The biology of the plant parasitic nematodes *Paratylenchus nanus* and *Paratrichodorus minor* in soil under pasture

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ERRATA

Line numbers are counted so as to include full and part lines of text, section headings, Table headings and contents and Figure legends. For changes, additions appear in italics, deletions as strikethroughs and comments are not enclosed in quotation marks.

Page	Line	Change
2	10	"Mono*n*chida"
12	4	"by sheep *and* by cattle"
12	10	"Haplo~~u~~dand"
12	14	"Haplohum*u*lt"
19	17	Replace "Decreamer" with "Decraemer"
22	18	Replace "Decreamer" with "Decraemer"
23	5	Replace "Decreamer" with "Decraemer"
23	13	Replace "*P. minor*" with "*Paratrichodorus minor*"
24	28	"The*re* was no significant"
35	3	"explanation of ~~other~~ symbols"
39	20	"which result in change*s*"
42	12	"Haplo~~u~~dand"
43	26	"(except %~~T~~N)"
59	28	Italicise "Metatetranychus ulmi" and "Aporroectodea caliginosa"
61	1	Italicise "P. nanus"
63	28	Transpose Boag & Alphey (1988) with Blakemore *et al.* (1987)
81	11	Replace "*P. nanus*" with "*Paratylenchus nanus*"
83	17	Replace "*Rotylenchus fallorobustus"* with "*Rotylenchus robustus*"
83	20	"(Yeates, 1973a)"
86	18	Replace "*P. nanus*" with "*Paratylenchus nanus*"
86	32	Replace "*Rotylenchus robustus*" with "*Rotylenchus uniformis*"
91	15	"Revue *de* Nématologie"
96	19	"Haplo~~u~~dand"
98	14	"shoot base of *the* plant"
118	1	"Haplo~~u~~dand"
136	31	Replace "New Zealands" with "New Zealand's"
142	Fig. 2.	Bar legends should appear as in Fig. 4. (page 144)
153	25	Replace "*Rotylenchus robustus*" with "*Rotylenchus uniformis*"

MASSEY UNIVERSITY
APPLICATION FOR APPROVAL OF REQUEST TO EMBARGO A THESIS
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Name of Candidate: Nigel Bell ID Number: 08505683

Degree: PhD Dept/Institute/School: Institute of Natural Resources

Thesis Title: The biology of the plant parasitic nematodes *Paratylenchus nanus and Paratrichodorus minor* from soil under pasture.

Name of Chief Supervisor: Prof Ken Milne Telephone Extn: 5100

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Abstract

The plant parasitic nematodes *Paratylenchus nanus* and *Paratrichodorus minor* were identified from soils under grazed pasture in the Waikato region of New Zealand. Host range testing showed that all hosts of *P. nanus* were grasses, while *P. minor* hosts included both grasses and clovers.

Several variations to the Whitehead and Hemming tray extraction method were compared. The optimum variant was found to yield *ca* 75% of the total nematode fauna.

Sampling of *P. nanus* populations from 0–10 cm and 10–20 cm soil depth showed that the abundance of *P. nanus* peaked in summer. A Population Age Index, based on developmental stages, showed *P. nanus* population age increased from a minimum in spring to maximum in winter. Positive correlations occurred with soil temperature and negative correlations with soil moisture and rainfall. Accumulated temperature and rainfall (Activity Index) was correlated with *P. nanus* abundance. Evidence is presented for density-dependence in the *P. nanus* population at 0–10 cm depth. Multiple regression models were fitted and results are discussed in terms of population dynamics.

Seedlings of five grasses were inoculated with one of three rates of *P. nanus*. There was a deleterious effect of the high rate of *P. nanus* inoculum on shoot dry matter only for *Lolium perenne* infected with a selected *Neotyphodium* sp. endophytic fungus (AR37+). Sampling of soil beneath grazed pasture determined the relationship of *P. nanus* populations with mature *L. perenne* plants. For all samplings, AR37+ supported a consistently greater abundance of *P. nanus* than other plants. Dry matter production and root mass data suggest that greater root production by AR37+ was partly responsible for the greater abundance of *P. nanus* beneath these plants. Implications for field sowing of AR37+ in the presence of *P. nanus* populations are discussed.

Sampling in soil from a second grazed pasture which contained populations of both *P. minor* and *P. nanus* showed the *P. minor* population had no seasonal periodicity while *P. nanus* had distinct spring and summer peaks. *P. minor* abundance was correlated with rainfall and Activity Index. There was no evidence for competition occurring between these two nematodes at the population levels studied.

Preface

This thesis is written as a series of papers, which follow the format of the international journal *Nematology* (Koninklijke Brill NV, Leiden). Therefore, each chapter contains Summary, Keywords, Introduction, Materials and Methods, Results, Discussion and References. The General Introduction and General Discussion chapters are additional to this format.

Acknowledgements

I thank my supervisors, Mr Richard Watson (AgResearch, Hamilton), Dr Gregor Yeates (Landcare Research, Palmerston North) and Prof. Ken Milne (Massey University, Palmerston North) for their valuable contributions to all aspects of the preparation of this thesis. Richard was the 'man on the spot' who fielded practical and theoretical questions and encouraged me that what I was doing was worthwhile. He also supported my family and I in times of hardship and for this we are very grateful. Gregor has taught me much about nematodes and their ways – an education that has been valuable not only for this thesis but also for my future career. Ken has made my path through the PhD process so much smoother than it would otherwise have been and has worked hard to ensure that everything was 'on track'. Both Ken and Gregor have visited me at least twice each year and all three of my supervisors have been open and approachable throughout the course of this study.

Dr Neil Cox (AgResearch, Hamilton) answered many questions on statistical matters in a way that even I could understand. For this and his help with analyses I thank him very much. Thanks also to Frank Neville (deceased) who helped with some coring expeditions and with set-up of the damage experiment. The cheerful help and advice I received from Ken Jones (farm manager, Tokanui Research Station) was vital to site selection and maintenance and is very much appreciated. For help and advice on soil matters I thank Dr Peter Singleton (AgResearch, Hamilton). As a friend and colleague Sergio Marshall (AgResearch, Hamilton) provided expertise in endophyte detection along with his offbeat sense of humour.

I thank my wife Angela for her support and motivation and for being such a great mother to our son Jonty (born 3 November 1998, the most scary, amazing and ultimately wonderful day of my life).

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Chapter 1: General Introduction

NEMATODES

Nematodes are unsegmented, unpigmented worms that are usually circular in cross section. Their body is mainly bilaterally symmetrical except for the head and oesophageal /pharyngeal regions, which typically show tri-radiate symmetry. Adults range in length from 8.5 m (*Placentonema gigantissium*) to less than the (theoretically) lower functional size limit of 0.3 mm (Roggen, 1970) (e.g. *Desmoscolex aquaedulcis*; female length 0.26 mm), although most are in the range 0.5–1.5 mm. There are *ca* 15,000 currently described nematode species but the total number of species is estimated at 500,000 (Poinar, 1983).

Nematodes are the most numerous multicellular animals on Earth and occupy almost every conceivable habitat. They generally have four developmental stages before maturity. Developmental stages are separated by moulting of the cuticle. Some workers (Bird & Bird, 1991) term the sub-adult stages of nematodes larvae as for the holometabolous insect orders (e.g. Coleoptera and Lepidoptera). However, as nematode development lacks a metamorphosis the sub-adults are termed juveniles, as is in the mainstream of plant nematology. Successive stages are denoted J1, J2, J3, J4, female and male.

The phylum Nematoda has traditionally been divided into two classes and 19 orders (Table 1). This classification is on a morphological basis and may require dramatic alteration in the view of molecular studies such as that of Blaxter *et al.* (1998). Although aquatic, marine and terrestrial forms are listed in Table 1, strictly all nematodes are aquatic. Nematodes are dependent on free water for their movement (Jones *et al.*, 1969), feeding and reproduction (Baujard & Martiny, 1994). Terrestrial forms live in the water film between soil particles. Orders listed as aquatic in Table 1, then, are taken to be those whose habitats include bodies of freshwater (e.g. lakes, rivers and ponds).

As a group, nematodes labeled microbivores in Table 1 feed on the entire range of microbial organisms. For example *Plectus* and *Caenorhabditis* are bacterial feeders, *Tylencholaimus* feed on fungi, *Achromadora* algae, and *Diplogaster* protozoa (see

Yeates *et al.*, 1993). Within orders genera may utilise different types of microbes as a food source e.g. for Enoplida *Alaimus* feed on bacteria and *Tobrilus* on algae.

Table 1. *Classes and orders of the phylum Nematoda (after Maggenti, 1991, and Decraemer, 1995), the habitat, representative genera and their feeding types within each order. A = aquatic, M = marine and T = terrestrial.*

Class	Order	Habitat	Representative genera	Feeding type
Adenophorea	Dorylaimida	T	*Longidorus*	Plant parasite
		T	*Tylencholaimus*	Microbivore
	Triplonchida	T	*Paratrichodorus*	Plant parasite
	Monochida	T	*Mononchus*	Predator
	Trypylida	A, T	*Ironus*	Predator
	Stichosomida	T	*Mermis*	Invertebrate parasite
	Araeolaimida	A, M, T	*Plectus*	Microbivore
	Chromadorida	A, M, T	*Achromadora*	Microbivore
	Desmoscolecida	M, T	*Desmoscolex*	Microbivore
	Enoplida	A, M, T	*Alaimus*	Microbivore
	Monhysterida	A, M, T	*Monhystera*	Microbivore
	Oncholaimida	A, T	*Onchulus*	Microbivore
	Desmodorida	M	*Desmodora*	Microbivore
Secernentea	Tylenchida	T	*Paratylenchus*	Plant parasite
		T	*Heterodera*	Plant parasite
		T	*Meloidogyne*	Plant parasite
		T	*Pratylenchus*	Plant parasite
	Rhabditida	T	*Heterorhabditis*	Invertebrate parasite
		T	*Caenorhabditis*	Microbivore
	Diplogasterida	T	*Diplogaster*	Microbivore
	Ascaridida	T	*Ascaris*	Vertebrate parasite
	Spirurida	T	*Dracunculus*	Vertebrate parasite
	Strongylida	T	*Ostertagia*	Vertebrate parasite

The first record of a plant parasitic nematode was from 'smutty wheat' by Needham in 1743 (Thorne, 1961). He observed the anhydrobiotic stage of the wheat seed gall nematode *Anguina tritici*. It has been suggested (Thorne, 1961) that the damage and subsequent germination failure symptoms associated with this nematode were recorded by Shakespeare in "Love's labour's lost" as "sowed cockle, reap'd no corn". A review of the history of plant nematology is given by Barker (1998).

The feeding apparatus of plant parasitic nematodes is termed stomatostyle (Tylenchida), odontostyle (Dorylaimida) or onchiostyle (Triplonchida), stylet being used as a general term and the prefixes referring to its embryological origin. The stomatostyle and odontostyle are similar in gross morphology and function. Both consist of a hollow 'spear' the lumen of which is continuous with the oesophagus. The stomatostyle or odontostyle is thrust into plant cells by means of protractor muscles attached to the base (stylet knobs or flanges of the odontophore) of the feeding apparatus. Plant cell contents are drawn through the lumen of the spear and ingested via an oesophago-intestinal valve by the action of a muscular bulb.

The triplonchid onchiostyle differs in both morphology and function from the other two forms. It has a solid tip, which requires a different ingestion process. The onchiostyle is used for piercing plant cells but ingestion typically occurs in conjunction with a feeding tube which is formed by secretions (probably from gland cells within the oesophageal bulb) which rapidly harden and act as a suction tool (Wyss, 1982). Ingestion is accomplished using thrusting action of the onchiostyle to draw the oesophageal wall (which is fused to the onchiostyle) forward at the same time as the oesophageal lumen is dilated near its base. In contrast to the tylenchid and dorylaimid process, onchiostyle thrusting must continue throughout feeding as this action is an integral part of withdrawing plant contents.

Relationships between plant parasitic nematodes and roots take several different forms. All Adenophorean plant parasites are ectoparasitic; i.e. they feed from the root surface and do not normally enter the root tissue. Within the Secernentean plant parasites there are ectoparasites (e.g. *Paratylenchus*), semi-endoparasites i.e. a portion of the body enters the root tissue (e.g. *Helicotylenchus*), migratory endoparasites i.e. nematodes that enter root tissue but have the ability to move out again (e.g.

Pratylenchus), and sedentary endoparasites i.e. those nematodes that enter root tissue and form a permanent attachment to the cortex of the root (e.g. *Meloidogyne* and *Heterodera*). Most studies in plant nematology have concentrated on the sedentary and migratory endoparasites. Work on ectoparasites has largely dealt with the Adenophorean forms, some of which have the ability to transmit viruses (nepoviruses in Dorylaimida and tobraviruses in Triplonchida).

CLIMATE AND SOILS OF NEW ZEALAND

The climate of New Zealand can generally be described as temperate oceanic. Rainfall patterns are dominated by a westerly wind flow. This brings rain to the west coast of both islands, leaving the east coasts relatively dry (Table 2). New Zealand has a north–south gradient in annual air temperature of *ca* 5°C.

Table 2. *Mean annual rainfall (mm), air temperature (°C), and air temperature in January (summer) and July (winter) for eight regions (in approximately north–south order) from both the North and South Island of New Zealand. Data are averaged over 1961–1998 (National Climate Centre, National Institute of Water and Atmospheric Research Ltd, Wellington, New Zealand).*

Island	Climatic region[1]	Rainfall	Temperature		
			Annual	January	July
North	Northern	1322	15.2	19.4	11.1
	Western	1466	13.3	18.0	8.4
	Central	1232	12.8	17.9	7.7
	Eastern	923	14.4	19.3	9.3
	Southern	1012	13.2	17.7	8.5
South	Western	2555	12.2	15.9	8.0
	North Eastern	770	12.5	17.7	6.9
	South Eastern	819	10.2	13.5	4.6

[1]*Population centres which make up each region: (North Island) Northern – Kaitaia, Kerikeri, Dargaville, Auckland, Tauranga; Western – Taumaranui, New Plymouth; Central – Rotorua, Taupo, Hamilton; Eastern – Gisborne, Napier; Southern – Masterton, Palmerston North, Wanganui, Wellington; (South Island) Western – Westport, Hokitika; North Eastern – Nelson, Blenheim, Christchurch; South Eastern – Lake Tekapo, Timaru, Dunedin, Queenstown, Gore, Invercargill.*

There are *ca* 90 identified soils in New Zealand (Molloy, 1998). The soils of the South Island, southern and northern North Island are sedimentary whereas the central North Island soils are pumiceous and those of the Waikato and Taranaki (north central and western North Island) are derived from volcanic ash. The current soils are based on these broad characters but are differentiated according to the effect climate has had on the parent material. Local variation in rainfall and topography account for some of the differences in soil type observed throughout the country. Temperature controls the rate of chemical reactions so that weathering is more rapid in northern than southern parts of New Zealand.

New Zealand soils are grouped into 16 main classes whose names reflect colour, texture, parent material, climate or topography of the soil. In the current study three soil groups are encountered: Allophanic soil (Otorohanga silt loam – see Chapters 2–7), Granular soil (Hamilton clay loam – see Chapters 2, 3 and 8) and Pumice soil (Ohinepanea sandy soil – see Chapter 7). Distribution of these soils in the North Island is shown in Fig. 1. The soil descriptions that follow are from Molloy (1998).

Allophanic soils contain a large proportion of the clay mineral allophane and are derived from volcanic ash. Allophane has very high retention capacity for phosphorus, sulphur, molybdenum and selenium. The Allophanic soils occur mainly in North Island volcanic ash areas but also in small areas of South Island high country. They have a low bulk density, and the topsoil is stable so that erosion rates are usually low. The volcanic loams, (e.g. Otorohanga silt loam) are a subset of the Allophanic soils and they are characterised by a moderately deep topsoil (15–25 cm), black to brown in colour and a subsoil commonly yellow-brown in colour. They are very friable sandy or silt loams (clay content 10–25%) with high moisture and phosphate retention capabilities.

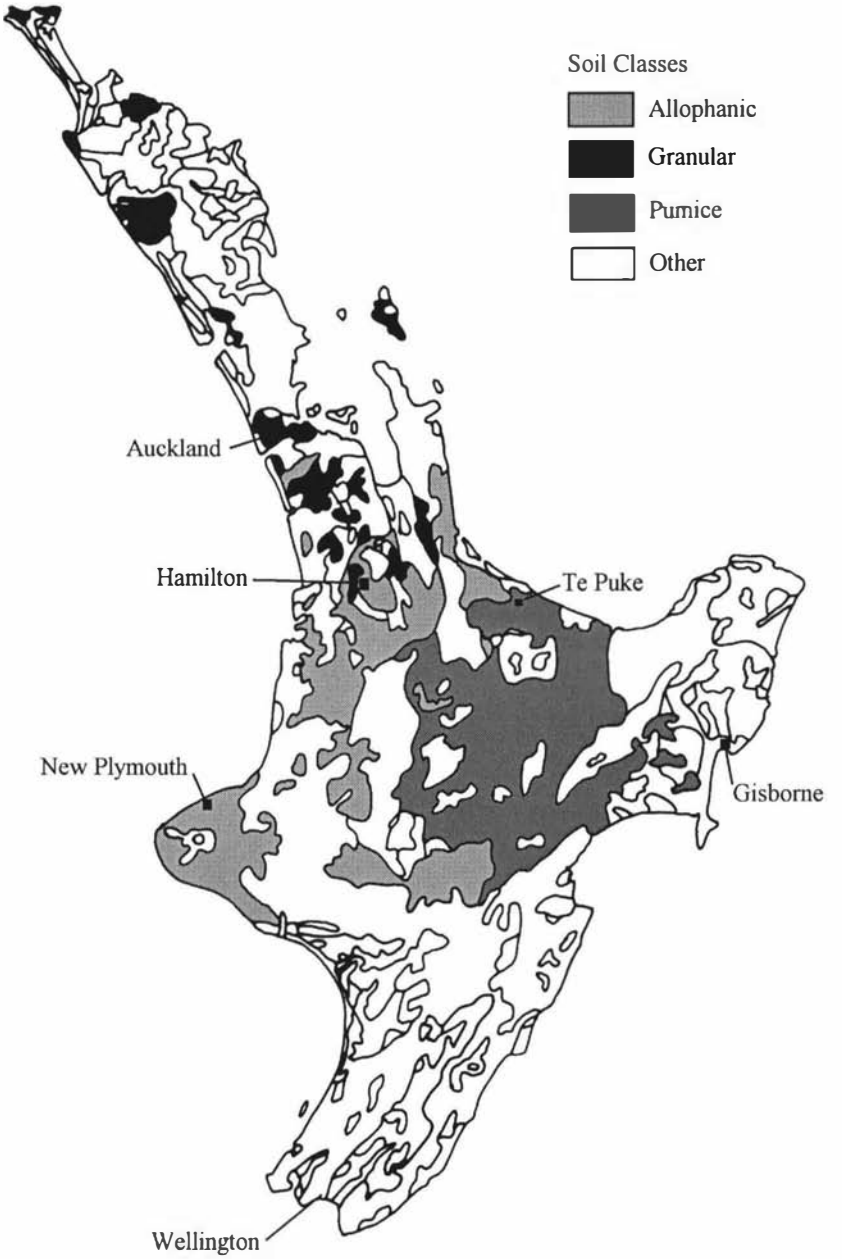

Fig. 1. *Distribution of Allophanic, Granular and Pumice soils in the North Island of New Zealand. Named localities are those that occur in the main body of the thesis along with some which appear in Table 2 (above). Map is after "Soil Map of New Zealand", 1997, Landcare Research, New Zealand Ltd.*

Granular soils are derived from strong weathering of volcanic rocks or ash. They are only slowly water permeable so have a limited plant rooting depth. Because they are strongly weathered, these soils have low nutrient reserves with low phosphorus and sulphate levels in the subsoil. The volcanic loamy clays (e.g. Hamilton clay loam) are granular in texture (clay content 50–80%) and become sticky when wet. They often have a high kaolin clay content that is characterised as having very low and low water and anion retention respectively. These soils are moderately free-draining which rewet easily but plants are prone to moisture stress after even short periods of drought.

Pumice soils have low soil strength, high macroporosity and deep plant rooting depth. They have low nutrient reserves with clay contents usually less than 10%. Pumice soils are generally coarse and plant available moisture is high with good aeration, but are also prone to drought and erosion.

AGRICULTURE AND PLANT PARASITIC NEMATODES IN NEW ZEALAND

The combination of relatively high rainfall and warm temperatures make the northern, western and central areas of the North Island, and western South Island (Table 2) the main dairying areas. Sheep farming and cropping predominate in the drier eastern areas of both islands. Agricultural production in New Zealand is largely based on permanent, perennial pastures sown with introduced grass and legume species. The most commonly sown pasture species are perennial ryegrass (*Lolium perenne* L.), annual ryegrass (*L. multiflorum* L.), cocksfoot (*Dactylis glomerata* L.), white clover (*Trifolium repens* L.) and red clover (*T. pratense* L.). Herbage yield from developed New Zealand pastures is typically in the range 10–20,000 kg dry matter /ha /year.

Twenty-two species of plant parasitic nematodes have been recorded from pastures or pasture plants in New Zealand (Table 3). Some occur only as localised populations e.g. *Longidorus*, *Paratrichodorus* and *Tylenchorynchus* (Yeates, 1975; Sturhan *et al.*, 1997), whereas others are virtually ubiquitous throughout the country e.g. *Heterodera* and *Paratylenchus* (Yeates, 1975; Skipp & Christensen, 1983).

Table 3. *Genera of plant parasitic nematodes in New Zealand associated with pasture plants and the number of species of each genus recorded (see Knight et al., 1997; Sturhan et al., 1997).*

Nematode genera	No. of species	Nematode genera	No. of species
Anguina	1	*Macroposthonia*	1
Aphelenchoides	1	*Meloidogyne*	4
Ditylenchus	1	*Paratrichodorus*	1
Geocenamus	1	*Paratylenchus*	1
Helicotylenchus	4	*Pratylenchus*	2
Heterodera	2	*Subanguina*	1
Longidorus	1	*Tylenchorhynchus*	1

Plant parasitic nematodes have the potential to damage both the grass and clover components in New Zealand pastures. Work carried out by Watson *et al.* (1985), using field nematicide applications, has shown that in most situations the white clover component of pastures is the most affected by nematode infestation with yield and nitrogen fixation increased by *ca* 40 and 50% respectively in the absence of plant parasites. The value of each 1% decrease in nitrogen fixation by white clover has been estimated at NZ$11–48 million when calculated across the country (see Mercer, 1994). Damage to grassland plants due to plant parasitic nematodes in temperate countries is reviewed by Cook & Yeates (1993).

Ectoparasitic nematodes can be the most numerous plant parasitic nematodes in some New Zealand pastures (Yeates & Barker, 1986, Yeates & Prestidge, 1986, Watson *et al.*, 1994, pers. obs.). During trials carried out in the Bay of Plenty district, to determine overall nematode effects on pasture, large populations of both *Paratylenchus* and *Paratrichodorus* were observed. At one site in particular *Paratylenchus* populations in grazed pasture were observed to reach *ca* 3 million /m^2 while *Paratrichodorus* populations reached *ca* 360,000 /m^2 (Watson *et al.* unpub. data).

The effects of *Paratylenchus* and *Paratrichodorus* have been studied in some crops overseas but their effects in pasture, either overseas or in New Zealand, are largely unknown. While both *Paratylenchus* and *Paratrichodorus* have the potential to cause direct feeding damage to pasture plants, *Paratrichodorus* could also potentially be a vector of plant viruses (Brown *et al.*, 1989). The impact of these two nematodes on pasture plants has to be quantified in order to assess the need for them to be incorporated into plant breeding or biological control programmes. Implicit in any investigation of plant damage by a pathogen is the need to specifically identify the organism and have an understanding of its spatial and temporal population dynamics. This thesis investigates these factors for both *Paratylenchus* sp. and *Paratrichodorus* sp. in two soils, under pasture grazed by sheep and cattle, near Hamilton in the Waikato region (central North Island, Table 2) of New Zealand.

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Chapter 2: Morphometric identification and host range assessment of the nematodes *Paratylenchus nanus* (Tylenchida: Tylenchulidae) and *Paratrichodorus minor* (Triplonchida: Trichodoridae)

Summary – Adult and juvenile characters were used to identify *Paratylenchus nanus* Cobb, 1923 and *Paratrichodorus minor* (Colbran, 1956) Siddiqi, 1974 from soils under pasture in New Zealand. The taxonomic status of *P. nanus* is discussed in relation to *Paratylenchus projectus* Jenkins, 1956 and synonymy of the species is accepted in addition to the possible existence of host races. Glasshouse testing of the host ranges of *P. nanus* and *P. minor* nematodes was carried out using 15 commonly occurring pasture plants. All good hosts (P_f : P_i >1.0) of *P. nanus* were grasses, namely *Dactylis glomerata, Lolium multiflorum,* and *L. perenne* (endophyte-infected and -free) all of which are new host records for this species. *P. minor* hosts included those listed for *P. nanus* plus *Festuca arundinacea* (endophyte-free), *Poa annua, Trifolium pratense, T. repens* and *T. subterraneum* were good hosts. With the exception of *T. repens,* these are all new host records for *P. minor* in New Zealand.

