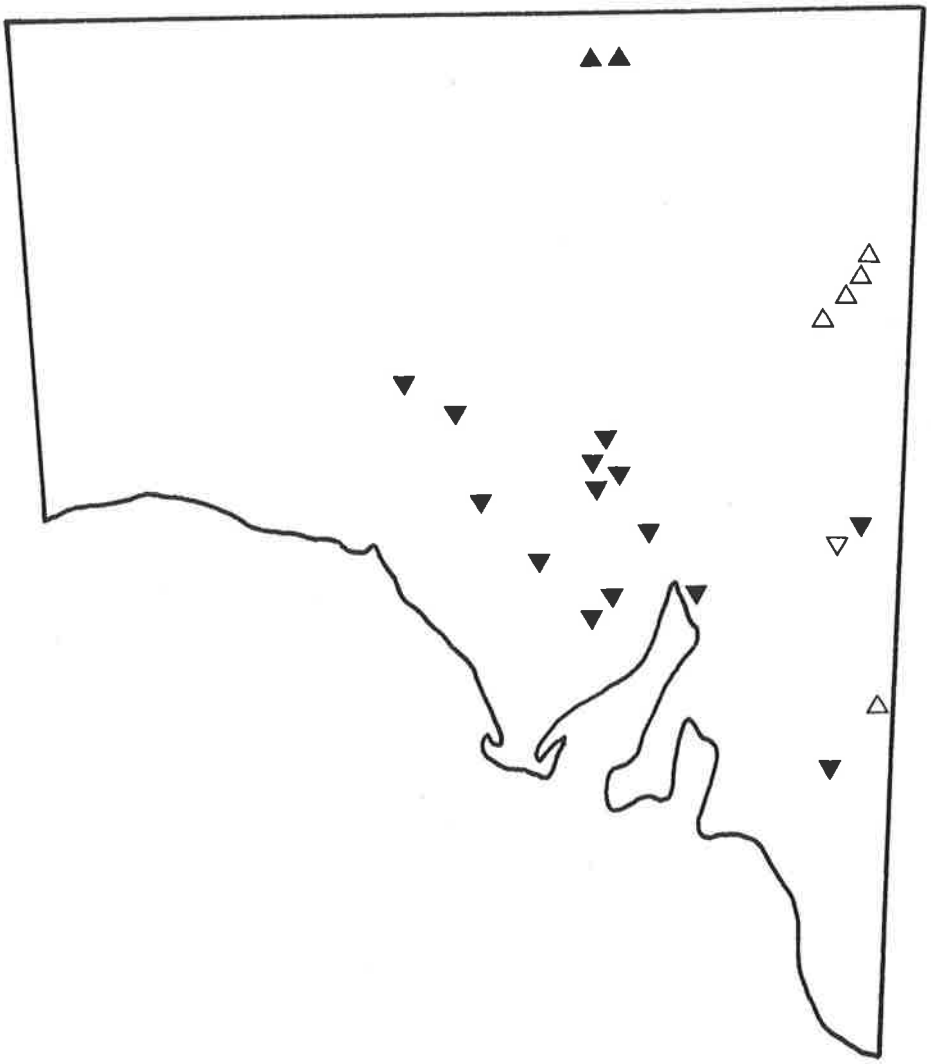


FIGURE 11 : THE DISTRIBUTION OF SPECIES OF Morulaimus AND Telotylenchus hastulatus WITHIN THE ARID REGION OF SOUTH AUSTRALIA.

M.geniculatus - △
M.simpsonii - ▲
Morulaimus spp. - ▼
T.hastulatus - ▽

Male : similar to female, but smaller. Spicules slender, gubernaculum protrusible and irregularly notched, both ventrally and dorsally. Caudal alae ending at tail terminus, tail tip prominent and tail shape broad.

Discussion.

This species belongs to a genus which is almost certainly endemic to the Australian continent. The distribution of this species appears to be confined to the eastern section of the arid region, associated with the large river systems of the Strezlecki Creek and the Murray River (Figure 11). Those sites near the Strezlecki Creek from which specimens were collected were dominated by a *Eucalyptus* Low Woodland.

Morulaimus simpsonii n.sp.

Measurements.

Holotype female : L=1.38mm; V=51%; a=42; b=10.9; b'=7.8; c=15.3; c'=3.9; stylet=53µm.

Paratype females (n=6); L=1.22-1.47(1.35)mm; V=51-54%(53); a=47-57(50); b=6.9-10.1; b'=6.1-8.3(6.9); c=15.1-24.0(18.2); c'=2.8-3.9(3.4); stylet=51-61µm(54).

Allotype Male : L=1.22mm; a=49; b=8.1; b'=6.6; c=12.1; c'=5.4; Spicules=35µm; Gubernaculum=17µm; stylet=50µm; T=51%

Paratype Males (n=6) : L=1.00-1.33mm(1.18); a=43-53(47); b=6.2-8.1(6.7); b'=5.2-6.6(5.9); c=10.9-12.1(11.2); c'=5.3-6.1(5.5); Spicules=31-38µm(35); Gubernaculum=15-18µm(17); stylet=50-54µm(52); T=45-56%(50).

Description.

Large nematode, body cylindroid, lip region set off, broad. Stylet long, well developed. Cuticle coarsely annulated, with four lateral lines, outer areolated. Dorsal oesophageal gland opening less than 1 µm behind stylet knobs. Oesophagus typical of genus, with slight dorsal overlap (Figure 10). Median bulb ovoid with well developed valve. Oesophago-intestinal junction anterior to mid-point of oesophageal lobe. Excretory pore parallel to oesopgago-intestinal

junction. Hemizonid present, 2 annules anterior to excretory pore and 3 annules long. Nerve ring just posterior to base of median bulb.

Female : reproductive tract didelphic, outstretched. Spermathecae present, full of sperm, set off. Vulva with prominent double epitygma, less than 5 µm, sloping anteriorly. Tail tapering to blunt rounded, annulated terminus. Rectum short, anus pore-like. Phasmid in anterior section of tail. Number of tail annules about 54.

Male : Smaller than female, similar. Spicules stout, gubernaculum with prominent notches on distal edge, protrusible. Tail very long (mean c'=5.5). Caudal alae enveloping tail terminus.

Discussion

There are seven named species of *Morulaimus* , excluding this one, only one of which has been found outside Australia. *M.simpsonii* is distinquished by having a small stylet (as compared to other species) and very long tail of both males and females (female *M.simpsonii* c'=3.4 compared to *M.simplex* Sauer and Annells, 1981, with c'=2.8). *M.simpsonii* was collected from soil from dunes on the western edge of the Simpson Desert. It is postulated that this species is distributed throughout the Simpson Desert, as it is closely associated with *Zygocloa paradoxa*, which is the major vegetation component on the crest of dunes (investigated in the next chapter). The distribution of sites from which *M.simpsonii* and other undescribed *Morulaimus* spp. is shown in Figure 11.

Dolichorhynchus (Neodolichorhynchus) sedecimstriatus n.sp.

Measurements.

Holotype Female : L=0.77mm; V=55%; a=30; b=6.0; c=14.0; c'=3.0; stylet=18µm.

Paratype Females (n=35) : L=0.59-0.83mm(0.74); V=52-58%(55); a=21-43(31); b=4.0-6.8(5.2); c=9.2-19.9(13.8); c'=2.0-3.3(2.9); stylet=17-20µm(18).

Allotype Male : L=0.67mm; a=30; b=6.8; c=12.8; c'=4.7; Spicules=27µm; Gubernaculum=7.5µm; stylet=16µm; T=63%.

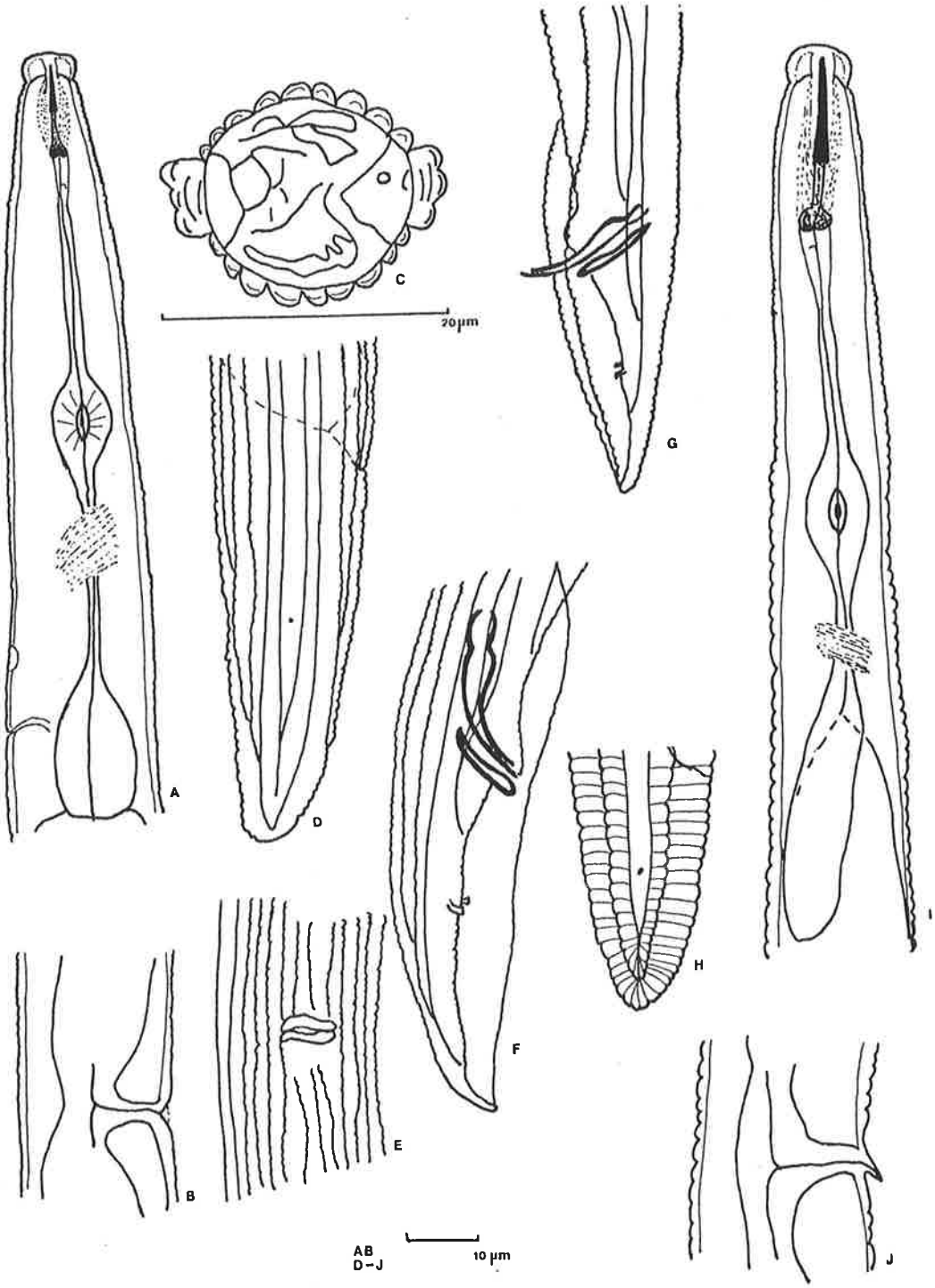


FIGURE 12 : Dolichorhynchus(Neodolichorhynchus)sedecimstriatus (A-F) ; oesophageal region (A), vulval region (B), transverse section through mid-body (C), female tail (D), ventral view of the vulval region (E), male tail (F).
Telotylenchus hastulatus (G-J) ; male tail (G), female tail (H), oesophageal region (I), vulval region (J).

Paratype Males (n=10) : L=0.66-0.74mm(0.69); a=28-34(31); b=4.9-6.8(5.3); c=10.7-15.2(12.7); c'=4.0-4.7(4.3); Spicules=25-27μm(26); Gubernaculum=7-14μm(10); stylet=15-18μm(16); T=61-77%(67).

Description.

Body straight, annules very fine (0.8μm), tail annules larger (1.0μm). Lip region set off, rounded, with three to four indistinct annules. Cuticle with 24 longitudinal ridges. There are nine dorsal and nine ventral and three raised above the others in each lateral field (Figure 12). The outer fields are indistinctly areolated, and fuse near tail terminus. The first 12 body ridges begin just behind the lip region and increase to 24 a short distance posterior to the stylet knobs. They decrease in number gradually from the anus to about 10 μm from the tail terminus, by fusing with each other and the lateral lines.

The oesophagus is typical of the genus (Figure 12) with the dorsal oesophageal gland opening 6 μm behind stylet knobs. The excretory pore is 15 annules anterior to the base of the terminal bulb. Hemizonid present, 5 annules long and between 5 to 16 annules anterior to excretory pore. Intestine typical, with some indistinct serpentine canals.

Female : Reproductive tract didelphic, outstretched. Spermathecae present full of sperm. Vulva a transverse slit, with no surrounding cuticular membranes and cuticle posterior to vulva with irregular depressions. Tail tapering to smooth terminus, about 50 annules on tail, phasmid in anterior section of tail.

Male : Similar to female, but smaller. Testis single, outstretched. Phasmid present on posterior section of tail. Caudal alae crenate, enveloping smooth tail terminus. Spicules delicate, gubernaculum protrusible, with smooth proximal end.

Juveniles found, similar to females except for reproductive tract.

Discussion.

The type specimens were collected from a site as indicated in Figure 9. Specimens were obtained from different sites, but the distribution appears to be confined to the south western section of the region, near Lake Gairdner in the Gawler Ranges. Most sites from which this nematode was collected were Low Woodland dominated by *Acacia papyrocarpa*

and with an *Atriplex vesicaria* understorey. More extensive field sampling was carried out and is presented in the following chapter.

The taxonomy of this genus has been partially resolved by Siddiqi (1986). The genus *Dolichorhynchus* was first proposed by Mulk and Siddiqi, 1982, to include those species formerly in *Tylenchorhynchus*, which have longitudinal ridges, the lateral ridges raised above the body ridges and the lip region offset from the body. Many of the species have a notched caudal ala, cuticular membranes surrounding the vulva and the gubernaculum with a flange.

In 1984, Jairajpuri and Hunt proposed the genus *Neodolichorhynchus* to include those species without a notched caudal ala or cuticular membranes surrounding the vulva and having a smooth gubernaculum. In 1986, Siddiqi placed the species with the above characters into the sub-genus *Neodolichorhynchus* of the genus *Dolichorhynchus* as it was considered that the presence of longitudinal ridges is a character at the generic level and the other characters are suitable for the sub-generic level. It is accepted here that this species will be placed in *Dolichorhynchus(Neodolichorhynchus)* .

Telotylenchus hastulatus (Colbran, 1960) Siddiqi, 1963

syn. *Belonolaimus hastulatus* Colbran, 1960

Morulaimus hastulatus (Colbran, 1960) Sauer, 1965

Measurements.

Females (n=6) : L=0.68-1.06mm(0.82); V=55-64%(59); a=32-43(36); b=5.3-7.2(5.8); c=15.8-25.0(21.2); c'=1.9-2.7(2.2); stylet=26-36µm(29).

Males (n=6) : L=0.81-0.92mm(0.88); a=35-44(40); b=5.4-7.9(6.4); c=13.0-17.1(15.3); c'=3.1-3.7(3.4); Gubernaculum=12-16µm(13; Spicules=24-28µm(25); stylet=31-34µm(32).

Description (amended from Colbran, 1960).

Body cylindroid, coarsely annulated. Four lateral lines, with outer bands areolated length of body. Lip region off set, 7 annules, indistinct. Oesophagus typical of the genus (Figure 12).

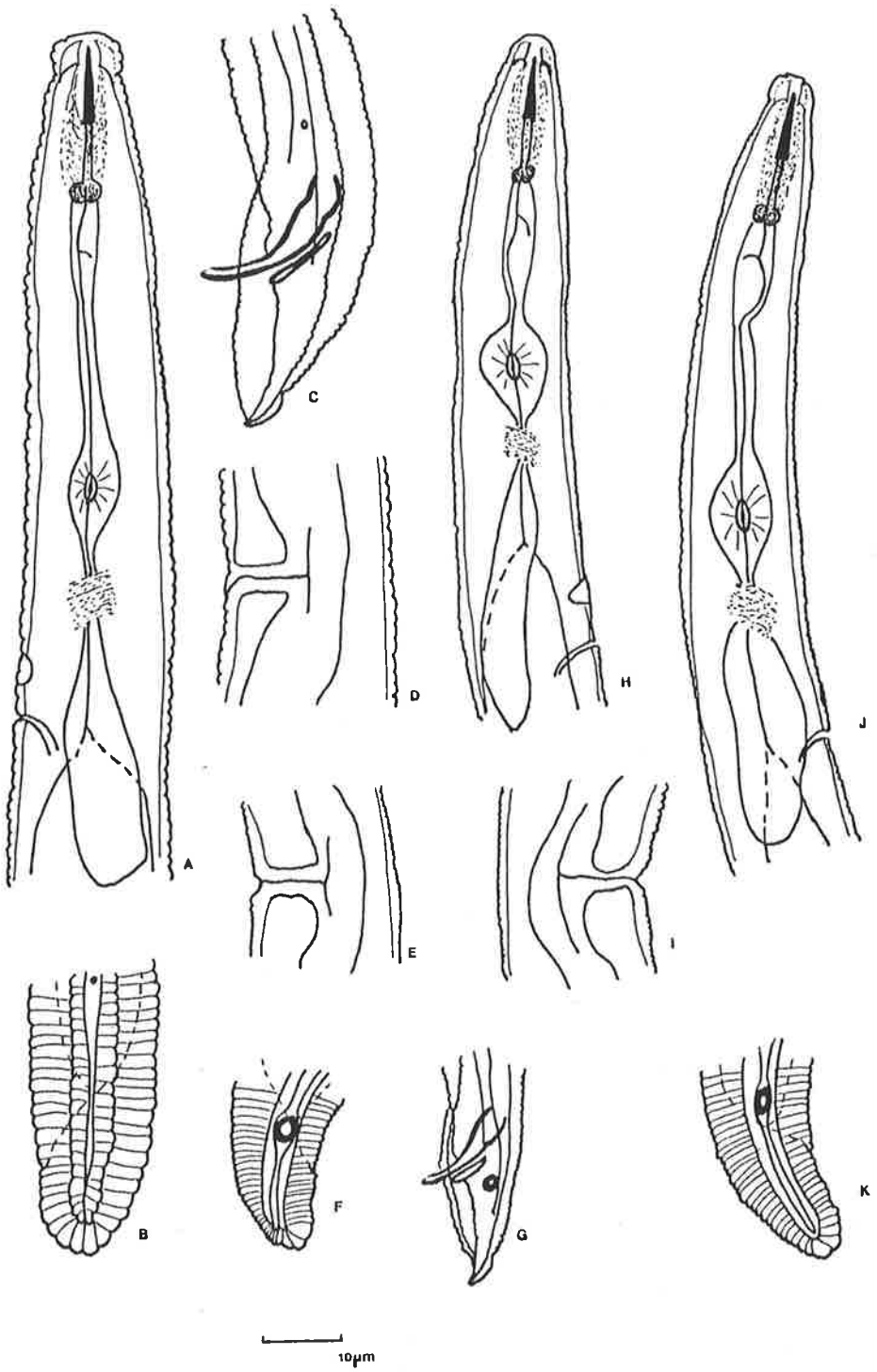


FIGURE 13 : Rotylenchus wallacei (A-D) ; oesophageal region (A), female tail (B), male tail (C), vulval region (D).
Scutellonema minutum (E-H) ; vulval region (E), female tail (F), male tail (G), oesophageal region (H).
S. laeviflexum (I-K) ; vulval region (I), oesophageal region (J), female tail (K).

Female : Reproductive tract didelphic, outstretched. Vulva with conspicuous epiptygma, double, 5µm long, sloping anteriorly. Rectum short, anus pore-like. Tail tapering to rounded terminus, 21-22 annules, coarser than body annules. Phasmid pore-like, posterior to anus by 6-8 annules.

Male : similar to females, smaller. Spicules stout, gubernaculum knobbed and distally flanged, protrusible. Caudal alae crenate, encompasses tail terminus.

Discussion.

T.hastulatus was identified from sites within a pastoral lease (sheep station) in the limestone based soils of the Murray Plains (Figure **11**). It is felt that this species is very closely related to the genus *Morulaimus*, as it was placed in this genus by Sauer in 1965. The main difference which places this species into *Telotylenchus* and not *Morulaimus* is the short stylet (21-36µm compared to 50-150µm, for the species in *Morulaimus*). A trend of increased body and stylet size within the family Dolichodoridae will be discussed in the concluding section of this chapter.

3.3.4. The Family Hoplolaimidae.

Rotylenchus wallacei n.sp.

Measurements.

Holotype Female : L=0.84mm; V=59%; a=33; b=7.6; b'=5.5; c=55.7; c'=0.8; stylet=28µm.

Paratype Females (n=12) : L=0.70-0.95mm(0.80); V=53-59%(56); a=25-33(29); b=7.1-10.3(8.1); b'=5.5-7.0(5.9); c=26.0-64.7(49.7); c'=0.6-1.2(0.9); stylet=22-27µm(25).

Allotype Male : L=0.78mm; a=31; b=8.4; b'=5.4; c=20.4; c'=2.2; Spicules=30µm; Gubernaculum=11µm; stylet=26µm; T=62%.

Paratype Males (n=15) : L=0.65-0.86mm(0.76); a=27-37(30); b=6.5-8.5(7.7); b'=5.0-6.0(5.5); c=16.7-26.9(19.1); c'=1.7-2.6(2.1); Spicules=25-30µm(26); Gubernaculum=11-16µm(13); Spicules=25-30µm(26).

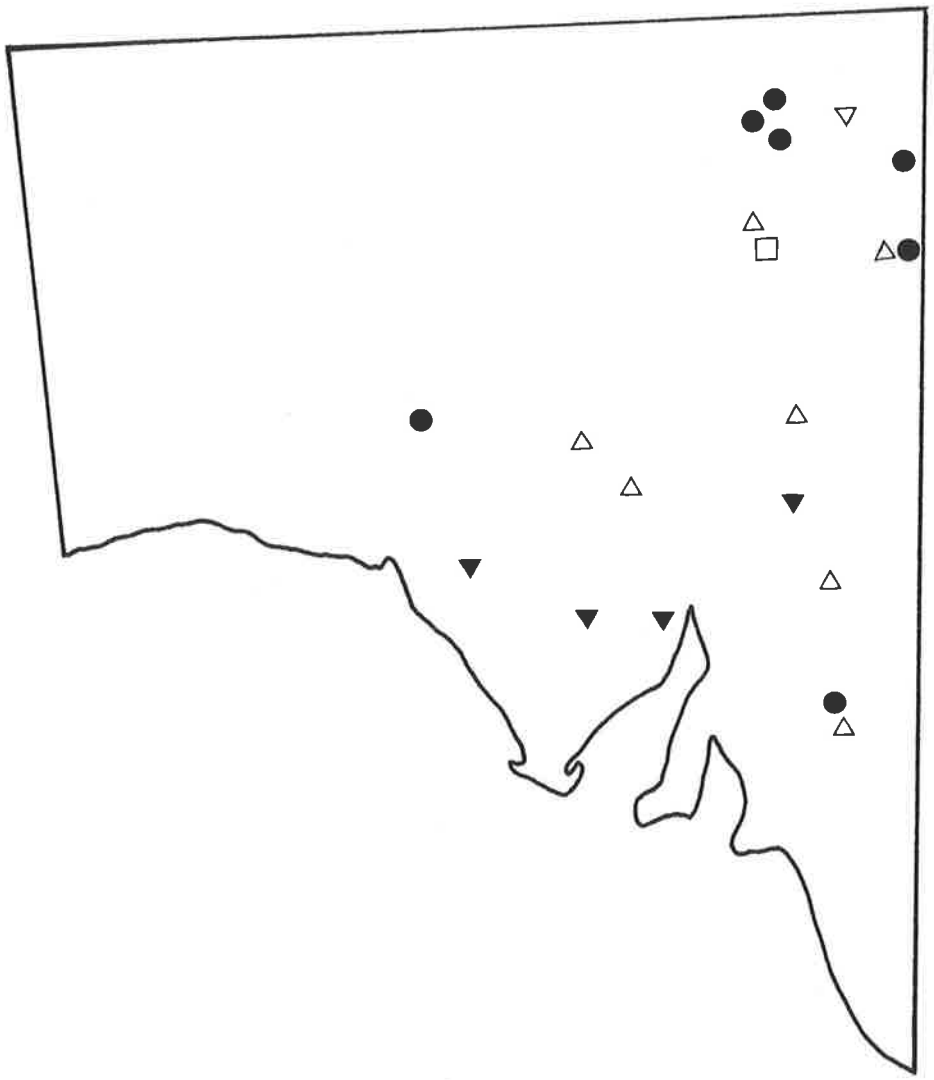


FIGURE 14 : THE DISTRIBUTION OF CERTAIN PLANT PARASITIC NEMATODES IN THE ARID REGION OF SOUTH AUSTRALIA.

Rotylenchus wallacei	-	□
Rotylenchus spp.	-	△
Hoplolaimus spp.	-	●
Paratylenchus spp.	-	▽
Paralongidorus spp.	-	▼

Description.

Body C-shape on relaxation, cylindroid. Cuticle coarsely annulated, lip-region set off, 3 annules, basal annule with some longitudinal striations. Lateral field with four incisures, areolated the length of the body. Labial framework lightly sclerotised, stylet strong, knobs sloping anteriorly. Oesophagus typical of the genus (Figure 13) with dorsal oesophageal gland opening 2 annules posterior to stylet knobs. Median bulb ovoid with sclerotised valve plates. Nerve ring posterior to beginning of oesophageal lobes. Oesophago-intestinal junction at mid-point of oesophageal lobes. Excretory pore opposite junction of intestine and oesophagus. Hemizonid present, 2 annules anterior to excretory pore, 2 annules long.

Female : Reproductive tract didelphic, outstretched. Vulva with epiptygma, double, 2 µm long. Vulva about 1/2 body width. Spermathecae present, full of sperm. Rectum long, anus pore-like. Tail hemispherical, bluntly rounded, curved more dorsally, short (c'=0.9) with 9 annules. Tail terminus annulated. Phasmid anterior to anus, 16 annules.

Male : Similar to female, slightly smaller. Spicules stout, gubernaculum smooth and stout, not protrusible. Phasmid anterior to cloaca. Caudal alae crenate, terminates at tail terminus, but not enveloping terminus. Last annule on tail larger than others.

Discussion.

R.wallacei was collected from one site from vegetation on the Birdsville track (Figure 14) and is placed in the genus *Rotylenchus,* because it has a small phasmid on the posterior section of the body and the oesophageal glands overlap the intestine more dorsally. It is distinguished from other species of *Rotylenchus* , except *R.ranapii* Darekar and Khan, 1982, by having the outer lateral fields completely areolated the length of the body. It differs from *R.ranapii* by being longer (0.70-0.95mm compared to 0.50-0.65mm), no small ventral projection present on tail terminus, number of tail annules greater (16 compared to 4-6) and the presence of males in the population.

Specimens of the genus *Rotylenchus* were identified from soil from many sites, but only occurred at this site in sufficiently high numbers to determine accurately the species. The distribution of sites from which specimens of the genus *Rotylenchus* were identified are presented in Figure 14.

Scutellonema minutum Sher, 1963

Measurements.

Females (n=17) : L=0.53-0.64mm(0.58); V=55-70%(62); a=20-31(26); b=5.2-8.2(6.7); b'=3.7-5.7(4.7); c=27.7-42.9(35.6); c'=0.9-1.6(1.1); stylet=21-25µm(23).

Males (n=6) : L=0.51-0.61mm(0.57); a=20-34(30); b=6.0-7.4(6.6); b'=4.1-4.8(4.6); c=10.5-21.7(15.6); c'=2.1-2.6(2.3); Spicules=19-22µm(20); Gubernaculum=7-10µm(8); stylet=19-20µm(19).

Description (amended from Sher, 1963).

Body assumes spiral shape on relaxation. Lip region hemispherical, continuous, with four indistinct annules. Oesophagus typical of genus (Figure 13).

Female : Reproductive tract didelphic, outstretched. Vulva occasionally (rare) with inconspicuous epiptygma. Tail tapering to bluntly rounded terminus. Phasmid scutella shaped, 1.5µm in diameter, 4 annules anterior to anus. Rectum short, intestine not overlapping, anus pore-like. Tail more curved dorsally, with 12 annules on ventral side.

Male : Different to males described by Sher (1963). Males similar to females, slightly smaller. Testis single, outstretched. Spicules stout, gubernaculum small, protrusible. Tail tapering to sharp terminus. Caudal alae not quite enveloping terminus.

Discussion.

It appears that the species, *Scutellonema minutum* , could be widely distributed in the north of the state, but this population may be confined to the Dalhousie Springs (Figure 16). Specimens of this species have been collected from other sites but in low numbers and often as juveniles. *S.minutum* was first described by Sher (1963) from a site in Queensland. The male specimens collected in this study from the Dalhousie Mound Springs, differed from the described species in tail shape, smaller spicules(19-22µm for the Dalhousie specimens compared to 22-24µm for the specimens of *S.minutum* described by Sher) and smaller stylet(19-20µm for the Dalhousie specimens compared to 21-25 µm for the specimens of *S.minutum* described by Sher). It is felt that since the females from the

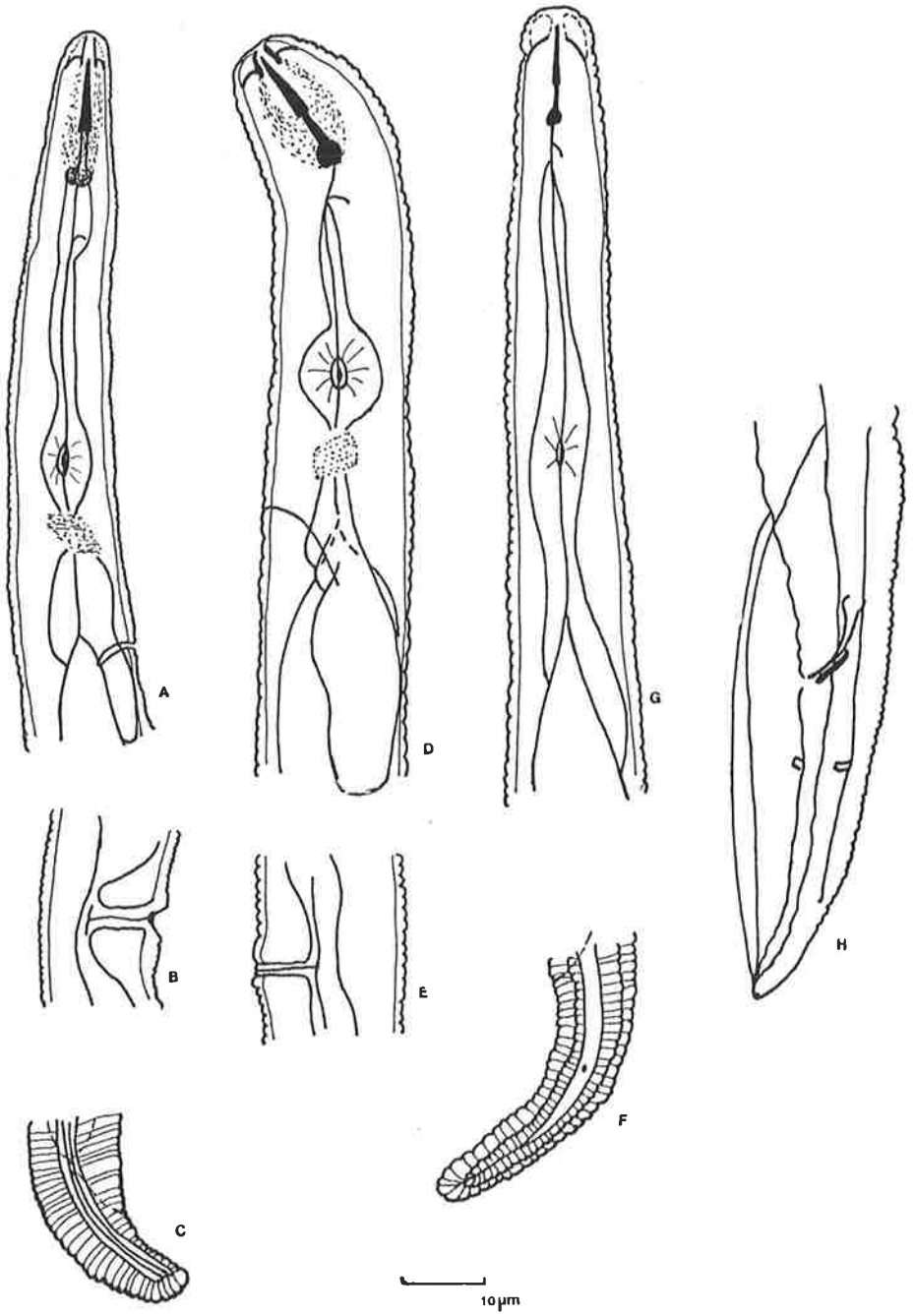


FIGURE 15 : Helicotylenchus variabilis (A-C) ; oesophageal regiob (A), vulval region (B), female tail (C).
Radopholus crenatus (D-H) ; female oesophageal region (D), vulval region (E), female tail (F), male oesophageal region (G), male tail (H).

springs are similar to the described females and that the males are slightly different, that the specimens from this site should remain in the species *S.minutum*.

Scutellonema laeviflexum Phillips, 1971.

Measurements.

Females (n=29) : L=0.53-0.88mm(0.66); V=55-68%(63); a=25-44(30); b=5.7-9.7(7.2); b'=4.2-7.2(5.2); c=30.4-62.3(43.0); c'=0.8-1.4(1.1); stylet=20-26µm(24).

Description (amended from Phillips,1971).

Body C-shaped. Lip region hemispherical, slightly set off, 6 annules. Oesophagus typical of the genus (Figure 13).

Female : Reproductive system didelphic, outstretched. Vulva with inconspicuous (rare) epiptygma. Scutella 1.5 to 1.8 µm (1.6) in diameter, 8 to 10 annules anterior to anus. Tail broadly rounded, slightly more curved dorsally, terminal striations slightly larger, but similar to other tail annules. No males were described by Phillips, and no males were found in the arid soils.

Discussion.

The original description by Phillips (1971), was from specimens collected from soil around native plants from Queensland. Measurements and ratios of the specimens collected from the field differed occasionally from those described by Phillips. This could be due to population differences due to isolation. The distribution of this species (Figure 16) appears to be fairly wide and also to be associated with growth of ephemerals.

Helicotylenchus variabilis Phillips, 1971.

Measurements.

Females (n=11) : L=0.53-0.80mm(0.71); a=30-40(35); b=5.9-12.3(9.7); b'=4.5-8.2(7.1); c=26.2-75.1(39.3); c'=0.8-1.8(1.4); V=63-69%(66); stylet=20-23µm(22).

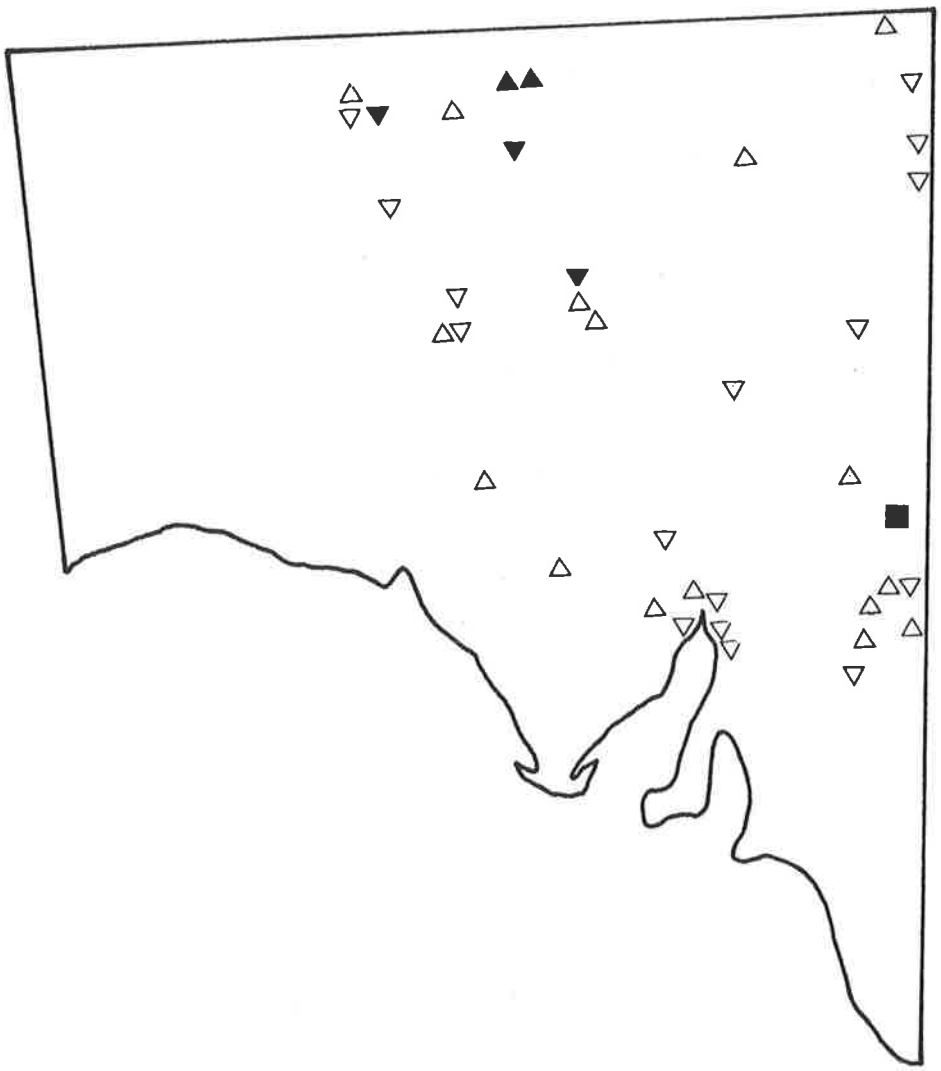


FIGURE 16 : THE DISTRIBUTION OF SPIRAL NEMATODES WITHIN THE ARID REGION OF SOUTH AUSTRALIA.

Helicotylenchus variabilis	-	■
Helicotylenchus spp.	-	△
Scutellonema minutum	-	▲
S.laeviflexum	-	▼
Scutellonema spp.	-	▽

Description (amended from Phillips 1971).

Body in loose spiral, lip region conoid-truncate, not set off, without distinct annules. Oesophagus typical of the genus (Figure 15).

Female : Reproductive tract didelphic, outstretched. Spermathecae round, not set off, no sperm. Phasmid 6-10 annules anterior to anus. Tail curved, more dorsally, 12-13 annules, terminus tapered to a rounded point, with varied shape and regularity of terminal striations. No males found in this population.

Discussion.

This species was collected from a site near Broken Hill, in the south eastern corner of the region (Figure 16). The specimens collected at this site were similar in measurement and morphology to those described by Phillips and so these have been called *H.variabilis*. Other specimens of the genus *Helicotylenchus* have been collected from other sites, but did not occur in large enough numbers (<4) for accurate identification to species. The distribution of the genus *Helicotylenchus* is presented (Figure 16).

Radopholus crenatus Colbran, 1970.

Measurements.

Females (n=3) : L=0.59-0.68mm(0.64); V=54-60%(57); a=24-35(28); b=7.2-8.2(7.7); b'=5.2-5.5(5.3); c=13.0-13.7(13.3); c'=2.5-4.1(3.1); stylet=20µm.

Males (n=2) : L=0.65-0.71mm(0.68); a=28-35(32); b=6.1-7.8(6.9); b'=3.8-4.5(4.1); c=10.9-11.0(10.9); c'=3.6-3.9(3.7); Spicules=15-17µm(16); Gubernaculum=7-9µm(8); stylet=15-17µm(16).

Description (amended from Colbran, 1970).

Females :- Body slender, cylindroid. Cuticle annulated, four lateral lines, outer areolated the length of the body. Lip region truncate-conoid, continuous. Stylet short, stout, knobs rounded. Oesophagus typical of the genus (Figure 15). Reproductive tract didelphic, outstretched. Vulva a small slit, with simple depression, with a lip on either side, anterior

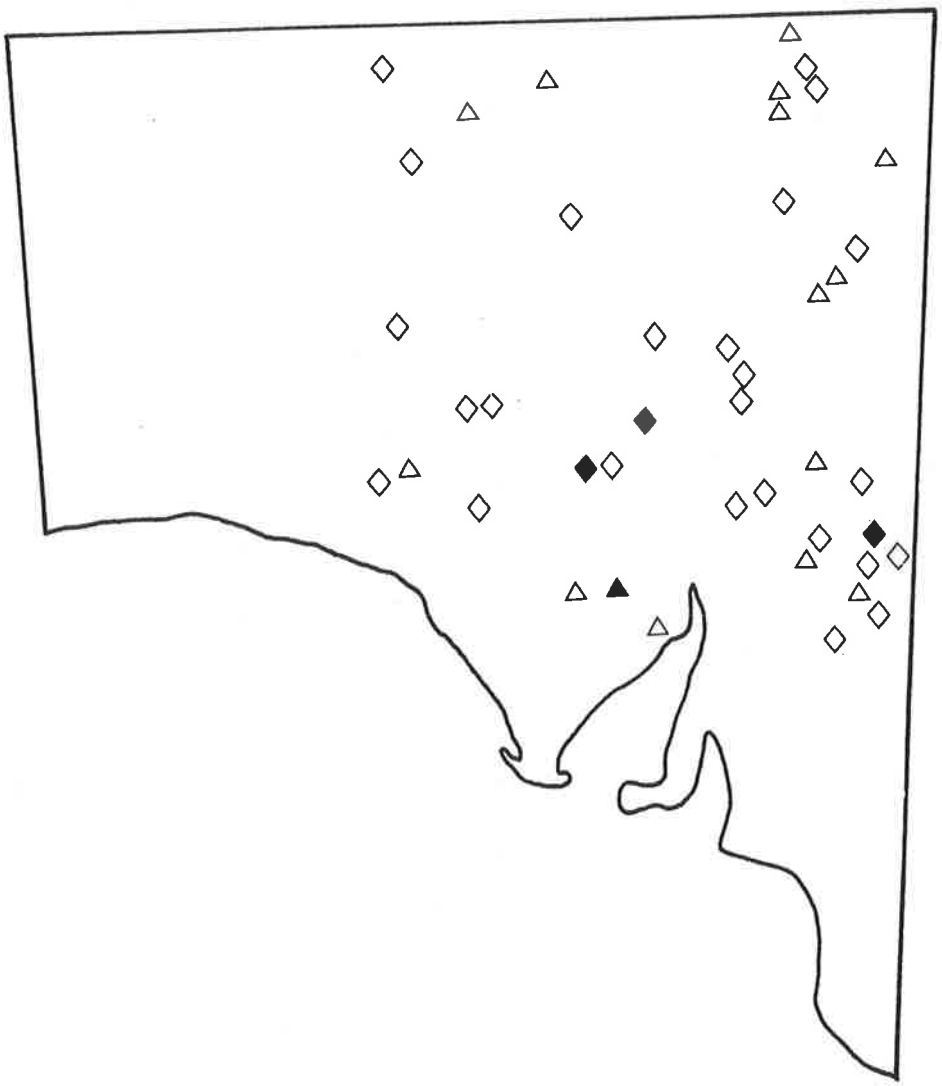


FIGURE 17 : THE DISTRIBUTION OF THE GENERA Radopholus, Pratylenchus AND Heterodera/Globodera IN THE ARID REGION OF SOUTH AUSTRALIA.

Radopholus crenatus	-	▲
Radopholus spp.	-	△
Pratylenchus spp.	-	◇
Heterodera/Globodera spp.	-	◆

and posterior. Tail tapering to rounded, annulated terminus, about 27 annules long. Rectum long, anus pore-like. Phasmid 11 annules posterior to anus, mid-point of tail.

Males :- Body cylindroid, head high, offset, different from female. Stylet and oesophagus degenerate. Testis single, outstretched. Tail long, terminus pointed. Spicules slender, arcuate, gubernaculum simple. Phasmid posterior to cloaca. Caudal alae enveloping terminus, crenate.

Discussion.

This species was collected from a site on the shore of Lake Gilles in the south central area of the state, just out of the marginal agricultural lands (Figure 17). Most specimens of *Radopholus* collected in the arid region of S.A. were juveniles. Only occasionally are there females and males. The specimens collected from the site described above closely resembled those specimens described as *R.crenatus*, except for female b and b' values, which could be due to environemntal factors affecting this ratio of oesophagus length to body length. The distribution of other *Radopholus* species and the genus *Pratylenchus* is presented in Figure 17.

3.3.5. Other plant parasitic nematodes.

The distribution of *Paratylenchus, Paralongidorus* and *Hoplolaimus* are presented in Figure 14. The distribution of *Heterodera/Globodera* is presented in Figure 17. Female specimens of these genera were rarely extracted from the arid region of South Australia, and so were not identified to species level.

3.3.6. Slide Collection.

Slides of all Holotypes and Allotypes are lodged at the South Australian Museum, Adelaide, South Australia. Slides containing paratypes are to be lodged at the following nematode collections :- Commonwealth Institute of Parasitology, St. Albans, Herts., U.K. ; University of California Nematode Collection, Davis, CA95616, U.S.A. ; Museum Nationale d'Histoire Naturelle, Paris, France ; the Waite Agricultural Research Institute, South Australia. The remaining paratypes are to be kept at the South Australian Museum,

TABLE 3 : NUMBER AND PERCENT OF SITES WITH DIFFERENT GENERA OF PLANT PARASITES (PP) EXTRACTED FROM SOIL SAMPLED IN THE YEARS 1983 TO 1985 INCLUSIVE.

	Sites with nematodes	
Genera	Number of Sites (n)	Percent (%) of Total Sites
Tylenchorhynchus	244	65
Dolichorhynchus	10	3
Radopholus	16	4
Morulaimus	29	8
Rotylenchus	8	2
Hoplolaimus	7	2
Scutellonema	17	5
Helicotylenchus	18	5
Pratylenchus	31	8
Paralongidorus	4	1
Sites with > 1 genera PP	97	26
Sites with PP	276	74
Sites without PP	98	26
No. sites sampled	374	100

(PP=plant parasites)

Adelaide, South Australia. The list of the slides which contain the new species is given in Appendix 6.

3.4. GENERAL DISTRIBUTION OF PLANT PARASITIC NEMATODES.

There were 13 genera of plant parasitic nematodes identified from soil collected from the arid region of S.A.. These were *Tylenchorhynchus, Morulaimus, Scutellonema, Telotylenchus, Hoplolaimus, Helicotylenchus, Pratylenchus, Radopholus, Rotylenchus, Paralongidorus, Paratylenchus, Hemicycliophora* and *Heterodera/Globodera* . Table 3 shows the genera of plant parasites present, the number and percent of sites sampled and the total number of sites sampled. The genera *Hemicycliophora, Paratylenchus* and *Heterodera/Globodera* are not included in this table because of the low numbers of sites from which these nematodes were identified (n<4).

The genus *Tylenchorhynchus* was the most abundant genus, as 65% of all sites sampled had at least one specimen, and it occured in 88% of sites which had plant parasites. Of all sites sampled, 26% had no plant parasites and 26% had more than one genus present. The other genera occurred at very low frequencies, so calculation of importance and prominence values for the different genera were not considered necessary or appropriate as the most important and abundant plant parasitic genus was obvious.

There were four species of *Tylenchorhynchus* identified from the arid soils, and over 95% of the sites with specimens of this genus were *T.tobari* (Table 4). *T.tobari* is clearly a widely distributed nematode within the arid region of S.A.. Analysis of the different vegetation, landform, dominant plant species, understorey components and host sampled for those sites with *T.tobari* , compared to the total sites within each catergory of the above was possible. The other species of *Tylenchorhynchus* did not occur in high enough numbers to allow significant associations to be found. This is also the case for the other genera.

From the Chi-square analysis (Appendix 2), there was no significant difference between the expected and observed frequency of *T.tobari* at specific vegetation, landform, dominant plant species and understorey components. There was a significant difference when the expected and observed frequency of *T.tobari* from specific plant species

TABLE 4 : SITES SAMPLED CONTAINING *Tylenchorhynchus* SPECIES (n=244). THE PERCENT OF TOTAL SITES SAMPLED CONTAINING *Tylenchorhynchus* AND PERCENTAGE OF TOTAL SITES SAMPLED (n=374) (data collected from 1983 to 1985 inclusive).

Species	Sites with *Tylenchorhynchus* Number	Percent (%)	Percent of Total Sites
T.tobari	237	98	63
T.velatus	12	5	3
T.annulatus	3	1	1
T.siccus	2	1	1
No *Tylenchorhynchus*	130	-	35

was assessed. This was due to a much higher observed frequency of *T.tobari* than expected when the Chenopod group was analysed. It is felt, therefore, a more intensive sampling of sites with different vegetation associations containing species of the family Chenopodiaceae could show more clearly any host/parasite relationship.

3.5. GENERAL CONCLUSION AND DISCUSSION.

No new families or genera were found in soil from native vegetation occurring in the arid region of South Australia. New species, as well as previously described species, were identified and described. Selection pressure appears to have operated on the nematodes occurring in the arid region, only at the species level. Morphological changes have been restricted to slight changes in body shape. The ability of these nematodes to survive desiccation for long periods may have caused changes at the physiological and biochemical levels, without affecting morphology.

Species of nematodes present today are believed to have evolved by successive adaptation and modifications from species that had primitive and simplified characters (Siddiqi, 1986). However, with the lack of fossil evidence the ability to formulate appropriate evolutionary trees is restricted. Therefore, to determine relationships between nematode species other methods need to be employed. Yeates (1986) showed some positive relationships between stylet and body lenght in females of 19 genera of plant parasitic nematodes from different families. When females of five species belonging in the family Dolichodoridae (all with epitygmas) were assessed for relationship between body and stylet length, there was no significant relationship (Figure 18). The regression lines all showed a positive relationship, but the hypothesis that there was a relationship between body size and stylet length was rejected as the slope of each line was not significantly different from 0. The number of individuals measured may have been too small to detect any significant associations. Simple comparisons of morphology may aid in determining relationships.

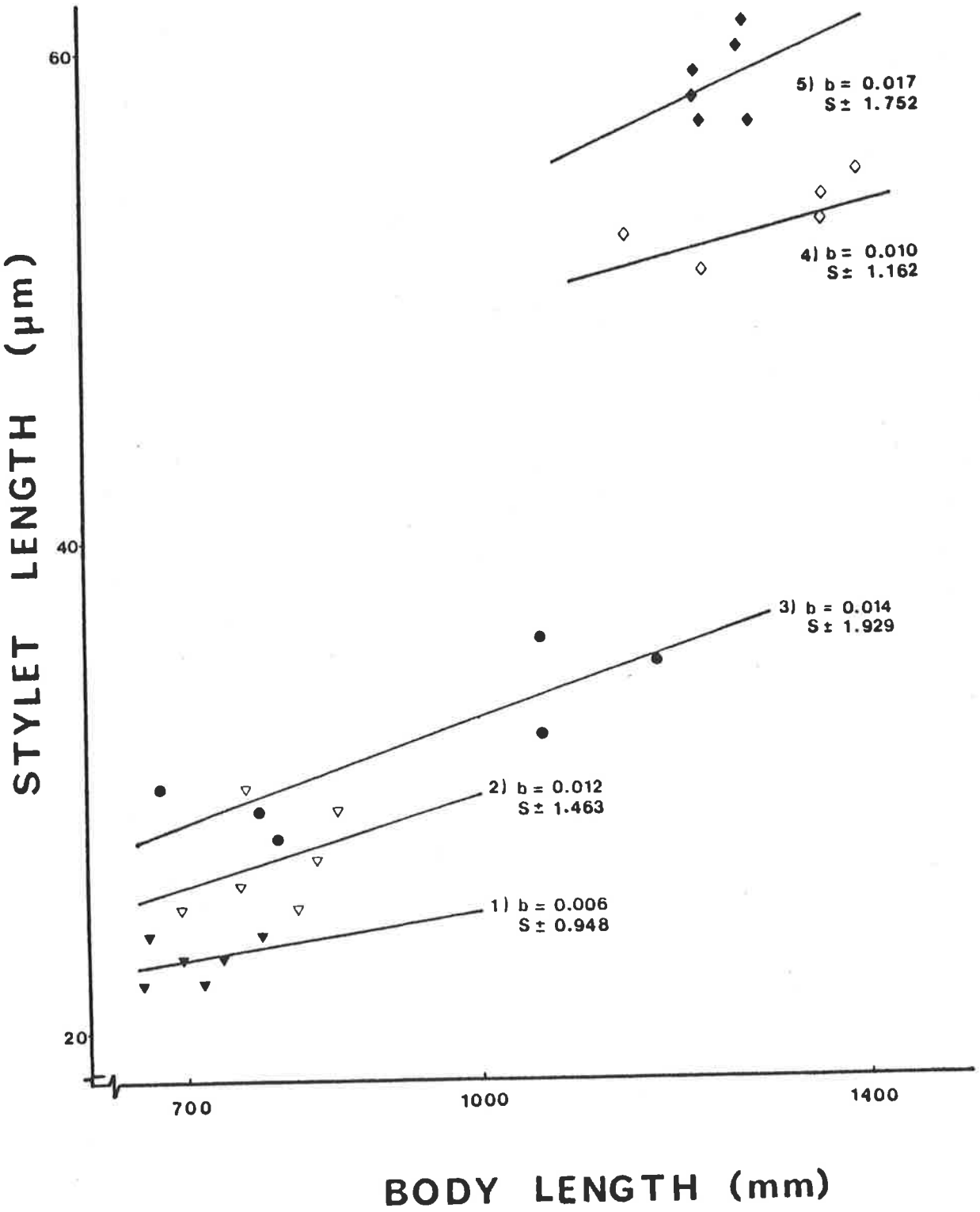
With the adaptation of nematodes within the Tylenchina towards plant parasitism, there has been an associated change in stylet length, thickness and/or shape (Fortuner and Luc, 1987), as well as a reduction in tail size. Within the family

FIGURE 18 : THE REGRESSION OF BODY AND STYLET LENGTH OF FIVE DIFFERENT SPECIES OF NEMATODES, ALL OF WHICH HAVE AN EPITYGMA, WITHIN THE FAMILY DOLICHODORIDAE.

1) Tylenchorhynchus velatus - ▼
2) Tylenchorhynchus siccus - ▽
3) Telotylenchus hastulatus - ●
4) Morulaimus simpsonii - ◇
5) Morulaimus geniculatus - ◆

(numbers preceeding species names correspond to numbers by regression lines).
(S= plus or minus the variation about the regression line).

(calculations were made from 6 individual nematodes for each species except Morulaimus simpsonii (n=5)).


STYLET LENGTH (µm)
60
40
20
700
1000
1400
BODY LENGTH (mm)
5) b = 0.017
S ± 1.752
4) b = 0.010
S ± 1.162
3) b = 0.014
S ± 1.929
2) b = 0.012
S ± 1.463
1) b = 0.006
S ± 0.948

Dolichodoridae there is an increase in stylet length from *Tylenchorhynchus velatus* (18 µm) to *Morulaimus geniculatus* (59µm). This could be due to the increased incidence of woody Angiosperms over the last 10 million years and adaptation in the nematodes that enabled them to penetrate roots of increased size. The basic morphology of these nematodes is very similar, the only major difference being size. In species of *Tylenchorhynchus* , such as *T.siccus* and *T.velatus,* the terminal oesophageal bulb has a stem-like projection overlapping the intestine and an epitygma. *Telotylenchus hastulatus, Morulaimus geniculatus* and *M.simpsonii* all have an epiptygma and an overlap of the oesophagus over the intestine. Further work using numerical taxonomy and levels of similarity may test the hypothesis that there is an evolutionary link between species within different genera of the family Dolichodoridae, but this was not attempted in this study.

The plant parasitic nematode *T.tobari* may be found to be associated with plant species of the family Chenopodiaceae, but further work on the relationship between the distribution of host plants in the field and particular plant parasites is needed and is presented in the following chapter.

CHAPTER 4 : THE RELATIONSHIP BETWEEN THE DISTRIBUTION OF PLANT SPECIES AND NEMATODES, WITH SPECIFIC REFERENCE TO THE TYLENCHID PLANT PARASITES.

4.1. INTRODUCTION.

The arid region of South Australia has a diverse vegetation which is mainly endemic to the Australian continent and has evolved over a period of fluctuating climate, with a predominately drying trend. The classification system for describing vegetation and landforms has been presented in Chapter 2 and will be used to classify sites from which soil was sampled for nematodes. Other considerations, such as growth of the host plant (Elton and Miller, 1954) will be considered, as well as the trophic structure of the nematode community, host roots, predators and parasites. In these studies of associations between nematodes and specific host plants, special reference is given to the plant parasitic nematodes.

Within the nematode community, there are several different trophic groups (Freckman and Caswell, 1985; Yeates, 1971) which are categorised by apparent food requirements. These food requirements are determined by the structure of the head, lip and oesophageal regions, which are easily identified under the dissecting microscope. In this study, four main groups are used :- 1) Omnivore/predators (mainly Dorylaimida); 2) Bacterial feeders (mainly Rhabditida); 3) Fungal feeders (mainly Aphelenchida and small stylet forms of Tylenchida); and 4) Plant parasites (Tylenchida). The composition of the nematode community within the arid (desert) regions of North America has been investigated (Freckman et al., 1975; Freckman and Mankau, 1977,1986; Parker et al., 1984; Whitford et al., 1981, 1983). From most studies, the bacterial feeders have been found to be the most abundant and widely distributed nematode trophic group within set areas. This study was designed to investigate the relationship between changes in the numbers of nematodes of the different trophic groups and the distribution of plant species.

Any extensive and intensive sampling of the total arid region of S.A. is difficult because of the distances involved, the diverse flora in the area, the time required to process soil and identify nematodes and the inacessibility of certain areas. Fortunately, vegetation close to the few tracks that were present, was undisturbed and so samples could be taken.

As shown in the previous chapter, there were a number of different plant parasitic nematode genera found in the arid region. However, the most abundant were those of the Family Dolichodoridae, especially the genus *Tylenchorhynchus*. The main purpose of the field work was to establish :- a) To what extent the distribution of particular host plants influence the distribution of nematodes and b) What changes occur in the numbers and proportion of nematodes of different trophic groups with time. The general distribution and classification of the Tylenchid plant parasites has been presented in the previous chapter. A more statistical assessment of their distribution is presented in this chapter.

4.2. DISTRIBUTION OF NEMATODES WITHIN DEFINED AREAS.

4.2.1. Introduction.

The association between the distribution of particular host plants and trophic groups of nematodes was investigated to answer the following questions :-

1) Is there a change in the structure and numbers of nematodes of the different trophic groups with changes in soil depth?

2) Are particular nematode species associated with particular types of vegetation and landform?

3) Are any particular nematode trophic groups or species associated with specific plant species within defined areas?

4) Is there any association between the presence of disease in plants found in the field and the presence of plant parasitic nematodes in the soil?

Some work has been done on the distribution pattern of nematodes within native vegetation, but a broad system of sampling only was used (Reay and Wallace, 1981a). In this project, an attempt was made to determine the spatial relationship between nematodes and host plants within small defined area.

4.2.2. Depth Analysis.

Introduction.

From work done in the U.S.A on nematodes present in soil under plants in desert regions (Freckman and Mankau, 1977, 1986), most nematodes of all trophic groups

TABLE 5 : MEAN NUMBERS OF NEMATODES OF DIFFERENT TROPHIC GROUPS EXTRACTED FROM SOIL COLLECTED FROM FOUR DIFFERENT SITES AT TWO DEPTHS (1-10cm and 11-25 cm) AND WITH LOW SHRUBLAND (dominated by *Atriplex vesicaria*).

		Trophic groups (50 ml of soil)					
Site	Depth	O/P	BAF	Aph	Tyl	PLP	Total
1	1-10	34.2	360.7	3.2*	9.5*	107.5	629.2
	sd	*11.9*	*77.1*	*1.9*	*4.5*	*55.5*	*217.1*
(n=4)	11-25	29.0	314.0	24.0*	27.0*	59.0	453.0
	sd	*10.2*	*56.2*	*8.2*	*10.4*	*25.1*	*52.9*
2	1-10	57.5	240.2	32.0*	19.2*	8.0	358.0
	sd	*18.0*	*71.6*	*17.5*	*9.9*	*5.0*	*94.7*
(n=4)	11-25	54.0	168.7	6.0*	41.5*	5.7	276.0
	sd	*14.5*	*30.7*	*2.7*	*23.7*	*2.5*	*41.7*
3	1-10	30.0	96.7	1.0	0.7*	39.3	167.7
	sd	*15.9*	*48.2*	*0.5*	*0.8*	*32.3*	*94.8*
(n=3)	11-25	18.0	73.0	0.0	4.3*	5.7	101.0
	sd	*10.5*	*12.8*	*0.0*	*2.9*	*4.1*	*6.9*
4	1-10	17.7	264.7	5.3*	23.0*	20.0	331.0
	sd	*6.2*	*32.0*	*4.6*	*4.1*	*7.4*	*42.1*
(n=3)	11-25	9.0	167.3	0.0*	11.0*	45.3	432.7
	sd	*3.3*	*33.2*	*0.0*	*1.7*	*15.1*	*59.2*

(*=significantly different, P=0.05, df=6 or 4, Standard t-test of means within each site). (numbers in *italic* =standard deviations)
(O/P=omnivore/predators, BAF=bacterial feeders, Aph=Aphelenchidae fungal feeders, Tyl=tylenchid fungal feeders, PLP=plant parasites, mainly *T.tobari*).

occur close to the plant root system and in the top 1 to 10 cm of the soil profile ; the least number of nematodes occur further away from the plant and over 30cm down the soil profile. There was also no significant effect of plant species on total nematode numbers. The top 10 cm is the area considered to have the highest level of microbial and plant root activity after rainfall. It is important, for logistic reasons, to determine if there are similar differences in the vertical distribution of particular nematode groups in the soil profile surrounding plant species in arid South Australia.

Materials and Methods.

Four sites, with Low Shrubland vegatation type dominated by *Atriplex vesicaria* (family Chenopodiaceae), were selected to study the variation in the composition and numbers of nematodes of the different trophic groups within the soil profile. At least six plants from each site was selected and soil taken to two depths; 1-10cm and 11-25cm. The nematodes were extracted from 50 ml of soil sample by the Baermann's Funnel technique and the different trophic groups identified and counted under the dissecting microscope. Sites from which *T.tobari* was the most abundant plant parasite were chosen from previous sampling of Low Shrublands present within the Plumbago Station pastoral lease.

Results.

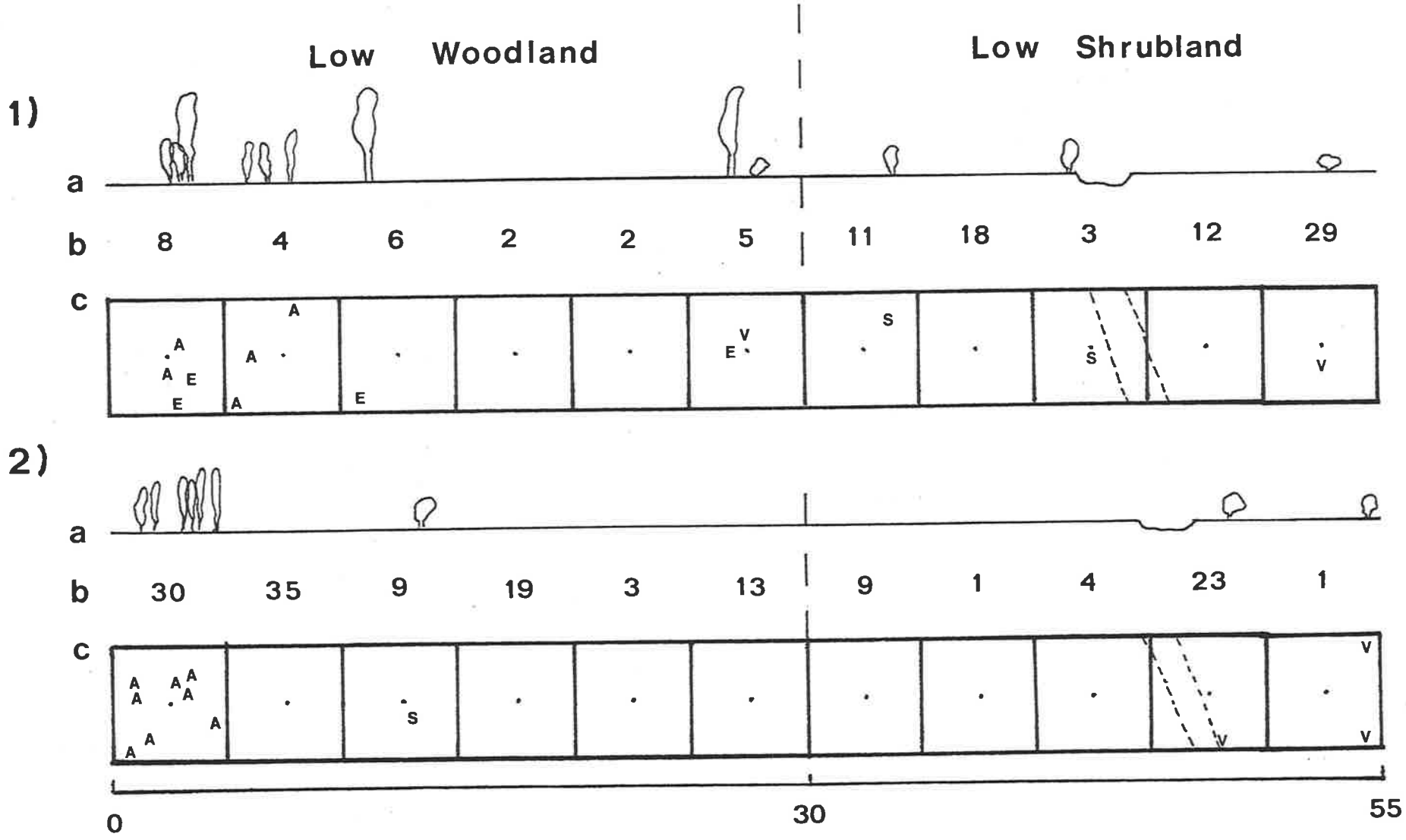
The number of nematodes of different trophic groups extracted from soil from different depths are shown in Table 5 and there was great variability in numbers of nematodes extracted from soil within each site. Greater numbers tended to occur in the upper layer of soil, but there were no significant differences between soil depths.

Conclusions.

These results suggest that nematodes of all trophic groups are evenly distributed in the upper 1-25cm of soil. To maintain a level of conformity, soil was taken to a depth of 25cm in further investigations within defined areas of vegetation.

FIGURE 19 : THE TRANSVERSE SECTION OF PLANT SPECIES (a), NUMBERS OF PLANT PARASITIC NEMATODES (Tylenchorhynchus tobari) EXTRACTED FROM 50 mls OF SOIL (b) AND THE POSITION OF PLANT SPECIES WITHIN INDIVIDUAL QUADRANTS (5 metre square) (c) WITHIN TWO ADJACENT TRANSECTS ENCOMPASSING A LOW WOODLAND AND LOW SHRUBLAND WITHIN THE CREEK BED OF THE STREZLECKI CREEK.

A = Acacia spp.
E = Eucalyptus spp.
V = Atriplex vesicaria
= Dry, shallow creek
• = soil sampled from
--- = change in vegetation type


Low Woodland
Low Shrubland
1)
a
b
8 4 6 2 2 5 11 18 3 12 29
c
2)
a
b
30 35 9 19 3 13 9 1 4 23 1
c
0
30
55
DISTANCE (metres)

4.2.3. Distribution of Nematode Trophic Groups within Areas of Different Vegetation and Landforms.

Introduction.

Community analysis, using importance and prominance values was used by Norton and Schmitt (1978) in the study of the plant nematodes occurring in the Kalso Prairie in Iowa, U.S.A.. They found that differences in the diversity of plant parasitic nematodes could be related to changes in landform and vegetation over defined areas. The Kalso Prairie appears to have a much more diverse community of plant nematodes than the South Australian arid region, and so use of importance and prominance values was not considered appropriate. However, an attempt was made to correlate the changes in vegetation and landforms with changes in the distribution of particular nematode species. Stowe and Wade (1979) showed that positive and negative associations could be found between species of plants within specified regions. The question posed was :- Are nematodes distributed randomly across a defined area of differing vegetation and landform or is there an association between particular vegetations or landforms and nematodes?

Materials and Methods.