Keywords: *Paratylenchus nanus, Paratrichodorus minor*, *Paratylenchus projectus,* host plants, species identification, P_f: P_i ratio.

There is little information from New Zealand on either the identity of *Paratylenchus* Micoletzky or *Paratrichodorus* Siddiqi species present in soils under improved pasture or their pasture plant host ranges. Information on both aspects is critical to understanding the role these nematodes play in agricultural systems.

According to Knight *et al.* (1997) five species of *Paratylenchus* have been identified in New Zealand: *P. halophilus* Wouts, 1966 from the coastal succulent *Sarcocornia quinqueflora*; *P. minutus* Linford, 1949 from beneath oats (*Avena sativa)*; *P. nanus* Cobb, 1923 from barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*); *P. projectus* Jenkins, 1956 from pasture grasses and legumes, tussock (*Festuca novae-zelandiae*), carnation (*Dianthus caryophyllus*), *Chrysanthemum* sp. and *Gypsophila paniculata*; and *P. tateae* Wu & Townshend, 1973 from beneath mixed ryegrass (*Lolium* sp.) and brome (*Bromus* sp.). *Paratrichodorus minor* (Colbran, 1956) Siddiqi, 1974 is the only species of the genus recorded from New Zealand (Yeates & Prestidge, 1986)

and has been associated with a wide variety of hosts (see Knight *et al.*, 1997; Sturhan *et al.*, 1997).

This chapter gives morphometric data of adult and juvenile stages of *P. nanus* and *P. minor* from two soils under pasture grazed by sheep by cattle. The host ranges of these nematodes were examined on a range of pasture grasses, clovers and weeds commonly found in New Zealand (see Lambrechtsen, 1975; Roy *et al.*, 1998).

Materials and methods

IDENTIFICATION

Paratylenchus sp. specimens were collected in February and May 1996 from Otorohanga silt loam (Typic Haploudand) under perennial ryegrass (*Lolium perenne*) /white clover (*Trifolium repens*) pasture rotationally grazed by sheep and beef cattle at Tokanui AgResearch Station (lat. 38° 5.0' S 174 ° 19.5' E, altitude 40 m above mean sea level). *Paratrichodorus* sp. specimens were collected in July 1995, February and May 1996 from Hamilton clay loam soil (Typic Haplohumlt) under pasture dominated by perennial ryegrass, rotationally grazed by sheep at Ruakura Agricultural Centre (lat. 37° 47'S, 175° 19'E, altitude 40 m above mean sea level). Specimens were processed to glycerol by the glycerol-ethanol method of Seinhorst (1959) after fixation with hot FA 4:1 (see Southey, 1986). They were mounted on glass slides in glycerol under a coverslip (supported by glass fibres) and sealed with 'Glyceel' (BDH Chemicals Ltd).

Measurements were made of the following nematode characters: Body length (L), stylet /onchiostyle length, maximum body width, oesophagus /pharynx length, tail length, position of vulva, length of gonads or genital primordia, distance from anterior end to excretory pore (EP) and, in males, length of spicules and gubernaculum. Measurements were made using a Zeiss drawing tube attached to a Zeiss ST14 compound microscope using 10, 25, 40 and 100× objectives; drawings were measured using 15 amp. fuse wire bent to the correct shape and length. From the measurements de Man formulae used for species differentiation, a, a', b, c, V and V^{exp} (anterior and posterior), were calculated (see Southey, 1986).

HOST RANGE ASSESSMENT

For host plant assessments, 80 clear polythene sleeves (8.5 cm dia. × 20 cm long) were each heat sealed at one end and used to line a PVC pipe (of the same internal dimensions as the sleeves) with a base of 1 mm stainless steel mesh, to form a lined PVC tube. Each tube was then filled with 5 mm grade pumice, watered to 30% moisture by weight and placed into a glasshouse waterbath at 24°C. Nutrients were supplied by an initial application of 1 g Osmocote® granules(18: 2.1: 9.1: 4: 0.5 N:P:K:S:Ca, Sierra Chemical Europe) per tube. Tubes were arranged in the waterbath in a randomised block design with each replicate comprising a block. Five seeds from 15 pasture plants (Table 1) commonly found in New Zealand pasture (and a fallow control) were sown into the tubes and watered daily. There were five (*Paratylenchus* sp.) and four (*Paratrichodorus* sp.) replicates respectively.

Inoculum was from cultures of each source population (above) which were maintained on roots of cocksfoot grown in pots (16.5 × 16.5 × 19.0 cm) in a glasshouse waterbath at 24°C.

For *Paratylenchus* sp., *ca* 150 individuals (comprising *ca* 6% J2 + J3, 41% J4, 43% females and 11% males) were inoculated into each sleeve 18 days after sowing seeds. Watering to weight was continued until 91 (one replicate), 98 (three replicates) and 105 (one replicate) days after inoculation; numbers of *Paratylenchus* sp. were then assessed. For *Paratrichodorus* sp. *ca* 50 individuals (comprising *ca* 96% juveniles and 4% females) were used as inoculum for each tube 70 days after sowing, with assessment of individual replicates carried out 61, 81, 97 and 102 days after inoculation.

For nematode assessment roots were shaken free of excess pumice and extracted using a variant of the Whitehead & Hemming (1965) extraction method; the remaining pumice was subject to a decant and sieve extraction (Brown & Boag, 1988) (see Chapter 3 for extraction efficiency of these methods).

Table 1. *Pasture plants used for host range testing of* Paratylenchus *sp. and* Paratrichodorus *sp.*

Common name	Botanical name	Cultivar	Accession number[1]
Grasses			
Annual poa	*Poa annua* L.	—	BP 1495
Cocksfoot	*Dactylis glomerata* L.	Grasslands Wana	K 2620
Italian ryegrass	*Lolium multiflorum* L.	Concord	B 3687
Paspalum	*Paspalum dilatatum* Poir.	Grasslands Raki	BO 303
Perennial ryegrass[2]	*L. perenne* L.	Yatsyn 1	A 8263
Perennial ryegrass[3]	*L. perenne*	Yatsyn 1	(Commercial)
Summer grass	*Digitaria sanguinalis* (L.) Scop.	—	BZ 2614
Tall fescue[1]	*Festuca arundinacea* Schreb.	Grasslands Advance	T 2509
Clovers			
Caucasian clover	*T. ambiguum* Bieb.	Endura	(Commercial)
Red clover	*Trifolium pratense* L.	Grasslands Colenso	F 2657
Sub clover	*T. subterraneum* L.	Bacchus Marsh	AK 518
White clover	*T. repens* L.	Grasslands Huia	C 7544
Weeds			
Chickweed	*Stellaria media* (L.) Vill.	—	O 702
Plantain	*Plantago lanceolata* L.	Grasslands Lancelot	O 1138
Yarrow	*Achillea millefolium* L.	—	O 1098

[1] = Margot Forde Germplasm Centre, AgResearch, Palmerston North
[2] = endophyte-free (E-), [3] = endophyte-infected (E+)

Results and Discussion

IDENTITY OF *PARATYLENCHUS* SP.

As the females were slender to slightly swollen with a stylet between 23–30 µm they clearly belong to *Paratylenchus* sp. rather than *Gracilacus* (Raski & Luc, 1987). Individuals were assigned to a stage based firstly on stylet length, then on the relationship between body length and gonad length (Fig. 1). No J1 individuals were observed, typical of many Tylenchida which hatch from the egg as J2 (see Maggenti, 1991).

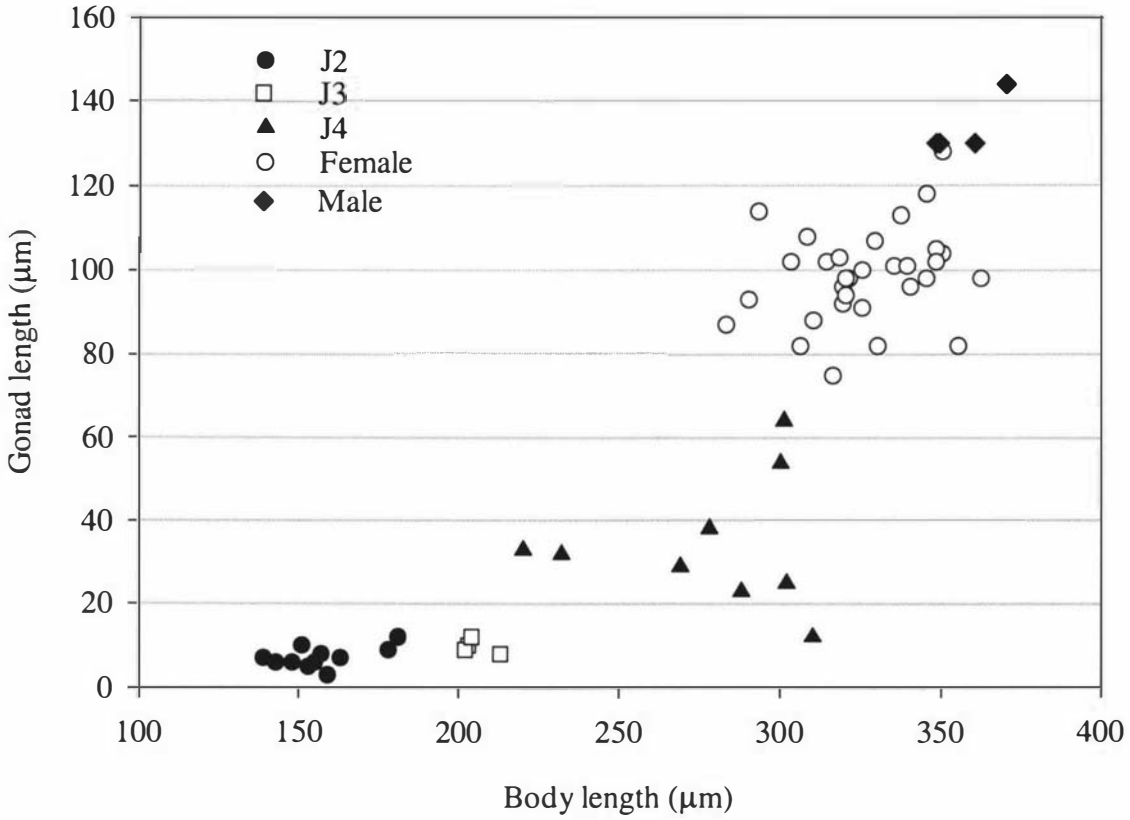

Fig. 1. *Gonad length in relation to body length for* Paratylenchus *sp. from silt loam.*

The morphometric characters of the adult *Paratylenchus* sp. specimens are presented in Table 2. As the anus of these specimens was always very difficult to discern, the 'c' value has not been used as a specific character. The morphometric characters of juvenile stage *Paratylenchus* sp. specimens are presented in Table 3.

Table 2. *Mean ± standard deviation (range) for morphometric characters of adult* Paratylenchus sp. *from silt loam.*

Character	Female (n = 33)	Male (n = 4)
L (μm)	328.9 ± 23.8 (283–405)	356.8 ± 10.4 (348–370)
Stylet (μm)	27.0 ± 1.7 (23–30)	—
a	22.3 ± 1.7 (18.1–24.9)	24.2 ± 0.7 (23.3–24.9)
b	3.7 ± 0.2 (3.2–4.5)	3.8 ± 0.4 (3.2–4.2)
c	18.9 ± 4.0 (13.5–31.4)	25.8 ± 1.9 (23–27)
V	83.2 ± 1.4 (79.9–86.1)	—
Gonad length (μm)	98.6 ± 11.2 (75–128) [1]	133.5 ± 7.0 (130–144)
V^{exp} ant.(T) (%)	26.8 ± 3.3 (20.3–34.1) [1]	(37.4 ± 1.2 (36.1–38.9))
V^{exp} post. (%)	3.5 ± 0.8 (2.2–5.2) [1]	—
Spicule (μm)	—	19.5 ± 0.6 (19–20)
Gubernaculum (μm)	—	4.0 ± 0.8 (3–5)
EP (μm)	73.5 ± 6.4 (47–83)	76.5 ± 2.6 (74–80)

[1] n=31

Table 3. *Mean ± standard deviation (range) for morphometric characters of juvenile* Paratylenchus sp. *from silt loam.*

Character	J2 (n = 11)	J3 (n = 9)	J4 (n = 9) †
L (μm)	157.0 ± 13.1 (139–181)	208.2 ± 5.0 (202–216)	277.8 ± 32.2 (220– 310)
Stylet (μm)	10.7 ± 1.4 (8–13)	12.0 ± 2.0 (9–15)	13.9 ± 2.5 (8–16)
a	18.1 ± 1.2 (15.9–19.9)	20.0 ± 3.2 (15.7–25.8)	19.4 ± 2.6 (15.8–23.2)
b	2.7 ± 0.4 (2.3–3.4)	3.3 ± 0.5 (2.9–4.3)	3.7 ± 0.3 (3.1–4.2) [3]
c	15.3 [1]	12.6 [1]	16.5 ± 4.0 (11.1–21.6)
V (%)	—	—	83.8 ± 1.8 (81.4–86.2) [3]
Gonad length (μm)	7.2 ± 2.5 (3–12)	9.8 ± 1.7 (8–12) [2]	35.9 ± 16.4 (12–64) [3]
V^{exp} ant. (%)	4.5 ± 1.4 (1.9–6.6)	4.8 ± 0.9 (3.8–5.9) [2]	11.5 ± 5.0 (3.9–19.3) [3]
V^{exp} post. (%)	—	—	1.8 ± 0.8 (0.7–2.9) [3]
EP (μm)	48.4 ± 2.9 (43–52)	52.7 ± 3.8 (45–55)	62.9 ± 5.1 (54–70) [3]

† Includes one potential male J4 which had no stylet and no visible oesophagus, excretory pore or vulva (L= 288 μm, genital length = 23 μm).
[1] n=1, [2] n=4, [3] n=8

In addition to the morphometric characters, female specimens had: slightly rounded head; EP position from mid-isthmus (24% of all specimens) to posterior third of isthmus (29%) or at the junction of isthmus and basal bulb (47%); basal bulb overlapping intestine; ovary single and outstretched with small post vulval sac (see V^{exp} post. in Table 2); spermatheca rounded (filled with globular spermatozoa in eight specimens); and tail pointed.

Body and stylet length of the specimens were compared to each of the species given by Raski (1975) and the four species most closely resembling these specimens are *P. mexicanus* Raski, 1975, *P. nanus, P. nawadus* Khan, Prasad & Mathur, 1967 and *P. projectus.*

These species can be differentiated from the present material as follows: *P. mexicanus*, head bluntly rounded, tail bluntly rounded, male anal sheath protrudes, V^{exp} too large (44 µm); *P. nanus*, male characters generally agree but specimens described here have 'a' and spicule length values which are below or just equal to the lowest values of this species; *P. nawadus*, no males, EP distance too short (66 µm); *P. projectus*, no males, spermatheca lacking or poorly developed.

Thus, the individuals described here are closest to *P. nanus*. However, it should be noted that these J4 individuals have stylets, whereas Brzeski (1995) considered J4 individuals of *P. nanus* to be devoid of a stylet. According to Brzeski (1995) only four *Paratylenchus* spp. have J4's with stylets: *P. baldaccii* Raski, 1975, *P. bukowinensis* Micoletzky, 1922, *P. projectus* and *P. similis* Khan, Prasad & Mathur, 1967. Brzeski (1995), however, did not refer to Fisher (1966) who includes photographs of *P. nanus* in which he describes the J4s as being potential males or females based on whether or not they have stylets.

If Brzeski (1995) is followed, the specimens described here could be attributed to *P. projectus* except that no males have apparently been described for that species (Raski, 1975; Brzeski, 1995). Tarjan (1960), in his review of *Paratylenchus*, included the measurement of a male *P. projectus* from a subculture of Jenkins' (1956) original population. Jenkins (1956) also found a male belonging to *P. projectus* (pers. comm. to

Tarjan). Both Raski (1975) and Brzeski (1995) agree that the specimens examined by Tarjan (1960) were *P. projectus* but neither mentions the males he observed.

Of the two described species to which these specimens most likely belong, *P. nanus* is described as having J4 with no stylet and males present, and *P. projectus* in which J4 specimens have a stylet but males not described. Fisher (1965) studied the effects of environmental variation on morphometrics of *P. nanus* and *P. projectus* and suggested the two species should be synonymysed based on female and male characters and the extent of variation under different conditions. Neither Raski (1975, 1991) nor Brzeski (1995) have synonymysed the species and they list the major differences between the two species (apart from J4 stylet and male presence) as being stylet and body lengths along with a presence of a well-developed spermatheca containing sperm in *P. nanus* but not in *P. projectus*. Tarjan (1960) includes male and female *P. projectus* measurements from a population on sweetpea roots in which the females had a well-defined spermatheca and he speculated that spermathecal development may be influenced by male presence, thus questioning the validity of separating species based on this character. As for stylet and body length, the work of Fisher (1965) would suggest that environmental differences could account for differences in these characters between *P. nanus* and *P. projectus*.

Populations of both *P. nanus* and *P. projectus* have been reported from New Zealand. Raski (1975) included in his description of *P. nanus* a population from the top of Porters Pass in New Zealand, the mean (and range) measurements from this population are: 11 ♀ L = 310 (280–350) µm , stylet = 27 (23–32) µm, a = 23 (18–26), b = 3.7 (3.3–4.0), V = 83 (82–85) %, EP = 69 (65–73) µm, 2♂ L = 310–320 µm, a = 25–28, spicules = 24 µm, gubernaculum = 4 µm, EP = 57 µm. The measurements of the Porters Pass females agree quite closely with those observed here, but the male measurements are somewhat different. For example, the males Raski (1975) described have shorter body lengths, longer spicules and a shorter EP length (shorter EP could be due to shorter body length) than the males described here. Raski (1975) notes that the body of females in the Porters Pass population curl more tightly and the tail outline is more acute than other populations described for the species, but considers that these differences do not warrant assignment to a different species.

Wood (1973) described *P. projectus* from tussock grassland in the Castle Hill Basin (adjacent to Porters Pass), the mean (and range) measurements of which are: 10♀ L = 395 (328–445) µm, stylet = 28.5 (28.0–29.5) µm, a = 23.8 (20.5–25.4), b = 3.6 (3.1–4.1), V = 85.5 (84.4–86.8) %, 10 J4 L = 310 (270–340) µm, stylet = 11.5 (10.0–13.0) µm, a = 17.7 (16.6–19.8) µm, b = 4.3 (4.1–4.5) [no V value given]. Both the female and J4 of Wood (1973) are longer than the specimens described here, and no males were described.

The morphometric characters and their variability under different environmental conditions support the proposition of Fisher (1965) that *P. projectus* be made a junior synonym of *P. nanus*. Further, if the grass-only host range of the population described below is confirmed, distinct races may have to be identified as was also suggested by Fisher (1966). Host races are, of course, well known for other tylenchid nematodes (e.g. *Meloidogyne hapla*: Ogbuji & Jensen, 1972; Sasser, 1972; Kirby *et al.*, 1975). The population described herein is therefore ascribed to *P. nanus* and this designation is used for the *Paratylenchus* population used in assessing host range.

IDENTITY OF *PARATRICHODORUS* SP.

Following Decreamer (1995) these nematodes have been placed in *Paratrichodorus* because the vulva lies near mid-body, the reproductive system consists of two opposing branches, and the cuticle is swollen after fixation.

Adult and juvenile *Paratrichodorus* sp. are clearly separated based on the relationship between body and gonad length (Fig. 2), and oesophagus length can be used to distinguish developmental stages (Fig. 3). Although trichodorids emerge from eggs as J1 (see Maggenti, 1991) no individuals were observed which could be attributed to this stage. Bird & Mai (1968) also failed to find identifiable J1 *P. minor* (=*Trichodorus christiei*) from soil samples. The reason for this is probably explained by the observation of Morton & Perry (1968) that J1 *P. minor* moulted soon after emerging from the egg.

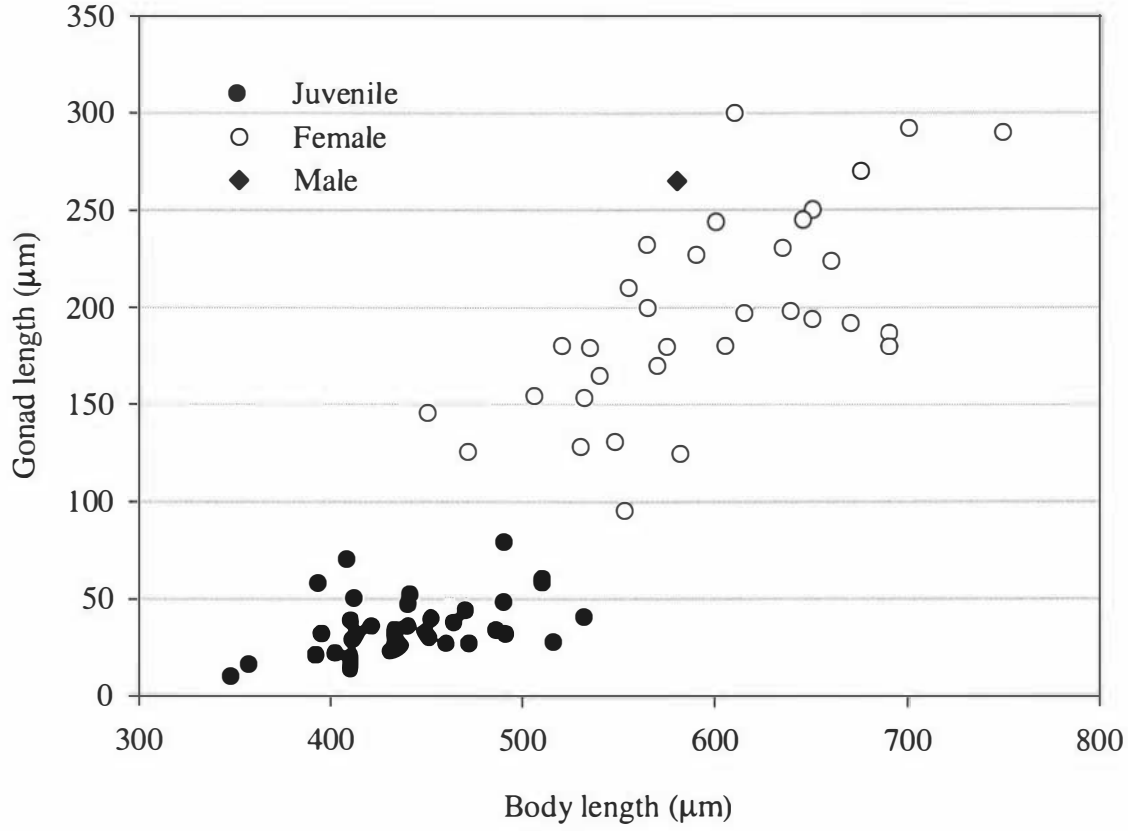

Fig. 2. *Gonad length in relation to body length for* Paratrichodorus *sp. from clay loam.*

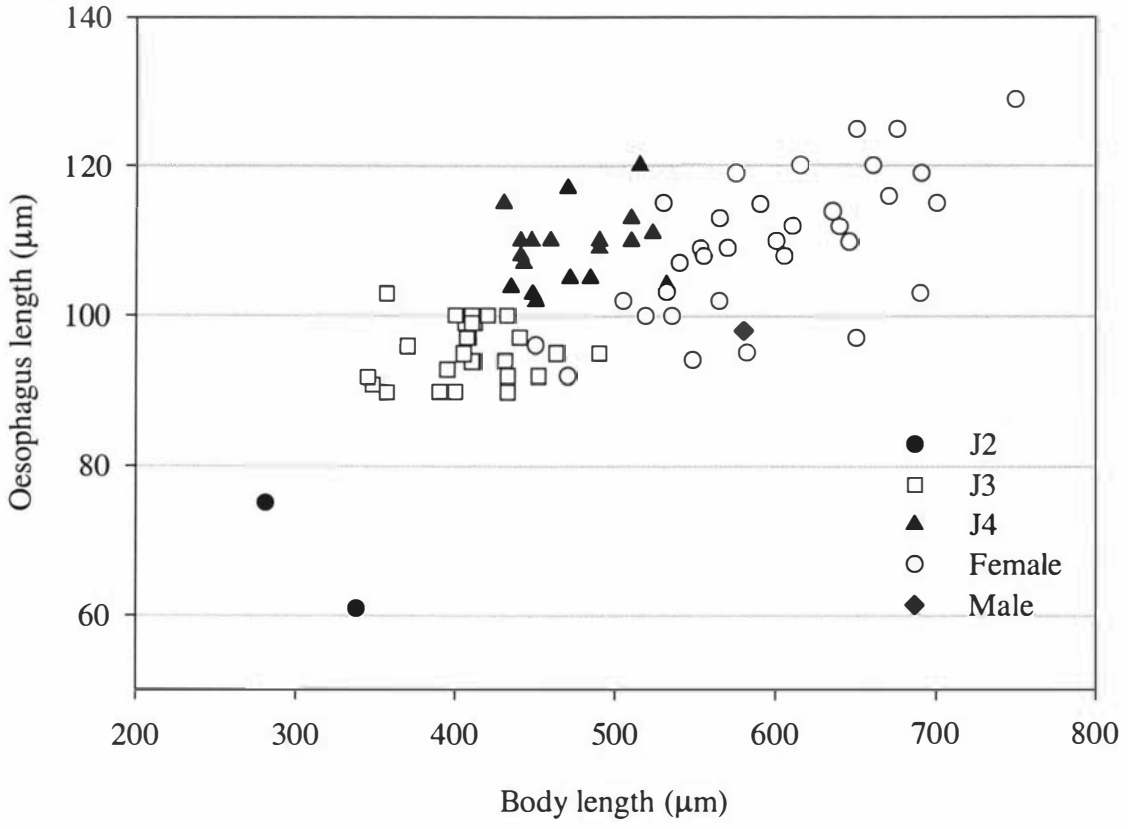

Fig. 3. *Oesophagus length in relation to body length for* Paratrichodorus *sp. from clay loam.*

The values for morphometric characters of life stages, excluding J1, of the *Paratrichodorus* sp. material are given in Tables 4 and 5. Additional observations included: inner onchium in two females, ovaries paired and outstretched, vulva a transverse slit, vaginal sclerotization rod-shaped and parallel to longitudinal body axis, spicule slightly curved.