Several methods were devised to investigate the distribution of nematodes over a variety of vegetation and landforms. Some methods were confined to small areas (a) and others included larger areas ((b) and (c)). Nematodes were extracted from 50 ml of soil by the Baermann's funnel.

a) Strezlecki Crossing (55 x 10 m transect)

Eleven, five metre square quadrats were marked out along each of two adjacent transects, encompassing a 55 x 10 m area in which the vegetation changed from a Low Woodland (*Eucalyptus* and *Acacia* spp.) to Low Shrubland (*Atriplex vesicaria*). Soil was sampled from the centre of each quadrat and the distribution of perennial plants noted (Figure 19). There was also an ephemeral herbland present as an understorey component of both vegetation types which had been actively growing for 2 months but was not assessed. *Morulaimus geniculatus* and *Tylenchorhynchus tobari* had previously been identified from

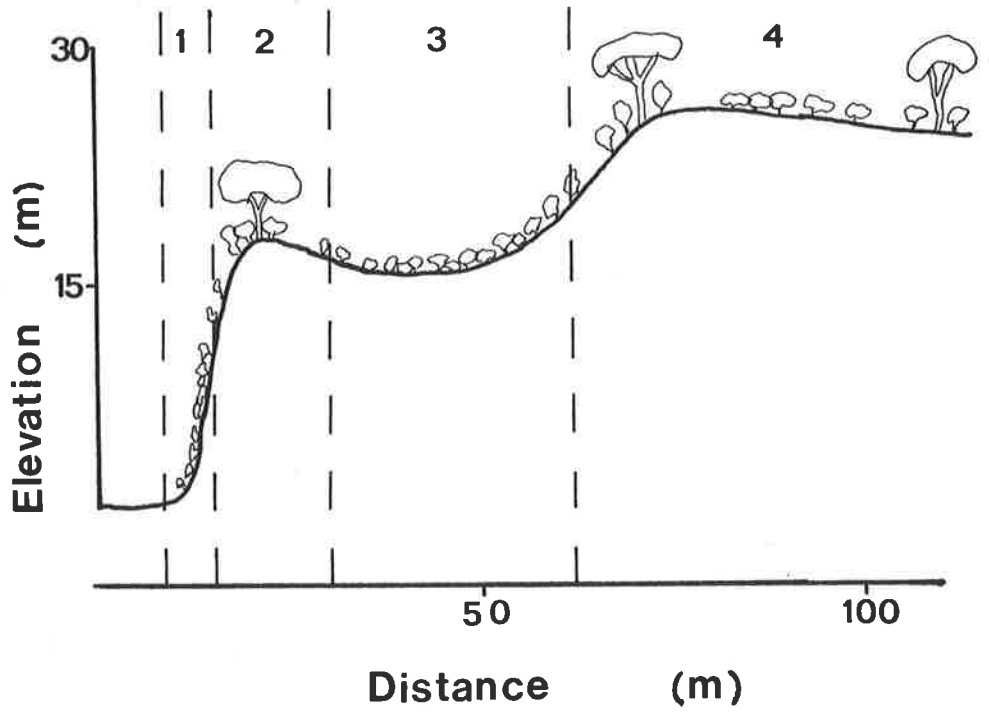


FIGURE 20 : THE DIFFERENT ZONES OF VEGETATION / LANDFORM ASSOCIATIONS SURROUNDING A SALT LAKE (Lake Gilles) IN THE ARID REGION OF SOUTH AUSTRALIA.

Zone 1) Slope of first dune, Salicornia spp. based Low Shrubland

2) Crest of first dune, Acacia papyrocarpa dominant Low Woodland with Rhagodia spp. understorey.

3) First swale after first dune, Low Shrubland with Atriplex vesicaria dominant and Salicornia spp. present.

4) Plain/dune after first swale, Low Woodland (Sparse) with Acacia papyrocarpa and mixed chenopod understorey.

this site (Strezlecki Creek crossing). Analysis of Variance (ANOVA) was used to determine whether there was any interaction between the different vegetation, rows and the different nematode trophic groups.

b) Zone transects.

Work by Norton and Oard (1981) showed that there were specific associations between specific landforms and particular plant parasitic nematodes. The sample area involved had uniform vegetation and so different results may be found in areas in which vegetation changed with landform. To reduce the numbers of plants sampled from different vegetation, areas were divided into zones and samples were taken at random within each zone. Two sites were selected, each starting with a water source (as the initial sampling zone) and progressing over a distance of about 150 m; this encompassed between 3 to 4 different zones based on vegetation and landform differences. ANOVA was used to test if there was any significant differences in the number of nematodes and the different vegetation/Landform zones. A description of each site is presented below :-

Site 1 : Lake Gilles (a salt lake near the Gawler Ranges, part of a Conservation Park). The site was divided into four zones starting at the foreshore (Figure 20), these are ;

Zone 1 : a Low Shrubland dominated by *Salicornia* spp.

Zone 2 : a Low Woodland on the first dune dominated by *Acacia papyrocarpa* with *Atriplex vesicaria* as part of the understorey.

Zone 3 : a Low Shrubland dominated by *Atriplex vesicaria* and *Salicornia* spp. on a shallow saline swale.

Zone 4 : a sparse Low Woodland dominated by *Acacia papyrocarpa* with *Atriplex vesicaria* as the understorey on the following dune systems.

The plant parasitic nematode species present were unknown before sampling.

Site 2 : Purni Bore (an artesian bore in the Simpson Desert). The vegetation surrounding the bore involved three zones (Figure 21) ;

Zone 1 : a Tall Shrubland with *Acacia* spp. dominant and a reed/mixed understorey.

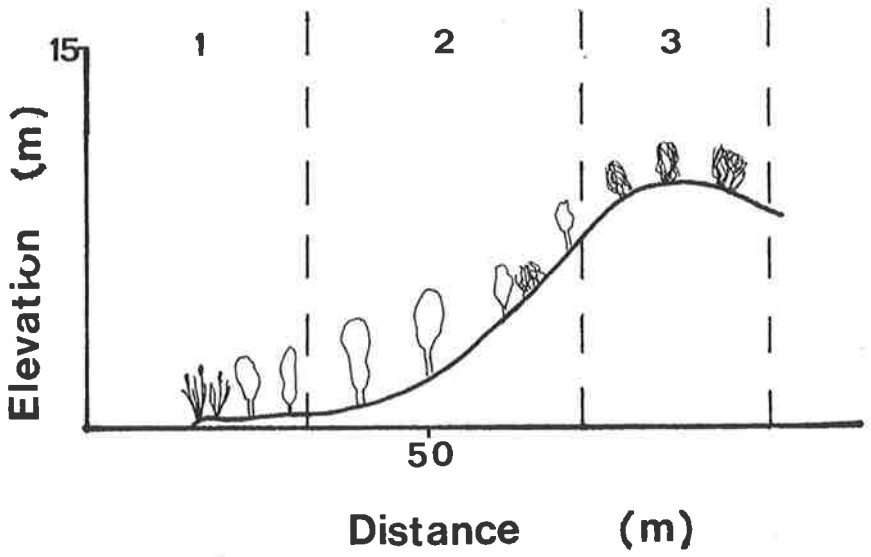


FIGURE 21 : THE DISTRIBUTION OF DIFFERENT ZONES OF VEGETATION / LANDFORM ASSOCIATIONS SURROUNDING AN ARTESIAN BORE IN THE SIMPSON DESERT.

Zone 1) Water's edge, Tall Shrubland with Acacia spp. and with a reed understorey.

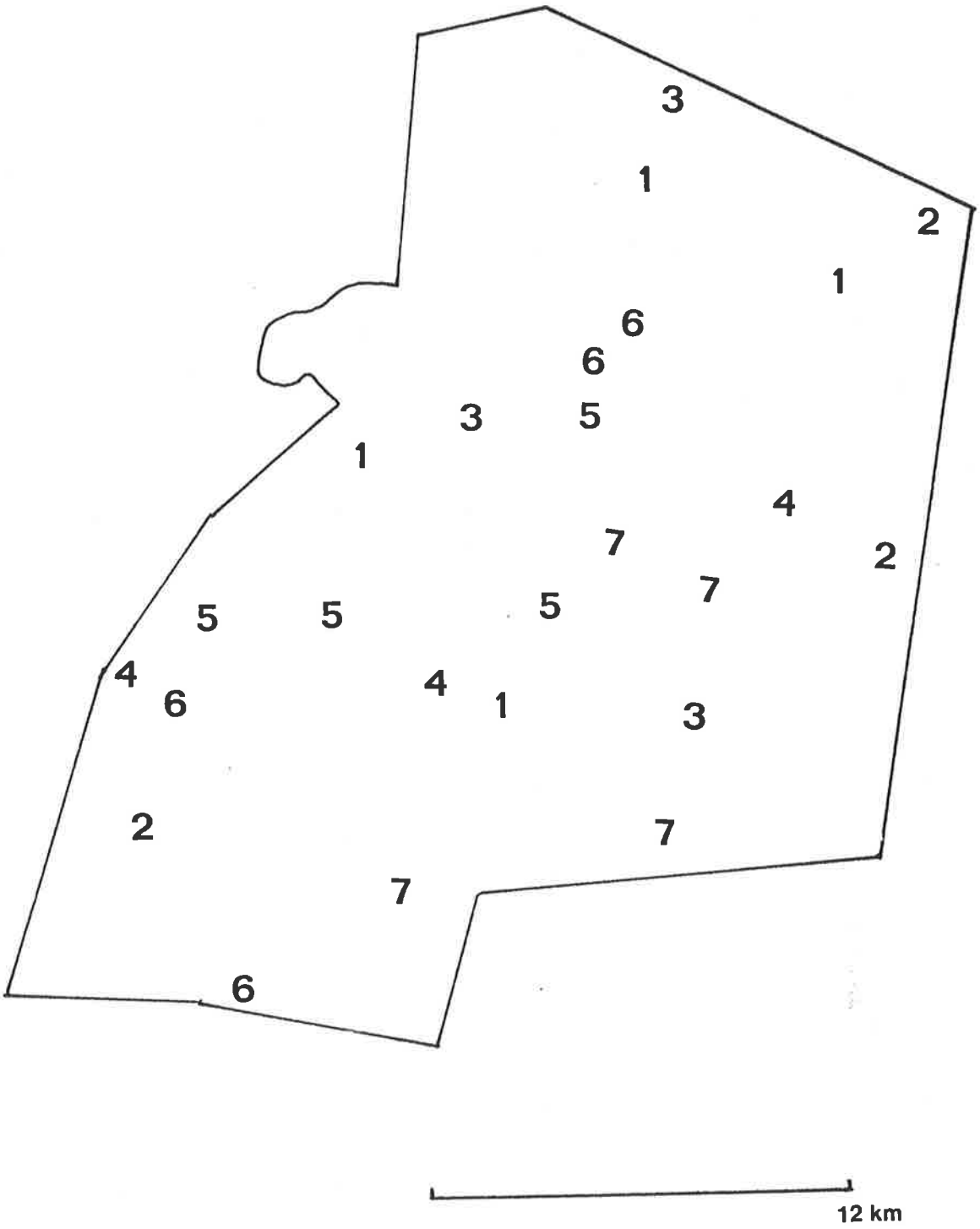
2) Slope of the first dune, with Acacia spp. dominant and Zygocloa paradoxa understorey.

3) Crest of dune, with a Hummock Grassland and Zygocloa paradoxa dominant and no understorey component.

FIGURE 22 : THE DISTRIBUTION OF VEGETATION / LANDFORM ASSOCIATIONS WITHIN A DEFINED AREA OF UNIFORM MANAGEMENT (Plumbago Station).

Vegetation/Landform Association

1) Sedementary Hills, shallow soil and sparse ephemeral plant cover
2) Flood-plain/Drainage area, undulating, with Low Shrubland and Low Woodland (Acacia and Chenopods).
3) Watercourse with Acacia victorii Tall Shrubland and varied understorey.
4) Casuarina cristata Low Woodland on an undulating plain, Chenopod understorey.
5) Low Granitic Hills, with Low Open Woodland or Tall Shrubland with Acacia spp., Cassia spp., Dodonea spp. and Eremophila spp..
6) Chenopod Low Shrubland / Plain, with Maireana spp. dominant.
7) Chenopod Low Shrubland / Plain, with Atriplex vesicaria dominant.


3
1
2
1
6
6
3
5
1
4
7
2
7
5
5
5
4
6
4
1
3
2
7
7
6
12 km

Zone 2 : a Shrubland dominated by *Acacia* spp. with *Zygocloa paradoxa* / mixed understorey on the slope of the dune.

Zone 3 : a Hummock Grassland (*Zygocloa paradoxa*) on the crest of the swale.

The plant parasitic nematode species present were unknown before sampling. The artesian water flowing from the bore had a very high concentration of Magnesium Sulphate. ANOVA was used to determine significant differences between zones and nematode trophic group.

c) A region of different vegetation/landform associations (Plumbago Station).

A large area which had been subjected to the same management practice of extensive livestock grazing was sampled using a system based on different vegetation/landform associations. A station (Plumbago) was selected, from which previous samples had shown the presence and identity of a number of plant parasitic nematodes but *T.tobari* was the most common. Seven different vegetation/landform associations could be identified : -

1) Hills of metamorphic rocks with shallow soil and sparse ephemeral cover.

2) Flood-plains/Drainage area-undulating, with a Low Shrubland and Open Woodland (*Acacia* spp. and Chenopods).

3) Water-course with *Acacia victorii* Tall Shrubland and varied understorey.

4) *Casuarina cristata* Low Woodland on undulating plains.

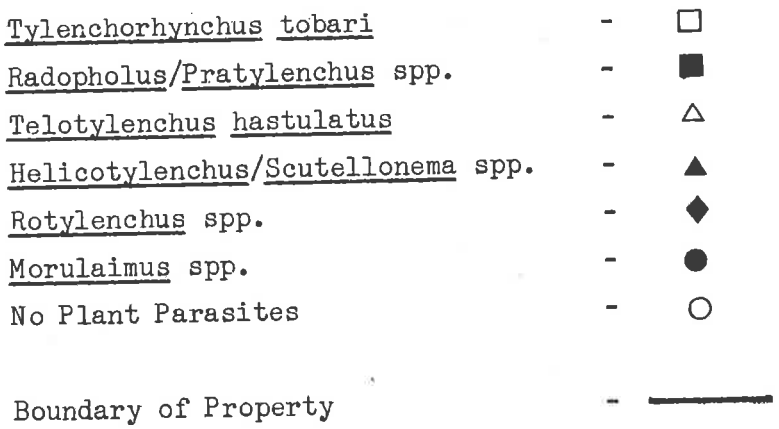
5) Low Granitic Hills with Low Open Woodland or Tall Shrubland with *Acacia*, *Dodonea*, *Cassia* and *Eremophila* spp..

6) Chenopod Low Shrubland/Plain with *Maireana* spp. dominant.

7) Chenopod Shrubland/Plain with *Atriplex vesicaria* dominant.

Sites were selected from areas of the above associations, at least 6 soil samples taken and the plants that were sampled recorded; nematodes were extracted and the different trophic groups identified and counted from a 50 ml sample of soil. Analysis of variance (ANOVA) was used to test if there were any differences between the number of nematodes of the different trophic groups and the different vegetation types. The position of each site was recorded (Figure 22) and the distribution of the different plant parasitic genera and species

FIGURE 23 : THE DISTRIBUTION OF THE PLANT PARASITIC NEMATODES WITHIN A DEFINED AREA OF UNIFORM MANAGEMENT (Plumbago Station).



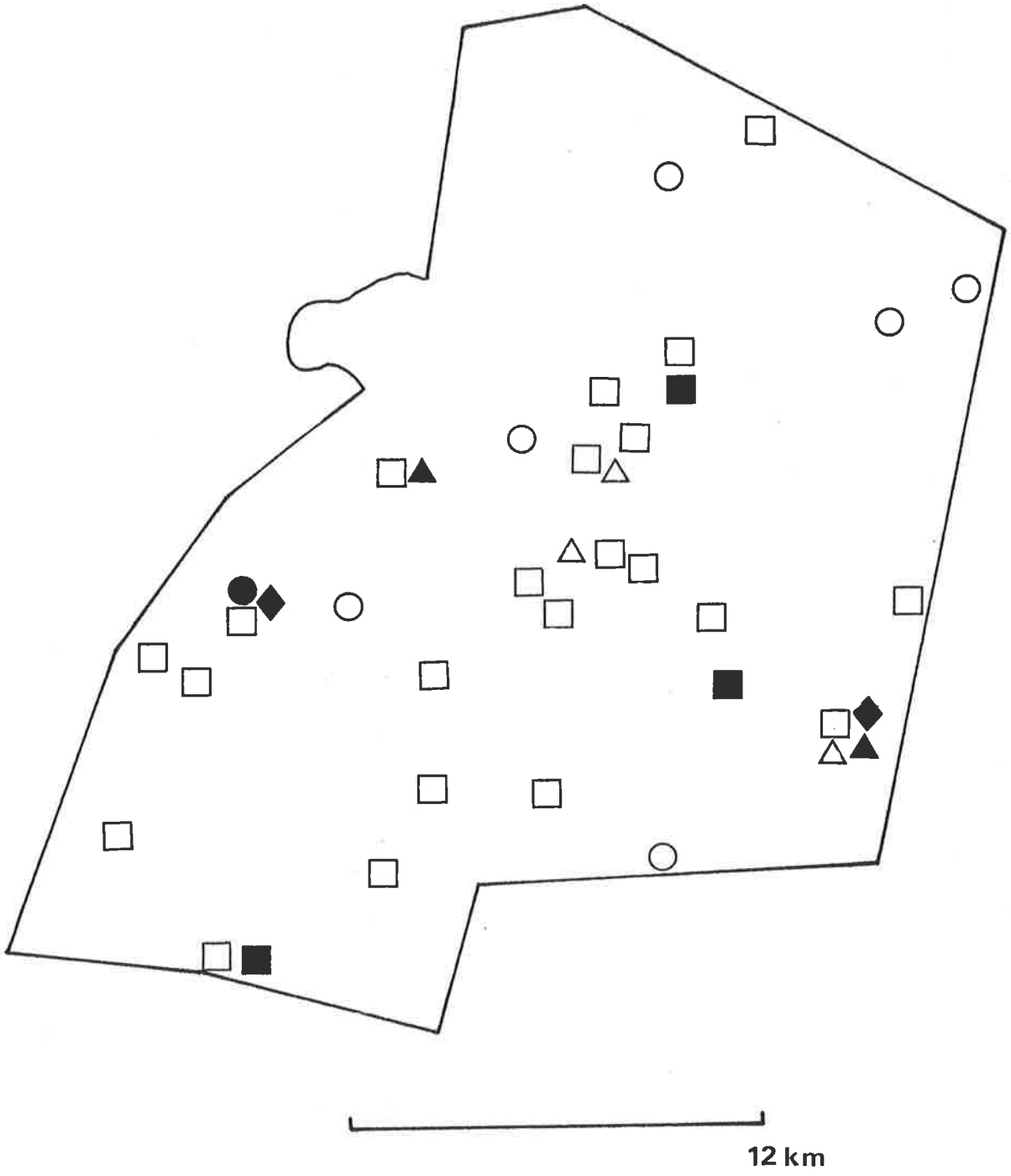

12 km

TABLE 6 : MEAN NUMBERS OF NEMATODE OF DIFFERENT TROPHIC GROUPS FROM A 55 X 10 METRE GRID, DIVIDED INTO 5 METRE SQUARE QUADRATS. THE VEGETATION CONSISTED OF A CHANGE FROM A LOW WOODLAND (dominated by *Eucalyptus* spp.) TO A LOW SHRUBLAND (with *Atriplex* spp. and ephemerals). Rows and vegetations corresponds to Figure 19.

Vegetation Type	Row	Nematode Counts (/50 ml of soil) Different Trophic Groups O/P	BAF	FUF	PLP	TOT
Low Woodland	1	10.2	113.5	10.7	4.5	138.9
	sd	*3.3*	*34.4*	*1.6*	*1.2*	*43.8*
	2	6.0	93.2	11.2	18.2	128.6
	sd	*2.9*	*12.2*	*4.4*	*6.2*	*6.8*
Low Shrubland	1	8.0	80.4	8.8	14.6	111.8
	sd	*4.4*	*14.1*	*4.0*	*4.8*	*14.8*
	2	6.4	105.0	13.2	7.6	132.2
	sd	*1.9*	*40.9*	*3.6*	*4.6*	*43.6*
Analysis of Variance						
Vegetation		ns	ns	ns	ns	ns
Row		ns	ns	ns	ns	ns
Vegetation x Row		ns	ns	ns	*	ns

(n=not significant, *=significant interaction, ANOVA, df=1,15, P=0.05)
(O/P=omnivore/predators, BAF=bacterial feeders, FUF=fungal feeders, PLP=plant parasites, TOT=total nematodes)
(numbers in italics, represent plus or minus standard deviation)

plotted (Figure 23). The information from this survey allowed permanent sites to be established in which changes in the numbers of nematodes over a season could be monitored.

Results.

a) Strezlecki Crossing (55 x 10 m transect) (Figure 19).

ANOVA of the number of nematodes of each trophic group and of total number of nematodes showed no significance within and between the different vegetation types and rows of quadrants. *T.tobari* was the only plant parasitic nematode identified. There was a significant interaction between the different rows and vegetation and the numbers of *Tylenchorhynchus tobari* (Table 6). However, looking again at Figure 19, there was no clear pattern, as the highest numbers of *T.tobari* were found in row 2 of the Low Woodland and row 1 of the Low Shrubland. The relationship between the differences in number of *T.tobari* within the different vegetation and rows was not consistent and is possibly influenced by other factors not assessed at the time of sampling (e.g. distribution of ephemerals).

b) Zone transects.

Site 1 : Lake Gilles (Figure 20) :-

The number, trophic group and specific species of plant parasitic nematodes are presented in Table 7. Using analysis of variance, there was no significant difference between the different zones of vegetation/landforms and nematode trophic groups. Within each zone and trophic group, there were high standard deviations, possibly causing the lack of significance. However, some trends between zones and different plant parasitic nematode species can be seen. *Radopholus crenatus* appeared to be closely associated with the presence of *Salicornia* spp. growing in saline soils. This is indicated by the higher numbers of *R.crenatus* present in zones 1 and 3 (these were the foreshore and saline swale behind the first dune). *Tylenchorhynchus tobari* was not present on the foreshore and was most abundant in zone (3) where *Atriplex vesicaria* was the dominant plant species.

TABLE 7 : AVERAGE NUMBERS OF NEMATODES OF DIFFERENT TROPHIC GROUPS IN DIFFERENT ZONES OF VEGETATION/LANDFORM ASSOCIATIONS SURROUNDING A SALT LAKE (Lake Gilles). Zones correspond to Figure 20.

	Numbers of nematodes (/50 ml of soil)				
	Zone				
Trophic group	1	2	3	4	
Omnivore/predator	3.0	63.3	13.3	10.3	ns
sd	*1.5*	*38.9*	*11.5*	*4.1*	
Bacterial Feeders	85.7	144.7	34.0	158.0	ns
sd	*47.8*	*48.8*	*9.1*	*34.1*	
Fungal Feeders	53.3	38.3	18.3	26.0	ns
sd	*17.6*	*15.5*	*6.3*	*1.8*	
R.crenatus	6.0	0.7	1.7	0.0	ns
sd	*2.8*	*0.3*	*0.4*	*0.0*	
T.tobari	0.0	15.0	96.0	37.0	ns
sd	*0.0*	*7.5*	*40.5*	*2.8*	
Total	148.8	261.6	163.3	218.0	ns
sd	*62.5*	*57.6*	*55.1*	*19.3*	
Leaf Litter (cm)	0.0	7.0	0.0	6.7	ns
sd	*0.0*	*2.6*	*0.0*	*5.8*	

(ns=not signficantly different, ANOVA, df=3,6, P=0.05).
(numbers in *Italics*, represent plus or minus standard deviations).
(zone 1= slope of first dune, zone 2=crest of first dune, zone 3=swale behind first dune, zone 4=dune/plain behind first swale)
(*R.crenatus* = *Radopholus crenatus* ; *T.tobari* = *Tylenchorhynchus tobari*)

TABLE 8 : AVERAGE NUMBER OF NEMATODES OF DIFFERENT TROPHIC GROUPS IN DIFFERENT ZONES OF VEGETATION/LANDFORM ASSOCIATIONS SURROUNDING AN ARTESIAN BORE (Purni Bore). Zones correspond to Figure 21.

	Numbers of nematodes (/50 ml of soil) Zone			
Trophic group	1	2	3	
Omnivore/predator	10.0	17.7	4.0	ns
sd	*6.5*	*2.4*	*2.0*	
Bacterial feeders	30.0	185.0	129.3	*
sd	*9.8*	*10.5*	*46.6*	
Fungal feeders	59.7	34.0	20.7	ns
sd	*50.4*	*10.5*	*5.1*	
T.tobari	0.0	11.7	13.0	ns
sd	*0.0*	*9.2*	*2.5*	
M.simpsonii	0.0	0.0	2.0	ns
sd	*0.0*	*0.0*	*1.3*	
Total	99.7	238.4	169.7	ns
sd	*60.4*	*12.0*	*55.9*	

(ns=not significantly different, *=significantly different, df=2,5, P=0.05)
(numbers in *italics* represent plus or minus standard deviation)
(zone 1=shore of artesian bore lake, zone 2=slope of dune, zone 3=crest of dune)
(*T.tobari* = *Tylenchorhynchus tobari*, *M.simpsonii* = *Morulaimus simpsonii*)

Site 2 : Purni Bore (Figure 21) :-

There was a significant difference in the numbers of Bacterial Feeders and vegetation zones (Table 8). There was an overall increase in numbers the further from the water's edge the soil sample was taken. No other trophic groups showed any significant associations, due to high standard deviations within zones, although the plant parasites showed some trends of association with specific plant species. *M.simpsonii* appeared to occur only on the crest of the sand dune surrounding the bore. This is associated with the Hummock Grassland, dominated by *Zygocloa paradoxa*. *T.tobari* occurred in Zones 2 and 3, away from the water's edge.

Both these sites show some trends in distribution between certain vegetation/landforms and the distribution of plant parasitic nematodes. However, to accurately determine the association between plant species and nematodes trophic groups, more intensive sampling and analysis is required. *R.crenatus* and *M.simpsonii* may be considered to be associated with *Salicornia* spp. and *Zygocloa paradoxa* respectively. The distribution of *T.tobari* needs to be more extensively surveyed.

c) Plumbago Station (Different vegetation/landform associations) :-

When the different vegetation types were compared using ANOVA, there was a significant difference between the numbers of Omnivore/predators present and the vegetation type (Table 9). Vegetation type 3 had significantly higher numbers than all other types. The other trophic groups showed no significant relationship, although the highest number of plant parasites were found under vegetation type 4 and 7. This finding was useful when establishing sites for regular sampling over a 14 month period.

Conclusions.

Investigations of associations between the distribution of different nematode trophic groups and vegetation/landform types needs to be more thoroughly sampled, to reduce the variability within the different types. Use of strictly defined sample points appears to be less accurate than sampling directly under plants, as spacing between plants is highly variable, and the root systems do not spread randomly about the plant. Trends in the

TABLE 9 : THE AVERAGE NUMBER OF NEMATODES OF DIFFERENT TROPHIC GROUPS, SAMPLED FROM SEVEN DIFFERENT VEGETATION/LANDFORM ASSOCIATIONS FOUND ON PLUMBAGO STATION.

Vegetation/ Landform	Mean Number of nematodes (/50 ml of soil) O/P	BAF	FUF	PLP	TOT
1	14.9	42.4	26.8	0.0	84.3
sd	*6.0*	*15.9*	*16.0*	*0.0*	*31.9*
2	10.5	86.3	10.8	1.6	109.5
sd	*4.7*	*34.1*	*6.3*	*1.7*	*36.0*
3	27.9#	56.6	38.7	0.9	127.3
sd	*9.2*	*18.4*	*43.9*	*1.2*	*51.2*
4	14.3	117.6	8.4	10.3	148.2
sd	*3.9*	*48.3*	*3.6*	*7.0*	*53.6*
5	11.2	96.0	8.0	0.8	117.1
sd	*5.3*	*33.6*	*2.9*	*2.2*	*40.0*
6	13.0	80.1	9.9	5.2	107.2
sd	*7.8*	*32.6*	*4.5*	*3.4*	*37.5*
7	11.6	81.1	6.4	10.5	107.3
sd	*3.1*	*18.7*	*2.2*	*6.1*	*22.3*
	ns	ns	ns	ns	ns

(ns=not significantly different, *=significantly different, ANOVA, df=6,12, P=0.05)
(#=significantly different, standard t-tests, df=4, P=0.05)
(numbers in *italics* are plus or minus standard deviations)
(O/P=omnivore/predators, BAF=bacterial feeders, FUF=fungal feeders, PLP=plant parasites, TOT=total nematodes)
(Vegetation/landform associations (1-7) are described in the text)

distribution of particular plant nematode species have been noted, but more intensive sampling is required to determine the specific host plants on which plant nematode species feed.

4.2.4. Association between the Distribution of Plant species and the Different Nematode Trophic Groups within a Particular Vegetation and Landform Type.

Introduction.

The association of nematodes between different vegetation/landform types has been indicated in the previous section. To answer the question :- Is there any association between the distribution of plant species and particular nematode trophic groups within a small defined area? vegetation of relatively uniform composition was selected. Also to be investigated was the association between poor growth of plants in the field and the presence of plant nematodes. Other workers (Goodell and Ferris, 1980) have undertaken very extensive samplings of uniform vegetation (agricultural crops) using a complex grid system. A less complex grid system was used to attempt to identify any associations between changes in the distribution of plant species and the different trophic groups. More extensive sampling would be required to attempt the modelling of population changes of the different trophic groups with changes in vegetation.

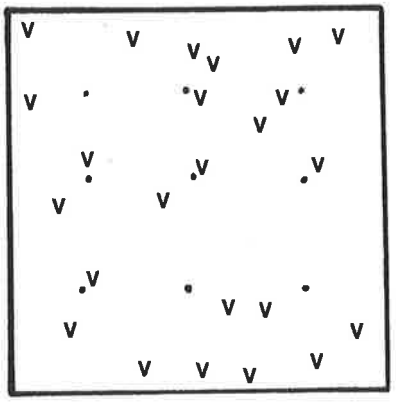
Differences and associations between the distribution of plant parasitic species (*Tylenchorhynchus* spp. and the related species *Dolichorhynchus sedecimstriatus*) and plant species were sought. It was considered that some indication of host specificity might enable these species to be cultured in the laboratory and so aid in understanding part of the ecology of nematodes within the arid region of South Australia.

Materials and Methods.

a) Distribution of nematodes within a small area (5x5 metres).

Two sites of Low Shrubland (dominated by *Atriplex vesicaria*) were selected, one having good growth of ephemeral and perennial plant species and the other having very

A)



B)

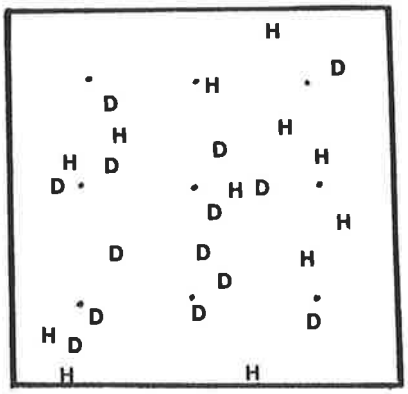


FIGURE 24 : THE DISTRIBUTION OF PLANTS WITHIN TWO 5 METRE SQUARE GRIDS WITH VARIED STATES OF GROWTH OF A LOW Atriplex vesicaria SHRUBLAND.

A) Nonning Station

B) Middle Back Station

V = Atriplex vesicaria plants

• = position from which soil was sampled

H = 'Healthy' plants (Atriplex vesicaria)

D = 'Dead' or 'Diseased' plants

• = position from which soil was sampled

poor growth of the perennial species present and few ephemerals. Both sites had had recent rainfall. At each site a 5 metre square grid was set up and 9 soil samples were taken at set coordinates (near host plants) and the distribution and nearest plant to sampling point was recorded (Figure 24). The nematodes were extracted (Baermann's funnel) from 50 ml of soil collected from each sample point and the different trophic groups identified and counted. The plant nematode species present was *T.tobari*. ANOVA of the data was used to indicate if there were significant differences between the different sites and growth habits. Standard test of means was used to establish where the differences occurred.

b) Distribution within a 25 m^2 grid.

Three sites were selected, one with known populations of *Tylenchorhynchus tobari*, one with *T.siccus* and the other with *Dolichorhynchus sedecimstriatus*. The three sites had their own vegetation type :-

Site 1 Kokatha :- *D.sedecimstriatus* - Low Woodland dominated by *Acacia papyrocarpa* with a mixed chenopod understorey

Site 2 Plumbago :- *T.tobari* - Low Shrubland dominated by *Atriplex vesicaria* with ephemeral understorey.

Site 3 Oakden Hills :- *T.siccus* - Low Woodland dominated by *Acacia papyrocarpa* with a mainly *Maireana sedifolia* understorey. *T.tobari* and a *Morulaimus* spp. were also present.

At each site the 25 m^2 grid was set up and divided into 25, 5 m^2 quadrants. The number of host plants within each quadrant was counted and the total canopy cover estimated (Waring, 1983). Soil samples were taken from under plants at random within each quadrant and the species of plant recorded. Nematodes were extracted from 50 ml of soil by the Baermann's funnel technique, taken from each sample point and the different trophic groups identified and counted. Multiple regression analysis was used to identify any associations between distribution of canopy cover, numbers of plant species and the number of nematodes of the different trophic groups within each quadrant.

The distribution of the different plant species and nematode trophic groups was plotted using a computer programme which estimated the distribution patterns using contour intervals within the 25 m^2 grid (Appendix 5). The contour intervals were estimated using

TABLE 10 : MEAN NUMBERS OF NEMATODES OF DIFFERENT TROPHIC GROUPS EXTRACTED FROM SOIL (50mls) FROM A 5 METRE SQUARE GRID AT SET COORDINATEs. TWO SITES OF LOW SHRUBLAND (dominated by *Atriplex vesicaria*) WITH DIFFERENT GROWTH CONDITIONS (/9 samples/grid) WERE SAMPLED.

	Numbers of nematodes of different trophic groups (/50 ml of soil)				
Site type	O/P	BAF	FUF	PLP	TOTAL
Good Growth	9.9	55.7	62.9	24.3	155.7
sd	*3.1*	*18.8*	*24.8*	*8.6*	*31.2*
Poor Growth	3.7	56.6	30.0	16.3	106.6
sd	*1.1*	*15.6*	*11.6*	*7.2*	*25.0*
	*	ns	*	*	*

(*=significantly different values of nematode trophic groups between the two sites, ns=not significantly different, standard test of means, P=0.05, df=16)

(O/P=omnivore/predators, BAF=bacterial feeders, FUF=fungal feeders, PLP=plant parasites)

(numbers in *italics* represent standard deviation, plus or minus)

TABLE 11 : MEAN NUMBERS OF NEMATODES OF DIFFERENT TROPHIC GROUPS IN SOIL SAMPLES FROM 'HEALTHY' AND 'DEAD' *Atriplex vesicaria* PLANTS SAMPLED AT SET COORDINATES WITHIN A 5 METRE SQUARE GRID IN A LOW SHRUBLAND.

	Numbers of nematodes of different trophic groups (/50 ml of soil)				
Plant type	O/P	BAF	FUF	PLP	TOTAL
'Dead' (n=4)	4.0	51.0	30.5	4.0	89.5
sd	*0.8*	*22.3*	*13.7*	*3.2*	*33.2*
'Healthy' (n=5)	3.4	61.0	29.6	26.2	120.2
sd	*1.3*	*10.0*	*11.2*	*5.8*	*17.0*
	ns	ns	ns	*	ns

(*=significant different values of nematode numbers between the different growth of the plants sampled, ns=not significantly different, standard test of means, P=0.05, df=7)
(numbers in italic represent standard deviation)
(O/P=omnivore/predators, BAF=bacterial feeders, FUF=fungal feeders, PLP=plant parasites)

the numbers from each 5 m^2 quadrant and depended on overall numbers and distribution within the grid. The factor by which the contour intervals increased is indicated and varies with high or low numbers of plant species or nematode trophic groups. With the Oakden Hill site, the discovery that three different species of plant parasitic nematodes were present promted a second extraction from the same soil. The soil had been kept at 4 ^oC for 7 days.

Results.

a) Distribution within a small area.

Within the area of poor growth, there were fewer nematodes than in the area in which the plants were well grown, with a significant decrease in number of fungal feeders, plant parasites and omnivore/predators compared with those in a well grown stand (Table 10). The number of Bacterial feeders showed no significant difference. Within the poor growth grid, significantly fewer plant parasites were obtained from the 'unhealthy' (no foliage) plants compared to the 'healthy' (some foliage) plants. Numbers of other trophic groups did not significantly differ (Table 11). The low numbers of plant parasitic nematodes associated with the 'unhealthy' plants is possibly due to their poor root systems, reflected in the poor growth of the plant.

b) Distribution of nematodes within a 25 m^2 grid.

The results for the three sites sampled are given below :-

Site 1 : KOKATHA - Low Woodland (dominated by *Acacia papyrocarpa*) with a mixed chenopod understorey. Regression analysis of numbers of nematodes of the different trophic groups and plant host parameters showed some useful correlations (Table 12). However, not all are relevant to the present study. There are some correlations which are useful in establishing the possible host of the plant nematode present in this site. For example, there are correlations between *Acacia papyrocarpa* and *Atriplex vesicaria* and the plant nematode *Dolichorhynchus sedecimstriatus*, suggesting a possible host/parasite relationship. The lack of significant correlation between the distribution of other plant species present and *D.sedecimstriatus*, suggests that these plant species is not a host. The significant correlation

TABLE 12 : THE CORRELATION MATRIX OF NUMBERS OF SPECIFIC PLANT SPECIES, CANOPY COVER AND NEMATODE TROPHIC GROUPS IDENTIFIED FROM A LOW WOODLAND DOMINATED BY *Acacia papyrocarpa* AND WITH A MIXED CHENOPOD UNDERSTOREY. THE AREA WAS SAMPLED USING A 25 METRE SQUARE GRID DIVIDED INTO 5 METRE SQUARE QUADRANTS. THE PLANT PARASITIC NEMATODE SPECIES PRESENT WAS *Dolichorhynchus sedecimstriatus* (Site 1 : Kokatha).

Variables (no./quadrant)	1	2	3	4	5	6	7	8	9	10	11	12
% Canopy Cover (1)	1.0											
Total Plants (2)	.73	1.0										
Acacia papyrocarpa (3)	-	-	1.0									
Atriplex vesicaria (4)	.70	.75	.55	1.0								
Maireana spp. (5)	-	-	-	-	1.0							
Zygophyllum spp. (6)	-	.55	-	-	-	1.0						
Dead Plants (7)	-	.41	-	-	-	.43	1.0					
Omnivore/Predators (8)	-	-	-	-	-	-	-	1.0				
Bacterial Feeders (9)	-	-	-	-	-	-	-	-	1.0			
Fungal Feeders (10)	.40	-	.48	-	-	-	-	.50	.74	1.0		
Plant Parasites (11)	.53	-	.79	.60	-	-	-	-	.55	.60	1.0	
Total Nematodes (12)	-	-	.40	-	-	-	-	.43	.99	.79	.61	1.0
	1	2	3	4	5	6	7	8	9	10	11	12

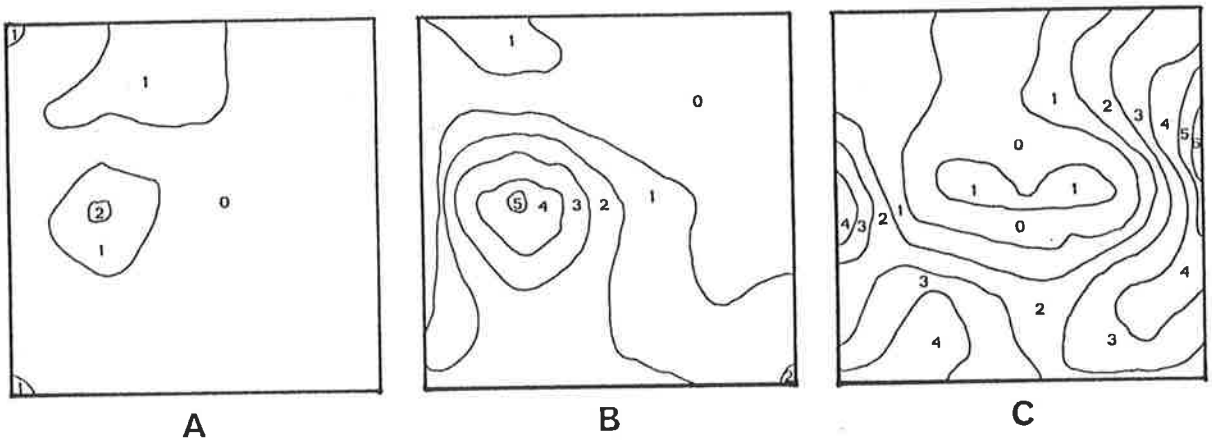
(The numbers shown in this table represent significant correlations between specific plant species, canopy cover and nematode trophic groups. Regression analysis, df=23, P=0.05).
(numbers in brakets after variable name correspond to numbers above and below numbers in the table).
(Nematodes were extracted from 50 ml of soil using the modified Baermann's Funnel technique).

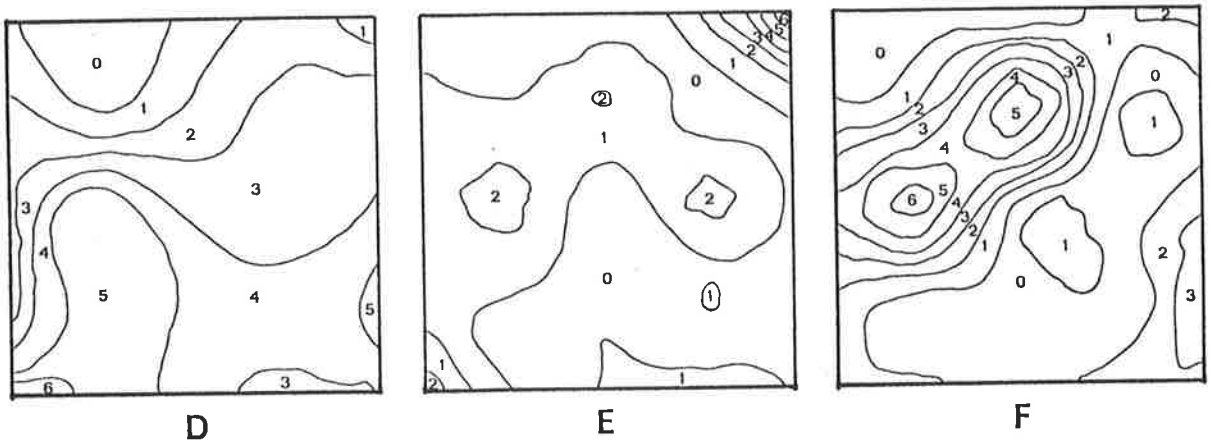
FIGURE 25 : THE DISTRIBUTION OF PLANT SPECIES AND NEMATODE TROPHIC GROUPS WITHIN A 25 METRE SQUARE GRID ENCOMPASSING A LOW WOODLAND DOMINATED BY *Acacia papyrocarpa* AND WITH A MIXED CHENOPOD UNDERSTOREY (the contours are calculated by the VAX computer system using the programme described in Appendix 6). Site 1 ; Kokatha Station.

Map	Organism	Factor by which contours increase
A	*Acacia papyrocarpa*	x 1.0
B	*Atriplex vesicaria*	x 3.0
C	Dead plants	x 1.0
D	Total plants	x 3.0
E	Omnivore/predators	x 10.0
F	Bacterial Feeders	x 75.0
G	Fungal Feeders	x 6.0
H	Plant Parasites	x 6.0

(*Dolichorhynchus sedecimstriatus* was the main plant parasitic species present).

(the factor by which the contours increase are calculated from the data collected from the field and reflect the multiple by which numbers increase, for example, in Map H where the factor is 6.0, 5 corresponds to 30 nematodes, 4 to 24 nematodes etc. etc.).


A
B
C


D
E
F

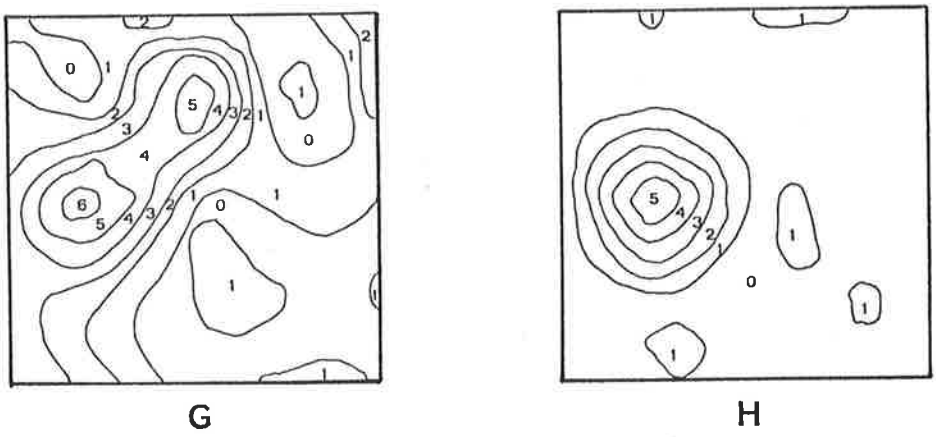

G
H

TABLE 13 : THE CORRELATION MATRIX OF THE REGRESSION ANALYSIS OF CANOPY COVER, NUMBERS OF PLANTS AND NEMATODE TROPHIC GROUPS FROM A LOW SHRUBLAND DOMINATED BY Atriplex vesicaria AND WITH A MIXED EPHEMERAL UNDERSTOREY AND SAMPLED USING A 25 METRE SQUARE GRID DIVIDED INTO 5 METRE SQUARE QUADRANTS. THE PLANT PARASITIC NEMATODE SPECIES PRESENT WAS Tylenchorhynchus tobari (Site 2 : Plumbago Station).

Variables (No./quadrant)	1	2	3	4	5	6	7	8	9	10
% Canopy Cover (1)	1.0									
Atriplex vesicaria (2)	.62	1.0								
Maireana spp. (3)	-	-	1.0							
Dead Plants (4)	-	-	-	1.0						
Total Plants (5)	.64	.91	.53	-	1.0					
Omnivore/Predtaors (6)	-	-	-	-	-	1.0				
Bacterial Feeders (7)	.47	.45	-	-	-	-	1.0			
Fungal Feeders (8)	-	-	-	-	-	.45	-	1.0		
Plant Parasites (9)	-	-	-	-	.40	-	-	-	1.0	
Total Nematodes (10)	.47	.52	-	-	.40	-	.94	-	-	1.0
	1	2	3	4	5	6	7	8	9	10

(numbers included in this table indicate signficant correlations between canopy cover, numbers of plant species and nematode trophic groups. Regression analysis, df=23, P=0.05).

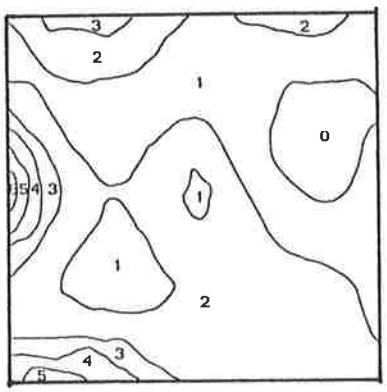
(numbers in brakets after variables names correspond to numbers above and below numbers in the table).

(nematodes were extracted by Modified Baermann's Funnel technique from 50 ml of soil).

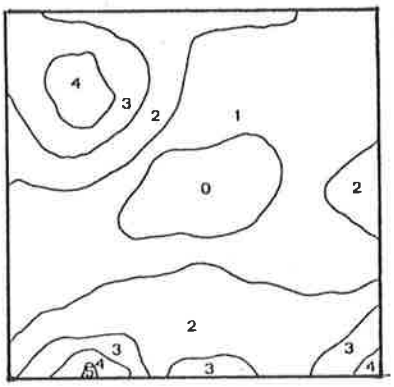
FIGURE 26 : THE DISTRIBUTION OF PLANTS AND NEMATODE TROPHIC GROUPS WITHIN A 25 METRE SQUARE GRID ENCOMPASSING A LOW SHRUBLAND DOMINATED BY Atriplex vesicaria WITH AN EPEHMERAL UNDERSTOREY (the contours are calculated by the VAX computer system using the programmes described in Appendix 6). Site ; Plumbago Station.

Map	Organism	Factor by which contours increase
A	Atriplex vesicaria	x 5.0
B	Maireana spp.	x 2.0
C	Dead Plants	x 1.0
D	Omnivore/predators	x 6.0
E	Bacterial Feeders	x 75.0
F	Fungal Feeders	x 15.0
G	Plant Parasites	x 7.0

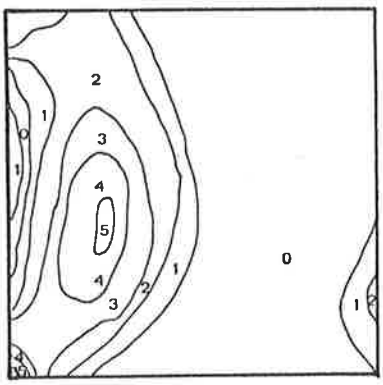
(Tylenchorhynchus tobari main species of plant parasites present)
(the factor by which the contours increase are calculated from the data collected from the field and soil samples and reflect the multiplication by which the numbers increase).



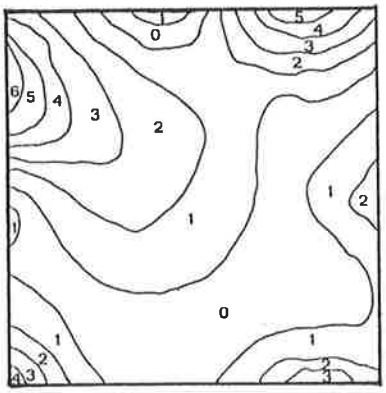
A



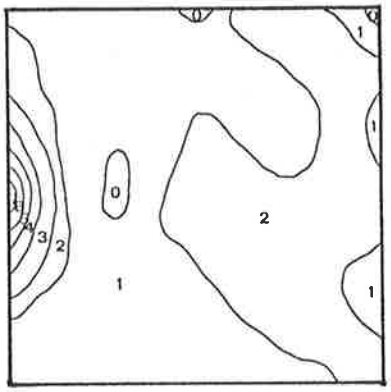
B



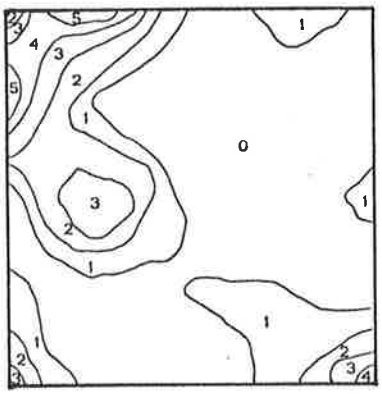
C



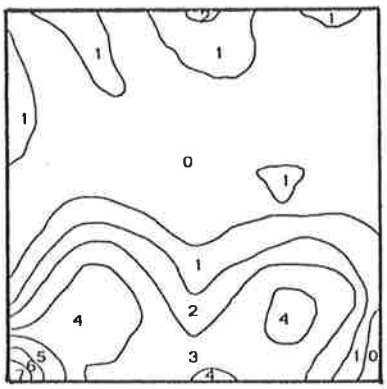
D



E



F



G

between plant parasite, fungal feeders and bacterial feeders, indicated a close relationship between these different forms. It seems possible that the close relationship between fungi, bacteria and the plant rhizosphere is the factor that binds these nematode groups together.

The distribution of the nematodes and plant parameters were plotted and are presented in Figure 25 as a contour map. The factor by which each contour line increases is represented by the contour intervals. A comparison of the distribution contours for *A.papyrocarpa* (A), *A.vesicaria* (B) and *D. sedecimstriatus* (H), show clear similarities, indicating a close association in space. Similarly the degree of congruence between the contours for the bacterial (F), fungal (G) and plant feeders (H) indicates their similar distribution in the Low Woodland site.

Site 2 : PLUMBAGO A Low Shrubland with *A.vesicaria* the dominant plant species present within each quadrant. The understorey consisted of an ephemeral herbland. There was a known population of *T.tobari* present at this site. The regression analysis of the data (Table 13), indicated that there were some significant correlations between the factors sampled. However, there was a need to concentrate on the factors which affected the distribution of the plant parasite, *T.tobari*. There are correlations between the numbers of *T.tobari* and the total number of plants per quadrant. The site was a fairly uniform Low Shrubland with *Atriplex vesicaria* the dominant plant species, which was significantly correlated with the total number of plants per quadrant. It is therefore possible to conclude that there may be a host/parasite relationship between *A.vesicaria* and *T.tobari*. There was no direct correlation between the different nematode trophic groups, and this is reflected in the distribution contours shown in Figure 26. The correlation matrix and contour maps indicate that there was no direct association between nematode distribution and that of any plant species. The lack of correlation could be due to the uniform dispersal of the root systems of the dominant plant species throughout the sample area.

Site 3 : OAKDEN HILLS STATION This site had a Low Woodland dominanted by *Acacia papyrocarpa* and with a mixed Chenopod understorey. The plant parasitic species present in the soil at this site included *Tylenchorhynchus tobari*, *T.siccus* and a *Morulaimus* species. The correlation matrix for plant parameters and total counts of the different nematode

TABLE 14 : THE CORRELATION MATRIX OF THE REGRESSION ANALYSIS OF THE NUMBERS OF PLANT SPECIES AND NEMATODE TROPHIC GROUPS IN A LOW WOODLAND DOMINATED BY Acacia papyrocarpa AND WITH A MIXED CHENOPOD UNDERSTOREY. THE AREA WAS SAMPLED USING A 25 METRE SQUARE GRID, DIVIDED INTO 5 METRE SQUARE QUADRANTS. THE PLANT PARASITIC NEMATODE SPECIES PRESENT WERE Tylenchorhynchus tobari, T.siccus AND A Morulaimus spp. (Site 3 : Oakden Hill Station).

Variables (no./quadrant)	1	2	3	4	5	6	7	8	9	10
Total Plants (1)	1.0									
Acacia papyrocarpa (2)	.51	1.0								
Maireana sedifolia (3)	.74	-	1.0							
Atriplex vesicaria (4)	.43	-	-	1.0						
Dead Plants (5)	-	-	.41	.44	1.0					
Omnivore/Predators (6)	-	-	-	-	-	1.0				
Bacterial Feeders (7)	-	-	-	-	-	-	1.0			
Fungal Feeders (8)	-	-	-	-	-	-	-	1.0		
Plant Parasites (9)	.62	-	.44	-	-	.41	-	-	1.0	
Total Nematodes (10)	-	-	-	-	-	-	.84	-	-	1.0
	1	2	3	4	5	6	7	8	9	10

(numbers present in this table represent significant correlations between plant and nematodes. Regression analysis, df=23, P=0.05).

(Numbers after variable names correspond to numbers baove and below numbers in the table).

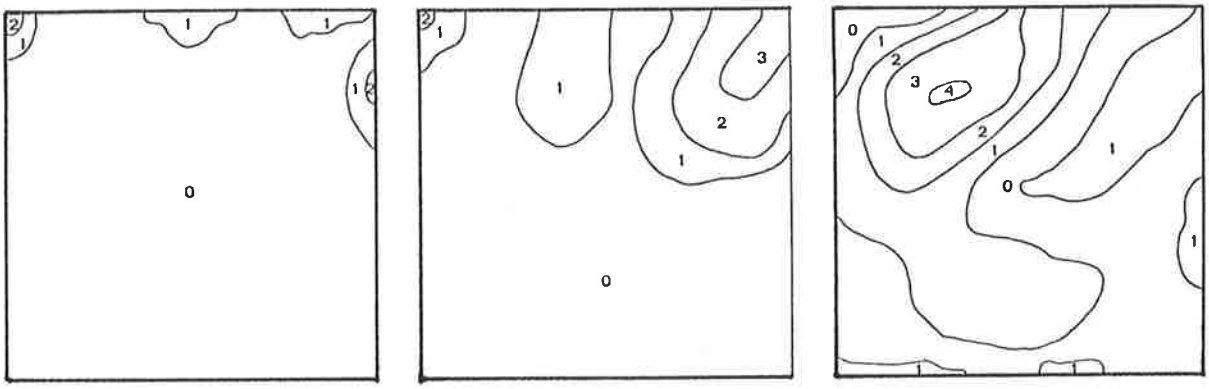
(nematodes extracted by modified Baermann's Funnel technique from 50 ml of soil)

(numbers of Plant Parasites represent pooled numbers for all species within this group)

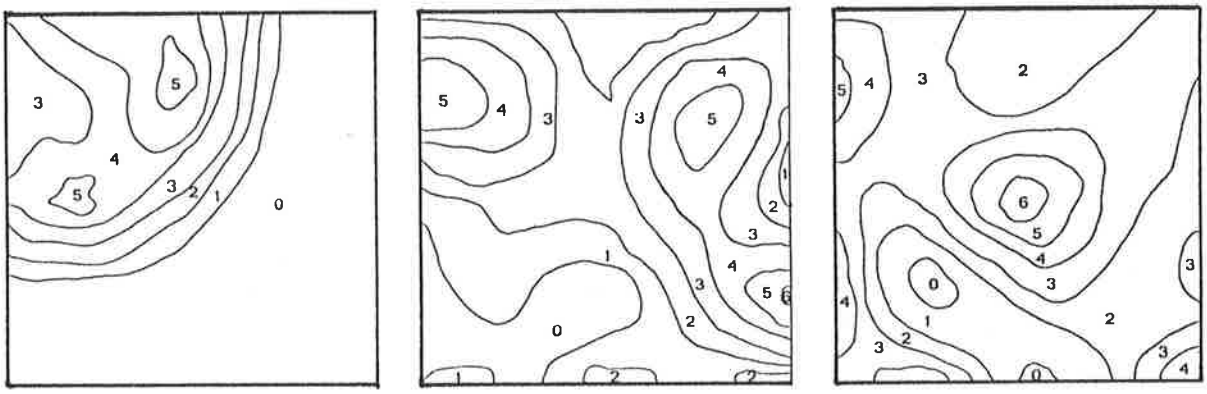
FIGURE 27 : THE DISTRIBUTION OF PLANTS, NEMATODE TROPHIC GROUPS AND DIFFERENT PLANT PARASITIC NEMATODE SPECIES WITHIN A 25 METRE SQUARE GRID ENCOMPASSING A LOW WOODLAND DOMINATED BY *Acacia papyrocarpa* AND WITH A MIXED CHENOPOD UNDERSTOREY (the contours are calculated by the VAX computer system using the programme described in Appendix 6). Site 3 : Oakden Hills Station.

Map	Organism	x Factor by which contours increase
A	*Acacia papyrocarpa*	x 1.0
B	*Maireana sedifolia*	x 4.0
C	Dead plants	x 1.0
D	*Atriplex vesicaria*	x 1.0
E	Omnivore/predators	x 5.0
F	Bacterial Feeders	x 50.0
G	Fungal Feeders	x 6.0
H	*Tylenchorhynchus siccus*	x 15.0
I	*T.tobari*	x 5.0

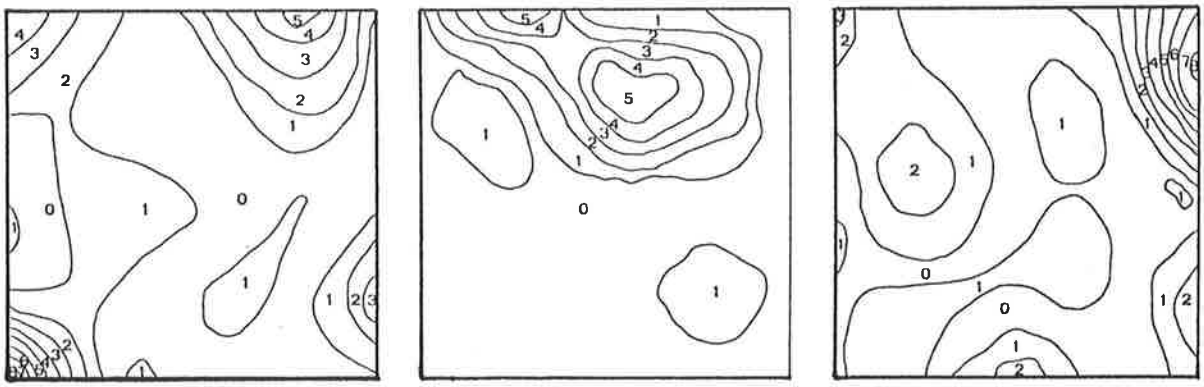
(the factor by which the contours increase are calculated from the data collected in the field and soil samples and reflect the multiple by which the numbers increase).



A B C



D E F



G H I

TABLE 15 : THE CORRELATION MATRIX OF THE REGRESSION ANALYSIS OF THE DIFFERENT PLANT SPECIES, NEMATODE TROPHIC GROUPS AND SPECIFIC PLANT PARASITE SPECIES FROM A LOW WOODLAND DOMINATED BY Acacia papyrocarpa, WITH A MIXED CHENOPOD UNDERSTOREY (Site 3 : Oakden Hill). The specific plant parasitic species were counted from a second extraction and compared with numbers of plants and nematode trophic groups extracted from a first extraction.

Variables	TPL	HO1	HO2	HO3	DEA	O/P	BAF	FUF	PLP	TOT	TOB	TSI	MOR	TNE
TOB	-	-	-	.53	-	-	-	-	-	-	1.0			
TSI	.50	.48	.63	-	-	-	-	-	.68	-	-	1.0		
MOR	-	-	-	-	-	-	-	-	-	-	.42	-	1.0	
TNE	.43	.40	.54	-	-.40	-	.55	-	.41	.66	-	.67	-	1.0
	TPL	HO1	HO2	HO3	DEA	O/P	BAF	FUF	PLP	TOT	TOB	TSI	MOR	TNE

(numbers represent significant correlations between numbers of specific plant parasitic species and the different plant species and nematode trophic groups, Regression analysis, df=23, P=0.05%)

(TPL=total numbers of plants/quadrant, HO1=number of Acacia papyrocarpa/quadrant, HO2-number of Maireana sedifolia/quadrant, HO3=number of Atriplex vesicaria/quadrant, DEA=number of dead plants/quadrant, O/P= number of Omnivore/predators from 1st extraction/quadrant, BAF=number of Bacterial Feeders from 1st extract /quadrant, FUF=number of Fungal Feeders from 1st extract/quadrant, PLP=number of Plant Parasic nematodes from 1st extract/quadrant, TOT=total numbers of nematodes from the 1st extract/quadrant, TOB=number of Tylenchorhynchus tobari from the 2nd extract/quadrant, TSI=number of T.siccus from the 2nd extract/quadrant MOR=number of Morulaimus spp./quadrant from the 2nd extract, TNE=total number of nematodes of all trophic groups from the 2nd extract/quadrant)

(nematodes were extracted by the modified Baermann's Funnel technique from 50 mls of soil, sampled at random within the field).

trophic groups is presented in Table 14. There is significant correlation between the numbers of *Maireana sedifolia* per quadrant and the numbers of plant nematodes. However, as there is a mixed population of plant nematodes within the boundaries of this site, a second count was done, in which the number of each plant nematode species was counted (Table 15). *T.siccus* is clearly correlated with the host plants, *Acacia papyrocarpa* and *Maireana sedifolia*. *T.tobari* is also clearly correlated with *Atriplex vesicaria*.

It appears, therefore, that there are differing associations between specific host plants and the different plant nematode species. This is indicated by the similarity between the distribution maps of *Acacia papyrocarpa* (A), *Maireana sedifolia* (B) and *Tylenchorhynchus siccus* (I) (Figure 27). However, no direct association between the distribution of *Atriplex vesicaria* (C) and *Tylenchorhynchus tobari* (H) can be observed. This suggests that contour mapping without further analysis is not accurate enough to base firm conclusions on the association of plants and nematodes. There was no clear association between any of the different trophic groups, although all had a positive effect on total numbers of nematodes. From both regression analysis and plotting of distribution patterns, clear associations between plant and nematode species have been found. These associations suggest that cultures of different plant nematodes could be established on the various hosts and would be helpful for testing host/parasite relationships. Little is known about the relationship between host and parasite that occur in natural ecosystems.

Conclusions.

There was an association between the state of growth of the plant species and the abundance of plant parasitic nematodes within the sampling area. If the plants were growing poorly, there were few plant parasitic nematodes extracted from soil from under such plants. It appears that the distribution of plants within a defined area does influence the distribution of nematodes of different trophic groups.

There was also association between specific plant species and particular plant parasitic nematodes. The distribution of *D.sedecimstriatus* was closely associated with that of both *Acacia papyrocarpa* and *Atriplex vesicaria*. In an area with a fairly uniform vegetation consisting of *Atriplex vesicaria*, there was no direct association between the plant species

PLATE 10 : 'HEALTHY' *Atriplex vesicaria* PLANT. This plant shows no disease symptoms, and so has a disease severity number of 4.

PLATE 11 : 'DISEASED' *Atriplex vesicaria* PLANT. This plant is severely affected by disease, with less than 25% of the stems with leaves. This plant has disease severity rating of 1.

PLATE 12 : LOW SHRUBLAND.

This is a view of the area of diseased vegetation found on the Middle-Back Station, near Whyalla.

and *T.tobari*. This could be due to the uniform spread of the root system throughout the sample area. There were clear differences in the distribution patterns of *T.tobari* and *T.siccus* when they occurred as a mixed population of plant parasites. The distribution of *T.siccus* was found to be closely correlated with *Maireana sedifolia* and *Acacia papyrocarpa* and *T.tobari* with *Atriplex vesicaria*. This was helpful in determining the appropriate host to be used in establishing a culture of the different nematode species.

4.2.5. Associations of Nematodes of Different Trophic Groups with Areas of Diseased Plants.

Introduction.

A site was selected within an area which showed die-back symptoms in *Atriplex vesicaria* . The native plants had received ample rainfall and had shown a flush of growth and then a rapid die-back, or loss of leaves. Plate 10, 11 and 12, indicates the symptoms of the die-back disease in the field and Appendix 4 shows the scale of disease severity used in the regression analysis of the different variables assessed. The leaves did not show evidence of fungal or bacterial disease or insect attack, so it was assumed that the disease causing agent occurred within the soil ecosystem. This die-back occurred at many different locations at different times of the year throughout the State, in Low Shrublands dominated by *Atriplex vesicaria* or *Maireana sedifolia*, but rarely in mixed stands of both species. It does not appear to be caused by overgrazing. An investigation into the cause of this disease was undertaken with special emphasis on the nematode fauna within the soil.

Materials and Methods.

Initial sampling had indicated that *T.tobari* was present at a site with disease problems present in the Middle-Back Ranges, west of Whyalla. A 50 m^2 sampling grid was set up within the diseased area and 22 plants were selected from within this area and soil samples taken from around the plant. An attempt was made to sample an even number of 'healthy' and 'diseased' plants. At every point of sampling, the coordinates were noted and a 2 m^2 quadrat was placed around the sampled plant. The total number and species of plants within the quadrat was counted and the % disease severity of the sampled plant assessed using

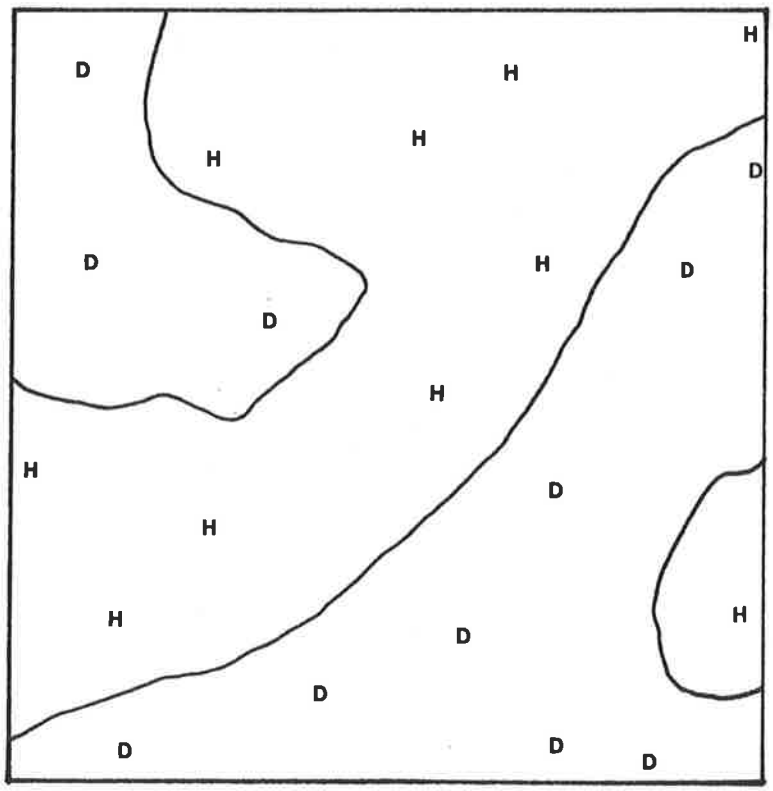


FIGURE 28 : THE DISTRIBUTION OF THE SAMPLED PLANTS WITHIN THE 50 METRE SQUARE GRID ENCOMPASSING AN AREA OF DISEASED AND HEALTHY PLANTS (Middle-Back Station). AN INDICATION OF THE DISTRIBUTION OF DISEASE IS ALSO SHOWN.

H - 'Healthy'

D - 'Diseased'

the systems stated in Appendix 4. The nematodes were extracted and number of fungal propagules were assessed (Appendix 3). The distribution of the disease within this 50 m2 grid was plotted and a pattern of disease incidence emerged (Figure 28).

Using regression analysis, several factors were assessed for correlation. These included :-

1) State of disease severity of the sampled plant.

2) Number of perennial plants within 2 m^2 of the sampled plant.

3) Number of fungal propagules present within the soil sample.

4) Number of nematodes of different trophic groups extracted from 50 mls of soil as an estimate of numbers of nematodes from the soil around the sampled plant.

Soil samples (n=22), from the same plants were taken 6 months later (March 1985), and the % disease severity, numbers of plants per 2 m^2 quadrat and the number of nematodes of different trophic groups was again assessed. There was no differences in state of disease severity between the two sampling time.

Results.

There were several significant correlations between number of plants/quadrant, numbers of nematodes of different trophic groups and the state of disease severity of the plants sampled and the number of plants present in the 2 m^2 quadrat (Table 16). These correlations indicate that :-

a) The state of disease severity of the plant sampled represented the state of the plants within the vicinity of the soil sample.

b) The disease severity of the plants within 2 metres of the soil sample was associated with higher numbers of *A.vesicaria* plants. Numbers of plant feeders are negatively correlated with numbers of *A.vesicaria.*

c) When there was a high percent of branches with leaves (representing well grown plants, disease severity = 0) there was also a high number of plant parasites.