Table 4. *Mean ± standard deviation (range) for morphometric characters of adult* Paratrichodorus *sp. from clay loam.*

Character	Female (n = 34)	Male (n = 1)
L (µm)	591.7 ± 73.1 (450–749)	580.0
Onchiostyle (µm)	27.4 ± 2.5 (21–33)	30.0
a	19.8 ± 3.0 (15.6–27.9) [1]	24.2
a'	25.2 ± 3.6 (19.1–34.5) [1]	30.5
b	5.4 ± 0.5 (4.6–6.7)	5.9
c	195.0 ± 66.7 (90.0–337.5)	—
V [T] (%)	56.7 ± 1.8 (53.8–60.8)	[45.7]
Gonad length (µm)	194.6 ± 51.5 (95–300)	265.0
V^{exp} (%)	32.7 ± 6.6 (17.2–49.2)	—
Spicule (µm)	—	58.0

[1] n=33

Table 5. *Mean ± standard deviation (range) for morphometric characters of juvenile* Paratrichodorus sp. *from clay loam.*

Character	J2 + J3 (n = 2)	J3 (n = 31)	J4 (n = 20)
L (µm)	309.5 ± 40.3(281.0–338.0)	407.4± 32.6 (345.0–490.0)	472.5 ± 32.3(430.0–532.0)
Onchiostyle (µm)	23.0 ± 4.2 (20.0–26.0)	23.9± 3.3 (18.0–30.0)	25.2 ± 2.0 (22.0–28.0)
a	18.3 ± 0.7 (17.8–18.7)	19.2± 2.4 (14.7–27.2)	20.6 ± 2.8 (16.0–25.8) [3]
a'	21.3 ± 1.7 (20.1–22.5)	24.4 ± 3.0 (18.8–35.0)	26.1 ± 3.5 (20.4–33.7) [3]
b	4.6 ± 1.3 (3.7–5.5)	4.3 ± 0.4 (3.5–5.2)	4.3 ± 0.3 (3.7–5.1)
c	126.6 ± 19.7 (112.7–140.5)	149.8 ± 43.1 (78.0–226.0)	172.7 ± 38.0 (122.5–257.5)
V (%)	—	58.1 ± 2.7 (47.9–61.3) [1]	57.9 ± 4.4 (48.2–66.5) [4]
Gonad length (µm)	—	33.8 ± 15.0 (10.0–70.0) [2]	39.6 ± 14.9 (26.0–79.0)
V^{exp} (%)	—	8.1 ± 3.5 (2.9–17.2) [2]	8.3 ± 2.9 (5.4–16.1) [4]

[1] n=23, [2] n=22, [3] n=19, [4] n=16

The measurements were compared to those for the 31 species of *Paratrichodorus* listed by Decraemer (1995). Body and stylet length of the female specimens provided the following "short-list" of possible species to which these specimens could be ascribed: *P. faisalabadensis* Nasira & Maqbool, 1994, *P. minor, P. mirzai* (Siddiqi, 1960) Siddiqi, 1974, *P. nanus* (Allen, 1957) Siddiqi, 1974 and *P. renifer* Siddiqi, 1974.

The present material differs from these taxa as follows: *P. faisalabadensis*, pore-like vulva, vagina small and trapezoid, male spicules too short (30–33.6 µm), T value too large (54–61.3%); *P. minor*, none; *P. mirzai*, vulva a longitudinal slit, small dot-like vaginal sclerotizations, male spicule too short (30–34 µm), T value too large (53–70%), males nearly as abundant as females; *P. nanus*, rounded triangular vaginal sclerotizations, male spicules too short (42–50 µm); *P. renifer*, males unknown.

From this comparison it seems clear that the individuals described here belong to *P. minor*. However, it should be noted that the only morphological differences between *P. minor* and *P. renifer* are the absence of males and a slightly shorter onchiostyle in *P. renifer*. Geographically, *P. renifer* has been recorded from Malawi, Western Australia, India, Florida, Pakistan, the U.K and China (Decraemer, 1995). *P. minor* has a worldwide distribution, including New Zealand (Dale, 1971, Yeates & Prestidge, 1986, Decreamer, 1995).

The key of Decreamer & Baujard (1998) uses female characters such as shape of vaginal sclerotized pieces, orientation of vaginal sclerotized pieces, occurrence of males, shape of sperm cells, shape of vagina, and vulva shape. Using their key and the measurements and description given here, the material described most closely matches *P. minor*.

The measurements from the specimens described here also agree quite closely to those given by Yeates & Prestidge (1986) for *P. minor* from Ruakura Agricultural Research Centre: 11 ♀ L = 587 (530–630) µm, V = 57.3 (57–59) %, stylet [onchiostyle] = 31.4 (30–33) µm and Wairakei Research Station: 12 ♀ L = 579 (520–630) µm, V = 58 (56–59) %, stylet = 31.9 (29–35) µm, 1 ♂ L = 530 µm, spicules = 59 µm, stylet = 34 µm. All measurements of body length and vulval position recorded by Yeates & Prestidge (1986) fall within the range of the present specimens and stylet range differs only

slightly. Importantly, the spicule length of the male *P. minor* from Wairakei is very close to that described here. The population of *Paratrichodorus* sp. used in host range assessments is therefore ascribed to *P. minor*.

Having assigned these specimens to *P. minor* it should be noted that the following are synonyms of *P. minor* according to Decraemer (1995) and Decreamer & Baujard (1998): *Nanidorus christiei, N. minor, P. christiei P.(N) christiei, P.(N) minor*, *P. (N) obesus*, *Trichodorus christiei, T. minor* and *T. obesus*.

HOST RANGE ASSESSMENT

The ratio of final to initial (inoculum) numbers ($P_f : P_i$) was used to determine good ($P_f : P_i > 1.00$), poor ($0.10 < P_f : P_i < 1.00$) and non ($P_f : P_i < 0.10$) hosts (e.g. Robinson & Percival, 1997). Grasses were the only hosts of *P. nanus* with Italian ryegrass, cocksfoot and perennial ryegrass good hosts, *Poa annua* a poor host and the two C4 grasses, paspalum and summer grass non hosts (Table 6). *P. minor* had a much wider host range. Ryegrasses, cocksfoot, *Poa annua*, tall fescue, and white, red and subterraneum clover were good hosts but paspalum, summer grass, caucasian clover and all three weed species were poor hosts (Table 6).

Table 6. *Ratio of final (P_f) to initial (P_i) numbers of* P. nanus *and* P. minor *per tube (E+ and E- denote endophyte-infected and free respectively).*

Common Name	*P. nanus*	*P. minor*
Grasses		
Annual poa	0.57	10.93 [1]
Cocksfoot	6.56 [1]	5.62 [2]
Italian ryegrass	1.56 [1]	3.72 [2]
Paspalum	0.00	0.14
Perennial ryegrass (E-)	1.59 [1]	1.22 [2]
Perennial ryegrass (E+)	2.11 [1]	2.14 [2]
Summer grass	0.00	0.16
Tall fescue (E-)	0.14	4.06 [2]
Clovers		
Caucasian clover	0.00	0.34
Red clover	0.00	10.90 [2]
Sub clover	0.00	15.42 [1]
White clover	0.00	11.48
Weeds		
Chickweed	0.00	0.18
Plantain	0.00	0.48
Yarrow	0.00	0.10
Fallow control	0.00	0.00

[1] = new host record
[2] = new host record for New Zealand

On average 59.1% of *P. nanus* and 30.1% of *P. minor* were recovered from roots and the balance from the pumice growth medium. There was no significant difference in the proportion of life stages between good and poor hosts of *P. nanus*; for all hosts these were 34.3% J2 + J3, 33.5% J4, 27.1% female and 5.1% male. The was no significant difference in the abundance of *P. nanus* on different sampling dates (Table 7).

The type host of *P. nanus* is an unspecified grass (Cobb, 1923). Other records for *P. nanus* include: grass (Tarjan, 1960), apple trees (*Malus* sp.) (Fisher, 1967) and cherry trees (*Prunus* sp.) (Arvesen, S. 1975, USDA Slide Collection, Slide Number G–4508). Solov'eva (1975) lists 22 additional hosts of *P. nanus* including grapes, potato, carrot,

celery, maize and notes that a further 35 woody and bushy plants are also hosts. Raski (1975) lists possible hosts from the USA and Canada including alfalfa (*Medicago sativa*).

P. minor numbers appeared to rise sharply between 81 and 97 DPI for good hosts, while numbers on poor hosts remained low over all assessment times (Table 7). The host range data supports the diagnosis of *P. minor* as a polyphagous species (see Rohde & Jenkins, 1957; Hooper, 1977; Decraemer, 1995). In New Zealand *P. minor* has been recorded from under white clover, cotula (*Leptinella* spp.), citrus and pome trees, fruit vines, tomato, tobacco and *Rhododendron* sp. (see Knight *et al.*, 1997).

Table 7. *Mean* P. nanus *or* P. minor *(± standard error) per tube at varying days post-inoculation (DPI) for pooled good (P_f: P_i > 1.0) and poor (P_f: P_i < 1.0) hosts.*

	P. nanus		*P. minor*	
DPI	Good hosts (n = 4)	Poor hosts (n = 2)	Good hosts (n = 9)	Poor hosts (n = 6)
61	—	—	73.2 (17.2)	10.7 (2.9)
81	—	—	100.0 (50.1)	22.7 (6.3)
91	408.0 (250.8)	20.0 (16.0)	—	—
97	—	—	572.4 (147.2)	10.7 (8.5)
98	189.0 (104.5)	62.0 (6.0)	—	—
102	—	—	709.3 (251.0)	2.7 (1.3)
105	466.7 (274.1)	63.3 (59.4)	—	—

Of the pasture plants tested only grasses supported *P. nanus* whereas *P. minor* had a much wider host range. There is scope to advance from the present host plant tests to assessment of plant yield reduction in the presence of *P. nanus* and *P. minor*. Given the frequent occurrence of *Paratylenchus* sp. in New Zealand [found in 42 of 77 pasture sites sampled throughout New Zealand by Yeates (1975)] such plant response assessments are important to determine the impact of these nematodes to pastoral agro-ecosystems.

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Chapter 3: Optimising nematode extraction from two soils under pasture

Summary – Several variations to the Whitehead and Hemming tray method, for extracting vermiform nematodes from soil samples, were compared using a silt loam and a clay loam soil under long-term pasture. Overall, the most efficient extraction was achieved when 50 g of soil was placed in a tray lined with 2-ply paper tissue and extracted for 48 h. Recovery of the total nematode fauna and plant parasitic nematodes extracted was significantly better ($P \leq 0.001$) using a 1 l straight-sided beaker than a 15 cm diameter filter funnel for settling. Nematode recovery using this method was 75% and 79% for the total nematode fauna from the two soils, where 100% were recovered in 144 h. Although there were differences in optimum extraction duration between plant parasitic nematode genera with different parasitic habits the overall recovery was 74% and 71% for the total plant parasites from the two soils respectively. Variations in the extraction method described resulted in changes in numbers and proportions of nematodes at the population and community level.

Keywords: Nematode extraction, extraction efficiency, tray method, plant parasitic nematodes, total nematode fauna.

Long-term studies of the population and community ecology of nematodes in soil require a method of extraction which is quantitative, simple, inexpensive and gives samples which are as clean as possible for accuracy of counting. The tray method described by Whitehead & Hemming (1965) fits all of these criteria for many soils. Recent studies (Brown & Boag, 1988; Bloemers & Hodda, 1995; Ruess, 1995) suggest that small changes in some extraction methods can result in large changes in total numbers of nematodes recovered and in the proportions of different nematode genera in the extract. Therefore, using soil from two long-term pasture sites, modifications to the Whitehead & Hemming (1965) tray method were investigated for their ability to extract various genera of plant parasitic nematodes and the total nematode fauna. Also, the Whitehead & Hemming (1965) method was compared to the decant and sieve method which has been shown to most efficiently extract large nematodes, such as trichodorids (e.g. *Paratrichodorus pachydermus*) (Brown & Boag, 1988) and longidorids (e.g. *Paralongidorus australis*) (Stirling & Vawdrey, 1985). Once the most efficient

extraction technique was established, the optimum duration of extraction was quantified for individual plant parasitic genera and the total nematode fauna.

Materials and methods

Silt loam and clay loam soils under pasture (from sites at Tokanui Research Station and Ruakura Research Centre respectively, see Chapter 2) were used for extraction experiments. Pasture was dominated by perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) and by ryegrass for the silt loam and clay loam soils respectively. Soil was collected by taking 19 cm spade squares to 20 cm depth.

Extraction methods

Five comparisons of nematode extraction were carried out, each using soil collected from contiguous soil samples at each site, during September and October 1995, and hand-mixed immediately prior to extraction. Each comparison consisted of five replicates of each factor, for each soil.

The basic extraction method used was the following variant of the Whitehead & Hemming (1965) tray method: plastic trays (26 × 21 × 6 cm) were lined with coarse (50 mm) and fine (1 mm) plastic mesh, then 2-ply paper tissue (Hygenex) placed on the 1 mm mesh. Fifty grams (approximately equivalent to a 2.5 cm diameter core to 10 cm depth) of hand-crumbled field moist soil were spread over the tissue and 500 ml of tap water poured into the tray. After 48 h extraction at ambient temperature (15–20°C), the soil and tissue were drained briefly, then discarded, and the nematode suspension was poured into a 15 cm diameter glass filter funnel (volume *ca* 700 ml), along with *ca* 100 ml water used to rinse the tray. Nematodes were allowed to settle overnight after which they were collected in 5 ml tubes and counted alive on glass slides using a stereomicroscope at 40 or 80 × magnification.

Variations to this basic method were: (1) 2-ply *vs* 1-ply tissue (2) 50 g *vs* 125 g or 250 g soil (3) 48 h *vs* 24 h extraction time, the same 50 g of soil being used for the 24 h and 48 h extractions, by removing and replacing the same volume of water from the

trays (4) the filter funnel was replaced with a straight-sided 1 l plastic beaker; nematodes were allowed to settle for 4 h in the beaker, the water in the beaker was reduced to 100 ml by suction and transferred to a 150 ml beaker; after a further 4 h the water was reduced to 10 ml and transferred to a 15 ml beaker; nematodes were counted after a minimum of 4 h further settling (Hooper & Evans, 1993) (5) comparison of the beaker settling variant with the decanting and sieving method for extracting trichodorids (CSDTF method) described by Brown & Boag (1988).

Extraction duration

Variant (4) above was used for extraction and every 24 h for 144 h, the samples were placed into fresh trays and the nematode suspension reduced before the nematodes were counted.

Statistical analyses

The significance of differences between extraction methods was determined by ANOVA using $\log_{10}(n+1)$ transformation of the nematode counts.

Results

Extraction methods

For silt loam soil, the use of 1-ply tissue did not significantly affect the number of any plant parasitic genera, total plant parasites or total nematode fauna (Table 1). However, significantly more *Paratrichodorus minor* and *Heterodera trifolii* were extracted from clay loam soil when 1-ply rather than 2-ply tissue was used (Table 2). These differences affected estimated community structure so that the percentage of *P. minor* and *H. trifolii* as a proportion of the total plant parasites increased from 1.5% and 1.3% respectively using 2-ply tissue to 4.9% and 5.3% respectively with 1-ply tissue. There was a consequent decrease in the proportion of all other nematodes e.g. *Pratylenchus* decreased from 91.4% to 84.1% (Table 2). The use of 1-ply tissue increased the number of total plant parasites extracted by 21.3% and 14.1% for silt loam and clay loam respectively (Tables 1 and 2). The number of total nematodes was increased by 7% and 20.6% respectively. However, samples extracted using 1-ply tissue

were more difficult to count due to the amount of soil particles that passed through the tissue.

Table 1. *Mean number of nematodes extracted using either the basic method (2-ply tissue, 50 g soil, 48 h extraction time) or variations to that method, from silt loam soil (means expressed per 50 g).*

	Comparison (1)		Comparison (2)			Comparison (3)	
	2-ply	1-ply	50 g	125 g	250 g	0–24 h	24–48 h
Paratylenchus nanus	50.4	75.4	24.2	34.5	25.8	57.4	15.0
Meloidogyne juveniles	392.6	456.2	106.2	101.8	69.7	195.6	160.2
Heterodera trifolii juveniles	43.0	57.2	13.4	17.5	10.4	17.0	4.0
Pratylenchus	10.6	14.0	14.2	13.9	7.4	35.4	23.0
Helicotylenchus	2.2	2.0	6.6	5.7	3.2	6.8	4.0
Total plant parasitic nematodes	498.8	604.8	164.6	173.5	116.5	312.8	206.2
Total nematodes	1529.6	1637.6	796.8	872.0	636.2	2349.6	794.4

There were large, non-significant differences between the numbers of some nematode genera extracted /50 g, for 50 g samples compared to 250 g samples from silt loam soil (Table 1). For *Meloidogyne* /50 g (the dominant plant parasitic nematode) this difference was 34.3% and was reflected in changes in *Meloidogyne* (64.5 *vs* 59.8% for 50 g and 250 g samples respectively) and *P. nanus* (14.7 *vs* 22.1% respectively) as a proportion of total plant parasitic nematodes (Table 1). The numbers of *Pratylenchus*, *Helicotylenchus* and total plant parasitic nematodes /50 g extracted were significantly higher in 50 g samples from clay loam soil (Table 2). Estimated community structure was also affected by sample size with a reduction in *Pratylenchus* (53.7 *vs* 43.0% for 50 g and 250 g samples respectively) and an increase in *P. minor* (10.7 *vs* 20.2% respectively) and *H. trifolii* (7.3 *vs* 14.9% respectively) as a proportion of total plant parasitic nematodes (Table 2). For both soils, the 250 g sample was the least efficient, with the 125 g sample being less efficient for clay loam and slightly more efficient (<10% for total plant parasites and total nematodes) for silt loam soil. Overall, nematode recovery was lower with larger soil samples.

For all nematodes discriminated in silt loam soil, over 50% of those extracted were recovered in the first 24 h (Table 1). For *P. nanus* and *H. trifolii*, 79.3% and 81.0%

respectively of the nematodes were extracted in the first 24 h. *Meloidogyne* had the lowest extraction rate, with 55.0% being extracted in the first 24 h (Table 1). For clay loam, *P. minor* and *Helicotylenchus* had the highest extraction rates with 100% and 76.9% respectively of these nematodes being extracted in the first 24 h (Table 2). *Pratylenchus* and *Meloidogyne* had only 44.1% and 45.5% respectively of the 0–48 h total being extracted in the first 24 h (Table 2).

Table 2. *Mean number of nematodes extracted using either the basic method (2-ply tissue, 50 g soil, 48 h extraction time) or variations to that method, from clay loam soil (means expressed per 50 g soil) (* and ** denote differences significant at P≤0.05 and 0.01 respectively).*

	Comparison (1)		Comparison (2)			Comparison (3)	
	2-ply	1-ply	50 g	125 g	250 g	0–24 h	24–48 h
Paratrichodorus minor	1.2	4.4*	3.8	4.5	4.6	1.8	0.0
Meloidogyne juveniles	0.4	0.0	0.0	0.0	0.0	30.2	36.2
H. trifolii juveniles	1.0	4.8*	2.6	2.7	3.4	35.6	26.6
Pratylenchus	72.6	76.2	19.0**	11.4	9.8	16.4	20.8
Helicotylenchus	4.2	5.2	10.0**	5.8	5.1	24.6	7.4
Total plant parasitic nematodes	79.4	90.6	35.4**	24.5	22.8	110.4	91.2
Total nematodes	1672.8	2017.6	916.0	666.2	666.9	1962.4	546.4

The beaker settling variant recovered significantly more *P. nanus*, *Meloidogyne*, total plant parasites and total nematodes compared with the funnel settling method for silt loam soil (Table 3). For clay loam soil significantly more *P. minor*, *Pratylenchus*, total plant parasites and total nematodes were recovered by the beaker variant (Table 4). This variant consistently recovered more of all nematode genera identified from both soils. The estimated abundance of the dominant plant parasitic nematode from each soil was significantly increased by the beaker settling method and this resulted in a large increase in the proportional contribution of these genera to the total plant parasitic nematode community. For silt loam soil, the proportion of *Pratylenchus* increased from 73.1 to 87.7% (Table 3) while for clay loam soil *Meloidogyne* increased from 47.8 to 67.9% (Table 4) when a beaker was used for settling, compared to funnel settling. Samples settled in beakers contained more soil particles than those settled using funnels, but not enough to slow counting.

Table 3. *Mean number of nematodes extracted using: funnel or beaker for settling, and beaker variant compared to decant and sieving method, from silt loam soil (50 g soil, 24 h extraction time) (*** denotes P<0.001, see Table 2 for explanation of other symbols).*

	Comparison (4)		Comparison (5)	
	Funnel	Beaker	Beaker	Decant
P. nanus	47.4	60.8*	39.8	52.2
Meloidogyne	108.0	358.6***	353.8	394.8
H. trifolii	58.6	87.6	25.4	33.0
Pratylenchus	6.4	12.0	116.4**	44.4
Helicotylenchus	5.4	8.0	6.4	10.6*
Total plant parasitic nematodes	225.8	528.0***	543.0	537.4
Total nematodes	945.6	2411.2***	3034.4*	2247.2

Decanting and sieving extracted significantly more *Helicotylenchus* than did the beaker settling method for silt loam soil (Table 3). However, the beaker method extracted significantly more *Pratylenchus* and total nematodes which resulted in the percent of *Pratylenchus* as a proportion of the total plant parasitic community being 21.4 *vs* 8.3% for beaker *vs* decant extraction respectively (Table 3). For clay loam soil the beaker variant extracted significantly more *H. trifolii* than did the decant method (Table 4). Overall, the beaker settling method recovered more plant parasitic and total nematodes than did decanting and sieving. Some samples extracted by decanting and sieving contained a large amount of debris and counting was more difficult in those samples. Further, extractions carried out using the decanting and sieving method took considerably longer than the beaker variant.

Table 4. *Mean number of nematodes extracted using: funnel or beaker for settling, and beaker variant compared to decant and sieving method, from clay loam soil (50 g soil, 24 h extraction time) (see Table 3 for explanation of other symbols).*

	Comparison (4)		Comparison (5)	
	Funnel	Beaker	Beaker	Decant
P. minor	5.2	8.8*	0.2	1.2
Meloidogyne	0.2	0.4	125.2	44.4
H. trifolii	3.8	6.4	100.8**	47.6
Pratylenchus	40.8	161.0***	8.0	6.0
Helicotylenchus	5.8	7.0	16.4	21.0
Total plant parasitic nematodes	55.8	183.6***	251.4	122.4
Total nematodes	663.2	2079.2***	1550.4	1492.8

EXTRACTION DURATION

After 48 h the beaker variant had extracted 73.5% of plant parasitic and 75.1% of total nematodes recovered over 144 h from silt loam soil (Fig. 1A). Of the plant parasitic nematodes identified, the recovery at 48 h was greatest for the ectoparasitic *P. nanus*, less for the migratory endoparasitic *Pratylenchus* and least for the endoparasitic *Meloidogyne* and *H. trifolii* (Fig. 1A). Hatching from eggs in soil may account for the longer extraction duration of *Meloidogyne* and *H. trifolii* juveniles (J2 being the only stage of these nematodes which are extracted from soil samples), while egg hatch and emergence from roots probably lengthened the duration of *Pratylenchus* extraction, compared to *P. nanus*.