The occurrence of the die-back symptom did not appear to be associated with *T.tobari* , which was the most common plant parasite present in the soil, as plant nematode numbers were not negatively correlated with severity of plant disease. The occurrence of

TABLE 16 : THE CORRELATION MATRIX OF THE REGRESSION ANALYSIS OF THE OCCURRENCE OF DISEASE SEVERITY, PLANT NUMBERS AND DIFFERENT TROPHIC GROUPS WITHIN A 50 METRE SQUARE GRID ENCOMPASSING A LOW SHRUBLAND DOMINATED BY *Atriplex vesicaria* WITH AN EPHEMERAL UNDERSTOREY (Middle-Back Station). Sampled in September 1984, from 22 different plants and an area of 2 metre square surrounding the sampled plant.

Variables	1	2	3	4	5	6	7	8
Disease severity(1)	1.0							
Atriplex vesicaria(2)	-.49	1.0						
Fungal Propagules(3)	-	-	1.0					
Omnivore/Predators(4)	-	-	-	1.0				
Bacterial Feeders(5)	-	-	-	.39	1.0			
Fungal Feeders(6)	-	-	-	-	-	1.0		
Plant Parasites(7)	.54	-.54	.40	-	-	-	1.0	
Total Nematodes (8)	-	-	-	.49	.91	.52	-	1.0
	1	2	3	4	5	6	7	8

(numbers in the table indicate significant correlations between different variables, Regression analysis, df=20, P=0.05)

(numbers after variables names correspond to numbers above and below numbers in the table)

(nematodes were extracted by the Baermann's Funnel technique from 50 ml of soil)

(disease severity assessed using method described in Appendix 4)

(soil samples and plant counts taken from selected sample plants within a 2 metre square quadrat).

TABLE 17 : THE CORRELATION MATRIX OF THE REGRESSION ANALYSIS OF DIFFERENT PLANT AND NEMATODE VARIABLES FROM A LOW SHRUBLAND DOMINATED BY *Atriplex vesicaria* AND WHICH SHOWED SOME SYMPTOMS OF DISEASES (Middle-Back Station). Sampled and assessed March 1985 from a 50 metre square grid in which 22 plants were sampled and the numbers of plant per 2 metre square quadrat assessed.

Variables	1	2	3	4	5	6
Disease Severity(1)	1.0					
Atriplex vesiaria(2)	-.63	1.0				
Omnivore/Predators(3)	-	-	1.0			
Bacterial Feeders(4)	-	-	.39	1.0		
Fungal Feeders(5)	-.40	.48	-	-	1.0	
Plant Parasites(6)	.76	-.64	-	-	-.34	1.0
	1	2	3	4	5	6

(numbers represent correlations between variables, Regression analysis, df=20, P=0.05).

(numbers in brakets after variables name correspond to the numbers above and below the numbers in the table)

(nematodes were extracted by the Baermann's Funnel technique from 50 ml of soil).

(variables were assessed from a 2 metre square quadrat surrounding a single plant from which soil was sampled)

disease appeared to be associated with the density of plants within a small area, as indicated by the negative correlation between the number of *A.vesicaria* within the 2 m^2 quadrat and the average state of foliage of these plants. Competition between plants for available nutrients and moisture may have been the major cause of the disease, rather than any organism.

A second sampling occurred 6 months after the first and after the summer season. Regression analysis of some of the factors assessed before, showed similar correlations (Table 17). However, numbers of *A.vesicaria* within each quadrat were positively correlated with numbers of fungal feeders and negatively correlated with numbers of plant parasites. The number of fungal feeding nematodes was positively correlated with factors, such as poor plant growth and high numbers of plants per quadrat, that were considered to be indicators of the die-back disease. This could be due to increased fungal activity during the decomposition of roots and other plant material from 'dead' plants. Whitford et al. (1982) indicated that bacteria were the first decomposers to operate on material in the soil and that fungi would act as secondary decomposers.

If numbers of nematodes of the different trophic groups are compared between the two sampling times, using ANOVA and a simple standard test of means (Table 18), it is found that the numbers of nematodes in all groups, except the omnivore/predator group, have significantly decreased over the summer season. This suggests that, during the drier seasons (December to March), survival of nematodes is reduced. The answer to the proposed question at the start of this section :- Does the plant nematode, *T.tobari,* cause disease symptoms in host plants in the field? is that there appears to be no effect of the nematode (*T.tobari*) in causing disease symptoms on plants in the field. Soil samples taken from under poorly growing plants tended to contain fewer numbers of plant parasitic nematodes than those under the healthy plants. To test further the effect of the nematode on host plants, laboratory and glasshouse experiments were conducted.

4.2.6. Conclusion.

TABLE 18 : MEAN NUMBER OF NEMATODES OF DIFFERENT TROPHIC GROUPS IDENTIFIED FROM SOIL FROM A 50 METRE SQUARE GRID SAMPLED IN SEPTEMBER 1984 AND MARCH 1985.

	Mean number		
Trophic group	1984	1985	
Omnivore/	23.9	18.5	ns
Predator *sd*	*10.0*	*7.3*	
Bacterial	174.6	92.2	*
Feeders *sd*	*62.3*	*28.5*	
Fungal	74.6	46.7	*
Feeders *sd*	*25.0*	*21.7*	
Plant	20.6	9.3	*
Parasites *sd*	*16.3*	*12.7*	
Total	293.6	166.7	*
Nematodes *sd*	*74.3*	*33.8*	

(*=significantly different values for nematode numbers between the two sampling times of the same soil collected in September 1984, P=0.05, standard test of means between the two different sample times, df=20)

The distribution of selected plant parasitic nematodes within defined areas, do show some clear associations with specific host plants. There is also an association between growth of the plant and numbers of plant parasites. The samples taken represent a single instance in the overall ecology of the site, in that numbers, proportion and distribution of the different nematode trophic groups, may change with time, growth phases of the plant and drying out of the soil. Further investigation on the change in numbers of nematodes of all trophic groups with time is to be investigated in the next section.

4.3. THE RELATIONSHIP BETWEEN CHANGES IN NUMBERS OF THE DIFFERENT TROPHIC GROUPS AND TIME.

4.3.1. Introduction.

As soil dries out it has been postulated that nematodes enter an anhydrobiotic state in which there is no detectable metabolism (Demeure, 1980; Demeure et al., 1979b; de Guiran, 1979; Van Gundy, 1965;). It is considered that coiling is a trait of nematodes that enables them to tolerate dehydration for some period of time and that tolerance to drought is a general phenomena in soil and plant nematodes (Simons, 1973). Investigation of the stimulus for inducing coiling (Demeure et al. 1979a), showed that there were physical forces brought to bear on the surface of the nematode by the thickness of the water film surrounding the nematode, causing coiling in some species. The optimum thickness of this water film was found to be 6-9 monomolecular layers. Not all nematodes survive desiccation by coiling. Parasites of above ground parts of plants tend to form aggregates of straight nematodes (Huang and Chiang, 1975). The nematodes of other desert systems have been found to coil in response to the drying out of the soil (Freckman et al., 1977) and it was considered important to determine whether nematodes in the arid region of S.A. coil to survive desiccation. The questions asked were :-

a) Is there a relationship between numbers of nematodes extracted from soil and the proportion of coiled and straight nematodes?

b) Are there changes in the number and proportion of nematodes of the different trophic groups with time?

c) Are there differences in numbers and proportions of nematodes between sites of different Vegetation?

4.3.2. Materials and Methods.

Two methods were used to investigate the changes in numbers, proportion and form of nematodes over a set period of time in the field. These two methods were :-

Method A) Extraction of Active nematodes (Baermann's Funnel Method).

Soil (50ml) from each sample was placed on a single ply tissue paper in a Baermann's Funnel, water added to the base of the petri-dish until the soil became wet and left for three days. The nematode suspension was then reduced in volume by passing through a 525µm mesh sieve into a measuring cylinder and the sieve carefully washed out to remove all nematodes. A proportion of no less than 25% was then taken from the measuring cylinder and the numbers of the different nematode trophic groups counted. The extraction efficiency of this method was assessed. When the nematode suspension was washed through the sieve the water was kept and the number of nematodes passing through the sieve counted. The nematodes remaining in the soil after three days were also assessed by placing the soil in a conical flask, adding 100 ml of water plus 5 ml of a solution of 0.75g of Separan NP-10® (Dow Chemical Ltd) /1,000ml. The concentration of the Separan solution was decided upon by comparing different concentration of Separan and the ability of the solution to precipitate soil particles within 3 minutes. The Separan solution precipitated the soil particles while the nematodes stayed suspended. The extraction efficiency of this method was found to be about 75%.

Method B) Extraction of coiled and straight nematodes (Hot Formalin Method).

There are several methods for the extraction of anhydrobiotic nematodes from soil. On experimentation, the Hot Formalin method was found to be the most suitable for the soils sampled because it enabled rapid, efficient and easy counting. The method used was :-

a) 50 ml of soil was placed in a 250 ml conical flask and about 100ml of hot formalin (1.5% at 90 ^{o}C) added and mixed in the fume hood.

b) The solution was left to cool and could be kept for some time.

To sample, 5ml of 0.75 g of Separan/1000ml water solution was added to the flask and the soil stirred and allowed to settle for 30 seconds. The nematode suspension was then decanted through a 525 µm aperture sieve three times. It was found that after washing the soil through the sieve three times, 75 % of the nematodes could be accounted for. This was assessed by counting the nematodes each time the soil was decanted and comparing the % nematodes accounted for each time.

The Hot Formalin Method (Freckman et al., 1977) was shown to me on a visit to Dr. Freckman at Riverside, California in January 1986. The formalin was used to preserve the nematodes until counting, the nematodes could then be kept for several months.For the *Casuarina cristata* Low Woodland, the soil samples collected before January 1986 were resampled (Baermann's Funnel technique) in March 1986 and the total number of nematodes present compared with the original counts. The soil samples had been kept at 4 ^{o}C from the time of removal from the field site. Those soils collected in August and October 1985, were found to have significantly fewer nematodes and were not included in the Tables and Figures. Those samples taken from the *Atriplex vesicaria* Low Shrubland were also resampled. Those samples collected in August 1985 were found to be significantly different when tested again, and so were not included. The soil samples collected in February 1986 were lost before counting of coiled and straight nematodes could proceed.

a) Assessment of the Different Methods of Extraction.

Before proceeding with the field trials, the similarity in the numbers of nematodes extracted by both methods was investigated. Soil samples from 2 different sites signified by (MR 12/2 and MR 11/2) with the same vegetation were tested by extracting nematodes from 50 ml of soil (3 replicates/soil sample) by both methods and comparing the number of nematodes extracted. Standard tests of means were used to analyse the data (Table 19).

b) Field Trials.

Two sites of different vegetation type, Low Woodland dominated by *Casuarina cristata* and the Low Shrubland dominated by *Atriplex vesicaria*, with known populations of

TABLE 19 : ASSESSMENT OF THE TOTAL NUMBERS OF NEMATODES EXTRACTED FROM DRY SOIL BY TWO METHODS (Baermann's Funnel or Hot Formalin Method)

Site	Total numbers of nematodes extracted from 50 ml of soil by Baermann's Funnel		Hot Formalin
MR12/2	415.3	ns	379.0
sd	*15.3*		*75.2*
MR11/2	296.0	ns	401.0
sd	*17.2*		*26.8*

(ns= no significant difference between the two extraction methods, P=0.05, df=4, standard test of means)

(numbers in *italics* represent plus or minus standard deviations)

(numbers are means of 3 samples)

TABLE 20 : MEAN NUMBERS OF NEMATODES IN DIFFERENT TROPHIC GROUPS FROM SOIL FROM A LOW WOODLAND DOMINATED BY *Casuarina cristata* AND SAMPLED EVERY TWO MONTHS FROM AUGUST 1985 TO OCTOBER 1986 (extracted by the modified Baermann's Funnel method).

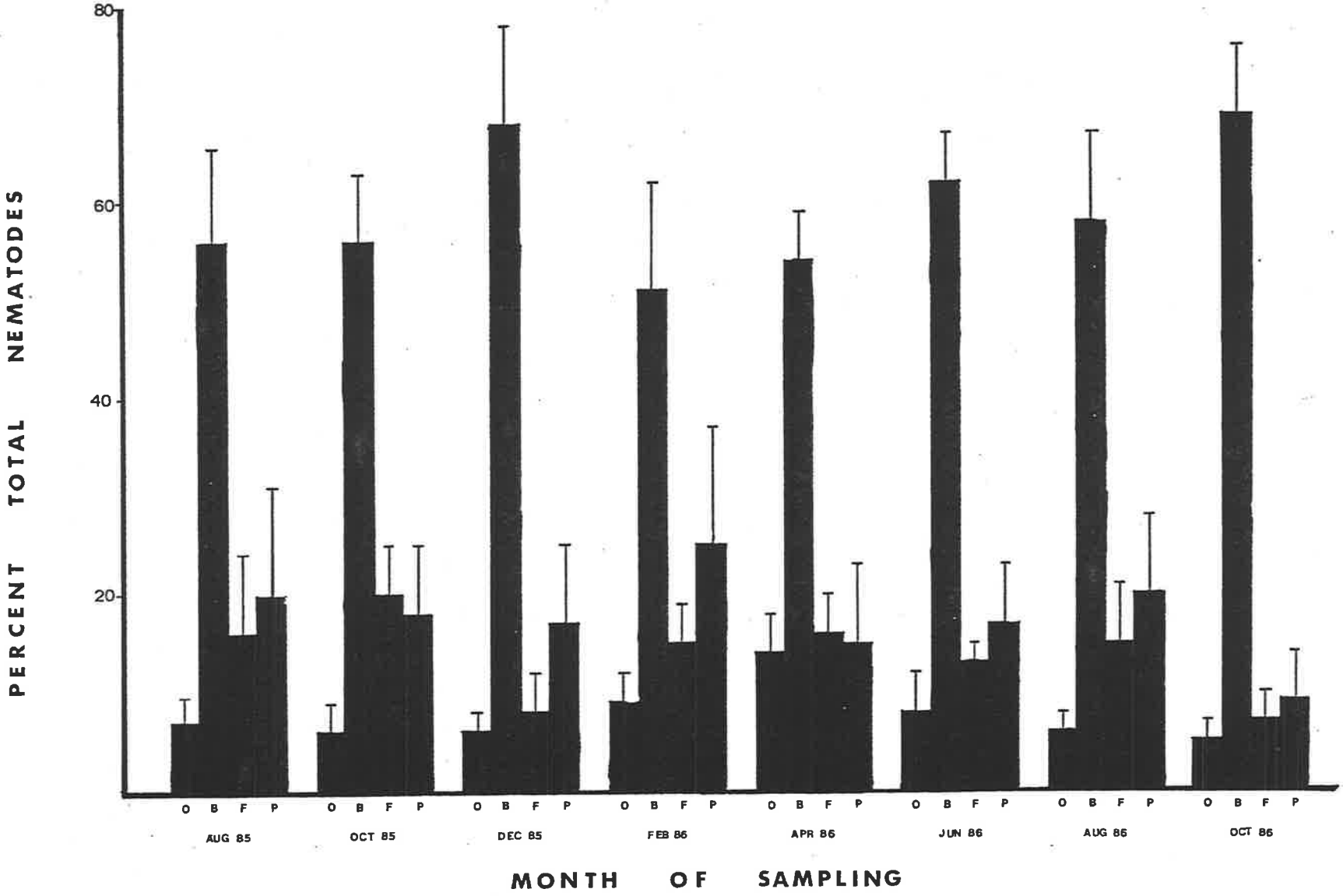
Mean numbers of nematodes (/50 ml of soil)

Trophic group	M1	M3	M5	M7	M9	M11	M13	M15	
Omnivore/ Predator	10.4	7.4	10.0	11.0	21.2	13.2	16.6	28.0	ns
sd	*4.9*	*2.9*	*2.5*	*4.5*	*7.9*	*7.2*	*8.3*	*9.3*	
Bacterial Feeders	78.0	72.4	135.0	105.4	87.2	107.2	162.2	451.8	*
sd	*24.8*	*24.1*	*35.8*	*26.1*	*27.5*	*32.1*	*35.1*	*114.1*	
Fungal Feeders	25.4	25.3	13.3	24.8	27.2	22.4	43.9	69.4	*
sd	*18.9*	*9.8*	*3.7*	*7.4*	*13.1*	*7.2*	*19.5*	*14.1*	
Plant Parasites	22.5	19.6	25.1	35.7	18.7	26.7	56.9	101.3	*
sd	*11.3*	*6.6*	*8.8*	*12.0*	*7.3*	*13.5*	*25.6*	*54.5*	
Total Nematodes	136.3	124.7	183.4	176.9	154.3	169.5	279.6	650.5	*
sd	*28.2*	*28.5*	*31.1*	*35.3*	*45.8*	*42.6*	*51.8*	*137.9*	

(*=significant difference between the different months of sampling, ns=not significant, ANOVA, P=0.05, df=14)
(numbers in *italics* represent plus or minus the standard deviation)
(M1-15=month of sampling from August 1985 (M1) to October 1986 (M15)).
(numbers are means of 8 samples).

FIGURE 29 : THE PROPORTION OF NEMATODES OF DIFFERENT TROPHIC GROUPS SAMPLED FROM A LOW WOODLAND DOMINATED BY Casuarina cristata OVER A 15 MONTH PERIOD FROM AUGUST 1985 TO OCTOBER 1986.

(O=Omnivore/predators, B=Bacterial Feeders, F=fungal Feeders, P= Plant Parasites, bar=plus standard deviation)
(Tylenchorhynchus tobari was the main plant parasitic species)
(month of sampling :- AUG85=August 1985, OCT85=October 1985, DEC85=December 1985, FEB86=Febuary 1986, APR86=April 1986, JUN86=June 1986, AUG86=August 1986, OCT86=October 1986)
(percentage of total nematodes of each of the four different trophic groups calculated from numbers of nematodes from 8 different soil samples and averaged over the sample time).


PERCENT TOTAL NEMATODES
80
60
40
20
O B F P
AUG 85
OCT 85
DEC 85
FEB 86
APR 86
JUN 86
AUG 86
OCT 86
MONTH OF SAMPLING

T.tobari were sampled every two months from selected host plants over a 15 month period (August 85 to October 86). The soil was sampled from around 4 *Atriplex vesicaria* plants or 3 *Casuarina cristata* plants and from bare soil. Two soil samples were taken from opposite sides of the same plants and bare soil between plants, every sampling time, therefore, from the Low Shrubland, 10 samples were taken, and from the Low Woodland 8, samples were taken. The nematodes were extracted using the two methods described, depending on whether active or coiled nematodes were to be counted. The final soil samples (October 1986) were stored at 4 C for 6 months and then sampled using both extraction methods. The numbers and form of the nematodes extracted were compared with the initial extraction using ANOVA and standard test of means.

4.3.3. Results.

a) Assessment of the different methods of extraction.

There was no significant difference between the two methods when mean total number of nematodes from each method was compared (Table 19). The amount of variation within the Hot Formalin method is much higher than that for the Baermann's Funnel method, so direct comparison between the numbers of nematodes obtained from soil samples in the field trials by either method was not attempted.

b) Field trials.

Site 1 : Low Woodland (dominated by *Casuarina cristata*)

There was a significant increase in numbers of nematodes of all trophic groups, except the Omnivore/Predator group, in the last 2 months of sampling (Table 20). However, there was no corresponding change in the relative proportion of trophic groups (Figure 29). The omnivore/predator group occurred in very low numbers and the bacterial feeders were the most abundant trophic group present. These results suggests that some factor, possibly increased precipitation, has caused the significant increase in numbers of nematodes, without affecting the proportion of nematodes of the different trophic groups.

There was also a significant change in the numbers of coiled and straight nematodes over the period in which the soil was sampled (Table 21). The coiled nematodes

TABLE 21 : MEAN NUMBERS OF COILED AND STRAIGHT NEMATODES EXTRACTED FROM A LOW WOODLAND DOMINATED BY *Casuarina cristata*. NEMATODES EXTRACTED USING THE HOT FORMALIN METHOD FROM SOIL COLLECTED AT DIFFERENT TIMES.

	Mean numbers of nematodes(/50 ml of soil) Sample months					
Type of nematode	DEC85	APR86	JUN86	AUG86	OCT86	
Coiled	246.0	78.0	124.0	47.0	86.0	*
sd	*64.5*	*31.9*	*25.5*	*12.7*	*60.5*	
Straight	25.0	6.0	30.0	335.0	439.0	*
sd	*8.5*	*1.8*	*5.3*	*119.7*	*100.8*	
Total	271.0	84.0	155.0	382.0	525.0	*
sd	*81.2*	*33.3*	*48.2*	*130.6*	*140.9*	

(*=significant difference between the proportion of nematodes coiled and straight and the time of sampling, ANOVA, P=0.05, df=14)
(numbers in *italics* represent standard deviation)
(DEC85=sampled in December1985, APR86=April 1986, JUN86=June 1986, AUG86=August 1986, OCT86=October 1986).
(numbers are means values of 8 samples)

FIGURE 30 : THE PROPORTION OF STRAIGHT TO COILED NEMATODES EXTRACTED FROM SOIL BY THE HOT FORMALIN METHOD WHICH HAD BEEN COLLECTED FROM A LOW WOODLAND DOMINATED BY Casuarina cristata AT DIFFERENT TIMES FROM DECEMBER 1985 TO OCTOBER 1986.

□ - Coiled

▨ - Straight

I - indicated plus or minus standard deviation

(soil sampled from DEC85=December 1985, APR86=April 1986, JUN86= June 1986, AUG86=August 1986, OCT86=October 1986).

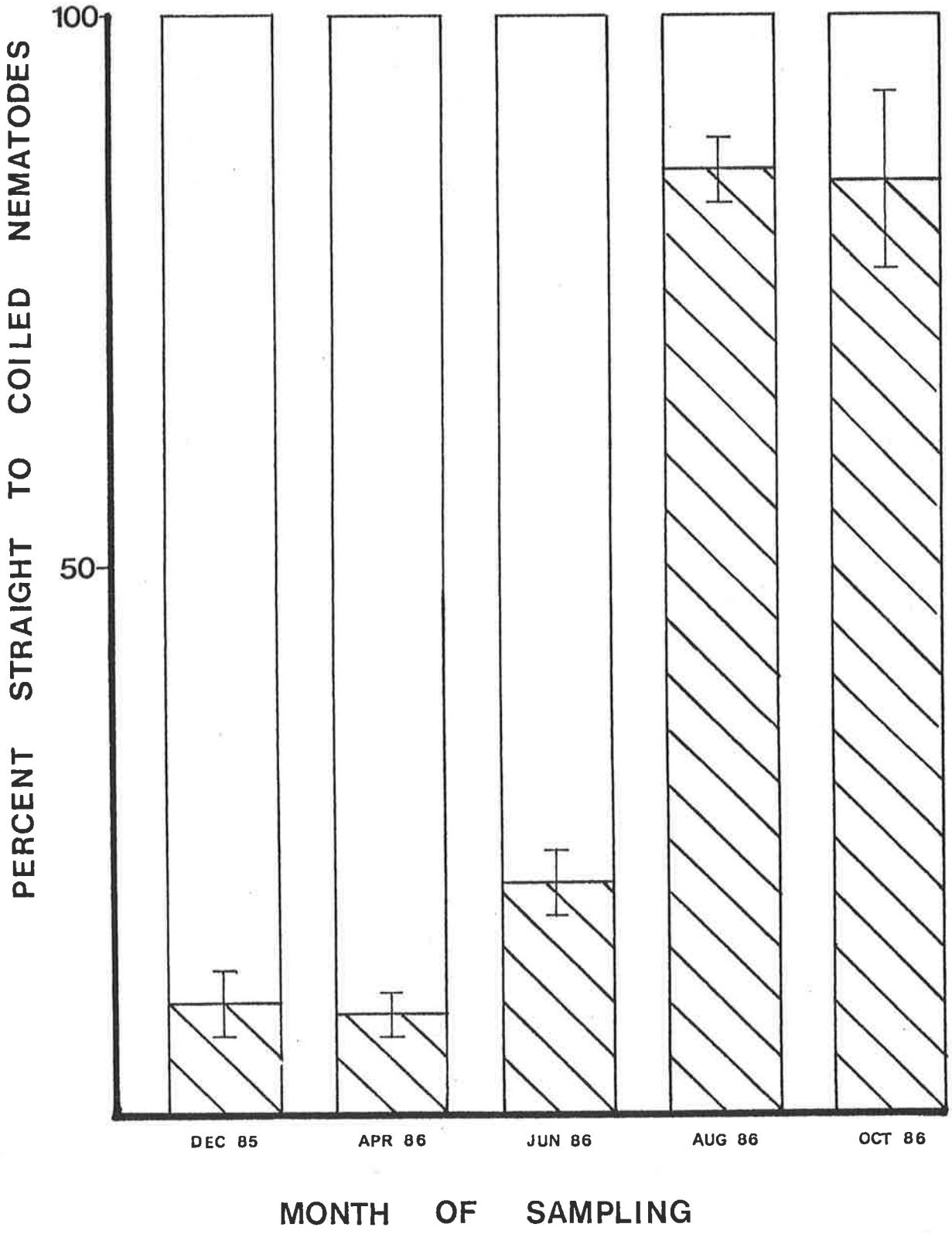

100
50
PERCENT STRAIGHT TO COILED NEMATODES
DEC 85
APR 86
JUN 86
AUG 86
OCT 86
MONTH OF SAMPLING

TABLE 22 : RAINFALL DATA AND THE TOTAL AMOUNT FALLEN TO THAT MONTH FOR THE HOMESTEAD OF PLUMBAGO STATION COMPARED WITH THE TOTAL NUMBER AND PROPORTION OF COILED TO STRAIGHT NEMATODES EXCTRACTED FROM TWO SITES OF DIFFERENT VEGETATION/LANDFORM ASSOCIATIONS LOCATED ON THE PLUMBAGO STATION PROPERTY.

	Rainfall		Total Number of Nematodes in Vegetation dominated by			
		Total to	*C.cristata*		*A.vesicaria*	
Month	Rainfall (mm)	Month (mm)	No.	C:S	No.	C:S
July 85	2.6	46.3	#	#	#	#
August 85	37.9	84.3	136.3	#	218.6	#
September 85	3.3	87.5	#	#	#	#
October 85	18.1	105.6	124.7	#	166.0	82:18
November 85	6.7	112.3	#	#	#	#
December 85	17.0	129.3	183.4	91:1	187.0	#
January 86	0.3	0.3	#	#	#	#
Febuary 86	4.2	4.5	176.9	#	161.0	#
March 86	0.0	4.5	#	#	#	#
April 86	0.0	4.5	154.3	93:7	114.0	89:11
May 86	17.8	22.3	#	#	#	#
June 86	11.0	33.3	169.5	80:20	216.0	28:72
July 86	17.7	51.0	#	#	#	#
August 86	25.9	76.9	229.6	12:88	544.0	8:92
September 86	11.1	88.0	#	#	#	#
October 86	44.5	132.2	650.5	16:84	622.0	6:94

(*C.cristata* = *Casuarina cristata* based Low Woodland, *A.vesicaria*= *Atriplex vesicaria* based Low Shrubland)
(number after month indicates year, 85=1985, 86=1986)
(C:S=proportion of coiled to straight nematodes, Hot Formalin Method)
(no. = mean total number of nematodes extracted from 50 ml of soil, by the modified Baermann's Funnel technique).
(#= missing values)

TABLE 23 : NUMBERS OF NEMATODES OF DIFFERENT TROPHIC GROUPS AND FORM WITH RESPECT TO SOIL COLLECTED IN OCTOBER 1986 AND EXTRACTED AT THE TIME OF SAMPLING AND 6 MONTHS AFTER.

	Time of counting				
	Sampling (October)		after 6 months (March)		
Extraction method	Numbers of nematodes (/50 ml of soil)				
Baermann's Funnel	No.	*sd*	No.	*sd*	
Trophic groups					
Omnivore/predators	28.0	*9.3*	28.0	*10.0*	ns
Bacterial Feeders	451.8	*114.1*	312.0	*45.8*	*
Fungal Feeders	69.9	*14.1*	71.7	*45.8*	ns
Plant Parasites	101.3	*14.1*	55.6	*17.4*	*
Total nematodes	651.0	*54.5*	467.3	*68.8*	*
Hot Formalin					
Form					
Coiled	86.0	*60.5*	97.7	*44.7*	ns
Straight	439.0	*100.8*	105.6	*19.9*	*
Total	525.0	*140.9*	202.3	*49.2*	*

(*=significant difference between the numbers of nematodes extracted from 50 ml of soil by both methods at the time of sampling the vegetation and 6 months stored at 4 ^{o}C, ns=not significantly different, ANOVA, P=0.05, df=13)

(numbers in *italics* represent plus or minus standard deviation)

(numbers are means of 8 samples)

were the major form of nematodes found in the soil in December 1985, April 1986 and June 1986. In August 86 and October 86, straight nematodes were the major form of nematodes. This is reflected in the proportion of coiled to straight nematodes shown in Figure 30. The increase in numbers of straight nematodes may reflect the increased activity of fungi, bacteria, algae and plants associated with increased moisture within the soil. It seems likely that in the months between June 86 and October 86, the nematode fauna was active and multiplying in reponse to some combination of factors, including recent rainfall. No rainfall figures could be collected directly from this site, due to the distance from settled areas, but data was collected from the nearby homestead are presented in Table 22. This table shows that there was increased rainfall within the area during the period from May 1986 to October 1986 compared with the previous 4 months, which corresponds to a change in the proportion of coiled to straight nematodes and increased numbers of total nematodes (Baermann's Funnel).

The soil from the last sample (October 1986) was stored at 4 ^{o} and then assessed 6 months after sampling for numbers of nematodes in the different trophic groups. When the numbers were compared with the numbers initially extracted, there was a significant decrease in numbers of bacterial feeders, plant parasites and total nematodes (Table 23). There was no significant difference between the numbers of fungal feeders and omnivore/predators. There was also a significant decrease in the numbers of straight and total nematodes extracted using the hot formalin method and no significant effect on the numbers of coiled nematodes. There are two explanations for this :-

a) The nematodes that are present in the soil in a straight form may only survive short periods of desiccation. The coiled nematodes are able to survive longer.

b) That both forms die at an equal rate, but that those originally found as straight, have become coiled.

From previous studies, by other workers, the first possibiltiy seems to be most likely.

Site 2) Low Shrubland (dominated by *Atriplex vesicaria*).

There was a significant difference between time of sampling and the numbers of nematodes of all trophic groups and total nematode numbers except numbers of plant

TABLE 24 : MEAN NUMBER OF NEMATODES OF DIFFERENT TROPHIC GROUPS EXTRACTED (Baermann's Funnel) FROM A LOW SHRUBLAND (10 samples) DOMINATED BY *Atriplex vesicaria* , EVERY TWO MONTHS FROM AUGUST 1985 TO OCTOBER 1986 (inclusive).

Trophic Groups		Mean Number of Nematodes (/50 ml of soil) Time of sampling M1	M3	M5	M7	M9	M11	M13	M15	
Omnivore/ Predator		22.0	10.0	6.0	10.0	11.0	17.0	48.0	33.0	*
	sd	*8.7*	*14.8*	*4.3*	*3.8*	*6.7*	*5.5*	*24.8*	*8.6*	
Bacterial Feeders		158.0	134.0	166.0	130.0	93.0	175.0	443.0	517.0	*
	sd	*22.7*	*26.6*	*40.6*	*26.9*	*31.4*	*37.2*	*154.2*	*139.0*	
Fungal Feeders		25.0	17.0	10.0	10.0	6.0	21.0	39.0	60.0	*
	sd	*11.2*	*8.4*	*2.1*	*2.1*	*2.4*	*7.4*	*12.0*	*19.4*	
Plant Parasites		13.0	5.0	5.0	11.0	4.0	3.0	14.0	12.0	ns
	sd	*6.6*	*0.5*	*1.5*	*6.6*	*7.5*	*1.4*	*5.6*	*6.2*	
Total Nematodes		218.0	166.0	187.0	161.0	114.0	216.0	544.0	622.0	*
	sd	*41.1*	*23.0*	*33.5*	*49.9*	*34.9*	*45.2*	*181.6*	*132.2*	

(*=significantly different values between the different sampling times, ns=not significantly different, ANOVA, P=0.05, df=18)
(numbers in *italics* represent plus or minus standard deviation).
(M1-15= month of sampling from August 1985 (M1) to October 1986 (15))
(numbers are means of 10 samples)

FIGURE 31 : THE PROPORTION OF NEMATODES OF DIFFERENT TROPHIC GROUPS SAMPLED FROM A LOW SHRUBLAND DOMINATED BY Atriplex vesicaria OVER A 15 MONTH PERIOD FROM AUGUST 1985 TO OCTOBER 1986.

(O=omnivore/predators, B=bacterial feeders, F=fungal feeders, P=plant parasites, bar=plus or minus standard deviation).
(Tylenchorhynchus tobari was the main plant parasite present).
(AUG85=August 1985, OCT85=October 1985, Dec85=December 1985, FEB86=Febuary 1986, APR86=April 1986, JUN86=June 1986, AUG86=August 1986, OCT86=October 1986).
(The proportions are mean values of the percent of the trophic group from 8 samples).