For clay loam soil the beaker variant recovered 70.6% of plant parasitic and 78.9% of total nematodes after 48 h (Fig. 1B). The extraction duration of *Pratylenchus* was similar to that from silt loam. *H. trifolii* had shorter extraction duration in clay loam than silt loam soil. In comparison with silt loam, extraction rate of total plant parasitic nematodes after 48 h was slightly lower (–3.5%) and total nematodes slightly higher (+3.8%) for clay loam soil (Figs 1A and B).

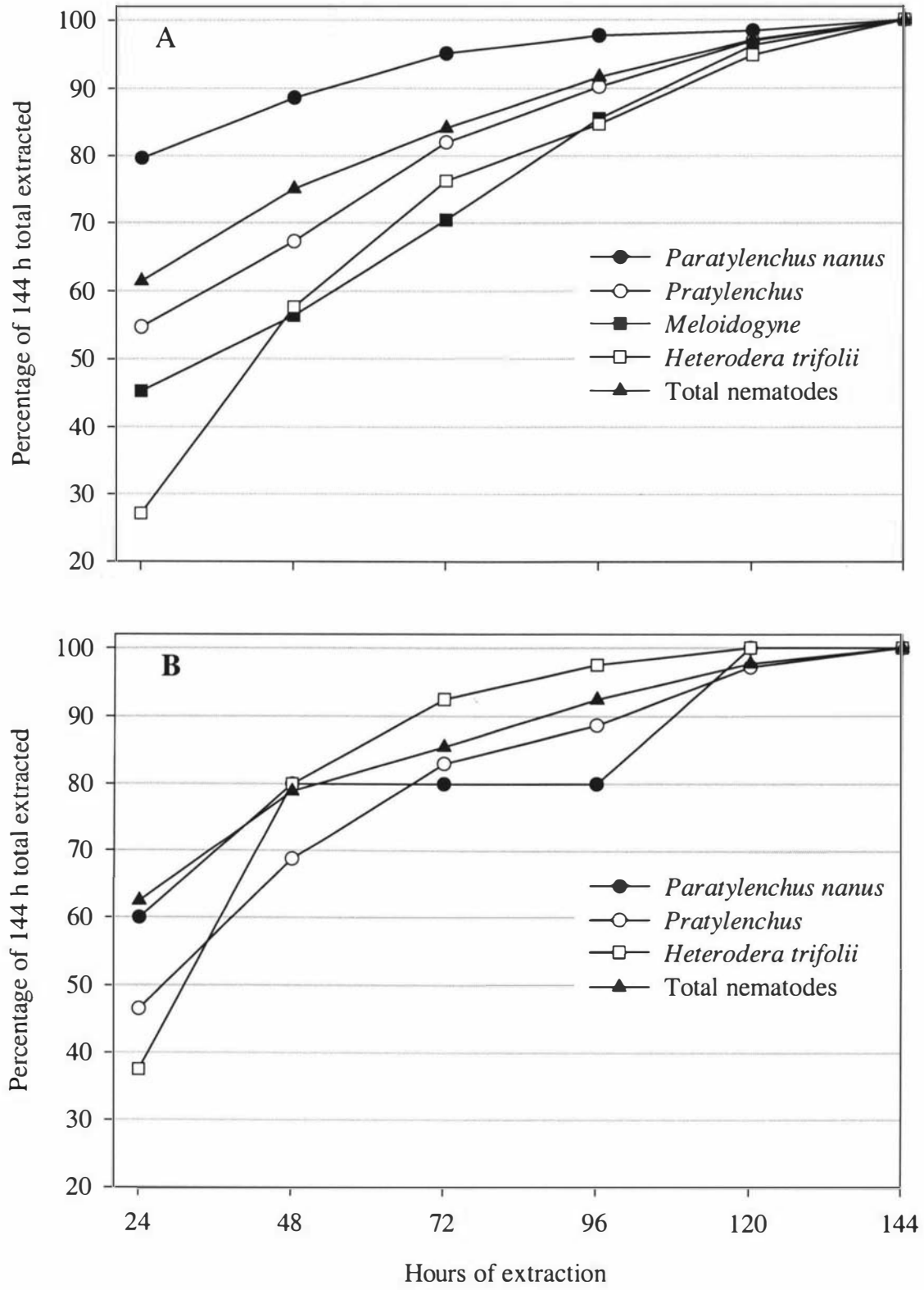

Fig. 1. *Cumulative nematodes extracted per 24 h period expressed as a proportion of the 144 h total, using A) silt loam and B) clay loam soils.*

Extraction duration of the J2 and J3 juvenile stages of *P. nanus* was similar to that of the J2 juvenile stage of *Meloidogyne* and *H. trifolii* (Figs 1 and 2).

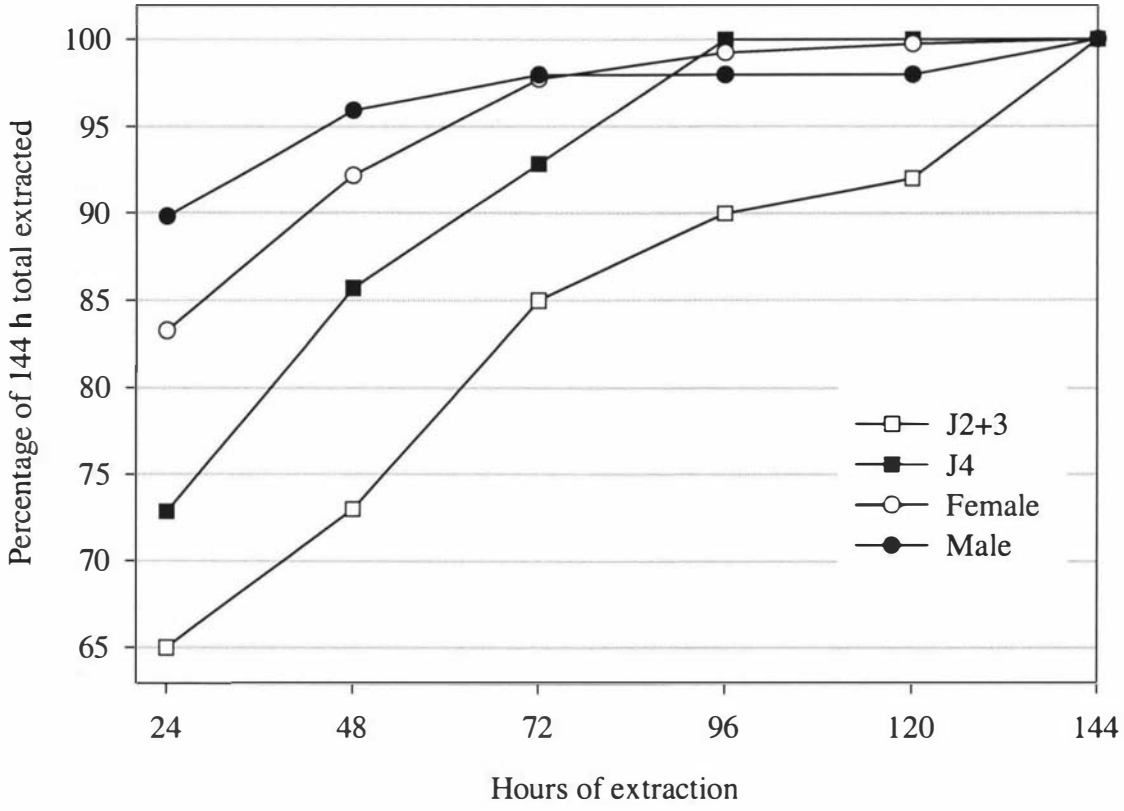

Fig. 2. *Cumulative* P. nanus *life stages extracted per 24 h period expressed as a proportion of the 144 h total, using silt loam soil.*

Discussion

This study has confirmed published observations that small variations to extraction method can make large differences to the number of nematodes extracted from soil. For both soils investigated, the recovery of both plant parasitic and total nematodes were increased when 1-ply tissue was used (Tables 1 and 2). However, the amount of debris which passed into the final sample meant that counting time was increased by a similar proportion to the increase in nematodes extracted, thus making it no more efficient than 2-ply tissue.

Counting time was least for the 50 g sample at both sites (due to less nematodes overall being extracted) and was therefore the most efficient sample size to extract and

count. If bulk nematodes are required for experimental inoculum a 250 g sample could be used but for ecological or survey-based studies, a 50 g sample would appear to be the best sample size for this method.

The time for which samples were extracted appeared to affect nematode taxa differently (Tables 1 and 2). In 24 h a large proportion of some taxa (*P. nanus*, *P. minor* and *Helicotylenchus*) were recovered but less than 60% of *Meloidogyne* so that, if comparisons are to be drawn between different genera, a 48 h extraction time is more likely to reflect the true relationships. This is true for individual plant parasitic genera, total nematodes and total plant parasites.

Using a beaker rather than a funnel for settling nematodes resulted in an increase of 2–3 × in total plant parasitic nematodes and total nematodes collected from each soil (Tables 3 and 4). Due to the very fine soil particles that were collected in beaker samples, but not funnel samples, it appears that nematodes may settle onto the sloping sides of the funnels and become trapped in fine soil. Therefore the nematodes will not settle down to the stem of the funnel and cannot be collected and counted. Similar results were obtained by Nakasono *et al.* (1987) who found that approximately 50% of nematodes they extracted using a Baermann funnel (including *Pratylenchus coffeae* and *Meloidogyne* sp.) were trapped on the wall of the funnel when fine soil particles were present. Ruess (1995) found that nematode extraction using the Baermann funnel was as efficient as direct examination of soil. The sample size used in her study was small (*ca* 20 ml soil) so the problem of nematodes becoming lodged in soil particles on the side of the funnel may not have arisen. If larger sample sizes are to be extracted the lodging problem would make funnel settling unsuitable for ecological studies.

When compared to the beaker method, the decant and sieving method extracted proportionally less total plant parasites than total nematodes from silt loam; in contrast to clay loam soil where proportionally less total nematodes than total plant parasites were extracted (Tables 3 and 4). Brown & Boag (1988) suggested that the method requires modification in humic or clay soils compared to sand, loamy sand and sandy loam soils. Spaull & Braithwaite (1979) used different extraction methods for sand and clay soils. This would give rise to difficulty in comparing nematode recovery from soils

of different texture due to generic biases. The present study has used silt loam and clay soils which represent *ca* 80 % of New Zealand soils (P. L. Singleton, AgResearch, pers. comm.) and is therefore of general applicability.

Overall, this study suggests that the best of the methods tested, for these soils, is that using 2-ply tissue, a 50 g soil sample, extracted for 48 h and settled in a beaker. The differences between this method and that of Whitehead & Hemming (1965) are the 2-ply tissue and the amount of soil used for extraction. The beaker settling is equivalent to the "bulk concentration apparatus" described by Whitehead & Hemming (1965), with the beaker having the advantage of lower cost.

The recovery over 24 h (as a proportion of 144 h extraction) obtained for total number of nematodes using the method described here were very similar for the two soils (75.1% and 78.9% respectively) (Fig. 1). Differences observed in the extraction duration of different genera were at least partly due to differences in parasitic habit (i.e. ectoparasitic *vs* endoparasitic), an effect that can also be seen between different types of extraction method (e.g. Barker *et al*, 1969). Extraction duration of the J2 and J3 juvenile stages of *P. nanus* was similar to that of the J2 juvenile stage of *Meloidogyne* and *H. trifolii*, with sequential egg hatch probably accounting for the similarity. This hatching effect may account for the unusual extraction pattern of *P. nanus* observed in the clay loam soil (Fig. 1B).

Changes in extraction method which result in change in the number of individuals of any nematode taxon lead to changes in estimated community structure, as shown here for the plant parasitic nematode community. This has implications for comparative ecological studies and for investigations into population interactions. To compare ecological studies that use different extraction methods, it may be necessary to "calibrate" the methods for the soil of interest, enabling calculation of a correction factor between methods. When determining population interactions such as resource competition and predator /prey interactions, it may be necessary to optimise the extraction method for the populations of interest as small changes in extraction technique can have a large effect on population numbers.

Overall the tray and beaker method is simple, inexpensive and provides relatively clean samples that are easily counted. It extracts a large proportion of the plant parasitic and total nematode fauna from soils of different texture. Accordingly, the method has been adopted for this study on the population and community ecology of nematodes in soils under pasture.

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Chapter 4: Population dynamics of *Paratylenchus nanus* in soil under pasture: I. Abiotic factors

Summary – Data from seasonal and monthly sampling of *Paratylenchus nanus* from a grazed pasture on silt loam soil in Waikato, New Zealand were used to investigate the relationships between this nematode and some abiotic factors. Abundance of *P. nanus* was greater at 10–20 cm than 0–10 cm soil depth and peaked in summer. For *P. nanus* populations significant positive correlations occurred with soil temperature and significant negative correlations with soil moisture (seasonally) and rainfall (monthly). A combination of accumulated temperature and rainfall was also significantly correlated with monthly *P. nanus* abundance. Soil phosphorus and percent total soil nitrogen were significantly negatively correlated with *P. nanus* populations on one occasion each, and it is suggested that these associations were mediated through host plant abundance.
Keywords: *Paratylenchus nanus*, population dynamics, rainfall, temperature, Activity Index, soil nutrients.

Of the factors that determine the population dynamics of nematodes, environmental variables such as temperature and moisture are probably the easiest to measure. Temperature directly affects nematode developmental rates such as: hatching of eggs [e.g. *Meloidogyne chitwoodi* (Inserra *et al.*, 1983)]; moulting between stages [e.g. *Paratylenchus projectus* J4 to female (Rhoades & Linford, 1959)]; reproduction [e.g. *Pratylenchus brachyurus* (Olowe & Corbett, 1976)] and hence the length of the life cycle [e.g. *Heterodera trifolii* (see Mulvey & Anderson, 1974)]. Temperature can also affect survival of plant parasitic nematodes in the absence of hosts [e.g. *Tylenchorhynchus dubius* (Saynor, 1972)] and indirectly via host plant responses (growth, stress).

Nematodes are aquatic animals dependent on free water for their activity. Soil moisture content affects their movement (Jones *et al.*, 1969), feeding and reproduction (Baujard & Martiny, 1994). The interrelation between soil moisture and rainfall is complex because the influence of factors such as evaporation, infiltration, transpiration and runoff varies between soils and with vegetative cover.

The population dynamics of *Paratylenchus nanus* Cobb have not been specifically studied in New Zealand although many of the studies of total nematode fauna from soil under pasture have included *Paratylenchus* spp. (e.g. Yeates, 1978, Yeates, 1981, Yeates, 1984a). Population dynamics of *P. projectus* have been studied under alpine tussock (*Festuca novae-zelandiae*) in New Zealand (Wood, 1971). This chapter is the first of two dealing with factors which may influence the population dynamics of *P. nanus* in a soil under pasture and examines its relationship with soil temperature, rainfall, soil moisture and nutrient status.

Materials and methods

Site conditions

The population of *P. nanus* was from Otorohanga silt loam soil (Typic Haploudand, wilting point 32%; field capacity 65%) at Tokanui AgResearch Station (see Chapter 2). The site consisted of a *ca* 1 ha paddock under rotational grazing, sloping east-west. Climate data (daily grass minimum temperature, 10 cm and 20 cm earth temperatures and rainfall) were obtained from the nearest New Zealand Meteorological Service climate station (Waikeria station C185132, altitude 46 m above mean sea level), situated *ca* 5 km east of the trial site. Sward details are given in Chapter 5.

Sampling

The site was divided into a 40-plot grid, with each plot being 15 × 15 m. A preliminary sampling of 30 plots (one 2.5 cm diameter core to 20 cm depth /plot) was undertaken on 5 July 1995 and the sampling precision (standard error to mean ratio) (see McSorley, 1987) for *P. nanus* was determined. The mean abundance of *P. nanus* for 0–20 cm depth at the preliminary sampling was 374.6 x 10^3 /m^2 with 18.6% precision which was considered adequate for the study (see McSorley, 1987). The same 30 plots were sampled on a further eight occasions: 7 November 1995, 7 February, 6 May and 7 August 1996 (Year 1); 14 November 1996, 14 February, 6 May and 2 September 1997 (Year 2). The sample times corresponded to approximately mid-spring, mid-summer, mid-autumn and mid-winter, and are termed seasonal samples.

Additionally, from 7 August 1996 to 6 May 1997, samples were collected at monthly intervals from 10 of the 30 plots. Five of these 10 plots sampled were selected as having high numbers (> one standard deviation above the mean) of *P. nanus* with the other five points as having had low numbers (< half a standard deviation below the mean) over the previous two seasons (February and May 1996).

At each sample point, two 2.5 cm diameter cores were taken from 0–20 cm soil depth, each then divided into two equal strata and each stratum placed into a plastic bag. The two cores were taken within 2.5 cm of each other. One core was used for nematode extraction and the other for soil moisture determination. Successive sample points were 1 m apart.

Nematodes were extracted from the cores by a variation of the Whitehead & Hemming (1965) tray method as described in Chapter 3. Total nematodes and individuals of all plant parasitic nematode genera in each sample depth were counted in a Doncaster dish (Doncaster, 1962), 25% of each sample being examined. The numbers of individuals of *P. nanus* in four life stage categories (J2+J3, J4, female and male) were also determined. These counts and identifications were carried out on live specimens under a stereo-microscope at 40–80× magnification. Soil moisture was determined after drying cores for 48 h at 90°C and expressed as percent dry weight.

Nutrient analyses were carried out on the oven-dried soil moisture samples for the November 1996, February and May 1997 monthly samplings. Samples were submitted to a soil testing laboratory for analysis. Phosphorus (P) was determined by a modification of Olsen *et al* (1954), sulphate sulphur (S) by chromatography (Watkinson & Kear, 1991), pH by the water slurry method (Blakemore *et al*, 1987), percent total nitrogen (N) by the Kjeldahl method (Bradstreet, 1965) and the extractable cations magnesium (Mg), potassium (K), calcium (Ca), and sodium (Na) using a flame spectrophotometer (Davies 1952). Values for soil nutrient levels (except %TN) were converted from 'Ministry of Agriculture and Fisheries Quicktest' units to ppm dry soil (Anon., 1998).

STATISTICS

Nematode data was transformed using log (n+4) because four is the minimum count possible when 25% of the sample is counted. A bias-corrected estimate (Neyman and Scott, 1960) of the *P. nanus* population estimate was calculated for seasonal and monthly samplings, whereby log (n+4) data were back-transformed using 10 $\exp(\bar{x} + (s^2 / 2))$ where $\bar{x}$ was the mean *P. nanus* /m^2 and s^2 the variance of the mean. For graphical purposes a back-transformed standard error of the mean (SEM) was calculated so that:

$$\text{back-transformed SEM} = \bar{x}\left(\frac{10^{SEM} - 10^{-SEM}}{2}\right) \quad (1)$$

where $\bar{x}$ was the back-transformed mean and SEM was $\frac{s}{\sqrt{n}}$ (s is standard deviation and n the number of samples).

Student's T-test was used to determine the significance of differences in abundance of *P. nanus* /m^2 and soil moisture for each season between years and between successive months.

Spatial distribution of *P. nanus* at each sampling was determined using the k in the negative binomial:

$$k = \frac{\bar{x}^2}{s^2 - \bar{x}} \quad (2)$$

The k-value is considered an index of dispersion and the smaller the value of k the more aggregated the population (i.e. $k \geq 8$ gives a Poisson distribution and $k = 0$ gives a logarithmic distribution) (Barker & Campbell, 1981).

To derive a population-specific index of aggregation for *P. nanus* across all sampling times (n = 8) Taylor's Power Law (Taylor, 1961) was used. The equation for Taylor's Power Law is:

$$s^2 = a\bar{x}b \quad (3)$$

where a is a sampling factor and b is an index of aggregation. Using this equation, distribution is uniform as b approaches zero, random when $b = 1$ and highly aggregated as b approaches infinity. Equation (3) is readily solved by a log × log plot of s^2 on $\bar{x}$ to produce:

$$\log s^2 = \log a + b \log \bar{x} \quad (4)$$

Correlations were calculated amongst: mean log transformed *P. nanus* numbers /m^2 at each sampling; soil moisture on the day of sampling; daily grass minimum temperature, and 10 cm and 20 cm earth temperature averaged over the four weeks preceding sampling; daily rainfall summed for the four weeks prior to sampling; soil pH; and major nutrient levels at the time of sampling. Each calculation produced Pearson's correlation coefficient (r) with n–2 degrees of freedom (see Sokal & Rohlf, 1969).

The index of activity proposed by Jones (1975) [herein referred to as Activity Index (AI)] for ectoparasitic nematodes was calculated for seasonal and monthly samplings:

$$AI = \Sigma((T_n - T_b) \, x \, R_n) \quad (5)$$

where T_n is mean daily soil temperature; T_b was basal development temperature; and R_n was the daily rainfall in cm. As noted by Jones (1975), this equation ignores ineffective rainfall (R_i) which fails to penetrate the soil and runs off or evaporates from the soil surface. For this reason, AI is probably an overestimate but the degree of overestimation is difficult to define and is dependent on both soil type and time of year. For seasonal samplings, the temperature × rainfall product in equation (5) was summed for the four weeks prior to sampling and, for monthly sampling, the period between samplings. Correlations between *P. nanus* abundance and AI were calculated.

Results

Site conditions

During the sampling programme total rainfall was 1604 mm for Year 1 (October 1995 to September 1996) and 957 mm for Year 2 (October 1996 to September 1997),

while the 20 year average rainfall was 1221 mm (1977 to 1997; National Climate Centre, National Institute of Water and Atmospheric Research Ltd, Wellington, New Zealand) (Fig. 1). The mean 10 cm earth temperature for Year 1 was 13.6°C, for Year 2 was 12.9°C (Fig. 2) and the 20 year average 10 cm earth temperature was 13.5°C. Thus, Year 1 was wetter and slightly warmer than normal, whereas Year 2 was drier and cooler. The maximum soil temperature recorded was 25.5°C on 25 February 1997 (20 cm earth) and the minimum was 3.2°C on 20 July 1997 (10 cm earth). The largest daily rainfall (49.4 mm) occurred on 21 May 1996.

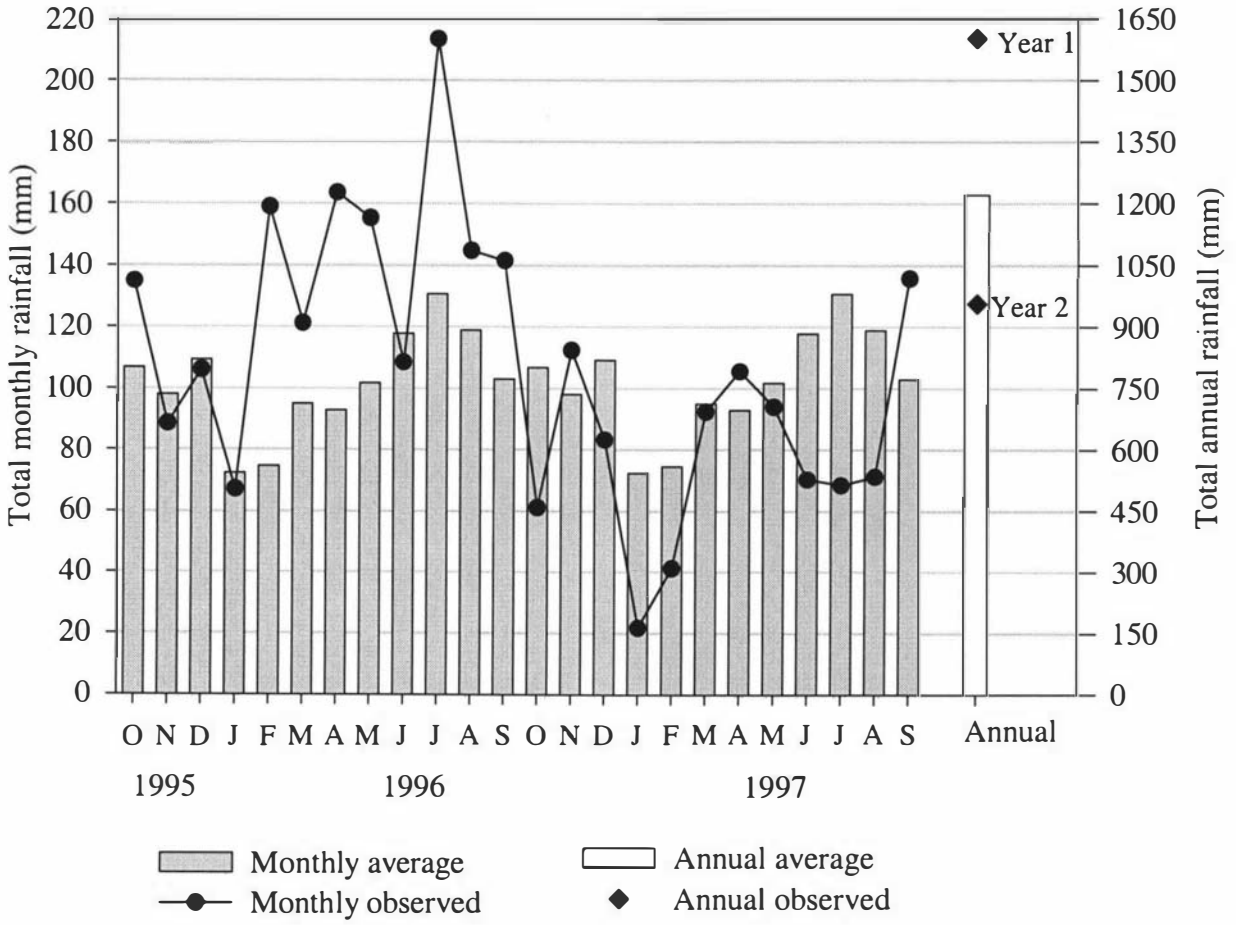

Fig. 1. *Observed mean monthly rainfall from 1 October 1995 to 30 September 1997 and calculated 20 year average.*

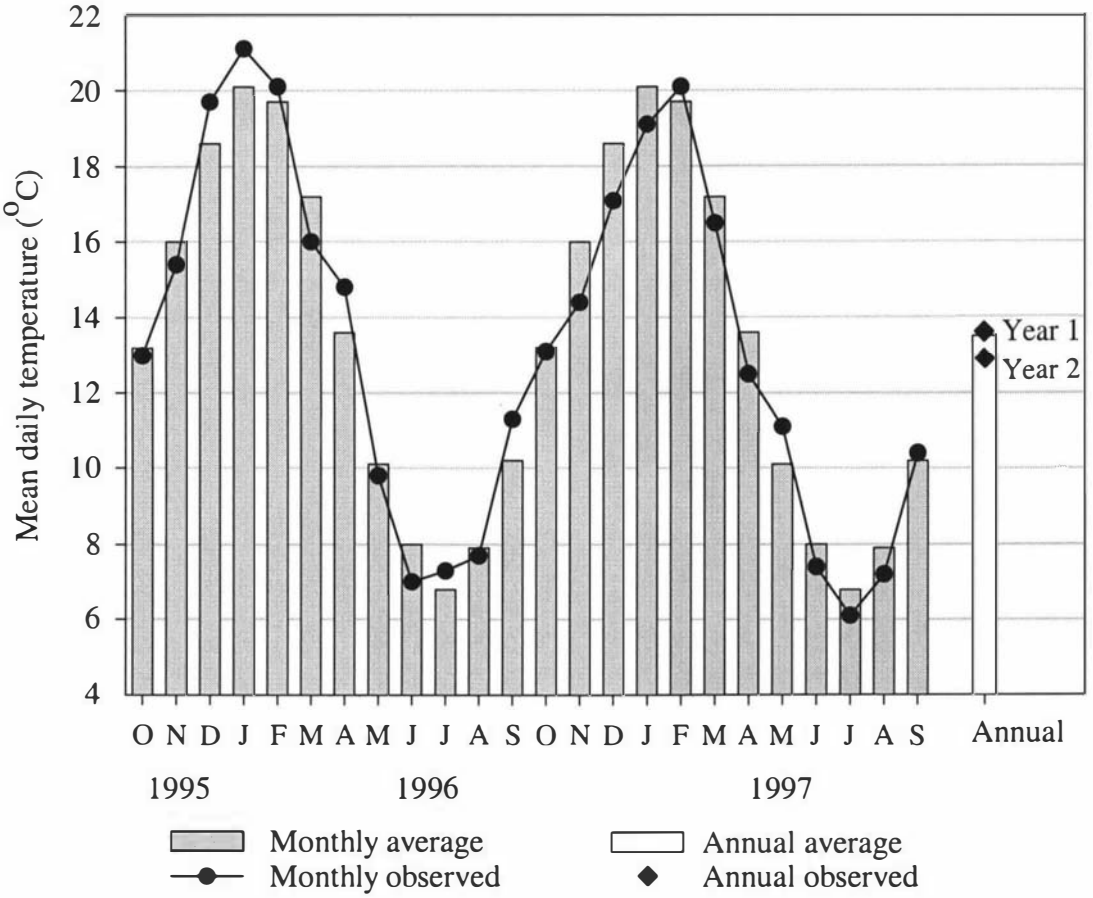

Fig. 2. *Observed mean monthly 10 cm earth temperature from 1 October 1995 to 30 September 1997 and calculated 20 year average.*

P. NANUS POPULATIONS

In both years, maximum total abundance of *P. nanus* in 0–20 cm soil was observed in summer (1376 and 1447 x 10^3 /m^2 respectively), with a minimum in winter (178 and 176 x 10^3 /m^2 respectively). There were significantly ($P<0.05$) less *P. nanus* in spring 1996 than spring 1995 in 0–10 cm depth, and significantly ($P<0.05$) more in autumn 1997 than autumn 1996 at both sampling depths (Fig. 3A). The summer and winter populations did not differ significantly between years. There was a greater abundance of *P. nanus* in 10–20 cm depth than in 0–10 cm depth (range: 1.6 – 4.7 × greater) at every sampling except in the spring of Year 1 (Fig. 3A). There were significant differences ($P<0.05$) in soil moisture between years for all seasons at both depths (Fig. 3B).

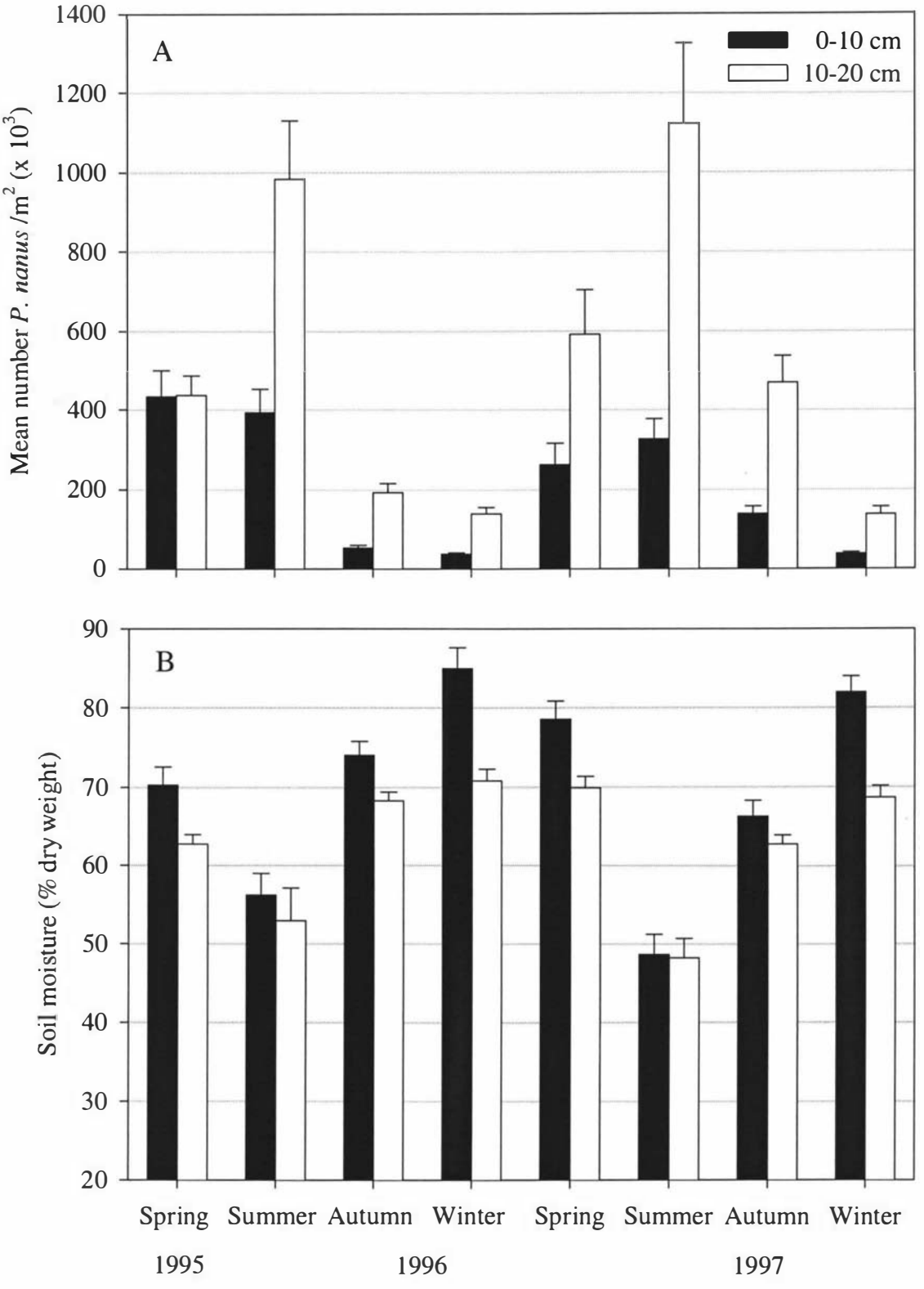

Fig. 3. *A) Seasonal abundance of* P. nanus *(± standard error) in 0–10 and 10–20 cm soil during the eight seasons sampled and B) soil moisture (± standard deviation) for the equivalent samples.*

Spatial distribution, as measured by the *k* value in the negative binomial, showed the *P. nanus* population was aggregated in all seasons but that the degree of aggregation varied between depths, seasons and years (Table 1). For 0–10 cm depth, winter of Year 1 had the lowest degree of aggregation and spring of Year 2 the highest . There was greater variation in *k* values in 10–20 cm depth, with spring samplings of Year 1 and Year 2 having the lowest and highest degrees of aggregation respectively (Table 1). The largest differences in values of *k* between Years 1 and 2, at 10–20 cm depth, were in spring and autumn (Table 1). These periods also showed the largest differences in abundance of *P. nanus* (Fig. 3A).

Table 1. *Values of the aggregation index* k *in the negative binomial for seasonal populations of* P. nanus *(spring 1995 to winter 1997).*

		Sample depth		
Year	Season	0–10 cm	10–20 cm	0–20 cm
1	Spring	0.76	1.27	1.45
	Summer	0.63	0.42	0.53
	Autumn	0.31	0.90	1.17
	Winter	0.91	0.68	0.91
2	Spring	0.15	0.12	0.21
	Summer	0.57	0.36	0.57
	Autumn	0.26	0.50	0.74
	Winter	0.35	0.81	0.75

The value for the sampling factor *a* in Taylor's Power Law for *P. nanus* was 0.21 and the population specific index of aggregation value *b* was 2.32 for 0–20 cm depth over all sampling times. This *b* value indicates an aggregated population.

At each depth, seasonal *P. nanus* abundance was significantly and positively correlated with mean 10 and 20 cm earth temperature for the four weeks before sampling (Table 2). In addition, at the 10–20 cm depth there was a significant positive correlation with grass minimum temperature. Abundance of *P. nanus* was not significantly correlated with cumulative rainfall for the four weeks preceding sampling

at either depth but was significantly negatively correlated with soil moisture at sampling.

Table 2. *Pearson's correlation coefficients (r) between log transformed seasonal abundance of* P. nanus *and observed environmental variables (spring 1995 to winter 1997) (n=8) (*, ** and *** denote significant correlations at P<0.05, 0.01 and 0.001 respectively).*

Environmental variable	Mean *P. nanus* abundance		
	0–10 cm	10–20 cm	0–20 cm
Grass minimum temperature[1]	0.663	0.750 *	0.674
Earth temperature (10 cm)[1]	0.783 *	0.898 **	0.832 *
Earth temperature (20 cm)[1]	0.774 *	0.905 **	0.838 **
Rainfall[1]	–0.462	–0.487	–0.482
Soil moisture (0-10 cm)[2]	–0.763 *	—	—
Soil moisture (10-20 cm)[2]	—	-0.848 **	—
Soil moisture (0-20 cm)[2]	—	—	–0.830 *
Activity Index (10 cm)[3]	0.521	—	0.567
Activity Index (20 cm)[3]	—	0.630	0.582

[1] Mean values for the four weeks prior to sampling
[2] Mean values at time of sampling
[3] Cumulative values for the four weeks prior to sampling, basal temperature (T_b) = 10°C

Although *P. nanus* was negatively correlated with soil moisture across all seasonal samplings, this association did not necessarily hold for each sampling (Fig. 4). At most samplings the value of correlations were similar for both depths (Fig. 4).

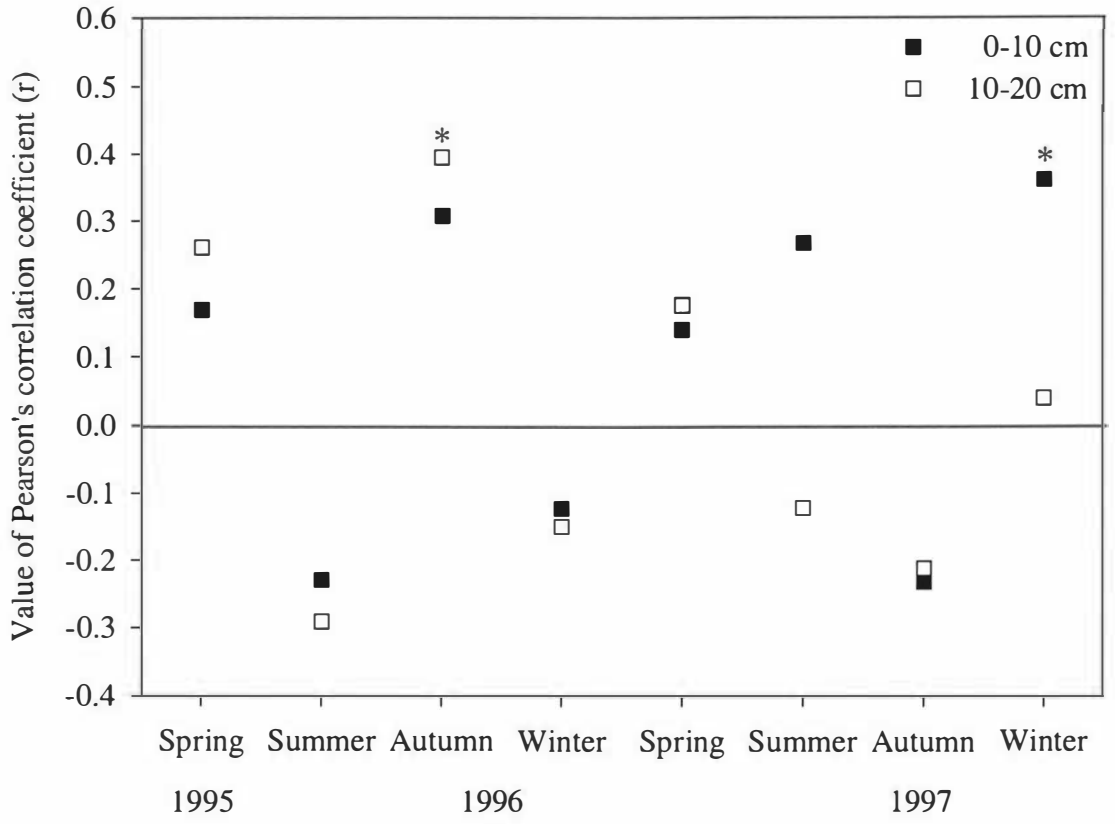

Fig. 4. *Value of Pearson's correlation coefficient (r) of log* P. nanus *vs soil moisture (spring 1995 to winter 1997). Values exceeding ± 0.361 are significant at P<0.05 and marked *.*

The abundance of the combined J2 and J3 stages of *P. nanus* for 0–20 cm depth was significantly correlated only with 20 cm earth temperature (Table 3). For other stages (J4, female and male) in addition to this correlation there was a negative correlation with 0–20 cm soil moisture (Table 3). Only male *P. nanus* were significantly correlated with grass minimum temperature. The significance of correlation values were the same for both 0–10 cm and 10–20 cm depths as for 0–20 cm depth (data not shown). Adult and J4 *P. nanus* showed similar relationships with seasonal environmental factors as did the population as a whole (Tables 2 and 3).

Table 3. *Pearson's correlation coefficients (r) between log transformed seasonal abundance of* P. nanus *life stages (0–20 cm depth) and observed environmental variables (spring 1995 to winter 1997) (n = 8) (see Table 2 for explanation of symbols).*

Environmental variable	Mean *P. nanus* /abundance			
	J2+J3	J4	Female	Male
Grass minimum[1]	0.545	0.704	0.626	0.787 *
Earth temperature (20 cm)[1]	0.717 *	0.874 **	0.769 *	0.902 **
Rainfall[1]	–0.536	–0.474	–0.410	–0.493
Soil moisture (0–20 cm)[2]	–0.616	–0.824 *	–0.927 ***	–0.859 **
Activity Index (10 cm)[3]	0.353	0.637	0.554	0.520
Activity Index (20 cm)[3]	0.352	0.650	0.584	0.513

[1] Mean values for the four weeks prior to sampling
[2] Mean values at time of sampling
[3] Cumulative values for the four weeks prior to sampling, basal temperature (T_b) = 10°C

During the monthly sampling, August 1996 to May 1997, the maximum number /m^2 of *P. nanus* was at 10–20 cm in the January sampling (Fig. 5A). There appear to be peaks of *P. nanus* numbers at 0–10 cm depth in January and April and at 10–20 cm depth in January and March (Fig. 5A). There were, however, no significant differences in *P. nanus* abundance between successive months for 0–10 and 10–20 cm depths. On no occasion did more *P. nanus* occur in 0–10 cm depth than in 10–20 cm depth. There was a significant difference ($P<0.05$) in soil moisture between successive months, with the exception of September and October (Fig. 5B).

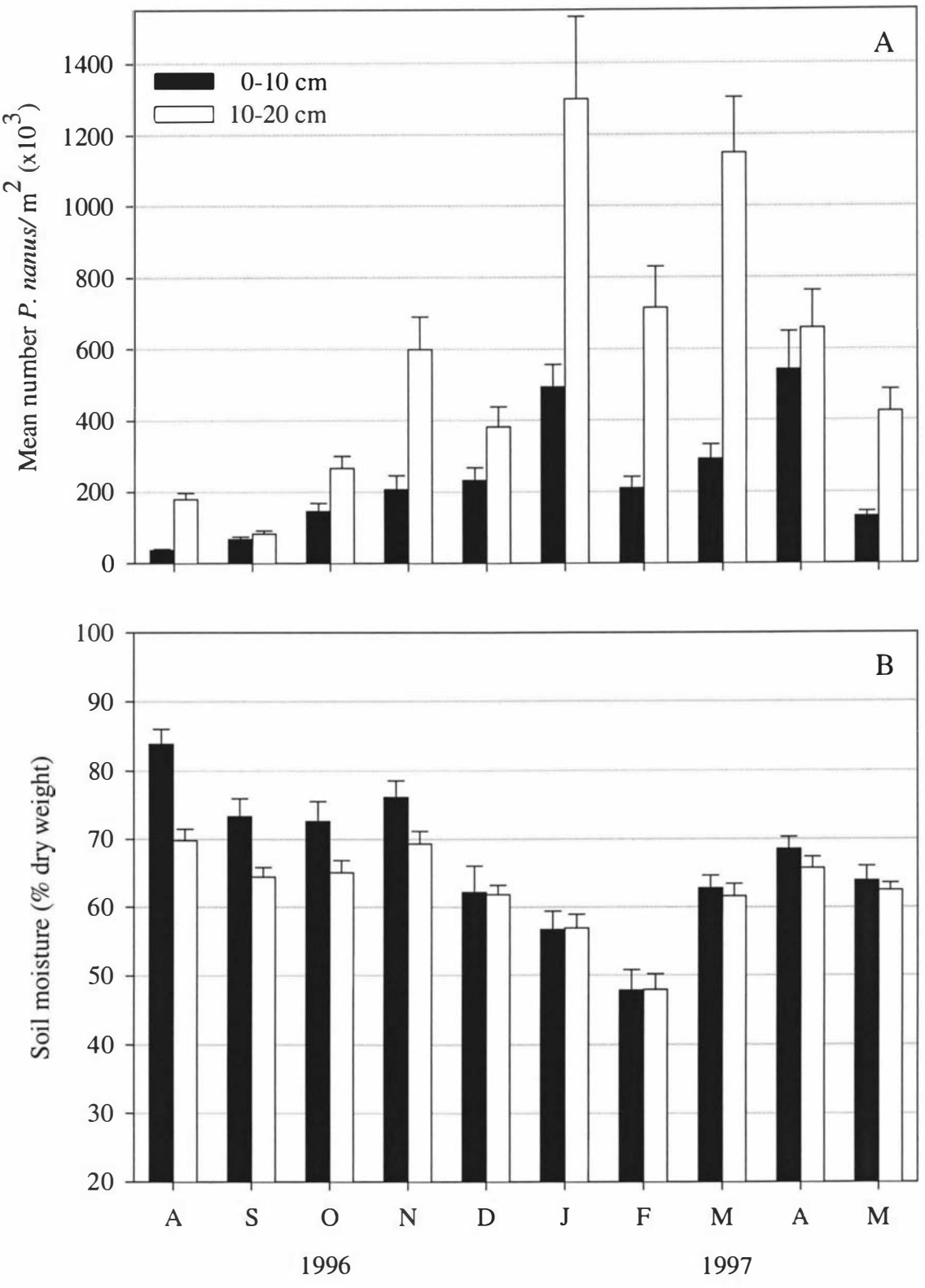

Fig. 5. *A) Monthly abundance of* P. nanus *(± standard error) in 0–10 and 10–20 cm soil during the ten months sampled and B) soil moisture (± standard deviation) for the equivalent samples.*

During the period of monthly sampling, the mean abundance of *P. nanus* in 0–10 cm depth was significantly correlated with all the environmental factors observed except rainfall (Table 4). In 10–20 cm depth, however, *P. nanus* was not significantly correlated with soil moisture but was significantly correlated with rainfall (Table 4). At 0–20 cm depth correlations more closely mirrored those in 10-20 cm depth, possibly due to the larger numbers of *P. nanus* at that depth.

Table 4. *Pearson's correlation coefficients (r) between log (n+4)* P. nanus *and observed environmental variables (August 1996 to May 1997) (n = 10) (see Table 2 for explanation of symbols).*

Environmental variable	Mean *P. nanus* abundance		
	0–10 cm	10–20 cm	0–20 cm
Grass minimum temperature[1]	0.732 *	0.792 **	0.761 *
Earth temperature (10 cm)[1]	0.791 **	0.826 **	0.816 **
Earth temperature (20 cm)[1]	0.801 **	0.848 **	0.842 **
Rainfall[1]	–0.592	–0.648 *	–0.658 *
Soil moisture (0–10 cm)[2]	–0.702 *	—	—
Soil moisture (10–20 cm)[2]	—	–0.379	—
Soil moisture (0–20 cm)[2]	—	—	–0.533
Activity Index (10 cm)[3]	0.887 ***	—	0.864 **
Activity Index (20 cm)[3]	—	0.730 *	0.832 **

[1] Mean values for the period between sampling times
[2] Mean values at time of sampling
[3] Cumulative values for the period between sampling times, basal temperature (T_b) = 10°C

The correlation between *P. nanus* and AI was greater than that for earth temperature for 0–10 cm but was slightly less so for 10–20 cm (Table 4). When viewed graphically, however, it becomes clear that AI describes the *P. nanus* population fluctuations more closely than does earth temperature alone (Fig. 6). The basal temperature of development of *P. nanus* (T_b) was estimated in two separate ways and the effects of changes in T_b estimation can be seen to make large differences in the utility of AI as a means of 'predicting' *P. nanus* population changes (Fig. 6). In one example in Fig. 6, T_b was assumed to be 10°C based on *P. nanus* population growth (i.e.

the approximate temperature at which *P. nanus* population increased in spring and declined in autumn, see Figs. 2, 3 and 4) rather than individual nematode growth threshold. A second T_b was calculated from the published results of the development time of *P. projectus* (Rhoades & Linford, 1961; Wood, 1973). By graphing temperature against the reciprocal of number of days to complete one generation and solving the regression equation for y = 0 (Trudgill & Perry, 1994), a basal temperature of 3.5°C was calculated when mean temperatures of 25 and 20°C were used for the results from Rhoades & Linford (1961) and Wood (1973) respectively (other combinations of mean temperature in the ranges published resulted in calculated basal temperatures below 0°C).