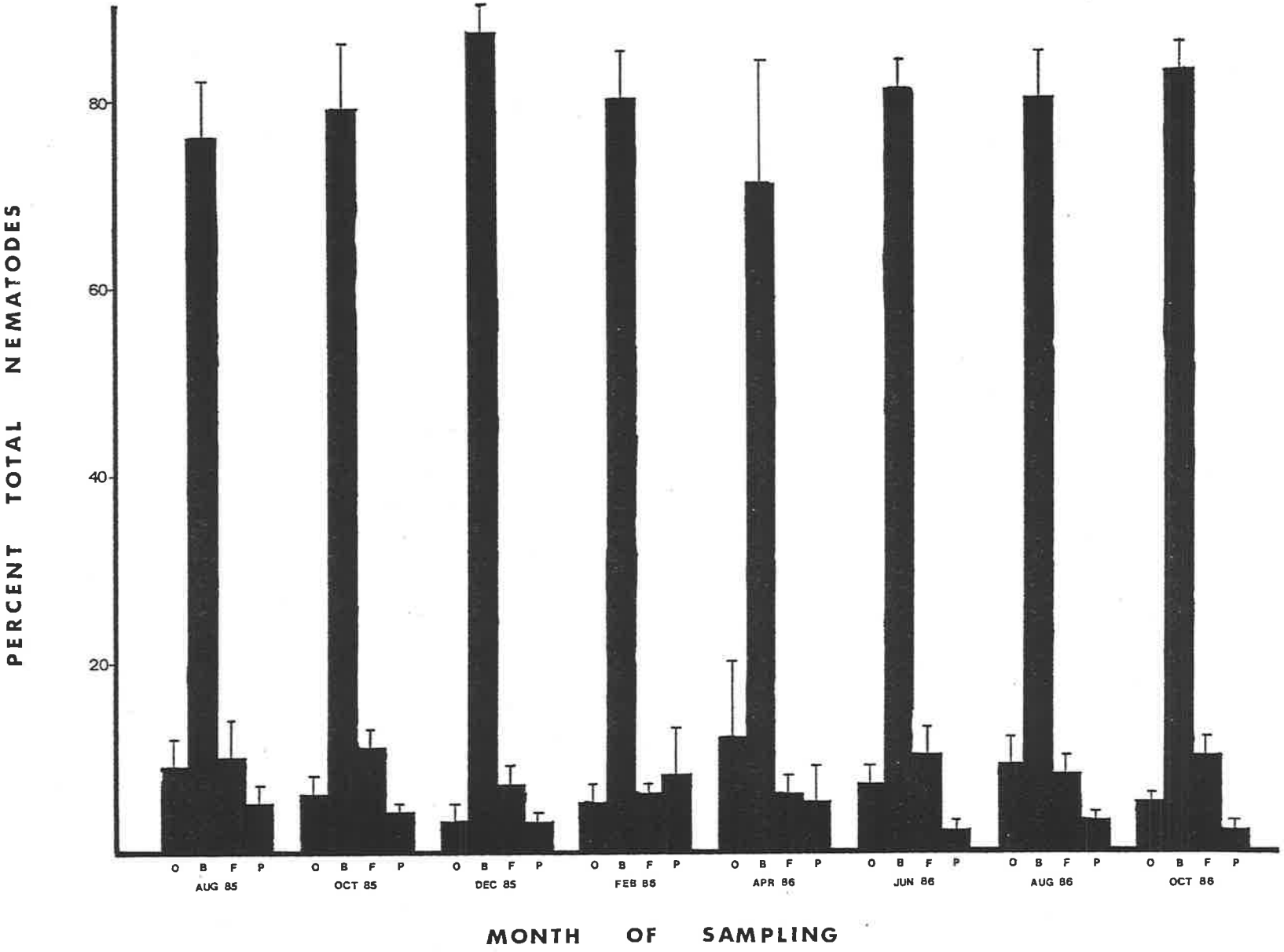
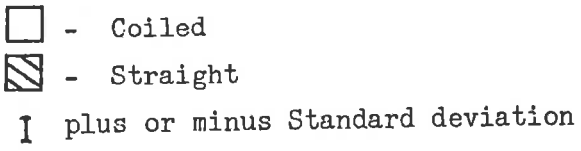

PERCENT TOTAL NEMATODES
80
60
40
20
O B F P
AUG 85
OCT 85
DEC 85
FEB 86
APR 86
JUN 86
AUG 86
OCT 86
MONTH OF SAMPLING

TABLE 25 : MEAN NUMBER OF COILED AND STRAIGHT NEMATODES EXTRACTED FROM SOIL (Hot Formalin Method) FROM A LOW SHRUBLAND DOMINATED BY *Atriplex vesicaria* , AT DIFFERENT MONTHS.

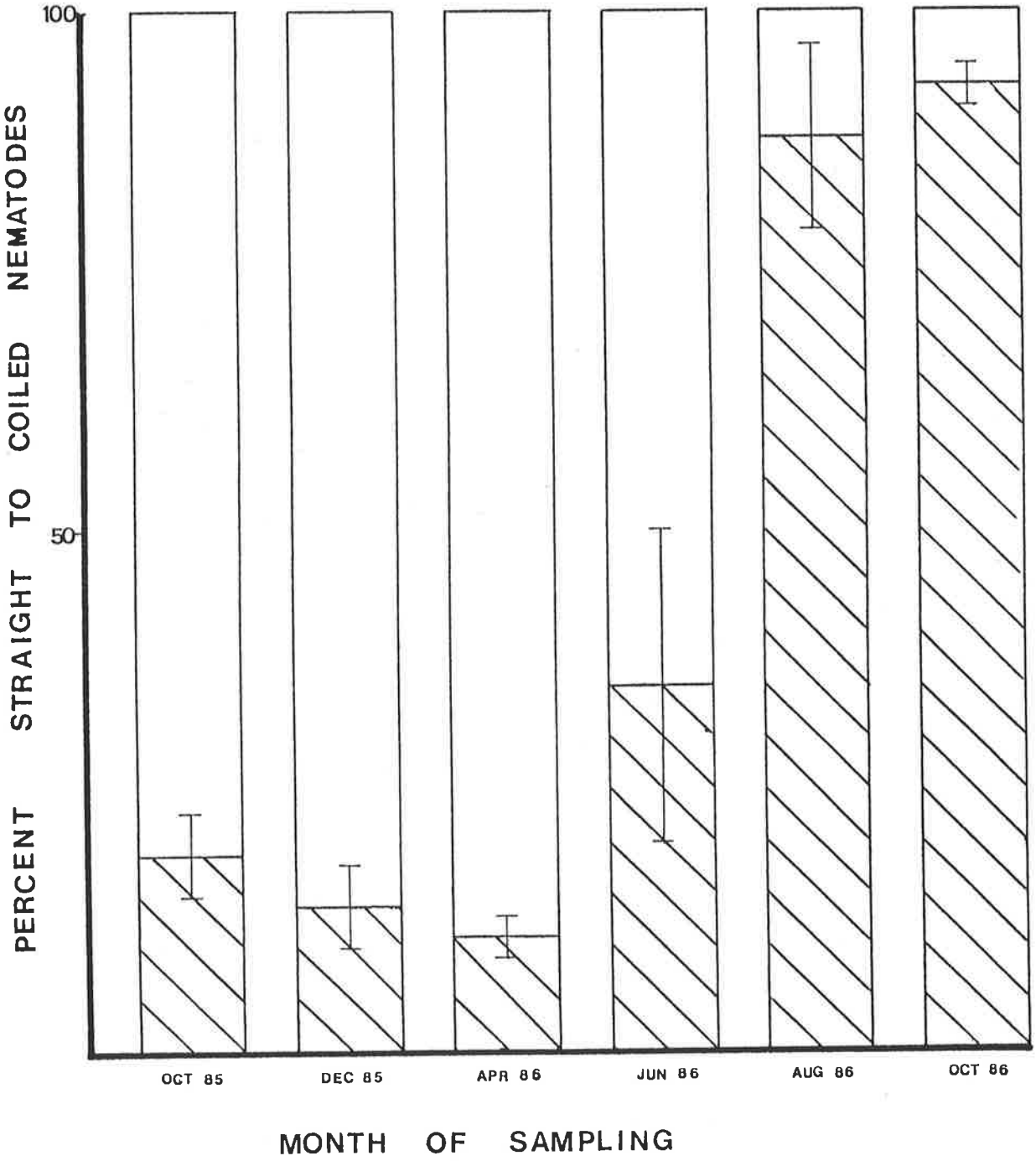
	Mean Numbers of Nematodes (/50 ml of soil)						
Type of	Month of sampling						
Nematode	OCT85	DEC85	APR86	JUN86	AUG86	OCT85	
Coiled	211.0	445.0	308.0	60.9	64.0	49.0	*
sd	*36.6*	*213.8*	*103.7*	*24.8*	*31.6*	*11.8*	
Straight	45.0	53.0	36.0	155.2	733.0	750.0	*
sd	*7.3*	*19.3*	*11.6*	*52.5*	*250.2*	*180.9*	
Total	256.0	482.0	344.0	216.1	767.0	808.2	*
sd	*40.4*	*231.1*	*113.8*	*29.7*	*271.6*	*183.2*	

(*=significant difference between the time of sampling and the numbers of nematodes extracted of different form, ANOVA, P=0.05, df=18)
(numbers in *italics* represent plus or minus standard deviation)
(OCT85= soil sampled in October 1985, etc.)
(numbers are means values from 10 samples)

FIGURE 32 : THE PROPORTION OF STRAIGHT TO COILED NEMATODES EXTRACTED FROM SOIL USING THE HOT FORMALIN METHOD COLLECTED FROM A LOW SHRUBLAND DOMINATED BY Atriplex vesicaria AND SAMPLED AT VARYING TIME FROM OCTOBER 1985 TO OCTOBER 1986.



(soil sampled at OCT85=October 1985, DEC85=December 1985, APR86= April 1986, JUN86=June 1986, AUG86=August 1986, OCT86=October 1986).


100
50
PERCENT STRAIGHT TO COILED NEMATODES
OCT 85
DEC 85
APR 86
JUN 86
AUG 86
OCT 86
MONTH OF SAMPLING

parasites (Table 24). The proportion of nematodes in the different trophic groups was not significantly different (Figure 31). The bacterial feeders were the most abundant trophic group, then the omnivore/predator group then the fungal feeders, with the plant parasites the least abundant. The highest numbers of nematodes of all trophic groups occurred in the last 2 months of sampling. This suggests that the factor influencing increased numbers, affects all trophic groups equally.

The numbers of coiled and straight nematodes also showed a significant change (Table 25). The coiled form was most abundant in the warmer and drier months from October 85 to April 86. From June 86 to October 86, a cooler and wetter time of the year, the straight form had significantly higher numbers than the coiled form. This is shown clearly when comparing the proportion of nematodes of each form at each sampling time (Figure 32). As for the previous site, rainfall figure from the homestead showed that there was increased rainfall in the months between June 86 to October 86 (Table 22).

The number of coiled and straight nematodes followed a similar pattern to that of the different trophic groups. There was a significant increase in numbers of nematodes in the last four months of sampling (Table 25). There was also a change in the proportion of straight nematodes to coiled nematodes (Figure 32), with a significant increase in the numbers of straight nematodes and the total number of nematodes with the number of coiled nematodes remaining fairly static, except over the drier months.

The Low Shrubland site differed from the Low Woodland site sampled previously as the soil, collected in the last month of sampling, kept at 4 C and sampled 6 months later, showed no significant differences in the total number of nematodes, numbers of bacterial feeders, omnivore/predators and plant parasites (Table 26). There was an increase in the number of fungal feeders over the six months. Although there was a significant decrease in the total number and number of straight nematodes extracted by the Hot Formalin method, there was no significant increase in the numbers of coiled nematodes. Many of the fungal and bacterial feeders extracted using the Baermann's funnel method were newly hatched, second stage juveniles, suggesting that the nematodes not only coil in response to desiccation, but can survive as eggs within the soil. Therefore, survival of nematodes at this site involved both coiling of adult and juvenile nematodes and as eggs. It

TABLE 26 : CHANGES IN THE NUMBER OF NEMATODES OF DIFFERENT TROPHIC GROUPS AND FORMS OVER A SIX MONTH PERIOD KEPT AT 10 ºC. AND SAMPLED FROM A LOW SHRUBLAND DOMINATED BY *Atriplex vesicaria*.

	Time of Counting				
	Sampling (October)		After 6 months (March)		
Extraction Method	Numbers of nematodes (/50 ml of soil)				
Baermann's Funnel	No.	*sd*	No.	*sd*	
Trophic groups					
Omnivore/predators	33.0	*8.6*	28.2	*14.3*	ns
Bacterial Feeders	517.0	*63.9*	478.2	*113.0*	ns
Fungal Feeders	60.0	*19.4*	107.2	*51.4*	*
Plant Parasites	12.0	*6.2*	8.2	*6.3*	ns
Total nematodes	622.0	*132.2*	621.8	*166.6*	ns
Hot Formalin					
Form					
Coiled	49.0	*11.8*	75.1	*39.5*	ns
Straight	750.0	*180.9*	456.7	*28.7*	*
Total nematodes	808.2	*183.7*	531.8	*116.3*	*

(ns=not significantly different, *=significant difference between the time of sampling the soil and the numbers of nematodes, standard test of means, P=0.05, df=16)
(numbers in *italics* represent plus or minus standard deviation).
(numbers mean of 10 values)

would have been useful to investigate the mechanism of survival of eggs, but this required more time than was available.

4.3.4. Conclusion.

There were some differences in numbers of different trophic groups of nematodes between the different sites. In the Low *Casuarina cristata* Woodland, the plant parasites were more abundant than the omnivore/predators. In Low Shrubland dominated by *Atriplex vesicaria*, the omnivore/predators were more abundant than the plant parasites. The fungal feeders were similarly abundant and the bacterial feeders were the most abundant group at both sites. The Low Shrubland also had higher proportions of Bacterial feeders (75-85%) compared to the Low Woodland (51-69%). The differences between the proportion of nematodes of the different trophic groups is a reflection of the type of vegetation sampled. The Low Woodland could possibly have a greater canopy cover, leaf litter and root system, protecting and allowing a larger area for growth of microorganisms and plants than the Low Shrubland.

To answer the questions posed at the start of this section, it appears that the numbers of nematodes in all trophic groups did change with time and were related to rainfall. However, the proportions of different trophic groups did not alter. When there was a significant increase in the total numbers of nematodes there was a corresponding increase in the number of straight nematodes and a decrease in numbers of coiled nematodes, indicating that coiling is a direct response to dehydration and that nematodes react to increased soil moisture by becoming active (straight). The numbers of each trophic group, as a percent of the total, did not change significantly throughout the sample period. This suggests that all nematodes groups within this arid region react similarly to desiccation and increased soil moisture content.

4.4. DISCUSSION.

Answers to the questions posed at the start of this chapter are as follows :

1) There was no consistent evidence that nematodes of any trophic group were more abundant at the depths of either 1-10 or 11-25 cm. Therefore, soil sampled in the field trials were taken to a depth of 25 cm.

2) *T.tobari* appeared to be most abundant under *Atriplex* spp. when soil samples were collected at random over different vegetations and landforms. *Radopholus crenatus* tended to be distributed with *Salicornia* spp. in saline soils and *Morulaimus simpsonii* tended to be distributed with *Zygocloa paradoxa* on the crests of sand dunes in the Simpson Desert. However, more extensive sampling was required to determine exact relationships between plant nematodes and their hosts.

3) Within a small area of uniform vegetation of Low Shrubland with *Atriplex vesicaria* the dominant plant species, *T.tobari* did not show any clear association with any specific plant species. Within Low Woodland with *Acacia papyrocarpa* as the dominant plant species, *T.siccus* was closely associated with the distribution of *Acacia papyrocarpa* and *Maireana sedifolia* and *Dolichorhynchus sedecimstriatus* with *Acacia papyrocarpa* and *Atriplex vesicaria*. *T.tobari* was found to be associated with *A.vesicaria* when present as a mixed population of plant parasites in an *Acacia papyrocarpa* Low Woodland with a mixed Chenopod understorey.

4) Attempts to diagnose the cause of areas of disease within the natural vegetation of the arid region of S.A., failed to implicate a living pathogen. It is possible that lack of nutrients, plant density and water stress were a contributing factor to disease severity.

5) Numbers of nematodes of different trophic groups show similar changes over a period of time. If there is an increase in soil moisture content, there appears to be a corresponding increase in the number and proportion of straight nematodes over coiled ones.

From these results it appears that the distribution and abundance of nematodes, especially *T.tobari*, are closely associated with the distribution of particular host plants. The discovery that *T.tobari* is clearly associated with *Atriplex vesicaria* , suggested that an attempt should be made to culture the nematode in the glasshouse, so that its biology and host/parasite relationships could be studied. Although the correlations established in this chapter are useful indicators of associations between plant parasitic nematodes and their hosts, they do not rigorously test hypotheses. Such correlative data are most useful in

producing hypotheses in the field. Subsequent glasshouse and laboratory experiments are clearly needed to test them.

The role of mycorrhizae has not been considered in this project as a substantial number of the plants sampled were chenopods and there is no evidence that they form mycorrhizal associations.

CHAPTER 5 : THE BIOLOGY AND HOST/PARASITE RELATIONSHIPS OF THE STUNT NEMATODE *Tylenchorhynchus tobari* SAUER AND ANNELLS, 1981.

5.1 INTRODUCTION.

Tylenchorhynchus tobari was first described by Sauer and Annells in 1981, from a site in the arid region of New South Wales. Few males have been found, so it is likely that reproduction is by parthenogenesis. No work had previously been done on the biology and host/parasite relationships of this nematode. *T.tobari* is the most common and widely distributed nematode within the arid region of S.A. and is closely associated with plants of the family Chenopodiaceae, especially the genus *Atriplex*. *Atriplex* species are important constituents of the native pasture used in extensive sheep production in the arid region of South Australia and other southern arid regions where sheep can be grazed. Investigation of the effect of *T.tobari* , a known parasite of Atriplex species, could be of importance to the Pastoral Industry.

In a previous chapter, this association was established in a general field survey of the region and in a more detailed analysis of the associations between plant species and nematode trophic groups. The first task was to determine the appropriate host, inoculation and extraction method, soil mixture and temperature regime so that the nematode could be cultured. Laboratory experiments could then elucidate a) the biology of the nematode b) the effect of the environment and plant on the nematode and c) the effect of the nematode on its host.

5.2. PRELIMINARY STUDIES ON *T.tobari*.

5.2.1. Establishment of a culture of *T.tobari*.

Soil from sites with known populations of the nematode were placed in small plastic trays, wetted and seedlings of either *Atriplex spongiosa* (an ephemeral saltbush) or *A.vesicaria* (a perennial saltbush) were planted in the soil. The plants were maintained for 3 months and then sampled. Nematodes were extracted from the soil using the Baermann's Funnel method and inoculated onto seedlings of *A.spongiosa* grown in an unsterilized potting mix soil (Appendix 7). Seeds of *A.spongiosa* (and other native plant species) were obtained

TABLE 27 : GERMINATION AND MATURATION OF FOUR SPECIES OF PLANTS OF THE FAMILY CHENOPODIACEAE, COLLECTED FROM THE FIELD AND KEPT AT 25 DEGREES CENTIGRADE.

Plant species	Germination Time(days)	%	Maturation
Atriplex spongiosa	3	40	6 weeks
A.lindleyii	7	20	12 weeks
A.vesicaria	7	20	no seed
Maireana sedifolia	14	<10	1% survival

from the field in large numbers and were successfully germinated and grown in the potting mix. The culture of *T.tobari* was originally established from soil from a site near the Strezlecki Crossing in the north-east of the region. No other soils were tested after this culture was established.

5.2.2. Selection of Host Plant Species.

Although *A.spongiosa* and *A.vesicaria* were used to 'trap' a culture of *T.tobari*, for experimental purposes the selection of an appropriate host was necessary. An appropriate host was considered to be one that was a native of the arid region of S.A., had a high germination rate, germinated promptly and readily set seed and allowed high numbers of nematodes to develop, ie. it was highly susceptible.

Seeds from four different species of Chenopodiaceae were collected from the field. Germination of seed from plants of this family required the fleshy pericarp surrounding the seed be removed. The seeds were then placed on the surface of a layer of wetted sand in a small petri-dish and kept at room temperature (Burbridge, 1945, 1946). The four species tested this way were *Atriplex spongiosa*, *A.vesicaria*, *A.lindleyi* and *Maireana sedifolia*. After the seedlings had germinated, two were sown in each of three pots for each plant species, using a sandy loam, and the time when seeds were set, recorded.

It was found that the seeds of *A.spongiosa* germinated in 3 days and took 6 weeks to reach maturity (Table 27). The other species had lower numbers germinate, were slower to germinate and tended to reach maturity (set seed) much later, if at all. The seeds produced by both *A.spongiosa* and *A.lindleyi* in the glasshouse had a germination rate of over 90%, in the same time as shown in Table 27. These two species, plus Barley (var. Clipper), Tomato and *Chenopodium quinoa* , were then tested for their ability to act as hosts to *T.tobari*.

Two pre-germinated seedlings of each plant species were planted into a small plastic pot, with a 1:3 soil:sand mixture (approximated the soil texture of soil from the arid region of S.A.). Each pot (3 / plant species) was inoculated with 50 nematodes and distributed randomly on the bench in the glasshouse. The nematodes were extracted after 2

TABLE 28 : NUMBER AND MULTIPLICATION RATE OF *Tylenchorhynchus tobari* FROM SOIL FROM FIVE DIFFERENT PLANT SPECIES THAT WERE INOCULATED AS SEEDLINGS WITH 50 NEMATODES AND EXTRACTED AFTER TWO AND A HALF MONTHS (3 replicates/plant species).

Host Plant	*T.tobari* Mean Number/Pot	Multi-Rate/Pot
Atriplex spongiosa	1283.0	25.7
sd	*224.6*	*4.5*
A.lindleyii	212.7	4.2
sd	*55.9*	*1.1*
Chenopodium quinoa	499.0	10.0
sd	*91.5*	*1.3*
Barley var Clipper	56.0	1.1
sd	*17.4*	*0.3*
Tomato	209.7	4.2
sd	*29.8*	*0.6*
	*	*

(*=significantly different, ANOVA, df=4,15, P=0.05)
(Multi-rate=Final density/Initial density of nematodes)
(numbers are means of 6 replicates)
(numbers in *italics* represent plus or minus standard deviation)

1/2 months using a combined Cobb's Sieving method and the Baermann's Funnel method (these are discussed later).

Significantly higher numbers of nematodes were extracted from soil in which *A.spongiosa* had grown than in soil from all other plant species (Table 28). *C.quinoa* had the next highest counts, followed by both *A.lindleyi* and the Tomato cultivar. The soil in which the Barley cultivar Clipper had grown had no significant increase in nematode numbers, although there was no decrease from the initial inoculum density. From these results it was clear that *A.spongiosa* should be used as the experimental plant in the investigation of the biology and host/parasite relationship of *T.tobari*. It is a native of the arid region of S.A., has a high germination rate so that the required numbers of plants for experiments could be produced, matures quickly and allows high numbers of nematodes to develop. Further preliminary experiments were now required to determine extraction methods, inoculation procedures, soil mixtures and temperature regimes which would optimise the growth of the plant and nematodes. Extraction efficiency was also examined.

5.2.3. Assessment of Extraction Method.

In most laboratory tests, small plastic pots (12 cm diameter by 12 cm depth) were used and contained a particular soil : sand mixture (1:3 potting soil mix to pure river sand). Extraction of the nematodes from this soil was not feasible using either centrifuge flotation or the Baermann's Funnel method alone, due to the bulk of soil to be extracted and the need to extract nematodes from the root system. Therefore, a combination of the Cobb's Sieving technique and the Baermann's Funnel technique was used as this reduced the amount of soil to be extracted by the Baermann's Funnel and was easily applicable for experimental purposes. The level of nematode loss was assessed by accounting for nematodes at each step of the extraction process. This was done by catching and counting the 'lost' nematodes and calculating the % lost from the total nematodes present. Each step had some loss of nematodes and these are detailed here as part of the description of the method used.

Method of Extracting Nematodes.

Step 1) The plant and attached soil were placed into a small plastic bucket, water added and the root system removed and washed over two sieves, the upper sieve with 1.0 mm aperture mesh and the lower with 45 µm aperture mesh. The nematodes still present on the root system and those washed through the sieve were counted; each accounted for about 1 % of the total numbers finally recovered.

Step 2) The soil was then mixed with water and decanted over the sieves three times. At each time, there was about 1% loss of nematodes. The soil plus nematodes trapped in the lower sieve were then placed on a single ply tissue paper laid ontop of a raised grid within a large glass petri-dish, water added until the tissue paper became wetted and the whole was kept for 3 days. The nematodes remaining in the soil in the bucket accounted for about 1 % of total nematodes.

Step 3) The water in the base of the petri-dish was then removed, washed through a 25 µm aperture mesh sieve and then decanted off into a measuring cylinder. Care was taken to wash all the nematodes into the cylinder, as a high proportion of nematodes could become trapped on the seive (as high as 22%). The number passing through the sieve was low (about 3%). Therefore, about 90% of nematodes present in the soil were counted from each pot sampled. This is a high proportion, and reflects the ability of Cobb's Seiving method to reduce the level of soil from which the Baermann's Funnel can successfully extract active nematodes. There was most probably a loss of eggs through the sieves, but counting the loss would have been impossible.

After extracting and counting the nematodes from each pot, the multiplication rate of the nematode for each pot was calculated and then an average was obtained for each treatment, allowing a standard deviation to be calculated.

5.2.4. Inoculation Methods.

The efficiency of several inoculum methods was investigated to determine if there were differences in numbers of nematodes able to infect the host plant (*Atriplex spongiosa*). Experiments involved the inoculation of *A.spongiosa* plants grown in small tubes of soil (1:3 soil:sand mixture) with 50 nematodes (*T.tobari*).

Two methods were finally shown to be the most efficient and easily manipulated. These were a) the hand inoculation method, and b) the aliquot method. The two methods are described below:-

a) Hand inoculation method. Nematodes were extracted by hand from a counting dish by a fine hair brush and placed in a glass vial containing approximately 3 ml of water. After the required number were extracted, the vial was upended over the plants to be inoculated and any nematodes remaining in the vial washed out.

b) Aliquot method. A nematode suspension from the Baermann's Funnel, was placed in a graduated measuring cylinder and the nematode density calculated by counting the number of nematodes present in 2 ml of suspension and determining the total numbers in the suspension remaining in the cylinder. The suspension was then adjusted to the required density.

With both methods, the numbers of nematodes surviving after being inoculated onto seedlings grown in soil for 7 and 14 days were 14 and 7 % respectively. Wiith both methods, there was a great decrease in numbers recovered from the soil after only a week, and there was no significant difference between the two methods. It was felt that any decrease in inoculum level below 50 would make the aliquot method more variable than the hand extraction method, Therefore, for inoculum densities below 50, the hand inoculation method was used, and for those above or equal to 50, the aliquot method was used (this saved time of preparation of inoculum for experiments). Further investigation, involving the inoculation of nematodes by both methods onto seedlings grown in an agar medium, were undertaken and will be described in the section on the biology of *T.tobari.*

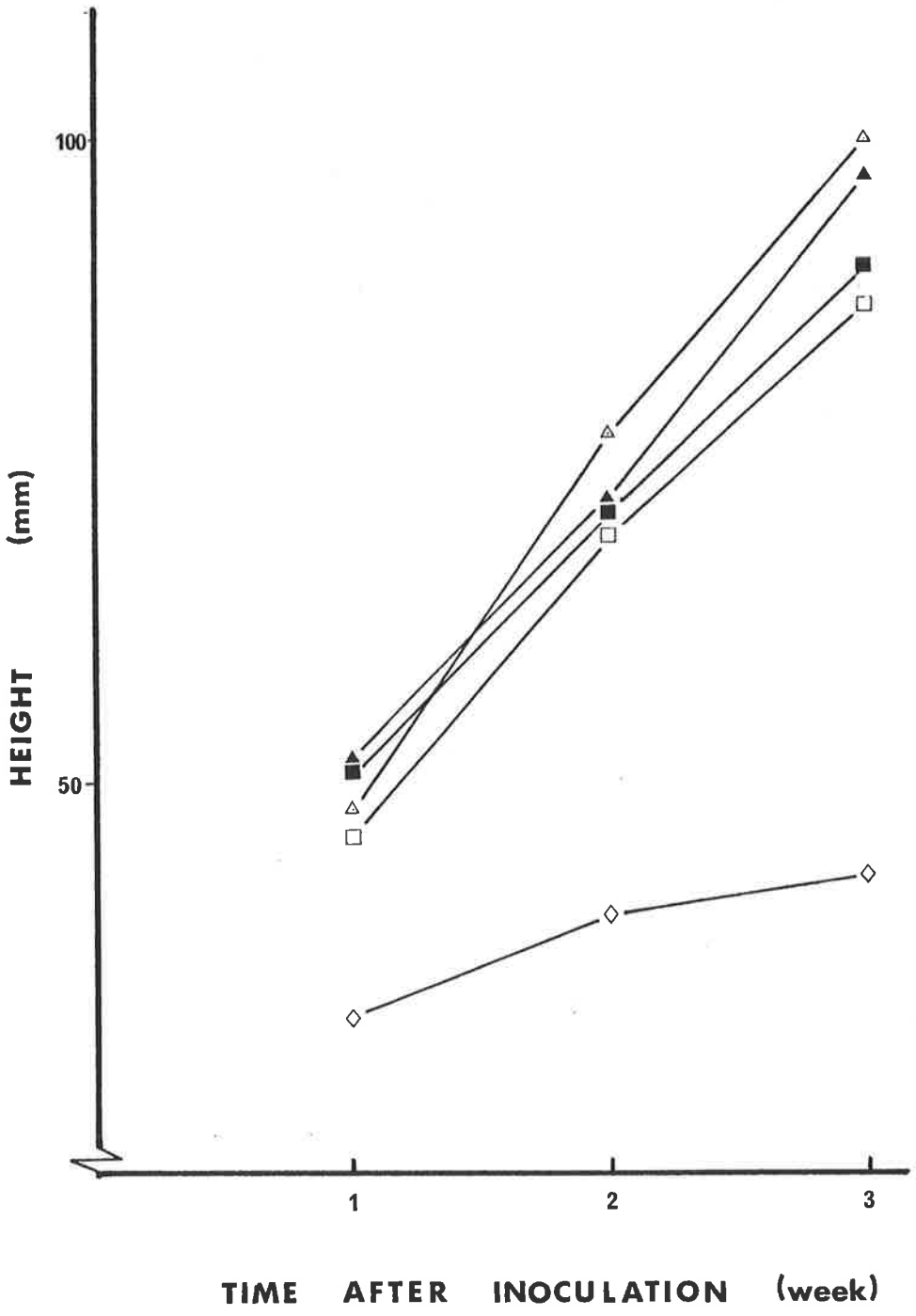
5.2.5. Culturing of *T.tobari* in Agar.

To obtain detailed information about the biology of *T.tobari* , the ability of the host to grow in an agar medium was tested. Other workers have been able to grow plants and infect them with *Tylenchorhynchus* species (Bridge, 1971). *A.spongiosa* was found to germinate and grow in a distilled water agar (1.5%) and when nematodes were inoculated onto the plants, feeding and some development occurred. Females were seen to produce eggs. The methods used were as follows :-

FIGURE 33 : THE GROWTH OF SEEDLINGS OF Atriplex spongiosa GROWN IN DIFFERENT SOIL : SAND MIXTURES OVER A 3 WEEK PERIOD (height of plants growing in each pot (2/pot) was measured every 7 days). EACH POT WAS INOCULATED WITH 50 NEMATODES.

Soil : Sand Mixtures	Symbols
4 : 0	□
3 : 1	■
2 : 2	△
1 : 3	▲
0 : 4	◇

(each measurement is an average height of 12 plants/soil mixture, with 2 plants grown per pot (6 replicates/soil mixture)).


100
50
HEIGHT (mm)
1
2
3
TIME AFTER INOCULATION (week)

Growth of Seedlings of *A.spongiosa* in Agar :

1) Seeds were surface sterilised in a 1.5% Sodium Hypochlorite solution for 5 seconds, excess water was then removed by tissue paper and the seed embedded in the agar under sterile conditions and left to germinate. There was no obvious difference in germination rate from those germinated on top of pure sand.

2) After the plant had germinated, nematodes were hand picked directly onto the root system of the seedlings or inoculated in an aliquot of 1ml of known numbers of nematodes

Using this method, the duration of the life-cycle, infection rate, fecundity and feeding behaviour could be investigated.

5.2.6. Soil Medium.

For further study of the host/parasite relationship between the nematode *T.tobari* and its host plant, *A.spongiosa*, pot experiments were required, as the growth of the host in agar was severely restricted and became contaminated after 2 weeks. Soil mixtures were investigated and certain criteria were needed to establish the one best suited for experimentation. The soil mixture should allow good growth of the host plant and high numbers of nematodes to develop.

Soil mixtures with ratios of 4:0, 3:1, 2:2, 1:3 and 0:4 potting mix soil (Appendix 7) to pure river sand were mixed and autoclaved (no extra nutrients were added). The soil was placed into small plastic pots (6 / soil mixture), planted with 2 seedlings of *A.spongiosa* and inoculated with 50 nematodes (aliquot method). The pots were watered constantly to ensure the surface of the soil did not dry out. Growth of the plants over the first 3 weeks was measured (Figure 33) and showed that the growth of plants in the pure river sand was significantly lower than for the other 4 soil mixtures. After 2 1/2 months, the nematodes were extracted and dry weight of shoots and roots were measured. This was done by separating the shoots from the roots, placing both into paper bags and placing in an oven at 60 ^{o}C for one day. The multiplication rate of the nematodes and the numbers of nematodes per g of root were calculated.

TABLE 29 : THE EFFECT OF DIFFERENT SOIL MIXTURES ON THE FINAL DRY WEIGHT OF *Atriplex spongiosa* AND NUMBERS AND MULTIPLICATION RATE OF *T.tobari* AFTER SEEDLINGS WERE INOCULATED WITH 50 NEMATODES AND KEPT FOR TWO AND A HALF MONTHS.

Soil Mixture	Dry Weight of Shoot	Dry Weight of Root	Nematode counts Final Numbers/Pot *T.tobari*	Nematode counts Final Numbers/Pot Multi-Rate	Nematodes /g root wt.
4:0	2.20	1.40	27.5	0.55	19.37
sd	*0.26*	*0.34*	*8.1*	*0.16*	
3:1	2.03	1.42	203.3	4.07	137.36
sd	*0.18*	*0.20*	*60.8*	*1.22*	
2:2	1.33	0.78	563.0	11.21	721.79
sd	*0.16*	*0.21*	*205.8*	*4.07*	
1:3	1.03	0.97	928.0	18.56	957.52
sd	*0.07*	*0.21*	*426.7*	*8.53*	
0:4	0.04	0.02	208.0	4.16	10400.00
sd	*0.01*	*0.01*	*101.3*	*2.03*	
	*	*	*	*	#

(*=significant difference between the different soil mixtures and different sampled variables, ANOVA, P=0.05, df=10)
(numbers in italics represent plus or minus the standard deviation)
(Weight of shoot and roots in gramme)
(#=not analysed)

Those plants growing in the pure soil and 3:1 soil:sand mixture had significantly higher weights of shoots and roots than those grown in other soil mixtures (Table 29). The plants grown in the 2:2 and 1:3 soil:sand mixtures had significantly higher weights of roots and shoots than those grown in pure sand. This greater growth of plants in the mixtures with a higher proportion of soil to sand did not increase numbers of nematodes or multiplication rate. Those plants grown in the 1:3 soil:sand mixture had significantly higher numbers of nematodes than all other soil mixtures. Those plants grown in pure potting soil had the significantly lowest numbers of nematodes of all soil mixtures.

Those plants grown in pure sand had a very high number of nematodes/g of root weight, suggesting that the development of the nematode *T.tobari* is greatly influenced by soil texture. It appears that the higher the silt/clay content of the soil, the lower the numbers of nematodes. It seems likely that the high degree of aeration and drainage associated with sandy soils favours *T.tobari*. The pure sand, although providing good aeration, would be low in nutrients and have a low water holding capacity, a handicap to both nematode and plant growth.

The 1:3 soil : sand mixture supported good growth of the host plant in the early stages of the experiment and so it was decided that some reduction in the time during which the experiment ran, could reduce some of the effects of reduced nutrients on the growth of the plants. This soil mixture was used in the experiments on host/parasite relationship as it allowed relatively good growth of the plant, especially at the start of the experiments and allowed good multiplication of the nematode

5.2.7. Temperature Effects.

The optimum temperature for host and nematode development was investigated using two controlled temperature growth rooms and the glasshouse. Pre-germinated seedlings of *A.spongiosa* were sown into a 1:3 soil:sand mixture, inoculated with 50 nematodes and then placed at 15 ^{o}C (12 hr light : 12 hr dark), 25 ^{o}C (24 hr light) or in the glasshouse where temperature had a diurnal variation. There were 6 pots/temperature and 2 seedlings/pot. The plants were sampled after 2 months for combined dry weight of the two plants and numbers of nematodes / pot. The multiplication rate and number of nematodes

TABLE 30 : THE INFLUENCE OF TEMPERATURE ON THE GROWTH OF Atriplex spongiosa AND THE FINAL NUMBERS AND MULTIPLICATION RATE OF T.tobari WHEN INOCULATED WITH 50 NEMATODES AND GROWN FOR 2 MONTHS (6 replicates/temperature).