From Fig. 6, it is apparent that a T_b of 10°C more closely resembles the fluctuations in abundance of *P. nanus* between months. Using T_b of 10°C, the effect of high rainfall on AI during the months of August and September are lessened because basal temperature is greater than mean soil temperature ($T_b > T_n$) and the value of AI becomes negative (Fig. 6). AI, using either value of T_b, accommodates the decrease in *P. nanus* abundance from January to February and the subsequent increase from February to March. Values of AI do, however, indicate that the abundance of *P. nanus* would be expected to be larger in March than January, which was not in accordance with this sampling (Fig. 6).

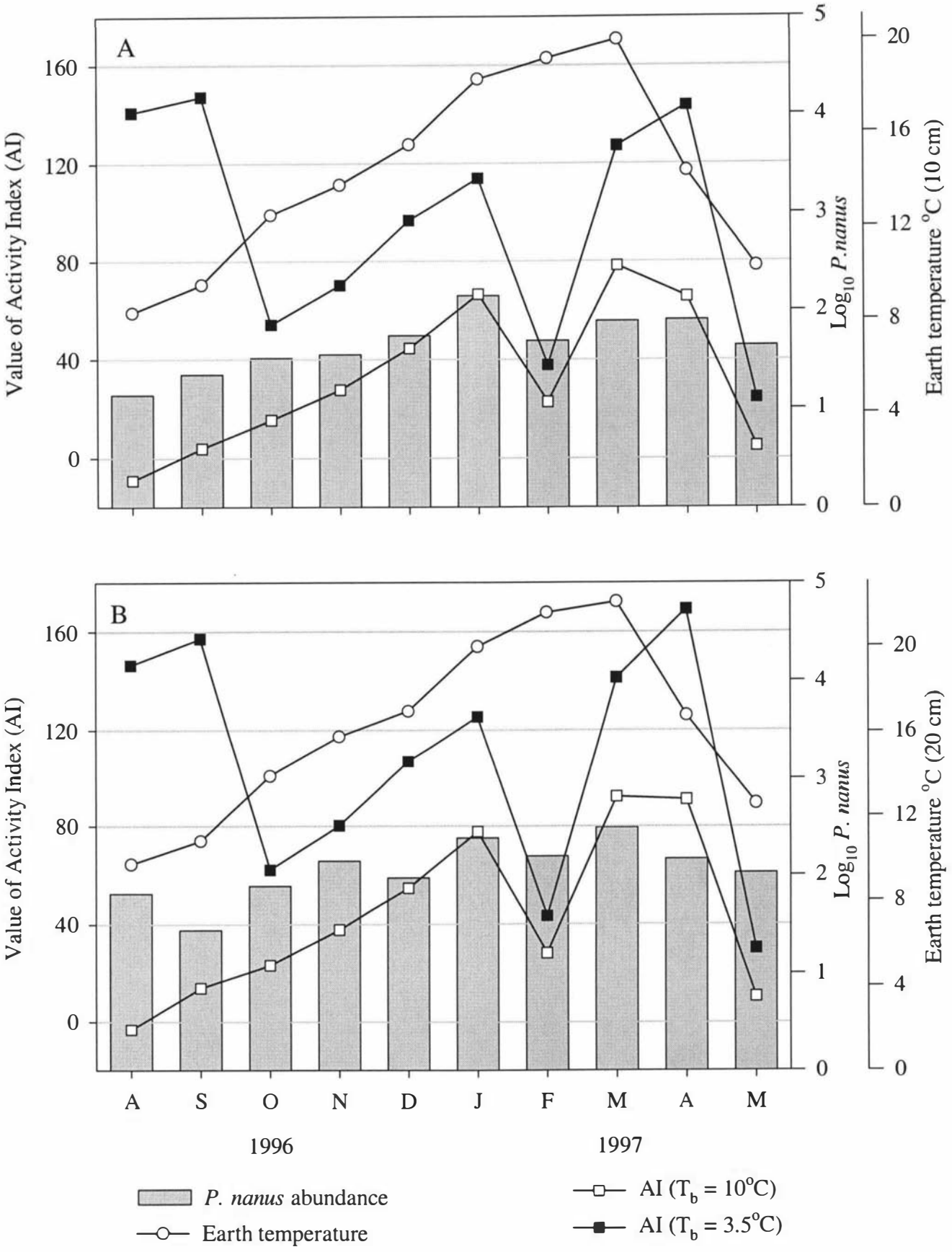

Fig. 6. *Monthly abundance of* P. nanus, *earth temperature and calculated values of the AI for A) 0–10 cm and B) 10–20 cm depths.*

The mean abundance of each stage of *P. nanus* for the months August 1996 to May 1997 was significantly correlated with grass minimum temperature (except females) and with 20 cm earth temperature (Table 5). There was a significant negative correlation between J2+J3 and J4 *P. nanus* and rainfall, but only males were significantly, negatively correlated with soil moisture (Table 5). The significance of correlation values were the same for 0–10 cm and 10–20 cm depths as for 0–20 cm depth (data not shown).

Table 5. *Pearson's correlation coefficients (r) between abundance of* P. nanus *life stages (0–20 cm depth) and observed environmental variables (August 1996 to May 1997) (n = 10) (see Table 2 for explanation of symbols).*

Environmental variable	Mean *P. nanus* /abundance			
	J2+J3	J4	Female	Male
Grass minimum[1]	0.723 *	0.726 *	0.574	0.880 **
Earth temperature (20 cm)[1]	0.787 **	0.833 **	0.675 *	0.949 ***
Rainfall[1]	–0.682 *	–0.702 *	–0.466	–0.610
Soil moisture (0–20 cm)[2]	–0.534	–0.533	–0.507	–0.645 *
Activity Index (0–10 cm)[3]	0.718 *	0.827 **	0.733 *	0.810 **
Activity Index (10–20 cm)[3]	0.675 *	0.792 **	0.723 *	0.765 **

[1] Mean values for the period between sampling times
[2] Mean values at time of sampling
[3] Cumulative values for the period between sampling times, basal temperature (T_b) = 10°C

There was little variation between samplings of mean values of pH or nutrients (Table 6). Yellow-brown loams, such as at this site, have high levels of the clay mineral allophane and therefore high P and S retention capability (see Molloy, 1998). It appears that P applied as fertiliser is retained in 0–10 cm depth whereas the more mobile S leaches to 10–20 cm depth (Table 6).

Abundance of *P. nanus* was significantly negatively correlated with soil P at 10–20 cm depth in spring (November) and with N at 10–20 cm depth in autumn (May) (Table 6).

Table 6. *Mean values (± standard deviation) (n=10) of pH and nutrient analyses for 0–10 and 10–20 cm depth soil sampled on 14 November 1996, 14 February and 6 May 1997. Values in bold are significantly (P<0.05) negatively correlated with* P. nanus *populations at the same dates.*

	Depth	Nov. 1996	Feb. 1997	May 1997
pH	0–10 cm	5.7 (0.2)	5.8 (0.1)	5.7 (0.2)
	10–20 cm	5.8 (0.2)	5.9 (0.1)	5.8 (0.2)
P (ppm)	0–10 cm	19.0 (3.6)	20.2 (5.1)	20.5 (3.4)
	10–20 cm	**7.7 (1.7)**	7.0 (2.3)	8.8 (2.2)
S (ppm)	0–10 cm	50.1 (24.8)	44.5 (18.5)	73.3 (34.2)
	10–20 cm	123.0 (89.5)	93.7 (46.3)	118.6 (59.9)
K (ppm)	0–10 cm	176.0 (83.2)	124.4 (76.0)	158.0 (91.6)
	10–20 cm	80.0 (57.3)	48.0 (48.3)	80.0 (63.9)
Mg (ppm)	0–10 cm	72.0 (16.7)	72.5 (18.7)	78.0 (19.0)
	10–20 cm	24.0 (6.1)	22.0 (5.4)	25.0 (7.1)
Ca (ppm)	0–10 cm	900.0 (241.5)	925.0 (146.7)	912.5 (236.1)
	10–20 cm	700.0 (237.2)	700.0 (134.4)	675.0 (134.4)
Na (ppm)	0–10 cm	31.0 (5.7)	32.5 (7.2)	31.5 (5.3)
	10–20 cm	26.0 (5.7)	25.5 (8.3)	27.0 (4.8)
N (%)	0–10 cm	0.954 (0.07)	0.992 (0.06)	**0.978 (0.07)**
	10–20 cm	0.536 (0.05)	0.523 (0.10)	0.561 (0.10)

To determine whether the associations observed between *P. nanus*, P and N (Table 6) were due to the nutrient levels themselves or to some other effect, correlations were calculated amongst *P. nanus* abundance, nutrients and all other abiotic and biotic variables measured. For the November 1996 sampling the variables that were also significantly correlated with log *P. nanus* and soil P were: log *Meloidogyne* juveniles /m^2 and other grass dry weight (Table 7). At this sampling other grasses comprised 29.0% of the total herbage and was made up of 86.1% *Poa annua*, 13.4% *Holcus lanatus* (Yorkshire fog) and 0.5% *Sieglingia* sp. (heath grass). Only log *Heterodera* juveniles /m^2 was significantly correlated with both log *P. nanus* /m^2 and N at the May 1997 sampling (Table 7).

Table 7. *Pearson's correlation coefficients (r) between: log (n+4)* P. nanus */m² (10–20 cm depth) and variables which were also significantly associated with: soil P for the November 1996 sampling (n = 10); and soil N for the May 1997 sampling (n = 10) (see Table 2 for explanation of symbols).*

	Log *P. nanus* /m²	P (ppm)	%TN
Log *Meloidogyne* /m²	–0.642*	0.632*	—
Other grass (dry weight, g)	0.731*	–0.801**	—
Phosphorus (ppm)	–0.871**	—	—
Log *Heterodera* /m²	–0.692*	—	0.663*
Nitrogen (%)	–0.692*	—	—

Discussion

The aggregated nature of the *P. nanus* population observed here (k = 0.12–1.45) is similar to that shown for other ectoparasitic plant parasitic nematodes (e.g. *Paratrichodorus minor k* value range 0.31–0.56 and *Helicotylenchus dihystera* 0.07–1.27) (see McSorley, 1987). While there were differences in degree of aggregation of the *P. nanus* populations between seasons, they were not great. The values of the indices a (0.21) and b (2.32) of Taylor's Power Law for *P. nanus* found here were similar to those reported for various plant parasitic nematode genera from a range of crops by McSorley & Dickson (1991). They showed that of six plant parasitic nematode species × five crops (including fallow) × 13 sample dates, only on three occasions did b values differ significantly between samplings. The a value of Taylor's Power Law was more variable with seven significant differences occurring between their samplings. The overall values calculated by McSorley & Dickson (1991) for all combinations of nematodes, crops and sampling times were a = 1.12 and b = 1.46.

Taylor (1961) listed b values for a wide range of vertebrate and invertebrate animal populations. The invertebrates which had populations aggregated to a similar degree to that of the *P. nanus* population observed here were red spider mite (Metatetranychus ulmi) (b = 2.19) from apple leaves; and earthworms (Aporroectodea caliginosa) (b = 2.54) from grassland (Taylor, 1961).

The depth distribution of *P. nanus* observed here, with maximum population at 10–20 cm (Fig. 3), agrees with the observations of Yeates *et al.* (1983) who sampled to 90 cm depth and found that (in sandy or gravelly loam soil under ryegrass and ryegrass /white clover pasture *ca* 25 km from the present site) *Paratylenchus* sp. had its maximum population below 10 cm depth (in one case at 40–50 cm depth) and was the dominant genus at depth in that soil. In other New Zealand soils where large (>200,000 /m^2) *Paratylenchus* sp. populations have been found, their distribution extended down to at least 20 cm, although maximum numbers sometimes occurred in shallower depths (Yeates, 1980). In Britain, Boag & Alphey (1988) sampled a sandy loam soil to 50 cm depth and found 76% of the *P. nanus* population occurred in 0–20 cm depth.

The seasonal population trend of *P. nanus* observed here (Fig. 3) shows an obvious summer peak and winter trough. The winter population was the same for both years of sampling despite significantly different autumn populations and somewhat different temperature and moisture conditions between years. As such winter, with it's cooler temperatures and higher soil moisture conditions, may represent an annual "bottleneck" in the development of *P. nanus* populations in the depths sampled at this site.

In common with the results found at this site (Fig. 5), Yeates (1984b) observed no significant difference in *Paratylenchus* abundance, in 0–10 cm soil, between months for seven sites over 13 "site-years". The monthly distribution of *P. nanus* at the present site, for 10–20 cm depth, showed maximum abundance when moisture was low and soil temperature high (January to March). Fisher (1967) observed a population peak of *P. nanus* females and males from an irrigated apple orchard in January at 10–20 cm depth when soil temperature was highest (*ca* 22°C minimum, 30°C maximum) with the minimum population occurring in August (*ca* 8°C min., 15°C max.). Fisher (1967) tested *P. nanus* survival in field soil within sealed glass jars and observed that survival was affected by soil temperature with good survival at 2, 5 and 25°C but a sharp decline in survival at 30 and –5°C. It would appear, therefore, that there was no direct temperature induced mortality of the *P. nanus* population at the present site as soil temperature neither exceeded 25°C, nor fell below 2°C. If soil temperature was not

limiting then the positive correlation that existed between temperature and P. nanus could be associative (e.g. reflecting host plant root growth rates) rather than causative.

At 0–10 cm depth maximum numbers occurred in January and again in April, when moisture had increased from its summer minimum (Fig. 5). Over the 36 months of sampling at 0–10 cm depth carried out by Yeates (1984b) in a summer moist soil, a "smoothed annual cycle" of *Paratylenchus* sp. showed a population peak during April and May with constant abundance over the remainder of the year. At the Kaitoke site of Yeates (1984b) mean annual abundance of *Paratylenchus* sp. was lower (ca. 150,000 *vs* 228,000 /m^2), annual rainfall was higher (1861–2277 mm), and 10 cm annual mean soil temperature cooler (11.1–12.4°C) than the site described here. A stable population (similar in magnitude to that observed here) was also observed for *Paratylenchus* at 0–10 cm depth in sandy loam at Rukuhia (*ca.* 25 km from this site) from nine months after sowing new pasture (Yeates and Barker, 1986).

It is possible that the negative correlation between *P. nanus* and soil moisture across seasons observed here (Table 2) is associative rather than causative, as correlations within some seasons were positive. Fisher (1967) observed a marked decline in survival of *P. nanus* at soil moistures below 8.4% in clay to clay loam soil. Similarly, Yeates (1978) observed in a summer dry site (Otikaieke, where soil moisture was below wilting point from January to April) that the lowest *Paratylenchus* sp. abundance (*ca* 50–100,000 /m^2) at 0–10 cm depth occurred from November to March before increasing in April. Irrigation at the Otikaieke site resulted in low numbers of *Paratylenchus* sp. (≥ 100,000 /m^2) occurring only in December and January (Yeates, 1978). Wood (1971) calculated a positive correlation between soil moisture and *P. projectus* and a negative correlation with soil temperature under tussock (*Festuca novae-zelandiae*) but this was due to the large proportion of the population which survived the cold intermontane winter (when soil moisture was high) as J4 individuals. Both seasonal and monthly sampling in the present work showed the various life stages of *P. nanus* were related to environmental variables in a similar manner to the population as a whole.

The observed correlations between *P. nanus* and some nutrients (Table 6) may have been mediated through plant growth (i.e. more soil P leads to increased root growth, increased root resource leads to an increase in root feeding biota). In the November sampling, for instance, the positive correlation between *P. nanus* and other grasses along with the negative correlation of other grasses with levels of soil P may have led to the observed negative association of *P. nanus* with this nutrient, especially as *Poa annua* was the dominant other grass and this is a host (although poor) for *P. nanus* (see Chapter 2). Yeates (1981) observed a significant positive relationship between total nematode numbers and soil P over eight sites and attributed the association to a causal relationship between soil P and herbage production and, via the size of root resource available, to nematode numbers.

The positive correlation of *Heterodera* juveniles with soil N (Table 7) could indicate an association between white clover (the principal host of *Heterodera* found in this pasture) and this nutrient. No significant correlation was observed between the migratory soil stage of *Heterodera* and clover dry weight at sampling (data not shown), suggesting that J2 *Heterodera* numbers were reflecting past clover levels. If abundance of juvenile *Heterodera* and *Meloidogyne* was indicative of white clover levels in pasture, it is possible the negative correlation between these nematodes and *P. nanus* could be the result of the non-host status of clover to *P. nanus* (see Chapter 2) rather than an inter-specific competition effect. It would appear likely that host root abundance, rather than nutrient status directly, is a determinant factor of *P. nanus* distribution where associations with nutrients exist.

The basal temperature for development (T_b) of 3.5°C calculated for *P. projectus* from published data appears to be too low for the *P. nanus* population observed in this study. When applied to activity index (AI) it produced a curve which was clearly overestimated in the cold and wet months of the year (August and September) (Fig. 6). It is likely that if more data were available on development rate of *P. nanus* or *P. projectus* then a more robust T_b could be calculated. A T_b of 10°C (estimated from the observed spring increase and autumn decrease in the *P. nanus* population), when applied to AI, gave a much better estimate of *P. nanus* population fluctuations. The error associated with calculating T_b from only two points, coupled with the observation that

the biological T_b is often several degrees higher than that calculated by back projection of the linear relationship between rate of development and temperature (see Trudgill, 1995a) suggests that a T_b of 10°C may be appropriate for *P. nanus*. Of the list of T_b given by Trudgill (1995b) for completion of one generation by plant parasitic nematodes the lowest and highest were 4.6 and 13.1°C for *Heterodera schachtii* and *Meloidogyne javanica* respectively.

Values of AI were strongly correlated with *P. nanus* for monthly samplings (Table 4) and shows that AI has utility as a partial means of understanding fluctuations in abundance from month to month. However, between years AI was less strongly correlated with *P. nanus* (Table 2), suggesting that other factors were also responsible for year to year variation. Jones (1975) related AI to the area of sugar beet stunted ("Docking disorder") by root ectoparasitic *Trichodorus* and *Longidorus* and found strong correlations across years. It is possible, in the present case, that damage due to *P. nanus* on pasture plants could similarly be predicted within a particular year if more accurate determinations of basal development temperature and plant damage functions were available (see McSorley & Phillips, 1993). Application of an AI to other ectoparasitic nematodes (*Paratrichodorus*, *Longidorus* and *Helicotylenchus*) in New Zealand pastures may also lead to predictions about their damage potential.

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Chapter 5: Population dynamics of *Paratylenchus nanus* in soil under pasture: II. Biotic factors and population modelling

Summary – Seasonal and monthly sampling of a *Paratylenchus nanus* population showed similar trends in biomass and abundance. A Population Age Index showed population age increased from a minimum in spring to maximum in winter. From mid-spring to mid-summer population age was less in 10–20 than 0–10 cm depth; this may have been due to a greater proportion of juveniles developing over this period at the deeper depth. Life stage data show a larger proportion of J4 *P. nanus* occurred at 10–20 cm than at 0–10 cm, which may reflect the smaller mass of host roots at 10–20 cm. Monthly sampling of patches of high *vs* low *P. nanus* density indicated that such patches retain their relative densities in most months. This may have contributed to the observed significant correlations between population numbers across some seasons and months. Evidence is presented for density-dependence in the *P. nanus* population at 0–10 cm and density-independence at 10–20 cm depth. Multiple regression models were fitted, taking into account all factors (abiotic and biotic) which were significantly associated with *P. nanus* abundance, and results are discussed in terms of population dynamics.

Keywords: *Paratylenchus nanus*, population dynamics, biomass, population age, life stages, regression model.

In common with all animals, nematode populations are constrained in their growth by factors internal and external to the population itself. Self-regulation of population growth in nematodes can include density-dependent effects such as reduced egg viability (Starr, 1988), competition for food (Boag, 1986; Noling & Ferris, 1986), overwinter survival (MacGuidwin & Forge, 1991), transition between development stages (Schmidt *et al.*, 1993), changed sex ratios (Trudgill *et al.*, 1967) and microbial parasitism (Ciancio & Bourijate, 1995). Production of non-feeding resistant stages in response to adverse conditions, e.g. J4 stage in *Paratylenchus projectus*, is another form of self-regulation (Rhoades & Linford, 1961). Biotic factors such as host plant availability, predation (Yeates & Foissner, 1995; Yeates & Wardle, 1996) and disease (Stirling, 1991) may each play a part in regulating nematode populations.

This is the second part of a study investigating a *P. nanus* population from soil under pasture. Internal and external biotic factors are examined and used, in conjunction with the significant abiotic factors already identified (Chapter 4), to develop regression models of the population.

Materials and methods

Sampling

Population dynamics of *P. nanus* were investigated from soil under grazed pasture. Details of site conditions and sampling methods are given in Chapter 4. Briefly, samples were collected on a seasonal (30 samples in each of eight consecutive seasons) and a monthly (10 samples in each of 10 consecutive months) basis. Plots for monthly samples were selected such that five plots had a high density of *P. nanus* over the previous two seasonal samplings, with the other five plots having had low density; further, all high density plots occurred on one half of the site and low density plots on the other.

Herbage was sampled from around each soil sampling point to estimate the amount of root from each herbage component that was present and available to plant parasitic nematodes. Herbage composition was determined at each sample point by cutting, at ground level, all foliage within a 15 × 15 cm quadrat. The foliage was separated into four categories: white clover (*Trifolium repens* L), ryegrass (*Lolium perenne* L), 'other grasses', and 'weeds'. It was then dried at 90°C for 48 h. Individual genera of 'other grasses' and 'weeds' were identified and their contributions to the total dry matter sample estimated visually.

Calculations

Mass of an individual nematode from each life stage of *P. nanus* was calculated using the formula of Andrássy (1956) (cited in Hooper, 1986):

$$\text{Body weight } (\mu g) = \frac{a^2 b}{1600000}$$

where a is greatest body width (μm) and b is body length (μm). Mean body dimensions of *P. nanus* were as calculated in Chapter 2. As the J2 and J3 stages were not separated during counting, the mean of J2 and J3 body weights was used in biomass calculations.

Biomass of the population at each sampling was calculated by multiplying the individual biomass for each stage by the number of individuals in that stage, then adding the biomass for each stage to obtain a population biomass.

As all post-hatching life stages of *P. nanus* were present at all sampling times the 'age' of the *P. nanus* population at each sampling was calculated using a Population Age Index (PAI). The mean number of individuals in each stage was multiplied by the stage number, the product from all stages added and then divided by the total number of individuals in the population i.e.:

$$\frac{([2.5(\text{no. J2+J3})] + [4(\text{no. J4})] + [5(\text{no. females})] + [5(\text{no. males})])}{\text{sum individuals in population}}$$

According to this index, populations comprised wholly of either J2+J3 or adults would have values of 2.5 and 5 respectively. Similar indices have been used for insect populations (e.g. Gerard & Burton, 1983) to attempt to compare different cohorts of larvae from the same population.

Data from sampling of high and low density patches were analysed by ANOVA after $\log_{10}$ (n+4) transformation. Horizontal distribution of *P. nanus* was analysed by calculating correlations amongst plots; abundance data was arranged in columns to reflect either east-west (upslope) or north-south (across slope) distribution. Interpolation was used to estimate abundance of *P. nanus* in unsampled plots, using the mean of the two immediately adjacent plots. Data were analysed using the autocorrelation function of Minitab (release 12.22).

Results

INTERNAL FACTORS

Stage-specific biomass estimates of *P. nanus* are presented in Table 1.

Table 1. *Mean maximum body width, body length and calculated body weight for* P. nanus *life stages*

Stage	Body width (μm)	Body length (μm)	Body weight (μg)
J2	8.7	157.0	0.007
J3	10.6	208.2	0.015
J4	14.4	277.8	0.036
Female	14.8	328.9	0.045
Male	14.8	356.8	0.049

Changes in the biomass (mg /m^2) of *P. nanus* for both seasonal and monthly samplings closely follows the abundance of *P. nanus* (see Figs 1, 2 and Chapter 4). Thus, biomass peaked in each summer (12.9 and 32.7 mg /m^2 in 0–10 and 10–20 cm depth respectively for summer Year 1) and was lowest in winter (2.1 and 5.9 mg /m^2 respectively for winter Year 1) (Fig. 1). In monthly samples the range of biomass of *P. nanus* was 2.1–18.1 mg /m^2 for 0–10 cm depth (August and April samples respectively) and 3.5–37.9 mg /m^2 for 10–20 cm depth (September and March samples) (Fig. 2).