Temperature ºC		Weight of Plants(g)	Nematode No./Pot	Multi-Rate/Pot	/g Plant
25		0.14 a	1131.4 a	22.6 b	8260.1
	sd	*0.03*	*363.8*	*7.3*	
15		0.07 b	48.5 b	1.0 a	630.9
	sd	*0.02*	*22.3*	*0.4*	
Glasshouse		0.68 c	1311.2 a	26.2 b	2125.0
	sd	*0.09*	*260.6*	*5.2*	
		*	*	*	#

(*=significant difference between the different temperatures, ANOVA, df=2,15, P=0.05)
(numbers with same letter, not significantly different, P=0.05%, df=10, standard test of means)
(numbers in *italics* represent plus or minus standard deviation)
(numbers are means of 6 replicates)
(#=numbers above calculated from means, therefore, not analysed)

/ g of plant weight were calculated. The results were analysed using analysis of variance to determine if there were any differences between treatments, and standard test of means to test where the differences occurred.

Those plants grown at 15 ^{o}C, had significantly fewer nematodes, lower multiplication rate and lower plant weight than those grown at either 25 ^{o}C or in the glasshouse (Table 30). While those grown at 25 ^{o}C were significantly lighter than those grown in the glasshouse, there was no signficant difference in number of nematodes or multiplication rate. The plants grown at 25 ^{o}C also had a higher mean number of nematodes / g roots, compared to the other temperature regimes. This confirms that temperature does have an important effect on the multiplication of the nematode and growth of the plant. The optimum temperature for the multiplication of the nematode appears to be about 25 ^{o}C, but the host grows more rapidly in the glasshouse for reasons not fully understood. Therefore, experiments on the host/parasite relationship of *T.tobari* and *A.spongiosa* were conducted in the glasshouse, which assured good multiplication of the nematodes, good growth of the host and facilitated experimentation, especially where numerous pots were involved (Table 35 for example).

5.3 BIOLOGY OF T.tobari.

5.3.1. Introduction.

Previous work on the life-cycle and biology of species of *Tylenchorhynchus* has shown some similarity. Most of the work has been done using plants grown in agar medium. *T.agri* was found to have a life cycle, from egg to adult, lasting 25 days (at 27 ^{o}C)(Coates-Beckford, 1983). The egg was laid as an unsegmented single cell and the nematodes were observed to feed ectoparasitically on the epidermal cells of the root hair zone. *T.claytonii* (Krusberg, 1959; Wang, 1971) was found to have a life cycle, from egg to egg, of about 31-38 days (at 28 ^{o}C) and there were four moults, the first being in the egg. Nand et al. (1982) found that *T.vulgaris* had a life cycle of 15-18 days at 30 ^{o}C and that there were four moults, the first in the egg. The duration of the life cycle of *T.tobari* was investigated, using plants grown in soil and on agar. It was felt that there may be differences

TABLE 31 : APPROXIMATE DURATION OF GROWTH STAGES OF *T.tobari* PLACED AT 25 °C.

STAGE	DURATION
Laid to Vermiform	2-3 days
1st Moult	3-4 days
Hatch	4-5 days
2nd Moult	11-12 days
3rd Moult	18-19 days
4th Moult	26-27 days
First Egg	30-31 days

between the above species and *T.tobari*, due to the different regions in which they occur and the host plants on which they feed.

Bridge (1974) found that the fecundity (number of eggs laid / female) of *T.claytonii* was 6.3 over a 19 day period. One female was observed to produce 14 eggs over the same period. The ability of females to produce eggs is an important part of understanding the rate at which nematodes can reproduce. The ability of *T.tobari* to reproduce is important in survival, as the periods in which reproduction can occur (wet periods) are severely restricted in the arid regions of the world. Therefore, investigation of the ability of this nematode to reproduce is important in understanding its role in the arid ecosystem.

5.3.2. Life-cycle.

The duration of the life-cycle of *T.tobari* was assessed first, by growing *A.spongiosa* seedlings in small tubes (12.5 cm length by 2.5cm width), filled with 1:3 soil:sand mixture and inoculated with 50 nematodes (aliquot method). The tubes were sampled at intervals after inoculation. The experiment indicated that juveniles were present in the soil after 2 weeks (third stage) and females after 4 weeks. For a more detailed study of fecundity, duration of stages, embryology and feeding behaviour, culturing of the plant and nematode in agar was used.

A hundred female nematodes were placed onto roots of *A.spongiosa* by hand and allowed to feed for 3 days. The gravid females were then transferred to a drop of distilled water on a microscope slide and the time at which eggs were laid recorded. The females were then removed and a coverslip placed over the drop of water. The eggs were laid as an undifferentiated single cell. The slide was kept at about 100 % relative humidity in a chamber at 25 ^{o}C until the time of hatching. The state of embryo development was noted at intervals. The newly hatched nematodes were then inoculated onto *A.spongiosa* and allowed to develop. Every second day about five juveniles were removed and their stage of growth assessed. There was a large amount of variability between nematodes sampled at each time, so assessment of the shortest time for development to maturity was necessarily approximate. The experiment was repeated twice and the results given in Table 31 are based on the combined results. When the eggs and juveniles were subjected to a lower

TABLE 32 : THE EFFECT OF INITIAL INOCULUM LEVEL ON THE SURVIVAL AND FECUNDITY OF *T.tobari* WHEN SINGLE SEEDLINGS OF *A.spongiosa* ARE GROWN ON AGAR AND INOCULATED WITH EITHER 2, 5, 10 OR 25 FEMALE NEMATODES AND LEFT FOR 5 DAYS.

Initial Inoculum	Final numbers of Females	Eggs	Survival FF/IF	Fecundity Eggs/FF
2	0.14	2.4	0.20	2.4 #
sd	*0.32*	*2.2*	*0.14*	*2.2*
5	1.60	17.6	0.32	11.4
sd	*0.27*	*3.5*	*0.06*	*1.8*
10	3.40	30.4	0.34	9.1
sd	*0.44*	*7.3*	*0.04*	*3.7*
25	7.60	73.6	0.30	10.3
sd	*1.03*	*16.1*	*0.04*	*2.5.*
	*	*	ns	*

(*=significant difference between the different inoculum levels, ns=not significantly different, ANOVA, P=0.05, df=8)
(#=significantly different, standard test of means, P=0.05, df=8)
(FF=final numbers of females, IF=initial number of females)
(numbers are mean values of 4 replicates)
(numbers in italics represent plus or minus standard deviation)

temperature, the rate of development was slowed and so time to complete the life cycle was increased.

5.3.3. Infection Rate and Fecundity.

An experiment involving the hand inoculation of a single host plant with 2, 5, 10 or 25 female nematodes was used to investigate the infection rate and fecundity of the nematode (Table 32). Plants were germinated in the agar medium as described and inoculated with the required density of nematodes (4 replicates per density) and kept at 25 ^{o}C. After 5 days, the final numbers of nematodes and numbers of eggs present in the root system were counted by direct observation through the agar. Fecundity (eggs/final number of females) and infection rate (final numbers of females/initial numbers of females) were calculated. There were four replicates per initial inoculum level.

The final numbers of nematodes are all significantly different between the 4 different inoculum levels. Not surprisingly, the highest number of females remaining after five days was found from plants inoculated with 25 females, the lowest was found from plants incoulated with 2 nematodes (few females remained at this inoculum level). A similar result was also obtained for number of eggs laid. There was, however, no significant effect on the percent number of nematodes surviving after 5 days at all inoculum levels. This indicates that the proportion of nematodes lost in 5 days is similar for all inoculum densities. The number of eggs produced per female inoculated onto plants at the lowest inoculum levels (2) was significantly lower than for plants inoculated at higher levels. Those females inoculated at higher inoculum levels (>2) produced similar numbers of eggs per female.

To ascertain whether higher inoculum levels did decrease survival, suspensions of 50 and 100 nematodes were inoculated onto a single seedling, the % surviving after 5 days were 22 and 16 respectively. These numbers were not significantly different from the survival rate of the other initial inoculum levels.

5.3.4. Feeding Behaviour.

Observations on the feeding behaviour of the nematode in agar culture were possible under the dissecting microscope. The nematode was observed to feed on the cell of

the root hairs, the junction between the root hair and epidermis and on cells of the epidermis. Cell destruction, abnormal cell growth or necrosis was not observed. There was also no aggregation of feeding nematodes around the root tip. This suggests that the feeding of the nematode causes little damage to the root system of the plant and little response of the plant to feeding, a characteristic that would have survival value for the nematode.

5.3.5. Summary of the Biology of *T.tobari*.

From these experiments on the biology of *T.tobari* on *A.spongiosa* in agar culture it has been found that :-

a) The minimum generation time from egg to egg is about 31 days at 25 ^{o}C.

b) The egg is laid as an undifferentiated cell and the first moult occurs in the egg.

c) Like other tylenchids, there are four moults, 3 outside the egg.

d) There is a high mortality (65-80%) of both females and juveniles when they are inoculated directly onto the root system.

e) The females can produce 2 eggs per day over at least 5 days.

f) The feeding of nematodes on the roots of the host plant appears to cause little damage.

It can also be postulated that at higher levels of inoculum there may be increased loss of nematodes due to overcrowding and a shortage of feeding sites, which would be greater when nematodes are inoculated into soil. Note of this needs to be taken when comparing different initial inoculum densities.

5.4 HOST/PARASITE RELATIONSHIPS.

5.4.1. Introduction.

Resistance of crop plants to nematodes that feed ectoparasitically is thought to be less likely than resistance to the sedentary endoparasites due to the complex nature of the host/parasite relationship between sedentary plant nematodes and their host (Cook, 1974). Damage caused by the stunt nematode (*Tylenchorhynchus claytoni*) has been observed in the field and glass house on corn (Nelson, 1956). However, high populations of the nematode had to be present in the soil surrounding seedlings to cause appreciable damage. As the plants

became older and had a more extensive root system, they could withstand high populations. The effect of high populations of T.tobari on a host plant (*Atriplex spongiosa*) can be investigated using small pot experiments and altering the initial density of nematodes. It is possible that, as with some crop plants, feeding by this nematode in high numbers may cause damage to the host plant. Accordingly, experiments were designed to investigate two hypotheses :-

1) As inoculum level of nematodes increases, the adverse effect on the growth of the host plant also increases and so the final numbers of nematodes are reduced.

2) The final numbers of nematodes increase as plant density and inoculum level increase.

5.4.2. The Effect of Different Initial Densities of Nematodes on the Final Numbers of Nematodes and the Growth of the Host Plant.

Introduction.

It is postulated that as nematode density increases, the adverse effect of the nematode increases. Also affected would be the growth of the nematode population or multiplication rate. The experiments presented in this section were designed to investigate the effect of the nematode on a set density of plants and to determine the effect of nematode density on their multiplication rate.

Materials and Methods.

Experiment 1 : The first experiment was designed to investigate the influence of initial inoculum on the multiplication rate and final density of the nematode . Seedlings were pre-germinated and sown into small pots which contained a pure river sand medium. The seedlings were inoculated with 5, 50, 100, 500 and 1000 nematodes and left for 2 months (6 replicates/pot per inoculum density, 2 seedlings per pot). Nematodes were extracted and counted using the method described. No plant variables were measured.

TABLE 33 : THE INFLUENCE OF THE INITIAL DENSITY OF *T.tobari* ON FINAL DENSITY OF NEMATODES WHEN SEEDLINGS OF *A.spongiosa* , PLANTED IN PURE SAND WERE INOCULATED WITH DIFFERENT DENSITIES OF NEMATODES (6 replicates/density).

Initial Density	Final density Numbers/Pot	Multi-Rate/Pot
5	160.8 b	32.3 a
sd	*77.7*	*12.9*
50	812.7 a	16.2 a
sd	*233.0*	*4.6*
100	1133.5 a	11.3 a
sd	*250.8*	*3.6*
500	393.7 b	0.8 b
sd	*52.5*	*0.1*
1000	355.3 b	0.4 b
sd	*49.0*	*0.04*
	*	*

(*=significantly different, ANOVA, P=0.05, df=4,24)
(numbers with same letter not significantly different, standard test of means, P=0.05, df=10)
(numbers in *italics* represent plus or minus standard deviations)

Experiment 2 : The second experiment investigated the effect of inoculum density on the growth of the plant, on final numbers and multiplication rate of the nematode. Pre-germinated seedlings were planted into a 1:3 soil:sand medium contained in a small plastic pot and inoculated with 0, 5, 20, 50 and 500 nematodes (lack of inoculum at the time of inoculation prevented higher numbers being used). There were 6 pots per inoculum density and 2 seedlings per pot. The nematodes were extracted after 2 months and counted and the plants separated into shoots and roots and dried for 1 day at 60 oC. The number of seeds per pot was also counted.

Results.

Experiment 1 : Plants grown in pure sand, inoculated with different densities of nematodes.

ANOVA indicated that there was a significant difference between the different initial inoculum levels and nematode numbers and multiplication rate (Table 33). When the mean numbers of nematodes and multiplication rates were compared between the different inoculum levels, there was evidence that an initial density of about 100, gave a maximum final population level, suggesting that feeding sites were limited in the higher populations. The multiplication rate decreased rapidly as initial inoculum increased. The results suggest that *T.tobari* is a nematode which survives best at population levels much lower than those encountered with other nematodes parasites of agricultural crops.

Experiment 2 : Plants grown in a 1:3 soil/sand mixture, inoculated with different densities of nematodes.

The overall relationship between initial inoculum and final numbers of nematodes (Table 34) was similar to that indicated in Experiment 1 (Table 33). ANOVA indicated that initial density had no influence on any of the plant variables. However, it showed that while the nematodes failed to survive and feed on the plant or that if feeding did occur, the plant was very tolerant of any damage. The clear relationship between initial density and multiplication rate, on the other hand, indicated that *A.spongiosa* is susceptible.

TABLE 34 : EFFECT OF DIFFERENT INOCULUM DENSITIES ON THE FINAL NUMBERS OF NEMATODES AND THE DRIED WEIGHT OF SHOOTS, ROOTS, SEEDS AND TOTAL PLANTS AND NUMBER OF SEEDS PRESENT WHEN PLANTS OF *A.spongiosa* ARE GROWN IN A 1 : 3 SOIL/SAND MIXTURE FOR 2 MONTHS.

Initial	Dry Weight of Plants (g)				Number of	Nematodes	
Density	Root	Shoot	Seed	Total	Seeds/Plant	Number	Multi-Rate
0	0.10	0.28	0.28	0.66	45	0.0	0.0
sd	*0.02*	*0.05*	*0.06*	*0.11*	*8.4*	*0 .0*	*0.0*
5	0.15	0.30	0.27	0.72	43	87.6	17.5
sd	*0.02*	*0.05*	*0.07*	*0.08*	*9.0*	*83.7*	*3.5*
20	0.11	0.27	0.30	0.68	42	178.8	8.9
sd	*0.01*	*0.02*	*0.03*	*0.04*	*4.6*	*83.7*	*4.2*
50	0.16	0.33	0.34	0.68	28	332.4	6.6
sd	*0.04*	*0.06*	*0.08*	*0.11*	*4.9*	*67.2*	*1.3*
500	0.19	0.25	0.25	0.65	35	536.4	1.1
sd	*0.11*	*0.08*	*0.06*	*0.11*	*7.5*	*84.7*	*0.2*
	ns	ns	ns	ns	ns	*	*

(ns=not significantly different, *=significant difference between the different inoculum levels, ANOVA, P=0.05, df=10)
(numbers calculated form 6 replicate pots)
(numbers in *italics* represent plus or minus standard deviation)

5.4.3. Interaction between different densities of nematodes and plants.

Introduction.

The hypothesis that the final numbers of nematodes increased as plant density and inoculum level increases, was tested using increasing inoculum levels and plant densities. The increasing plant densities could make available larger areas of root surface for colonisation by nematodes. From previous experiments, initial inoculum levels of about 50 or 100, produced the highest increase in numbers, when 2 seedlings were inoculated. It is postulated that by increasing plant density, the number of nematodes able to establish initially would also increase. Therefore, the population of nematodes would increase as plant density increases. The effect of feeding by nematodes on single or more plants may be different, and reflected in decreased root and shoot weight and in decreased production of seeds. The influence of plant and nematode on each other is sought.

Materials and Methods.

Pregerminated seedlings were planted into small plastic pots containing a 1:3 soil:sand mixture at 1, 2 and 4 plants/pot. These were inoculated with 0, 5, 50 and 500 nematodes. Each plant and nematode density had 6 replicates. The soil and plants were harvested 2 months after inoculation and the dry weight of shoots and roots assessed. The numbers of developed and undeveloped seeds per plant were counted and total seeds per pot assessed. The nematodes were extracted and the final number of nematodes and multiplication rate per treatment assessed.

Results.

ANOVA of the data showed there were no significant statistical interactions between any of the plant parameters and the initial and final number of nematodes. There was a marked effect of plant density on the growth of the plants which may have masked any effect of the nematodes on plant growth. If the different plant densities are considered separately, some effect of the initial number of nematodes may be evident. Looking at the growth variables for those plants grown at the three different plant densities (Table 35),

TABLE 35 : EFFECT OF DIFFERENT INOCULUM DENSITIES OF *T.tobari* ON THE GROWTH OF *A.spongiosa* PLANTS GROWN AT DIFFERENT DENSITIES IN SMALL PLASTIC POTS WITH A 1 : 3 SOIL/SAND MIXTURE FOR TWO MONTHS (6 replicates / plant and inoculum density).

Plant Density	Inoculum Density	Dry weight of (g) Shoot/Pot	Root/Pot	Numbers of Seed/Pot	U/seed/Pot
1	0	1.10	0.12	40.0	89.5
	sd	*0.27*	*0.01*	*6.8*	*16.1*
	5	0.87	0.14	30.2	83.7
	sd	*0.10*	*0.01*	*3.4*	*12.9*
	50	0.89	0.16	35.3	97.8
	sd	*0.13*	*0.02*	*4.5*	*7.3*
	500	0.78	0.11	35.5	79.8
	sd	*0.22*	*0.01*	*5.6*	*22.6*
		ns	ns	ns	ns
2	0	1.06	0.16	55.2	79.8
	sd	*0.06*	*0.01*	*11.8*	*12.3*
	5	1.04	0.17	55.2	85.8
	sd	*0.13*	*0.01*	*8.6*	*10.9*
	50	1.04	0.15	61.3	88.0
	sd	*0.09*	*0.01*	*4.5*	*9.4*
	500	0.99	0.17	49.2	88.0
	sd	*0.15*	*0.01*	*8.4*	*7.5*
		ns	ns	ns	ns
4	0	1.18	0.26	67.8	79.3
	sd	*0.16*	*0.03*	*6.1*	*6.0*
	5	1.18	0.25	90.8	78.8
	sd	*0.10*	*0.02*	*6.1*	*11.6*
	50	1.12	0.23	72.3	85.2
	sd	*0.14*	*0.03*	*8.0*	*14.9*
	500	0.98	0.23	68.7	70.2
	sd	*0.15*	*0.02*	*7.5*	*14.0*
		ns	ns	ns	ns

(ns=not significantly different, ANOVA, P=0.05, df=3,20)
(numbers in *italics* represent plus or minus standard deviation)
(U/seed = undeveloped florets or seeds)

TABLE 36 : EFFECT OF DIFFERENT PLANT DENSITIES ON FINAL NUMBERS AND MULTIPLICATION RATE OF *T.tobari* INOCULATED ONTO *A.spongiosa* AT DIFFERENT INOCULUM LEVELS (6 replicates/plant and nematode densities).

Initial Inoculum	Plant Density	Nematode Numbers/Pot	Multi-Rate/Pot
5	1	169	33.8
	sd	*84.1*	*17.2*
	2	115	23.0
	sd	*56.8*	*8.8*
	4	93	18.6
	sd	*33.6*	*6.7*
		ns	ns
50	1	1298	25.9
	sd	*232.7*	*4.6*
	2	1094	21.8
	sd	*311.1*	*3.8*
	4	1165	23.3
	sd	*379.9*	*7.6*
		ns	ns
500	1	606	1.2
	sd	*32.1*	*0.1*
	2	2114	4.2
	sd	*1234.5*	*2.4*
	4	1611	3.2
	sd	*279.1*	*0.6*
		ns	ns

(ns=not significantly different, ANOVA, P=0.05, df=3,20)
(numbers in *italics* represent plus or minus standard deviation)

there were no significant differences between the mean weight of the shoots and the initial inoculum densities. The weight of the roots and numbers of developed and undeveloped seeds also showed no significant differences with inoculum levels. If the weight of roots and shoots / plant / pot was assessed, the same result was found. From these results, inoculum density did not influence root growth, shoot growth and seed production, therefore, the hypothesis that *T.tobari* is a pathogenic nematode at the densities tested is rejected.

Assessment of the final number of nematodes extracted from soil in which different densities of plants were grown, showed that within each inoculum density there were no significant differences between numbers of nematodes at different plant densities (Table 36). There is, however, a very high standard deviation when 500 nematodes were inoculated onto 2 plants. This may account for the lack of significance at this level of inoculum and the different plant densities. More intensive work may need to be done on other methods of growing and inoculating these plants and nematodes, so that the variation within inoculum levels is reduced. Within the confines of the pot experiment, it appears that plant densitiy has little influence on nematode multiplication. This is possibly due to the poor survival rate of the nematode in soil and reflects the inoculation method. In the natural ecosystem, nematodes would be present near to root systems, are not traumatised by handling and so would be able to infect the plant more effectively. Studies on sowing seedlings into soil already containing the nematode may aid in determining the reasons behind the poor survival and infection rate.

5.4.4. Conclusions.

There was no apparent effect of the nematode on the growth and development of the host plant. This is possibly due to the poor infection and survival rate of the nematode and the tolerance of the plant. The various preliminary experiments enabled plant species to be selected on which high populations of nematodes could develop, so enabling experiments to be done using high numbers of nematodes. Therefore, there was selection away from intolerant and resistant native plants. From the information obtained in these experiments, further experiments can be undertaken to test other native plants of different genera or families.

5.5. DISCUSSION.

Bridge (1971) found that species of *Tylenchorhynchus* associated with natural grass pastures in England showed variable feeding behaviours, but most browsed among the root hairs and epidermis. Few species fed on the root tip, and those that did were found to cause root damage and an increase in the number of side roots produced. In the present study, *T.tobari* caused little, if any, damage to root growth of plants grown in agar or soil even when inoculum levels exceeded those usually found in the field, although changes in root morphology were not examined. *A.spongiosa* is clearly tolerant to *T.tobari*, a character common to many other native host plants. It would be of interest to investigate the reaction of other native plants to the nematode to assess if there are any intolerant native species. For example, species such as *A.lindleyi*, have been shown to be less susceptible to the nematode than *A.spongiosa* (this Chapter).

Multiplication and development of the nematode were favoured by particular environmental factors. The optimal temperature for nematode development was 25 ^{o}C even when the growth of the host plant was retarded. The composition of the soil:sand medium was also found to be important in both the growth of the plant and multiplication of the nematode. When the host was grown in a 1:3 soil :sand mixture, there were more nematodes than for any other mixture. Other workers have shown a similar requirement of species of *Tylenchorhynchus* for particular soil textures. *T.clarus* (Kheir et al., 1977a) had lower numbers in sandy and loamy sand soils, and higher in clay loamy soils. Intermediate densities of nematodes were found in intermediate soil types. Brzeski, 1971 found that a population of *T.dubius* on cabbage reached highest numbers in coarse sand rather than medium to heavy sand or silt. Multiplication of *T.tobari* was found to be favoured by soils with a high sand content, which was related to the type of soils in which it was found. The importance of soil moisture on nematode survival and reproduction is an aspect that needs further study. Use of relative humidity chambers to induce anhydrobiosis, was attempted, but the results proved inconclusive, possibly due to contamination and failure to induce correct relative humidities. These results are not included in this thesis.

As with most nematode species there was an effect of inoculum level on the final number of nematodes. For most nematodes, the rate of population increase decreases as the number of nematodes inoculated onto the plant is increased (Bridge, 1971; Green and Verdigo, 1985; Khan et al., 1986; Kheir et al., 1977b; La Mondia and Brodie 1986). The same occurs with *T.tobari* . Final numbers of nematodes increased as inoculum level increased, when the host was grown in pure sand, until the inoculum level reached over 100, then the final number of nematodes decreased until negative growth was reached. When plants were grown in a 1:3 soil:sand mixture, the final numbers of nematodes increased as the initial inoculum level increased and no real limit was reached when the growth rate became negative. The rate of population increase, however, decreased as initial inoculum increased. This indicated that there might be competition for feeding sites and reduced survival and fecundity for those nematodes inoculated at high initial densities.

The pot experiments did not reveal any influence of *T.tobari* on its host, suggesting the inoculum levels might have been too low to inhibit plant growth. However, the inoculum levels used were much higher than those occurring under natural conditions in the field. Consequently it is concluded that the host plant, *A.spongiosa* , is tolerant and is able to compensate for any damage caused to the root system by the nematode. The ability of roots to regenerate could be one such characteristic. The host plant is also susceptible to the nematode (i.e. the nematode reproduces well). This combination of tolerance and susceptibility confirms the notion that co-evolution between parasite and its host is a matter of balance with the minimum of selection pressure being exerted on either organism by each other. Environmental factors, such as drought, would exert a much greater selection pressure on both.

Plant Pathology is mainly concerned with the inhibitory effects of nematodes on yield in agricultural crops and data, showing decreased plant growth as nematode populations increase, are common. With natural ecosystems, like the arid regions of South Australia, such marked effects may be less common, due to the co-evolutionary aspect between host and parasite. Thus, in experiments on the influence of numbers of *T.tobari* on *A.spongiosa* , results that fail to show an inhibitory effect of the nematode on the host, support the hypothesis of a tolerant host and a nematode of low pathogenicity.

CHAPTER 6. DISCUSSION.

A major problem in any ecological survey undertaken over a large land mass, is sampling. With limited time and manpower, the ability to determine distribution patterns and associations of an organism is greatly restricted. Factors that affect the distribution of nematodes include competition between individuals, species and genera, predators and parasites, host distribution, growth of host and drying out of soil (Elton and Miller, 1954). It was felt that by assessing the distribution of topographical and vegetation features in relation to species of nematode present, it would be possible to establish some broad associations. Classification systems for vegetation and landform systems was possible using a system based on growth habit of the plants and simple topographical features. Broad associations between particular nematode species and sites were sought.

With ecological studies of large areas, it is important to identify individual nematode genera and species. In the present study it was necessary to establish what species were present, to describe them in detail if new and to indicate the broad distribution within the arid region. Having established which nematodes were present, processing of soil samples could be handled quickly and accurately. Such a goal was achieved in this study, aided by the fact that a limited number of genera were present. Surveys of this region in times of higher rainfall could reveal more genera and species than this study, which was undertaken over a drier than average period.

The data collected on the genera and species of plant nematodes provides some information for speculation on their evolution in the South Australian arid region. No new families or genera were found, and of the species recorded, only one occurs outside Australia. This suggests that there has been little selection pressure, at least at the morphological level, on the plant nematodes. Within the members of the family Dolichodoridae identified from the arid region of South Australia, a trend was observed towards increased stylet length from *Tylenchorhynchus* , through *Telotylenchus* to *Morulaimus* . *Morulaimus* is essentially an Australian genus and whatever factors that may have influenced the trend towards increased stylet length (with corresponding increased body length), they were probably unique to the Australian continent. The separation of the above genera may be artificial, but also may be due to ecological reasons. One possible factor influencing the trend

towards increased size could be the differences in the size of roots upon which the nematodes feed. The distribution of *Tylenchorhynchus siccus* closely follows that of *Maireana sedifolia* and *Acacia papyrocarpa*, while *T.tobari*, within the same site, showed an association with the distribution of *Atriplex vesicaria* , a much smaller plant. Consequently, it can be suggested that the greater stylet size of *T.siccus* (27µm) compared to *T.tobari* (18µm) may be related to the host upon which it feeds. The feeding site on roots of host plants on which species of nematodes other than *T.tobari* feeds is unknown, but should be investigated.

A study of the evolutionary trends in the Tylenchida has been investigated (Siddiqi, 1986; Luc et al., 1987). Within the arid regions, especially the isolated Australian continent, the diversity and distribution of nematodes has been greatly affected by climate. Even in wetter regions, climate plays an important role in the distribution of nematodes (Dao, 1970). It is possible that the period of diversification and speciation of the plant parasitic nematodes in the Australian arid regions occurred earlier during periods of wetter climates. Periods of intense aridity may have caused very high selection pressures on the plant parasitic nematodes (and other nematode trophic groups). Survival would then depend on the ability to tolerate dry soil conditions and to feed on a wide variety of plant hosts. The major period of emergence of the Australian flora occurred in the early Tertiary and it is possible that the plant parasitic nematode species and genera evolved soon after. The species of nematodes observed today are, therefore, likely to be either remnant populations of confined distribution, or widely dispersed species. Their distribution patterns indicate the ability of the nematodes to survive desiccation.

The means by which the different nematode species became dispersed throughout the region is reflected in the distribution of particular plant species. The distribution of *T.tobari* has been found to be closely associated with the distribution of plant species of the family Chenopodiaceae, but not limited to these host plants. The method of dispersal of the nematode is unknown but it can be postulated by considering those of other nematodes. There are two major forms of dispersal of nematodes (Wallace, 1963; Norton, 1978), active migration under their own locomotion, which occurs largely in the rhizosphere, and passive dispersal, in which external factors, such as water, wind, animals,

foliage and other factors transport the nematodes for long distances. It seems most likely that passive dispersal is the major mode of dispersal in the arid region.

Transport of nematodes by the wind has been investigated (Carroll and Viglierchio, 1981) and if dry larvae or eggs are present on the surface of the soil, lifting of these forms into the air is likely. The background atmospheric burden of nematodes in the air of the Sacramento Valley in California, was found to contain many nematode genera commonly found in the arid region of South Australia (Viglierchio and Schmitt, 1981). These nematodes were all revived before identification could take place and so were viable. The most commonly found nematodes were *Aphelenchus, Aphelenchoides*, *Tylenchus* and members of the Rhabditida. The least common, but still present, were *Ditylenchus*, *Tylenchorhynchus*, *Pratylenchus* or members of the Dorylaimida. None of these nematodes would be present in the atmosphere if they could not survive desiccation. It, therefore, seems possible that *T.tobari* can be dispersed by the wind, as it can survive desiccation and has a wide distribution within the arid area.

Transport by other means may account for the confined distrbution of certain plant parasitic nematodes. The localised distribution of *Scutellonema minutum* in the soil surrounding the Dalhousie Mound springs, could be due to transport by birds, but is most probably a remnant population, confined to the area when drought conditions restricted the distribution of the plant on which it feeds. The nematode *T.annulatus*, appears to be associated with the large river systems flowing into the Lake Eyre Basin. This nematode has been identified from native and crop plants in the south-west region of Queensland and so could have migrated down the drainage systems that feed Lake Eyre. The distribution of many other species of plant parasites is very localised, and may reflect the area in which they evolved. Even if the nematodes are transported by wind, water or animals, a suitable host plant is required before the nematode can become established. *T.tobari* appears to have a very wide host range a factor contributing to its wide distribution.

Exploratory excursions into the arid regions of South Australia identified sites in which specific plant nematode species occurred. A more intensive sampling programme enabled the relationship between the distribution of different plant species and the nematodes to be studied. Nematodes which had food requirements not directly related to the plant

TABLE 37 : THE ESTIMATED RATE OF POPULATION INCREASE, CALCULATED FROM SURVIVAL AND FECUNDITY DATA FOR 50 NEMATODES INOCULATED ONTO A SINGLE *Atriplex spongiosa* SEEDLING IN AGAR, AND ASSESSED AGAINST EXPERIMENTAL DATA FROM THE GLASSHOUSE.

Criteria for calculations :-
a) for each generation the mortality rate of females is about 62%
b) females that survive produce 24 eggs (2 eggs/day/2 weeks)
c) The generation time from egg to egg is 30 days
d) fecundity remains constant
e) assume all juveniles survive and mature.

Generation	Numbers of nematodes Start	Survive	Eggs	Actual numbers of nematodes
				50
1	50	19	456	
2	456	173	4159	
3	4156	1580	37932	1298

(Outlined numbers correspond to similar times after initial inoculation)

(omnivores/predators, bacterial feeders amd fungal feeders) were, nevertheless largely dependent on the distribution of the plants, presumably due to high activity of bacteria, fungi and algae in the rhizosphere that provided their food. Futhermore, it is considered that numbers of nematodes at the different sites would probably be affected by environmental factors, such as soil structure, salinity, water, and temperature, all of which fluctuate with time, and which also influence the activity, numbers and diversity of plant species. With the establishment of broad associations between vegetation, landforms and nematodes it was then possible to explore more precise relationships by determining associations between particular plant and nematode species. To understand the role of the nematode community in the soil, field and laboratory studies need to be implemented by further studies on individual genera and species (Yeates, 1979). As shown in Chapter Four, no detectable damage to the host plants in the field could be attributed to plant parasitic nematodes. A culture of *T.tobari* was successfully established enabling the host/parasite relationships to be investigated in pot and agar experiments. Hence the nematode was found to be a parasite rather than a pathogen, apparently living with their host plants in a balanced relationship.

Using the data for survival at the various stages of development, the theoretical rate of population increase was calculated for 50 *T.tobari* inoculated onto a single *A.spongiosa* plant grown in agar for 3 months (Table 37). The value of 1580 corresponds to the number 1298 actually found under controlled conditions in soil in the glasshouse. Thus the artificial environments of pots and petri-dishes, seem to yield data of a similar order and are probably useful tests of hypotheses developed in the field. In both pot and field experiments similar numbers of *T.tobari* had no effect on the growth of *Atriplex* species.

Yeates (1984), found that in pastures which were growing in a moist silt loam, the most abundant trophic groups were the plant feeding genera of *Helicotylenchus* and *Pratylenchus*. The Rhabditids, or bacterial feeders (so categorised in this study) survived in a 'dauer' larvae form, and the overall numbers of this group remained constant. The nematode fauna within soil of the arid region of South Australia, is exposed to long droughts and then short periods of abundant rainfall. The response of the nematode community to recent rainfall appears to be increased activity, reflected in increased numbers, with a

corresponding increase in numbers of straight nematodes over the coiled form. In the arid soils the major trophic group of nematodes was the bacterial feeder, the other groups varying from site to site. However, the response of each trophic group is approximately the same, with all types increasing with increased soil moisture. Further work is required to establish the relationship between drying of the soil and the ability of nematode species of the different trophic groups to survive. Both field and laboratory experiments were required but lack of time prevented accurate data being collected.

Plant feeding nematodes, in the agricultural context, often cause poor growth in plant hosts. However, such losses may arise because genes for tolerance have been lost in selecting for yield. There was no evidence in the arid regions of South Australia that nematodes inhibited plant growth and where extensive damage did occur (in *Atriplex vesicaria*), lack of water and nutrients appeared to be the probable cause. Pot experiments and direct observations confirmed the view that *T.tobari* , for example, caused little damage to the host plant. The host plant, *Atriplex spongiosa* , was both susceptible and tolerant to the nematode. However, the effect of different populations of the nematode on different host plant species has not been investigated and may affect the hypothesis that native plants are always tolerant and susceptible to native nematodes.

The results from this study confirm that the numbers and composition of the different nematode trophic groups differ between sites and change with time. Such variations reflect differences in vegetation between sites and the changes in the soil flora and fauna, with changes in climate and growth of host plants. Further studies to relate changes in bacterial, fungal and nematode populations with plant growth and soil conditions should throw further light on this aspect.

REFERENCES :

BAILEY N.T.J. (1976) ; **Statistical methods in biology** . Uni Books. Hodder and Stroughton. (9th edition) London, United Kingdom.

BOOMSMA C.D. and LEWIS N.B. (1981) ; **The native forest and woodland vegetation of South Australia .** Woods and Forest Dept. S.A. : Bulletin 25. South Australian Government Printers, Adelaide, South Australia.

BRIDGE J. (1971) ; **Ecology and biology of nematodes associated with grass** . Ph.D. Thesis, University of London.

BRIDGE J. (1974) ; **Hatching of *Tylenchorhynchus maximus* and *Merlinius icarus*** . Journal of Nematology 6 : 101-102.

BURBRIDGE N.T. (1945) ; **Germination studies of Australian Chenopodiaceae with special reference to the conditions necessary for regeneration. 1 *Atriplex vesicarium* Heward** . Transactions of the Royal Society of South Australia 69 : 73-84.

BURBRIDGE N.T. (1946) ; **Germination studies of Australian Chenopodiaceae with special reference to the conditions necessary for regeneration. 2 a) *Kochia sedifolia* F v M, b) *K.pyramidata* Benth, c) *K.georges* Diels** . Transactions of the Royal Society of South Australia 70 : 110-119.

BRZESKI M.W. (1971) ; **Nematodes associated with cabbage in Poland : Experiment with *Tylenchorhynchus dubius* Butschi** . Zesz. probl. Postep. Nauk roln No. 121 : 107-111 Reference found in Helminthology Abstracts. : Series B : Vol. 41 : No. 350.

CARROLL J.J. and VIGLIERCHIO D.R. (1981) ; **On the transport of nematodes by the wind** . Journal of Nematology 13 : 476-483.

COATES-BECKFORD P.L. (1982) ; **Developmental biology and feeding behaviour of *Tylenchorhynchus agri* on two hosts, *Trifolium repens* and *Poa pratensis*** . Nematropica 12 : 1-5 . reference found in Helminthology Abstracts : Series B : Vol 52 : No. 307.

COLBRAN R.C. (1960) ; **Studies of plant and soil nematodes. 3. *Belonolaimus hastulatus*, *Psilenchus tumidus* and *Hemicycliophora labiata*, three new species from Queensland** . Queensland Journal of Agriculture and Animal Science 17 : 175-185.

COLBRAN R.C. (1970) ; **Studies of plant and soil nematodes. 15. Eleven new species of *Radopholus* Thorne and a new species of *Radopholoides* de Guiran (Nematoda : Tylenchida) from Australia** . Queensland Journal of Agriculture and Animal Science 27 : 437-466.

COOK R. (1974) ; **Nature and inheritance of nematode resistance in cereals** . Journal of Nematotolgy 6 : 165-173.

COOPER A.F. and VAN GUNDY S.D. (1971) ; **Senescence, quiesence and cryptobiosis** . 297-318 in B.M. Zucherman and M.F. Mai (eds) **Plant Parasitic Nematodes** . Vol 2. Academic Press, New York, New York, U.S.A..

DAO F. (1970) ; **Climatic influence on the distribution pattern of plant parasitic and soil inhabiting nematodes** . Mededelingen Landbouwhogeschool Wageningen . Nederland 70 : 2

DAREKAR K.S. and KHAN E. (1982) ; ***Rotylenchus ranapoi*** **sp.n. (Nematoda : Hoplolaimidae) from Maharashtra** . Indian Journal of Nematology 12 : 183-185.

DAUBENMIRE R.F. (1974) ; **Plant and Environment :- A textbook of Autoecology** . John Wiley and Sons Inc., New York, New York, U.S.A.

DEMEURE Y. (1980) ; **Biology of the plant parasitic nematode, *Scutellonema cavenessi*, Sher, 1964 : Anhydrobiosis** . Revue de Nématologie 3 : 283-289.

DEMEURE Y, FRECKMAN D.W. and VAN GUNDY S.D. (1979a) ; ***In vitro*** **response of 4 species of nematodes to desiccation and discussion of this and related phenomena** . Revue de Nématologie 2 : 203-210.

DEMEURE Y., FRECKMAN D.W. and VAN GUNDY S.D. (1979b) ; **Anhydrobiotic coiling of nematodes in soil** . Journal of Nematology 11 : 189-195.

DROPKIN V.H. (1980) ; **Introduction to plant nematology** . John Wiley and Sons . New York, New York, U.S.A..

ELLENBY C. (1975) ; **A simple method of comparing the rate of drying of nematode worms** Journal of Nematology 21 : 115-116.

ELTON C.S. and MILLER R.S. (1954) ; **The ecological survey of animal communities : With a practical system of classifying habitats by structural characters** . Journal of Ecology 42 : 460-496.

FIELDING M.J. (1955) ; ***Tylenchorhynchus martini*, a new nematode species found in sugar cane and rice fields of Louisianna and Texas** . Proceedings of the Helminthological Society of Washington 23 : 47-48.

FERRIS H. (1982) ; **The role of nematodes as primary consumers** . 3-13 in D.W. Freckman (ed)**Nematodes in the Soil Ecosystem** University of Texas Press. Austin, Texas, U.S.A..

FORTUNER R. and LUC M. (1987) ; **A reappraisal of Tylenchina (Nemata) 6. The family Belonolaimidae Whitehead, 1960** . Revue de Nématologie 10 : 183-202

FRECKMAN D.W. and CASWELL F.P. (1985) ; **The ecology of nematodes in agroecosystems** . Annual Revue of Phytopathology 23 : 275-296.

FRECKMAN D.W., KAPLAN D.T. AND VAN GUNDY S.D. (1977) ; **A comparison of techniques for extraction and study of anhydrobiotic nematodes from dry soils** . Journal of Nematology 9 : 176-181.

FRECKMAN D.W. and MANKAU R. (1977) ; **Distribution and trophic structure of nematodes in desert soils** . Ecology Bulletin (Stockholm) 25 : 511-514.

FRECKMAN D.W. AND MANKAU R. (1986) ; **Abundance, distribution, biomass and energetics of soil nematodes in a Northern Mojave Desert [Nevada, U.S.A.] ecosystem.** Pedobiologia 29 : 129-142.

FRECKMAN D.W. MANKAU R. AND FERRIS H. (1975) ; **Nematode community structure in desert soils : nematode recovery** . Journal of Nematology 7 : 343-346.

GOODELL P.B. (1982) ; **Soil sampling and processing for detection and quantification of nematode populations for ecological studies** . 178-198 in D.W. Freckman .(ed) **Nematodes in the Soil Ecosystem** . University of Texas Press. Austin, Texas, U.S.A.

GOODELL P. and FERRIS H. (1980) ; **Plant parasitic nematode distribution in an alfalfa field** . Journal of Nematology 12 : 136-141.

GREEN C.D. and VERDIGO J. (1985) ; **Colonisation of *Pisim sativum* and *Phaseolus vulgaris* by *Pratylenchus penetrans* in sterile media** . Nematologica 31 : 452-457.

de GUIRAN G. (1979) ; **Survie des nematodes dans les sols secs et satures d'eau : oeufs et larves de *Meloidogyne incognita*** . Revue de Nématologie 2 : 65-77.

HOOPER D.J. (1978) ; **Structure and clasification of nematodes** . 3-45 in J.F. Southey (ed) **Plant Nematology** . Ministry of Agriculture, Fisheries and Food, Her Majesty's Stationary Office, London England.

HUANG C.S. and CHIANG Y.C. (1975) ; **The influence of temperature on the ability of *Aphelenchoides besseyi* to survive dehydration** . Nematologica 21 : 351-357.

HUANG C.S. and HUANG S.P. (1974) ; **Dehydration and the survival of rice white tip nematode, *Aphelenchoides besseyi*** . Nematologica 20 : 9-18.

JAIRAJPURI M.S. and HUNT D.J. (1984) ; **The taxonomy of Tylenchorhynchinae (Nematoda : Tylenchida) with longitudinal lines and ridges** . Systematic Parasitology 6 : 261-268.

JENKINS W.R. and TAYLOR D.P. (1967) ; **Plant Nematology** . Reinhold Publishing Corporation, New York, New York, U.S.A..

JOHNSON A. and BOOTH C. (1983) ; **Plant pathologists pocketbook** . Commonwealth Mycological Institute, Commonwealth Agricultural Bureau, Slough, England.

KHAIR G.T. (1986) ; **List of plant parasitic nematodes of Australia** . Department of Primary Industries (3rd edition). Australian Publishing Service, Canberra, Australia.

KHAN R.M., KHAN A.M. and KHAN M.W. (1986) ; **Interaction between *Meloidogyne javanica, Rotylenchulus reniformis* and *Tylenchorhynchus brassicae* on tomato** . Revue de Nématologie 9 : 245-250.

KHEIR A.M. SHOHLA G.S. AND ELGINDI D.M. (1977a) ; **Population behaviour of *Tylenchorhynchus clarus* infecting Egyptian cotton, *Gossypium barbadense*** . Zeitshrift fur Pflanzenkrankheiten und Pflanzenschutz 84 : 663-665.

KHEIR A.M., EL-SHERIF M.A. and ELGINDI D.M. (1977b) ; **Inoculation effects of *Tylenchorhynchus clarus* on cotton (*Gossypium barbadense*)** . Zeitshrift fur Pflanzenkrankheiten und Pflanzenschutz 84 : 666-670.

KRUSBERG L.R. (1959) ; **Investigation of the life cycle, reproduction, feeding habits and host range of *Tylenchorhynchus claytoni* Steiner** . Nematologica 4 : 187-197.

LaMONDIA J.A. and BRODIE B.B. (1986) ; **Effects of initial nematode density on population dynamics of *Globodera rostochiensis* on resistant and susceptible potatoes** . Journal of Nematology 18 : 159-164.

LASERON C.F. (1969) ; **Ancient Australia** . revised by R.V. Brunnschweiler. Angus and Robertson, Sydney, Australia.

LAUT P., HEYLIGERS P.C., KEIG G., LOFFLER E., MARGULES C., SCOTT R.M. and SULLIVAN M.E. (1977a) ; **Environments of South Australia : Province 5 : Eastern Pastoral**.Division of Land Use, CSIRO., Relim Technical Publication Pty., Ltd., Canberra, Australia.

LAUT P., HEYLIGERS P.C., KEIG G., LOFFLER E., MARGULES C., SCOTT R.M. and SULLIVAN M.E. (1977b) ; **Environments of South Australia : Province 6 : Flinders Ranges**.Division of Land Use, CSIRO., Relim Technical Publication Pty., Ltd., Canberra, Australia.

LAUT P., HEYLIGERS P.C., KEIG G., LOFFLER E., MARGULES C., SCOTT R.M. and SULLIVAN M.E. (1977c) ; **Environments of South Australia : Province 7 : Western Pastoral**.Division of Land Use, CSIRO., Relim Technical Publication Pty., Ltd., Canberra, Australia.

LAUT P., HEYLIGERS P.C., KEIG G., LOFFLER E., MARGULES C., SCOTT R.M. and SULLIVAN M.E. (1977d) ; **Environments of South Australia : Province 8 : Northern Arid**.Division of Land Use, CSIRO., Relim Technical Publication Pty., Ltd., Canberra, Australia.

LUC M., MAGGENTI A.R., FORTUNER R., RASLILI D.J.and GERAERT E.(1987) ; **A reappraisal of the Tylenchina (Nemata) 1. For a new approach to the taxonomy of Tylenchina** . Revue de Nématologie 10 : 127-134.

McCLEARY J.A. (1968) ; **The biology of desert plants** . 141 in G.W. Brown (ed) **'Desert Biology : special topic on the physical and biological aspects of arid regions'** . Academic Press, New York, New York, U.S.A..

McGINNIES W.G., GOLDMAN B.J. and PAYLORE P. (1968) ; **Deserts of the World : An appraisal of research into the physical and biological environment** . University of Arizona Press, Arizona, U.S.A..

MABBUTT J.A. (1971) ; **The Australian arid zone as a prehistoric environment** . 66-79 in D.J. Mulvaney and J. Golson (eds) **'Aboriginal Land and Environment in Australia'** Australian National University Press, Canberra, Australia.

MACKAY W.R., SILVA S., LIGHTFOOT D.C., PAGANI M.I. and WHITFORD W.G. (1986) ; **Effect of increased soil moisture and reduced soil temperatures on a desert soil arthropod community** . American Midland Naturalist 116 : 45-56.

MULK M.M. and SIDDIQI M.R. (1982) ; **Three new species of Hoplolaimid nematodes from South America** . Indian Journal of Nematology 12 : 124-131.

NAND S., SESHADRI A.R. and DASGUPTA D.P. (1982) : **Investigations of the life cycles of *Tylenchorhynchus vulgaris*, *Pratylenchus thornei* and *Hoplolaimus indicus*, individually and in combined infestations in maize** . Indian Journal of Nematology 12 : 272-276.

NELSON R.R. (1956) ; **Resistance to the stunt nematode in corn** . Plant Disease Reporter 40 : 635-639.

NORTON D.C. (1978) ; **Ecology of plant parasitic nematodes** . John Wiley and Sons Inc., New York, New York, U.S.A..

NORTON D.C. and OARD M. (1981) ; **Plant parasitic nematodes in loess toposequences planted with Corn** . Journal of Nematology 10 : 171-176.

NORTON D.C. and SCHMITT D.P. (1978) ; **Community analysis of plant parasitic nematodes in the Kalsow Prairie, Iowa** . Journal of Nematology 10 : 171-176.

PARKER L.W., SANTOS P.F., PHILIPS J. and WHITFORD W.G. (1984) ; **Carbon and nitrogen dynamics during the decomposition of litter and root of a Chihuahuan desert annual, *Lepidium lasiocarpum*** . Ecological Monographs 54 : 339-360.

PHILLIPS S.P. (1971) ; **Studies of plant and soil nematodes. 16. Eight new species of spiral nematodes (Nematoda : Tylenchoidea) from Queensland** . Queensland Journal of Agriculture and Animal Science 28 : 227-242.

REAY F. and WALLACE H.R. (1981a) ; **Plant nematodes associated with native vegetation in South Australia** . Nematologica 27 : 319-329.

REAY F. and WALLACE H.R. (1981b) ; **The Response of some Australian native plants to *Meloidogyne javanica*** . Nematologica 27 : 449-457.

ROESSNER J. and PERRY R.N. (1975) ; **Water loss and associated changes after desiccation in *Rotylenchus robustus*** . Nematologica 21 : 438-442.

SAFED M. and ROESSNER (1984) ; **Anhydrobiosis of plant associated nematodes** . Journal of Nematology 16 : 119-124.

SAUER M.R. (1965) ; ***Morulaimus*, a new genus of the Belonolaiminae** . Nematologica 11 : 609-618.

SAUER M.R. and ANNELLS C.M. (1981) ; **Three new Tylenchs (Nematoda) from Australia** . Nematologica 27 : 422-431.

SCHINDLER A.F. (1961) ; **A simple substitute for a Baermann funnel** . Plant Disease Reporter 45 : 747-748.

SHANKS R.E. (1954) ; **Plotless sampling trials in Appalachian forest types** . Ecology 35 : 237-244.

SHER S.A. (1963) : **Revision of the Hoplolaiminae (Nematoda). 111. *Scutellonema* Andrassay, 1958** . Nematologica 9 : 421-443.

SIDDIQI M.R. (1963) ; **On the identity of *Belonolaimus hastulatus* Colbran, 1960, and *Tylenchorhynchus indicus* Siddiqi, 1961 (Nematoda : Tylenchida)** . Current Science, Bangalore 32 : 550-551.

SIDDIQI M.R. (1986) ; **TYLENCHIDA : Parasites of plants and insects** . Commonwealth Agriculture Bureaux, Slough, England.

SIMONS W.R. (1973) ; **Nematode survival in relation to soil moisture** . Mededelingen Landbouwhogeschool Wageningen - Nederland 73 : 3

SOUTHEY J.F. (1986) ; **Laboratory methods for work with plant and soil nematodes** . Ministry of Agriculture, Fisheries and Food, Her Majesty's Stationary Office, London, England. Killing, Fixing and Processing 63 - 86,

SPECHT R.L. (1972) ; **The vegetation of South Australia** . S.A. Govt. Printers, Adelaide, South Australia

STIRLING G.R. and VAWDREY L.L. (1985) ; **Distribution of a needle nematode *Paralongidorus australis* in rice fields and areas of natural vegetation in North Queensland .** Australasian Plant Pathology 14 : 71-72

STOWE L.G. and WADE M.J. (1979) ; **The detection of small-scale patterns in vegetation** . Journal of Ecology 67 : 1047-1064.

TARJAN A.C. (1973) ; **A rapid lactophenol-glycerin method for preserving nematodes .** Proceedings of the Helminthological Society of Washington 40 : 296-297.

TOWNSEND J.L. (1984) ; **Anhydrobiosis of *Pratylenchus penetrans*** . Journal of Nematology 16 282-289.

TRUSWELL E.M. and HARRIS W.K. (1982) ; **The Cainozoic palaeobotanical record in arid Australia ; fossil evidence for the origin of an arid-adapted flora** . 67-76 in W.R.Barker and P.J.M. Greenslade (eds) **Evolution of the Flora and Fauna of Arid Australia** . Peacock Publications, Frewville, South Australia, Australia.

VAN GUNDY S.D. (1965) ; **Factors in survival of nematodes** . Annual Revue of Phytopathology 3 : 43-68.

VERMA K.K. and DEVENDRA P. (1980) ; **A simple method to prepare permanent mounts of nematodes** . Indian Journal of Parasitology : Abstract from 3rd National Congress. Parasitology 9 : 24-26.

VIGLIERCHIO D.R. and SCHMITT R.V. (1981) ; **Background atmospheric burden of nematodes in Californian Sacremento Valley** . Nematologica Mediteranean 9 : 111-116.

WALLACE H.R. (1963) ; **The biology of plant parasitic nematodes** . Edward Arnold., London, England.

WALLACE H.R. (1973) ; **Nematode ecology and plant disease** . . Edward Arnold, London, England

WALLACE H.R. (1983) ; **Interactions between nematodes and other factors on plants** . Journal of Nematology 15 : 221-227.

WALLWORK J.A., KAMILI B.W. and WHITFORD W.G. (1984) ; **Life studies of desert litter dwelling microarthropods : a reappraisal based on the reproductive behaviour of cryptostigmatid mites** . South African Journal of Science 80 : 163-169.

WARING R.H. (1983) ; **Estimating forest growth and efficiency in relation to canopy leaf area** . Advances in Ecological Research 13 : 327-354.

WANG L.H. (1971) ; **Embryology and life cycle of *Tylenchorhynchus claytoni* .** Journal of Nematology 3 : 101-107.

WHITFORD W.G., FRECKMAN D.W., ELKINS N.Z., PARKER L.W., PHILLIPS J.and TUCKER S. (1981) ; **Diurnal migration and responses to simulated rainfall in desert soil microarthropods and nematodes** . Soil Biology and Biochemistry 13 : 417-425.

WHIRFORD W.G., FRECKMAN D.W., SANTOS P.F., ELKINS N.Z. and PARKER L.W. (1982) ; **The role of nematodes in decomposition in desert ecosystems** . 98-116 in D.W. Freckman (ed) **Nematodes in the soil ecosystem** . University of Texas Press, Austin, Texas, U.S.A..

WHITFORD W.G. FRECKMAN D.W., PARKER L.W., SCHAEFER D., SANTOS P.F. and STEINBERGER Y.(1983) ; **The contribution of soil fauna to nutrient cycles in desert systems** . 49-59 in P.Lebrun ,Y. Stienberger ,W. Mackay,L.W. Parker,D. Freckman,J.A. Wallwork and D. Weems (Eds) **Proceedings V111 International Colloquium** Soil Zoology .

WHITFORD W.G., STIENBERGER Y., MACKAY W., PARKER L.W., FRECKMAN D., WALLWORK J.A. AND WEEMS D. (1985) ; **Rainfall and decomposition in the Chihuahuan Desert [New Mexico]** . Oecologia (Berl.) 68 : 512-515.

YEATES G.W. (1967) ; **Studies on nematodes from dune sands : Tylenchida** .New Zealand Journal of Science 10 : 280-286.

YEATES G.W. (1968) ; **A simple method of making sieves using nylon gauze and PVC tubing** Nematologica 14 : 456-458.

YEATES G.W. (1971) ; **Feeding types and feeding groups in plant and soil nematodes** . Pedobiologia 11 : 173-179.

YEATES G.W. (1979) ; **Soil nematodes in terrestrial ecosystems** . Journal of Nematology 11 : 213-229.

YEATES G.W. (1984) ; **Variations of pasture nematode populations over thirty-six months in a summer moist silt loam** . Pedobiologia 27 : 207-219.

YEATES G.W. (1986) ; **Stylet and body length as niche dimensions in plant parasitic nematodes** . Zoologische Anzeiger 216 : 327-337 . reference found in Biological Abstracts 83 : 4604.

ZAR J.H. (1984) ; **Biostatistical analysis** . Prentice-Hall Inc. (2nd edition),Englewood Cliffs, New Jersey, U.S.A..

APPENDIX 1 :- PERMANENT WAX MOUNTING METHOD FOR FIXED NEMATODES. (Verma and Devendra, 1980; Siddiqi, 1986).

1) After nematodes specimens have been processed through an alcohol series to pure glycerol, they are ready for mounting.

2) Place small drop of pure, dehydrated glycerol onto the centre of a glass slide.

3) Select nematode specimen, place in the drop of glycerol. Make sure the specimen is on the surface of the glass slide.

4) Place small pieces of Parafin wax (melting point = 56 ^{o}C) around the glycerol drop. Place a clean coverslip ontop of the wax pieces.

5) Heat the slide over a naked flame or hot plate, allow wax to melt and make sure the wax completely encircles the glycreol drop.

6) Observe the position of the specimen and seal the coverslip with glyceel or clear nail varnish.

This method can also be used with lactic-phenol replacing glycerol as the mounting fluid, except care needs to be taken that the nematodes do not shrink. Sheath nematodes may become distorted in the pure glycerol, so care needs to be taken on placing these nematodes into the glycerol drop. This method does not require glass rods to support the nematode specimens, and the specimens are confined to a small space and will not move to the side of the coverslip.

APPENDIX 2 :- ANALYSIS OF THE FREQUENCY OF OCCURRENCE OF *Tylenchorynchus tobari* SAUER AND ANNELLS, 1981 WITHIN THE ARID REGION OF SOUTH AUSTRALIA.

Having classified sites into specific groups of vegetation, landforms, dominant plant species, understorey components and plants sampled, the association between specific groups and the presence of *Tylenchorhynchus tobari* could be analysed. Chi-squared analysis was used to test the hypothesis that there was no association between classification and the occurrence of *T.tobari*. The observed frequency is compared with an expected frequency, calculated from the total number of sites sampled within specific groups. This expected frequency specifies the frequency with which observations should fall into certain classification groups (Bailey, 1976; Zar, 1984). For example, the Low shrubland vegetation type has the observed number of sites from which *T.tobari* was collected being 58. The expected frequency, 47.5, was calculated from the % of total sites sampled within the Low Shrubland vegetation group and adjusted to fit the total number of sites from which *T.tobari* was identified. The Chi-square value was calculated from the formula (O-E)2/E. For the vegetation types, the sum of all the chi-square values was 4.02 and was not significant at k-1 (5) degrees of freedom. The Null Hypothesis, used to calculate the expected frequencies, was retained, and there was no association between specific vegetation types and the occurrence of *T.tobari.*

NULL HYPOTHESIS : The Observed number of sites with *T.tobari* present within specific types of vegetation, landforms, dominant plant species, understorey components and plant species sampled is equal to the Expected frequency, calculated from the % of total sites of each type sampled.

VEGETATION:-

Type	Number of sites Observed	Expected	Chi-square value
Low Shrubland	58	47.5	2.37
Shrubland	61	71.3	1.48
Woodland	88	87.2	0.01
Grassland	2	2.6	0.14
Tall Shrubland	24	24.6	0.01
Herbland	4	3.8	0.02
Total	237	237.0	4.02 ns

(ns=not significantly differen, P=0.05, df=5, Chi-square analysis)
(Woodland includes Low Woodland and Woodland types)
Null Hypothesis retained.

LANDFORM:-

Type	Number of sites Observed	Expected	Chi-square value
Rise	27	22.0	1.12
Dune	49	52.6	0.25
Plain	85	68.3	4.10
Playa Lake & Surrounds	23	28.0	0.88
Water-course	39	50.0	2.42
Plateaux	8	9.0	0.11
Floodplain	6	7.1	0.17
Total	237	237.0	9.05 ns

(ns=not significant, Chi-square analysis, df=6, P=0.05)
Null Hypothesis retained.

APPENDIX 2 Cont.

DOMINANT PLANT SPECIES:-

Species/Groups	Number of Sites Observed	Expected	Chi-square value
Chenopods	58	45.5	3.43
Acacia spp.	78	75.1	0.11
Melaleuca spp.	6	5.7	0.01
Eucalyptus spp.	44	54.9	2.16
Ephemerals	3	3.3	0.03
Grasses	3	3.3	0.03
Zygocloa paradoxa	8	10.8	0.72
Salicornia spp.	0	1.9	1.90
Casuarina cristata	18	13.9	1.21
Trees	3	4.9	0.55
Shrubs	15	18.0	0.50
Total	237	237.0	10.65 ns

(ns=not significant, df=10, P=0.05, Chi-square analysis)
Null Hypothesis retained

(Chenopods include *Maireana* and *Atriplex* spp.)
(Trees include *Myoporum* , *Pittosporum* and *Callitris* spp.)
(Shrubs include *Dodonea*, *Cassia* and *Eremophila* spp.)
(Grasses include ephemeral and prennial spp., not *Zygocloa paradoxa* or *Triodia* spp.

UNDERSTOREY COMPONENTS:-

Species/Groups	Number of Sites Observed	Expected	Chi-square value
Chenopods	90	73.9	3.51
Grasses	14	22.8	3.39
Ephemerals	89	91.7	0.08
Salicornia spp.	4	7.7	1.78
Shrubs	14	13.4	0.03
Acacia spp.	6	5.7	0.02
Zygocloa paradoxa	13	12.2	0.05
Reeds	3	4.5	0.50
Triodia	3	5.0	0.80
Total	237	237.0	10.80 ns

(ns=not significant, df=8, P=0.05, Chi-square analysis)
Null Hypothesis retained.

APPENDIX 2 Cont :-

HOST PLANT SAMPLED:-

Species/Groups	Number of Sites Observed	Expected	Chi-square value
Chenopods	140	108.5	9.08 ##
Ephemerals	14	14.6	0.02
Eucalyptus spp.	27	36.6	2.52
Acacia spp.	71	76.8	0.43
Grasses	9	15.6	2.53
Shrubs	16	25.6	3.60
Trees	24	18.8	1.44
Salicornia spp.	8	7.8	0.01
Reeds	0	3.2	3.20
Zygocloa paradoxa	15	16.8	0.19
Total	324	324.0	23.02 **

(**=significant, df=9, P=0.01, Chi-square Analysis)
(Trees include *Myoporum* , *Pittosporum* and *Callitris* spp.)
(Shrubs include *Dodonea*, *Cassia* and *Eremophila* spp.)
(##=signficant, df=1, P=0.01, Chi-square analysis)
Null Hypothesis rejected.

APPENDIX 3 :- DILUTION PLATE TECHNIQUE FOR COUNTING NUMBER OF FUNGAL PROPAGULES IN SOIL :

(Reay F. pers. comms).

1) Weigh 10g of soil from each sample.

2) Add to each of 5 bottles containing 90 ml of sterile distilled water (dilution=1/10). Shake bottle vigorously for 1 minute, stand for 1 minute.

3) Transfer 10 ml from first dilution to 90ml of sterile distilled water using a pipette (sterile). Mix by blowing air through pipette (dilution =1/100).

4) Add 1 ml from each of the five bottles, to each of 2 petri-plates (2 reps/plate for each sample).

5) Add molten selective media (NDY/6 + Vancomycin and Streptomycin) to each plate and rotate to distribute soil evenly.

6) When agar has set, incubate at 25 ^{o}C.

7) After 3 days, count mean number of fungal colonies for each soil sample, and calculate the total number of fungal propagules in the soil.

APPENDIX 4 :- ASSESSMENT OF DISEASE SEVERITY WITHIN A LOW SHRUBLAND DOMINATED BY *Atriplex vesicaria.*

The growth of individual plants is influenced by external factors such as soil moisture, availability of nutrients and the activety of organisms within the soil (Wallace, 1983). Disease is therefore a reflection of the adverse effect of one or more of the above external factors. In the field, disease can be assessed using two different scoring methods methods (Johnson and Booth, 1983). These scoring methods use either 1) a descriptive type of key, which describes the different levels of disease and asigns a category, index, grade or percentage infection to each description or 2) a standard area diagram, in which the areas of disease on the infected part of the plant is described, again using a category, index, grade or percentage infection. The use of a percentage scale is the most favoured as it is universally known, has upper and lower limits, can be conveniently divided and scaled and can easily be transformed for any subsequent epidemiological analysis. A system of scoring disease severity in an infected field of Atriplex vesicaria was devised as % disease severity.

Scale	Description
0	no leaves present on branches of the sampled plant
1	< 25% branches of the sampled plant with leaves
2	25-50% branches of the sampled plant with leaves
3	50-75% branches of the sampled plant with leaves
4	> 75% branches of the sampled plant with leaves

This system enabled the sampled plant to be assessed for disease severity. Assessment of disease was done by eye in the field.

APPENDIX 5 :- GENSTAT PROGRAMME FOR CONTOUR MAPS.

Programme for the plotting of contour maps of the distribution of plant species and nematode trophic groups within a 25 metre square grid, encompassing different vegetation types. This programmes was originally designed by T.Hancock, in the Biometry Department of the Waite Institute, Adelaide and modified by J.Davidson and myself to fit the data.

```
$ Set Def [JNOBBS]
$ GEN SYS$INPUT,OUT=CON.RES
'REFE' GRID
"THIS PROGRAMME IS DESIGNED TO PLOT THE DISTRIBUTION OF VEGETATION AND NEMATODES
WITHIN A 25 METRE SQUARE GRID, DIVIDED INTO 5 METRE SQUARE QUADRANTS AND FOR 8
VARIABLES
    SUB1=variable 1, SUB2=variable 2, SUB3=variable 3, SUB4=variable 4,     SUB5=variable
5, SUB6=variable 6, SUB7=variable 7, SUB8=variable 8 "
'UNITS' $ 200
'MATRIX' X $ 40,5:SUB1,SUB2,SUB3,SUB4,SUB5,SUB6,SUB7,SUB8$5,5
'READ' X $ S ,X,8,/
'EQUATE'SUN1=X$(1,7X)25:SUB2=X(X,1,6X)25:SUB3=X(2X,1,5X)25:SUB4=X(3X,1,4X)25:
SUB5=X(4X,1,3X)25:SUB6=X(5X,1,2X)25:SUB7=X(6X,1,X)25:SUB8=X(7X,1)25:
'DEVALUE' X
'PRINT/S'SUB1,SUB2,SUB3,SUB4,SUB5,SUB6,SUB7,SUB8$25(5.0)
'VARI'X1$4=1,5,1,5:X2$4=1,5,1,5:X3$4=1,5,1,5:X4$4=1,5,1,5:X5$4=1,5,1,5:X6$4=1,5
,1,5:   X7$4=1,5,1,5:X8$4=1,5,1,5
'CONT/CI=1,BV=X1'SUB1
'CONT/CI=1,BV=X2'SUB2
'CONT/CI=1,BV=X3'SUB3
'CONT/CI=1,BV=X4'SUB4
'CONT/CI=1,BV=X5'SUB5
'CONT/CI=1,BV=X6'SUB6
'CONT/CI=1,BV=X7'SUB7
'CONT/CI=1,BV=X8'SUB8
'RUN'
```

The phrase CONT/CI=1 generates the contour intervals and can be adjusted to fit the data (i.e. if the majority of the values are greater than 5, then a contour interval can be adjusted by a factor of 5 (CONT/CI=5), to produce a simpler map of the distribution of the variable).

APPENDIX 6 :- SLIDE LIST AND DESCRIPTION OF SITES OF NEW SPECIES OF PLANT PARASITIC NEMATODES.

Holotype and Allotype specimens are lodged at the Waite Institute Nematode Collection, to be moved to the Adelaide Museum, when space is available. The remaining paratypes not sent to outside collections and specimens of named nematode species are to be kept at the Waite Institute under a numbering system prefixed by the letters JNO.

Slide Number	Description.
WINC 333	*Tylenchorhynchus siccus* :- Collected 17/8/85. Map Sheet TORRENS SH53-16 ; 686 495 Sampled taken from *Acacia papyrocarpa* and *Maireana sedifolia*. Site located on Oakden Hill Station
WINC 334	*Dolichorhynchus (Neodolichorhynchus) sedecimstriatus* :- Collected 12/7/83. Map Sheet GAIRDNER SH53-16 ; 534 656 Samples were taken from *Acacia papyrocarpa* and *Atriplex vesicaria*. Site was on Kokatha Station.
WINC 335	*Morulaimus simpsonii* :- Collected 17/5/85. Map Sheet DALHOUSIE SG53-11 ; 411 735 Samples were taken from *Zygocloa paradoxa*. Site was located in the Simpson Desert, near Purni Bore.
WINC 336	*Rotylenchus wallacei* :- Collected 23/5/84 Map Sheet KOPPERAMANA SH54-1 ; 154 462 Samples were taken from *Zygocloa paradoxa*. Site was located north of Copper Creek Crossing on the Birdsville Track.

Map references refer to the 1:250,000 series, from the Royal Australian Survey Corps.

APPENDIX 7 :- COMPOSITION OF POTTING MIX SOIL USED IN CHAPTER 5.

(University of California Mix (U.C.)).

1/4 cubic metre of coarse sand and 1/4 cubic metre of "Detorf" peatmoss (Sphagum moss peat) are mechanically mixed with the following nutrients :-

Potassium Nitrate	60 gm
Magnesite	120 gm
Reverted Super	700 gm
Plaster of Paris	460 gm
Potassium Sulphate	60 gm
Blood Meal	700 gm
Hydrated Lime	900 gm.

The mix is sterilized at 75 ^{o}C for 45 minutes using aerated steam.

ERRATA :-

PLATE 11 : '25% of the branches with leaves'

not '25% of the stems with leaves'