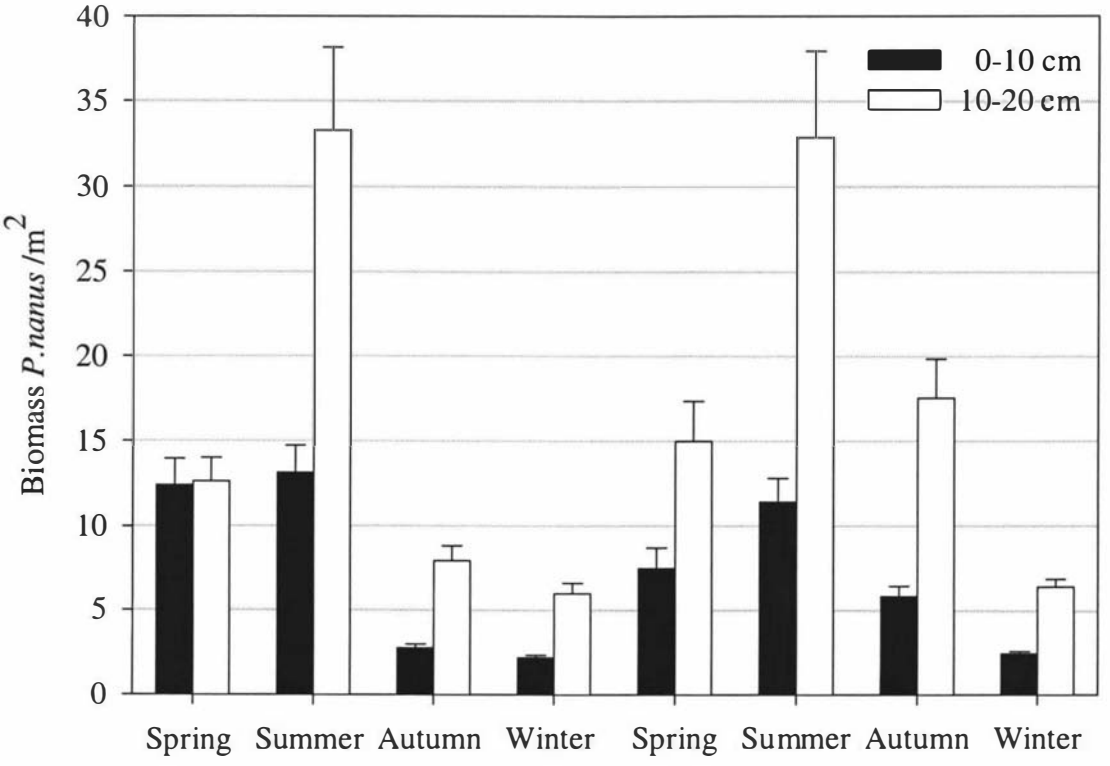


Fig. 1. *Seasonal biomass (mg) of* P. nanus /m^2 *in 0–10 and 10–20 cm depths from spring 1995 to winter 1997 (error bars are SEM).*

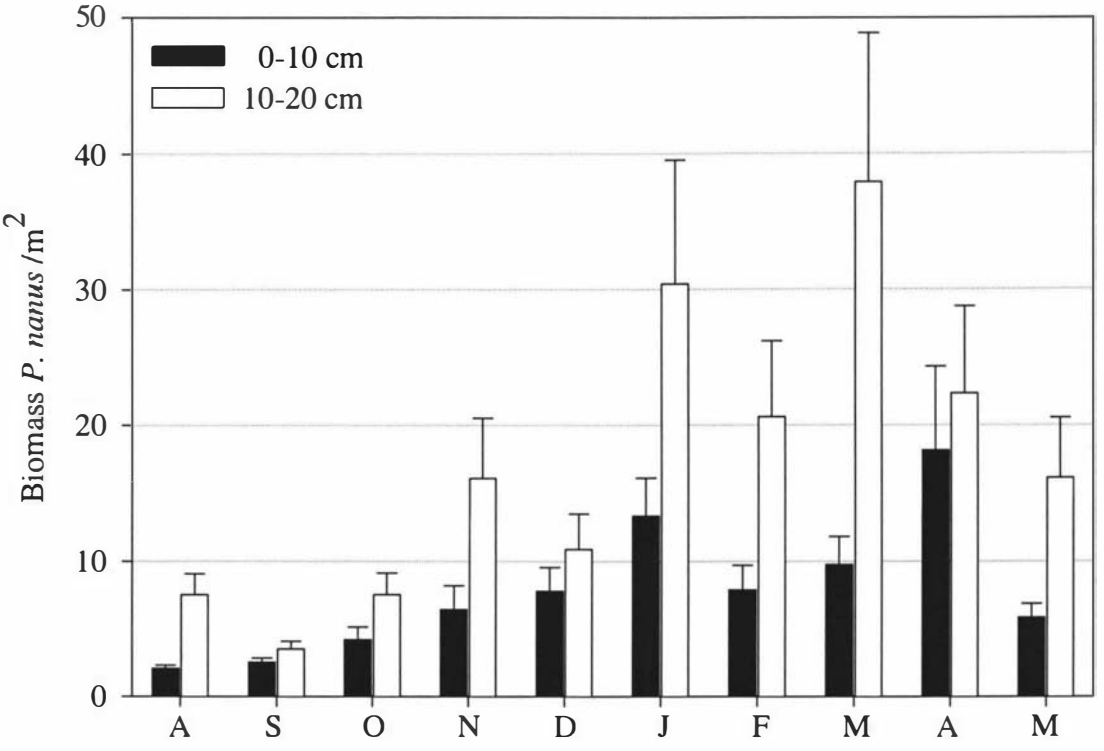


Fig. 2. *Monthly biomass (mg) of* P. nanus */m*^{2} *in 0–10 cm and 10–20 cm depths from August 1996 to May 1997 (error bars are SEM).*

The PAI of the *P. nanus* population observed here increased from a spring minimum to a winter maximum (Fig. 3); the value of the index is independent of population size. There was a large proportion of female *P. nanus* in winter populations and J2 and J3 stages in spring (data not shown). Population age was similar for 0–10 cm and 10–20 cm depths. Monthly samples show that population age decreased from an August (winter) maximum to a minimum from October (mid spring) to January (early summer) before increasing again through to May (late autumn) (Fig. 4). Population age in 0–10 cm depth was equal to or greater than that in 10–20 cm depth from November to February (late spring to mid summer) (Fig. 4).

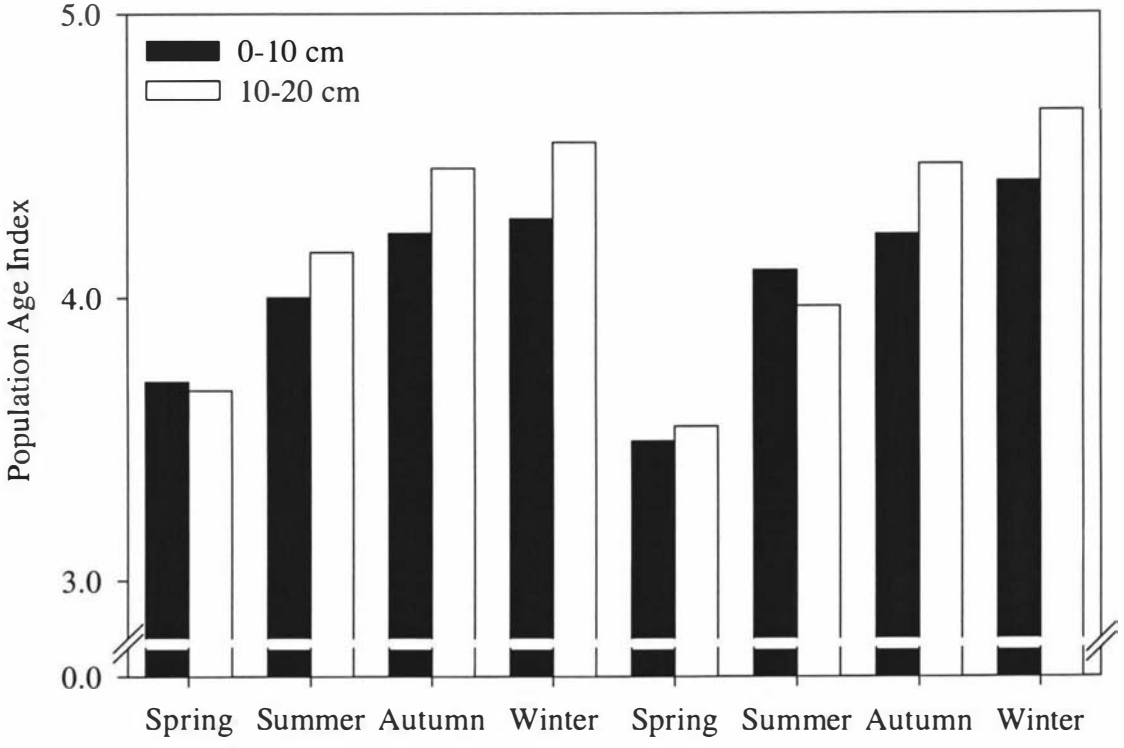


Fig. 3. *Seasonal* P. nanus *Population Age Index in 0–10 and 10–20 cm depths from spring 1995 to winter 1997.*

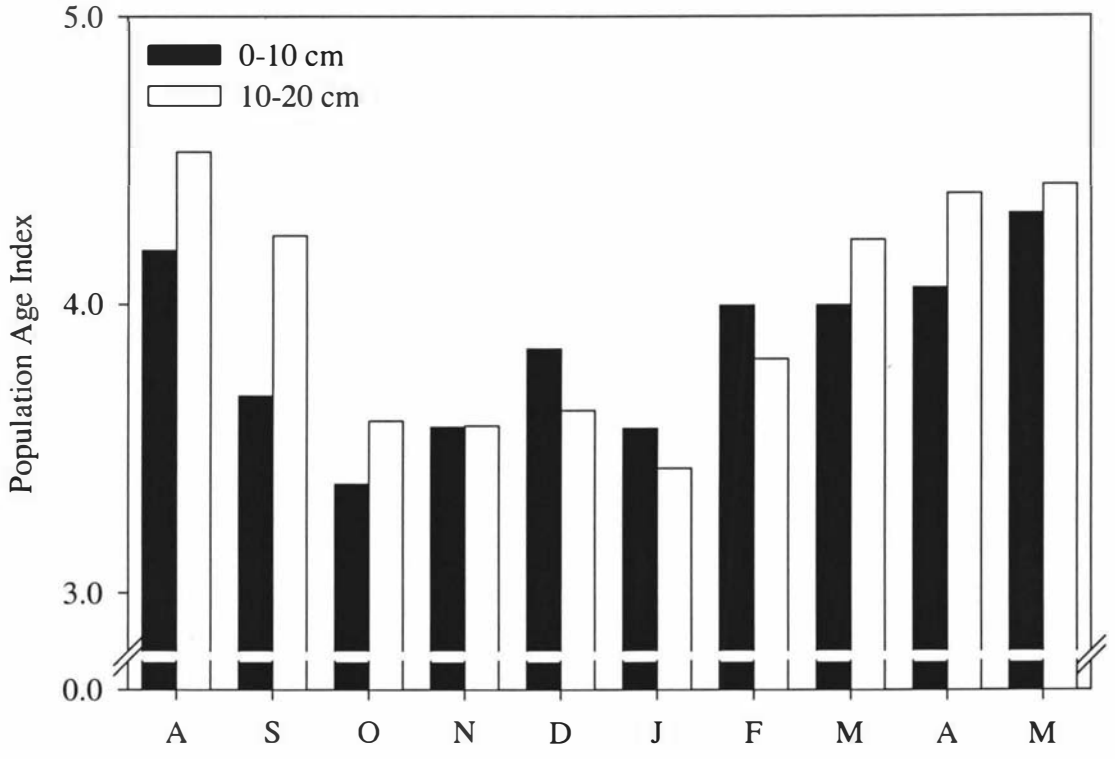


Fig. 4. P. nanus *Population Age Index in 0–10 cm and 10–20 cm depths from August 1996 to May 1997.*

Despite differences in numbers of *P. nanus* in each stage between years, the stage distributions were very similar within each depth in the two years. Therefore a generalised stage distribution was constructed (Fig. 5). Seasonally, there were differences in stage distribution between depths. For 0–10 cm depth there was a greater proportion of J2+J3 stages in autumn and winter compared to 10–20 cm depth (Fig. 5). There was a smaller proportion of J4's in spring, summer and autumn, and of female *P. nanus* in winter in 0–10 than 10–20 cm depth (Fig. 5). There was no discernable seasonal pattern in the proportion of males in the population. The mean male: female ratio for 0–10 cm depth was 1: 3.5 and for 10–20 cm depth was 1: 6.2. In contrast, the male: female ratio for monthly samples was higher in both 0–10 cm and 10–20 cm depths (1: 5.7 and 1: 11.5 respectively) (data not shown).

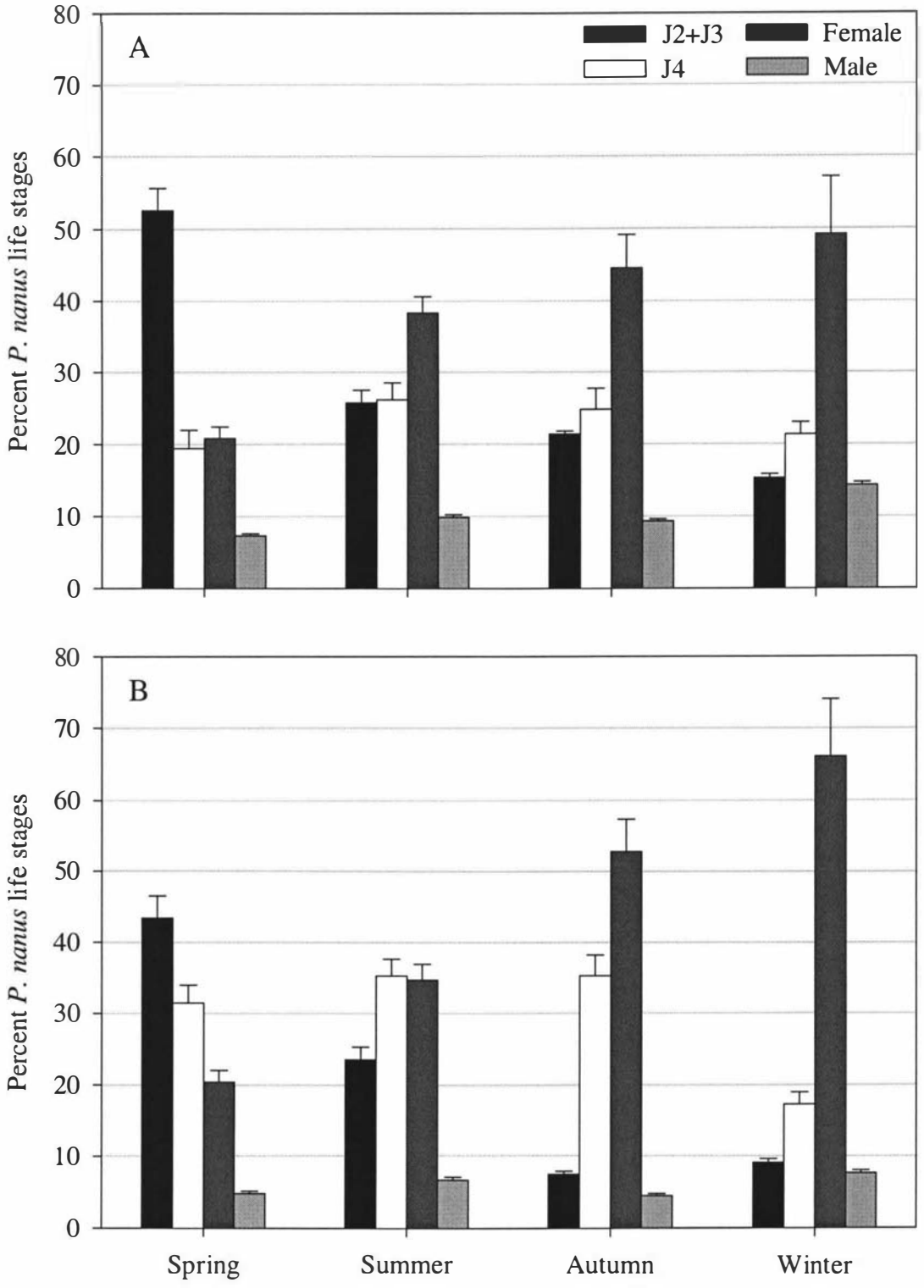
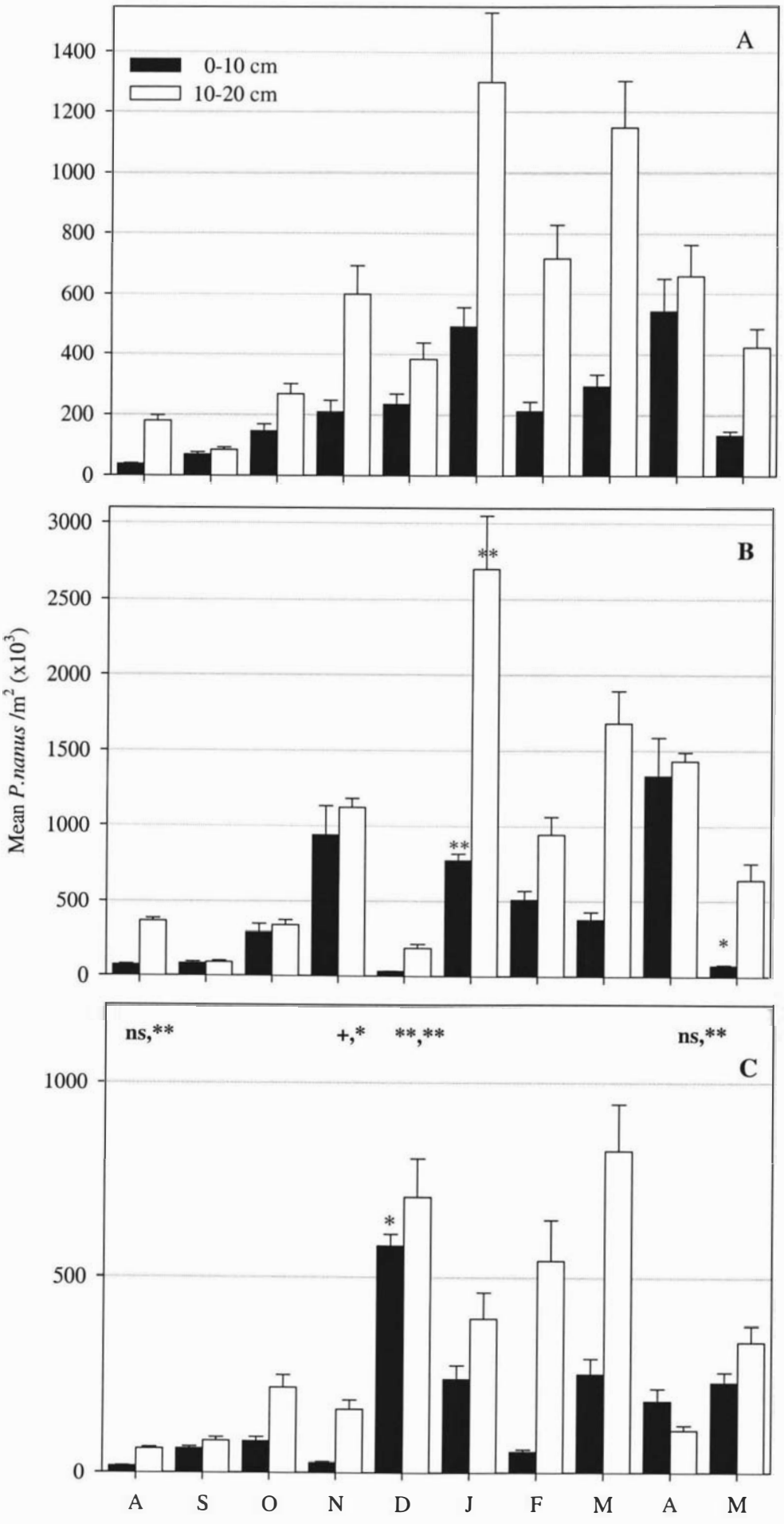


Fig. 5. *Generalised seasonal percentage of* P. nanus *in each life stage for: (A) 0–10 cm and (B) 10–20 cm depths (error bars are SEM).*

A large proportion of the monthly population arose from those plots initially selected as having high *P. nanus* density (Fig. 6). There were significantly more *P. nanus* in high density plots than in low density plots in August, November, February and April (Fig. 6B, C). Conversely, there was significantly more *P. nanus* in low density plots in December. Significant differences in numbers of *P. nanus* from one month to the next occurred in December–January and April–May for high density plots but only in November–December for low density plots (Fig. 6B, C). The significance of differences between high density and low density plots was the same for all life stages of *P. nanus* as for the total population (data not shown).

Fig. 6 (overleaf). *Mean monthly number of* P. nanus */m*^{2} *in 0–10 cm and 10–20 cm depths from August 1996 to May 1997 in: (A) all plots; (B) high density plots; (C) low density plots (error bars are SEM). (Note: symbols at head of Fig. 6C denote significance of differences between high density and low density plots for 0–10 cm, 10–20 cm depths. Symbols above error bars denote significance of differences between population at indicated month and the previous month).*


A
0-10 cm
10-20 cm
1400
1200
1000
800
600
400
200
0
B
3000
2500
2000
1500
1000
500
0
**
**
*
Mean P.nanus /m² (x10³)
C
ns,**
+,*
,
ns,**
1000
500
0
*
A
S
O
N
D
J
F
M
A
M

Analysis of the horizontal distribution of *P. nanus* by autocorrelation showed that abundance in one plot was more likely to be similar to an adjacent plot upslope than to more distant plots (Table 2) or to plots across slope (Table 3) (adjacent plots were separated by 15 m in each direction).

Table 2. *Correlation coefficients from autocorrelation of* P. nanus *abundance in 0–20 cm depth for nearest (Lag 1) and increasingly distant plots (Lags 2-4) in the east-west (upslope) direction (figures in bold are significant at P<0.05) (n=30).*

		Lag			
Year	Season	1	2	3	4
1	Spring	0.360	0.016	0.096	0.089
	Summer	**0.527**	**0.369**	0.175	–0.093
	Autumn	**0.475**	0.206	0.044	0.001
	Winter	0.256	0.131	0.078	0.064
2	Spring	0.342	–0.075	–0.093	–0.104
	Summer	0.256	0.026	0.055	0.012
	Autumn	**0.372**	0.102	0.003	–0.021
	Winter	**0.419**	0.131	–0.046	0.033

Table 3. *Correlation coefficients from autocorrelation of* P. nanus *abundance in 0–20 cm depth for nearest (Lag 1) and increasingly separated plots (Lags 2 and 3) in the north-south (across slope) direction (Note: lags 2 and 3 for winter Year 2 could not be calculated due to missing values) (n=30).*

		Lag		
Year	Season	1	2	3
1	Spring	–0.188	–0.167	–0.110
	Summer	–0.282	–0.166	–0.254
	Autumn	–0.109	–0.261	–0.128
	Winter	0.040	–0.234	0.093
2	Spring	–0.007	0.017	–0.062
	Summer	–0.040	–0.191	–0.115
	Autumn	0.073	–0.264	–0.008
	Winter	–0.086	—	—

Significant correlations between *P. nanus* populations at different sampling times were found (Tables 4 and 5). Some of the significant correlations were between one season and the next (e.g. summer–autumn and autumn–winter of Year 1) while others were between seasons with a large degree of temporal separation (e.g. spring Year 1–summer Year 2) (Table 4). The only contiguous monthly correlation was between November and December and this was the only negative association (Table 5).

Table 4. *Significant (P<0.05) Pearson's correlation coefficients (r) between abundance of* P. nanus *populations at various seasonal sampling times for 0–10 cm and 10–20 cm depths. Only rows and columns with significant r values are included.*

	Depth	Spring Year 1	Summer Year 1	Autumn Year 1	Winter Year 1
Autumn Year 1	10–20 cm	—	0.407	—	—
Winter Year 1	10–20 cm	—	—	0.415	—
Spring Year 2	0–10 cm	—	—	0.364	0.474
Spring Year 2	10–20 cm	—	0.461	—	—
Summer Year 2	0–10 cm	0.400	—	—	—
Summer Year 2	10–20 cm	—	—	0.441	—

Table 5. *Significant (P<0.05) Pearson's correlation coefficients (r) between* P. nanus *populations at various monthly sampling times for 0–10 cm and 10–20 cm depths. Only rows and columns with significant r values are included.*

	Depth	August	September	November	February	March
November	0–10 cm	0.891	—	—	—	—
December	0–10 cm	—	—	–0.634	—	—
March	10–20 cm	—	0.793	0.753	—	—
April	0–10 cm	—	—	—	0.744	—
April	10–20 cm	0.858	—	0.753	—	—
May	10–20 cm	—	0.798	—	—	0.700

The relationship between the population of *P. nanus* from one month to the next was further explored by plotting population at any one month (P_i) against the population at the next month (P_f) (Figs 7 and 8). For 0–10 cm depth the relationship is described by

a log normal curve (r = 0.948, P<0.01) such that: $Pf = Pf_0 + ae^{\left[-0.5(\ln(Pi/Pi_0)/b)^2\right]}$ and a = 432.64 (ln a = 6.07) and b = 0.25. The P_i : P_f relationship showed that P_f increased with increasing P_i up to a P_i of *ca* 300 *P. nanus* /m^2 after which P_f decreased with increasing Pi (Fig. 7). At very low P_i, there was no relationship between P_i and P_f. There was no significant relationship between P_i and P_f in 10–20 cm depth (Fig. 8).

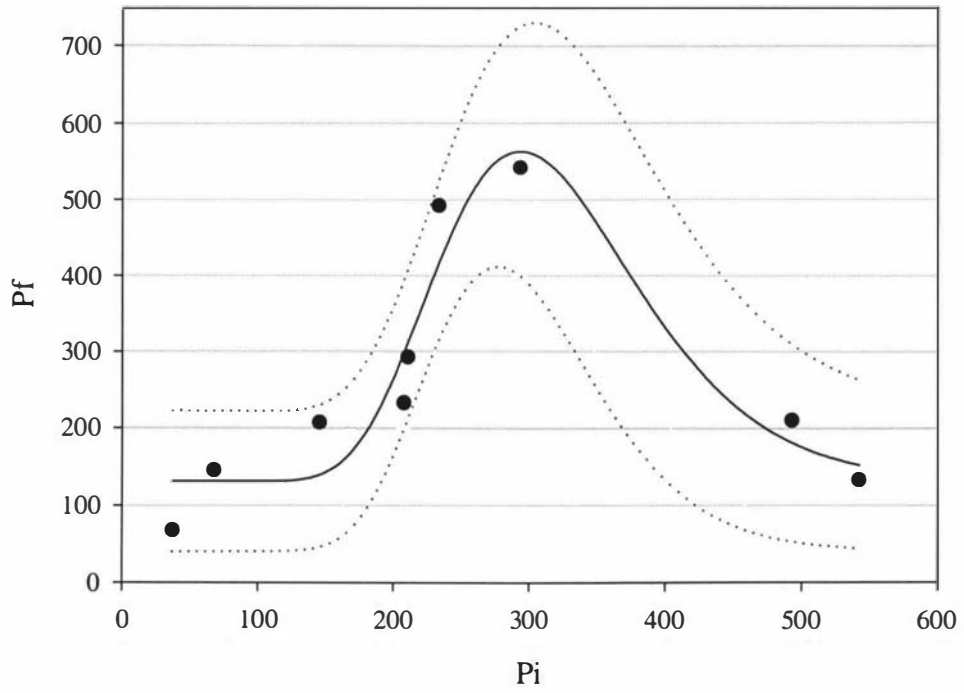


Fig. 7. *Log normal curve fitted to plot of* P. nanus *population numbers /m^2 (x10^3) in one month* (P_i) *and the next* (P_f) *in 0–10 cm depth from August 1996 to May 1997. Graph points are not sequential by month (dotted lines are 95% confidence interval).*

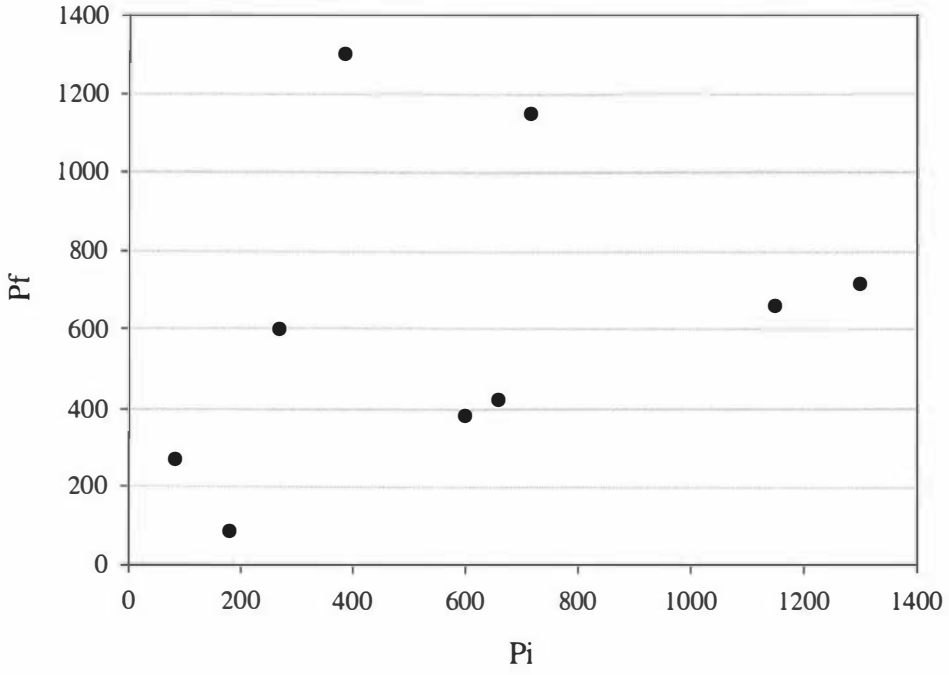


Fig. 8. *Plot of* P. nanus *population numbers /m^2 (x10^3) in one month* (P_i) *and the next* (P_f) *in 10–20 cm depth from August 1996 to May 1997.*

EXTERNAL FACTORS

Pasture composition was dominated by ryegrass (*L. perenne*) and 'other grass', although white clover (*T. repens*) was a major pasture component in 1997 (Fig. 9). 'Other grass' consisted of *Poa annua* L., *P. trivialis* L. and summer grass (*Digitaria sanguinalis* (L) Scop.) with *P. annua* comprising 69.7 to 97.0% of 'other grass' composition over all seasonal samplings, except for summer 1996 when summer grass constituted 90.7% of 'other grass'. The major components of the weed flora were chickweed (*Stellaria media* (L.) Vill.), dandelion (*Taraxacum officinale* Weber) and narrow-leaved plantain (*Plantago lanceolata* L.). Comparisons between seasons across years showed significant differences in the proportions of: white clover, ryegrass and 'other grass' in summer ($P<0.001$); white clover and ryegrass in autumn ($P<0.05$); and white clover and 'weeds' in winter ($P<0.001$) (Fig. 9).

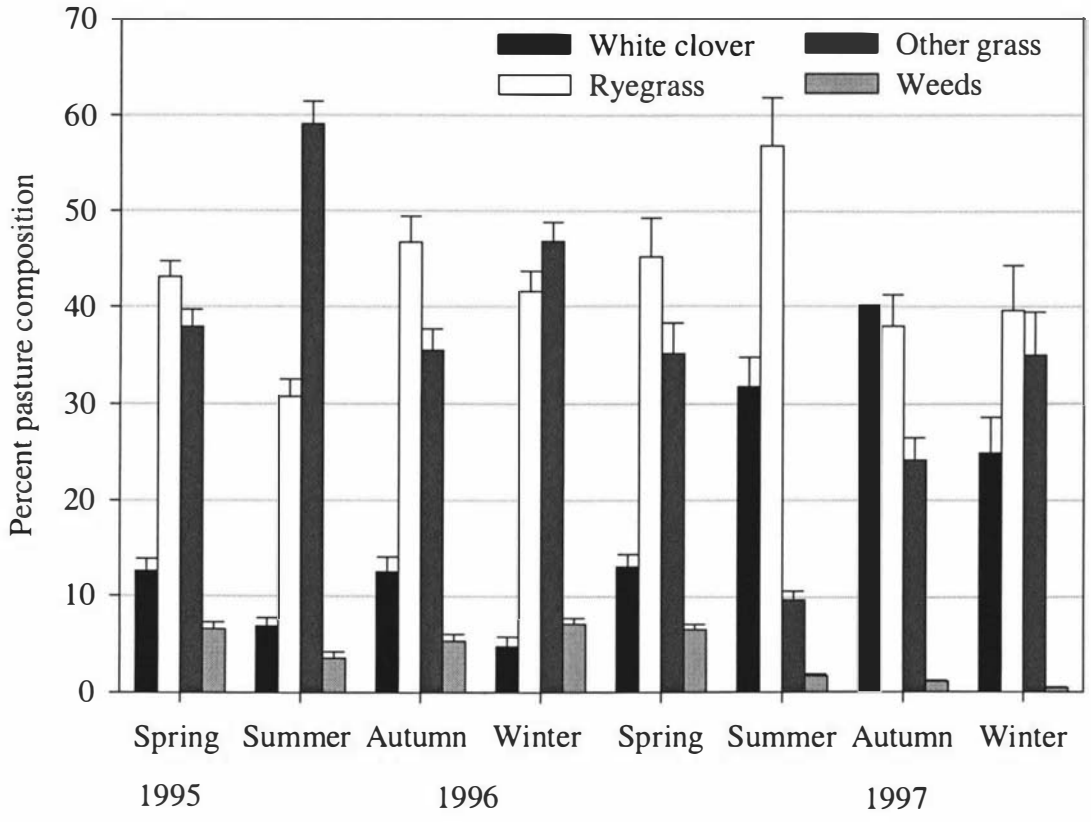


Fig. 9. *Seasonal pasture composition for the seasons spring 1995 to winter 1997 (error bars are SEM).*

The total herbage dry weights for the eight seasonal samplings were 1182, 1800, 800, 342, 1062, 1422, 618 and 262 kg /ha respectively. Although seasonal samplings were taken to coincide with mid-season rather than grazing time, sampling was delayed or advanced to avoid sampling immediately before or after grazing. Thus while not representing actual growth rates these results indicate the pasture growth patterns at this site during this period; greatest dry weights occurred in summer of both years and least in winter. Dry weight does not necessarily reflect pasture quality, as evidenced by the high proportion of poor quality summer grass in the summer of Year 1 (Fig. 9).

Numbers of *Pratylenchus*, non-plant parasitic nematodes (Appendix 1) and *Poa* spp. dry weight (as a proportion of total herbage dry weight) were significantly correlated with the abundance of *P. nanus* for both seasonal and monthly samplings (Tables 6 and 7). For seasonal sampling, ryegrass and total herbage dry weights were significantly correlated with *P. nanus* abundance (Table 6). Numbers of *Helicotylenchus* sp. and clover dry weight were significantly correlated with *P. nanus* abundance for monthly sampling (Table 7).

Table 6. *Pearson's correlation coefficient (r) between log (n+4)* P. nanus *and biotic factors for the seasonal samples spring 1995 to winter 1997 (n = 8)) (†, *, ** and *** denote P<0.1, 0.05, 0.01 and 0.001 respectively). Note: Percent* Poa *contribution = estimated* Poa *dry weight as a proportion of total herbage dry weight.*

Biotic variable	Log *P. nanus*	
	0–10 cm	10–20 cm
Log *Pratylenchus*	0.823 *	0.790 *
Non-plant parasitic nematodes	0.694 †	0.778 *
Ryegrass dry weight	0.846 **	0.870 **
Percent *Poa* contribution	–0.678 †	–0.819 *
Total herbage dry weight	0.918 **	0.940 ***

Table 7. *Pearson's correlation coefficient (r) between log (n+4)* P. nanus *and biotic factors for the months August 1996 to May 1997 (n = 10) (see Table 6 for explanation of symbols).*

Biotic variable	Log *P. nanus*	
	0–10 cm	10–20 cm
Log *Pratylenchus*	0.725 *	0.310
Log *Helicotylenchus*	–0.533	–0.745 *
Non-plant parasitic nematodes	0.674 *	0.789 **
Clover dry weight	0.673 *	0.791 **
Percent *Poa* contribution	–0.668 *	–0.740 *

POPULATION MODELLING

Multiple regression analysis was used to determine which of the abiotic and biotic factors mentioned above and in Chapter 4, best described the observed variation in the *P. nanus* population. Only factors shown above to be significantly correlated with *P. nanus* were considered. For seasonal *P. nanus* populations at 0–10 cm depth, once total herbage dry weight was included in the regression model no other factor added significantly to the proportion of variation described by the regression. Total herbage dry weight accounted for 86.4% (r^2) of the variation in *P. nanus* numbers and gave a significant ($P<0.001$) regression such that:

$$\log P.\ nanus = 1.04 + 0.28(\text{total herbage dry weight}).$$

A similar regression model applied to seasonal *P. nanus* populations at 10–20 cm depth, where total herbage dry weight gave a significant regression ($P<0.001$), accounting for 88.4% of *P. nanus* variation. No other factor could be added to the regression which had significant influence on the model. The regression equation for 10–20 cm depth is:

$$\log P.\ nanus = 1.58 + 0.23(\text{total herbage dry weight}).$$

For the monthly sampling at 0–10 cm depth four factors (10 cm earth temperature; 10 cm Activity Index (AI, a developmental temperature × rainfall index, see Chapter 4); *P. nanus* in 0–10 cm depth the preceding month (P_i); *Pratylenchus* at 0–10 cm depth) gave a significant ($P<0.001$) regression which accounted for 98.2% of the variation in numbers of *P. nanus*. As the AI was partly derived from earth temperature, temperature

was omitted from the analysis, in which case the remaining three factors gave a significant ($P<0.01$) regression accounting for 93.4% of variation. Each of the three factors had significant ($P<0.05$) influences on the regression:

log *P. nanus* = –0.487 + 0.007(AI) + 0.761(log *Pratylenchus*) + 0.448(*P. nanus* P_i).

For *P. nanus* at 10–20 cm depth only *Helicotylenchus* and non-plant parasitic nematodes contributed to the regression equation. Each individual factor had a significant ($P<0.05$) influence on the regression equation. The equation accounted for 80.6% of *P. nanus* variation and was significant ($P<0.01$):

log *P.nanus* = 3.12 –2.31(log *Helicotylenchus*) + 0.0003(non-plant parasitic nematodes).

Discussion

Biomass (mg /m^2) of *P. nanus* (Figs 1 and 2) in all seasonal and in all but one monthly sample closely followed abundance (see Chapter 4 and Fig. 6). For combined depths in the March monthly sample, biomass was higher than would be expected from abundance and this was due to the relatively high proportion of female *P. nanus* in the population (as indicated by Figs 4 and 5). The biomass of seven of the 15 most common nematode species recorded by Yeates (1973) from a beech forest followed abundance very closely over the course of 12 months. The plant parasite *Rotylenchus fallorobustus*, in common with *P. nanus*, had only one month when biomass varied from that expected by abundance and this was a month when the population contained a high proportion of juveniles (Yeates 1973a). Species that exhibited the greatest variation between biomass and abundance included the bacterial feeding *Plectus assimilis* and *P. parietinus*, and the omnivorous *Aporcelaimus superbus*.

The Population Age Index (PAI) described here for *P. nanus* (Figs 3 and 4) reflected life stage distribution independent of abundance, permitting comparisons between sample times where abundance differs greatly. On a seasonal basis, the population age calculated for *P. nanus* in this study increased from spring through to winter. In monthly samples, age declined through spring and summer then increased again from autumn. MacGuidwin & Stanger (1991) used a similar index (based on life stage proportions rather than abundance and termed Index of Population Maturity) to describe changes in the age of *Pratylenchus scibneri* from roots and soil under potato and corn. They found that, in general, population age was greatest early and late in the

growing season (i.e. spring and autumn respectively) and declined mid-season (summer), similar to results from the present study.

Both the monthly and seasonal PAI trends seemed to follow the likely trends in root growth (see Baars, 1976 for seasonal herbage data). Trudgill (1995) suggests that the stage structure of a nematode population is indicative of the status of their host plant as a food source i.e. when well fed the population will be dominated by juveniles as egg production will be high. On a monthly basis population age in 0–10 cm depth was higher in late spring and summer than for 10–20 cm depth. This may, therefore, be due to the roots at 10–20 cm being a superior resource to that at 0–10 cm, at least in terms of egg production.

Although all stages of *P. nanus* are present at all times of the year (Fig. 5) and the likely generation time is *ca* one month (see Rhoades & Linford, 1961 and Wood, 1973 for *P. projectus*), the population observed here displayed a generalised stage distribution akin to a population with one (or possibly two in 0–10 cm depth) generation per year. It may be that *P. nanus* population models could be developed which used parameters normally applied to univoltine or bivoltine populations. It is interesting to note the winter "bottleneck" in *P. nanus* abundance observed at this site (see Chapter 4) which may reflect adaptation to a continental climate where very cold winters would mitigate against population development from autumn to spring and hence the population would exhibit an obligatory winter decline. It may be that at the northern and southern limits of its worldwide distribution *P. nanus* has a single generation annual lifecycle.

The generalised seasonal *P. nanus* stage distribution (Fig. 5) showed a larger proportion of J4 individuals at 10–20 cm than 0–10 cm depth in spring, summer and autumn, and lower proportions of J2 and J3 stages in autumn and winter. If presence of host plant roots determine moulting of J4 to adults in *P. nanus*, as shown by Fisher (1966), it may be that there were fewer ryegrass roots at the deeper depth (see also Chapter 6). Lesser amounts of ryegrass roots in 10–20 cm depth may also explain the lower proportion of J2+J3 at this depth in the autumn and winter samples.

Fisher (1967) observed *P. nanus* around apple roots in South Australia and found a decrease in the proportion of adults in winter and a subsequent increase to almost 50%

of the population in summer. In the population observed here the proportion of *P. nanus* adults was lowest in spring with a consistent increase from spring to winter (Fig. 5). Fisher (1967) also found numbers of J4 stage *P. nanus* always exceeded numbers of adults but this was not the case here. Fisher (1967) suggested that the dominance by J4 in his population was due to dormancy of host roots so it may be that, in the population observed here, there were sufficient active host roots all year round to prevent dominance by J4 stages.

The percentage of males observed for *P. nanus* in this study was 6–17% in 0–10 cm and 4–8% in 10–20 cm depth for seasonal samples. Proportions of males greater than that of females have been recorded for *P. nanus*, and they may comprise up to 95% of the adult population (Fisher, 1965). If male *P. nanus* are produced in reaction to adverse conditions, as has been shown for other plant parasitic nematode genera (see Yeates, 1987), it would appear that, in the population observed here, such adverse conditions were not present, although there was a slight increase in proportion of males in winter at 0–10 cm depth.

From the present sampling programme it appears that patches of high and low *P. nanus* density were present consistently over the majority of a season (Fig. 6). This was apparent not only from the high and low density plots that were deliberately chosen but also by the correlations between *P. nanus* populations across seasons (Table 4). It also appears that high and low density patches were elongated in the upslope direction (Table 2), similar to the elongated horizontal distribution observed by Ploeg (1998) for *Longidorus africanus* on bermudagrass where patches were up to 15 m long. The 15 m separation between plots in the current study is greater than the 10 m used by Wallace & Hawkins (1994) but in their study nematode patches were observed to extend from 40 (for *Heterodera trifolii*) to 160 m (for *Aglenchus agricola*). The horizontal distribution of *P. nanus* observed in this study has implications for all studies on *P. nanus* in as far as the study area may need to be mapped before commencing study. Blocking and co-variance may need to be considered when designing and analysing field experiments with initial *P. nanus* density used as a covariate.

Sampling from an area that includes a wide range of population densities is an experimental technique to reduce the influence of sample error on density-dependence

calculations (Duncan & McSorley, 1987). The samples used for density dependence calculations in this study were deliberately selected to have the broadest population range for *P. nanus*. Duncan & McSorley (1987) used the logistic model: $P_f = cP_i^{(1-b)}$ to describe the effect of initial (P_i) on final (P_f) population abundance of *Tylenchulus semipenetrans* where the parameters *c* and *b* are scaling and rate determining variables respectively. The values of the parameters ln *a* (6.07) and *b* (0.25) from the current study (Fig. 7) are of a similar magnitude to ln *c* (1.28–4.03) and *b* (0.20–0.60) calculated by Duncan & McSorley (1987) for the total population range of *T. semipenetrans* on citrus roots.

The significant positive correlation between *P. nanus* and *Pratylenchus* at 0–10 cm depth in monthly samples (Table 6) may be due to both nematodes using the same food resource at least some of the time as in the district *Pratylenchus* has been reported to feed on both ryegrass and white clover roots (Yeates *et al.*, 1985). Density-dependence of *P. nanus* populations at 0–10 cm depth cannot, however, be wholly explained by increased interspecific competition at this depth as soil populations of *Pratylenchus* were similar at 0–10 and 10–20 cm depths (Appendix 1) and there was no significant difference in soil abundance of *Pratylenchus* between high and low density *P. nanus* patches (data not shown). It is possible that nematode-pathogenic microbes are, at least in part, responsible for the observed density-dependence at 0–10 cm. For example, non host specific microbes such as nematode trapping fungi (e.g. *Arthrobotrys* and *Monacrosporium*) (Viaene & Abawi, 1998) would be able to proliferate to a greater extent at 0–10 cm depth where the levels of organic matter and associated abundance of non-plant parasitic nematodes is 2-3 × those in 10–20 cm depth (Appendix 1). The significant positive correlation with non-plant parasitic nematodes at both depths (Tables 6 and 7) may be due to exploitation of some common abiotic factor such as moisture or soil pores. It is possible that lower numbers of total nematodes at 10–20 cm depth results in *P. nanus* being density-independent at that depth (Fig. 8), hence allowing greater numbers of *P. nanus* to develop, despite a smaller root resource than in 0–10 cm depth.

Interspecific competition may be an important determinant of *P. nanus* population increase. Boag & Alphey (1988) observed large increases in the numbers of *P. nanus* after nematicide application had eliminated its main competitor (*Rotylenchus robustus*)

from soil beneath a *L. perenne* /*T. repens* sward. Yeates & van der Meulen (1996) observed similar increases when *Paratylenchus* sp. recolonised a soil under pasture after methyl bromide fumigation reduced competition by *Helicotylenchus* sp. [probably *H. pseudorobustus*, see Yeates *et al.* (1991) and Yeates & Wouts (1992)]. Conversely, *P. nanus* populations have been prevented from recovering in a favourable soil planted to pasture after long term maize cropping, where high populations of five other plant parasitic nematode genera are present and in which *P. nanus* is the dominant plant parasitic nematode in adjacent long term pasture (Watson & Bell, unpub. data). Interspecific competition by *Criconemoides simile* and *H. pseudorobustus* has been shown to affect population growth of *P. projectus* (McGawley & Chapman, 1983) when inoculated into soil with soybean.

The main competitor of *P. nanus* (in terms of numbers present) in this soil was *Pratylenchus* (Appendix 1) which utilises some of the same food resource. *Pratylenchus* feeds on both ryegrass and white clover roots with clover probably being favoured due to the higher nitrogen content of its roots (Whitehead, 1983). Competition between *P. nanus* and *Pratylenchus* may therefore be mediated by pasture composition (i.e. host abundance). A trial to investigate the effects of phosphorus (low and high rates), lime or no lime and stocking rate (low and high) on pasture production (Bircham & Crouchley, 1976) was sampled for nematodes by Yeates (1976). He found that *Pratylenchus* abundance in soil increased fourfold under high rates of P and lime and by 1.5 × under high stocking rate. *Paratylenchus* abundance, however, increased only under the lime treatment (Yeates, 1976). The high P treatment increased pasture production but the composition remained ryegrass dominant (*ca* 48% content), high stocking rate increased ryegrass content from 37 to 49% and lime increased white clover content of the pasture from 20 to 26% (Bircham & Crouchley, 1976). It is suggested that the increased clover content (and presumably clover root resource) in the lime treatment was utilised preferentially by *Pratylenchus*, allowing *Paratylenchus* to feed on a proportionately larger ryegrass root resource. In the current study, the positive correlation between *P. nanus* and white clover on a monthly basis (Table 7) may be a reflection of a similar effect on resource competition with *Pratylenchus*.

Herbage was sampled in the current study to indicate the amount of plant roots present. Changes in seasonal pasture composition, and presumably host root abundance,

between years may influence plant parasitic nematode populations in succeeding seasons. Therefore, the observed significant differences in summer pasture composition between years, with summer grass dominance in Year 1 and ryegrass dominance in Year 2 (Fig. 9) may have contributed to the significantly increased autumn *P. nanus* population in Year 2 (see Chapter 4). Similarly, the increased abundance of *Pratylenchus* in the summer of Year 2 may have been due to the large increase in combined white clover and ryegrass content of the pasture compared to summer of Year 1 (see Appendix 1).

As expected from *P. nanus* host range testing which showed that grasses (including ryegrass) are the only pasture hosts of this nematode (Chapter 2), positive correlations between this nematode and ryegrass were observed in the seasonal samples (Table 6). Ryegrass root replacement rate exhibits significant seasonal fluctuations, with summer maxima and winter minima (Matthew *et al.*, 1991). This trend mirrors the temporal distribution of *P. nanus*. It was observed by Matthew *et al.* (1995) that ryegrass tiller weight, net leaf production and levels of herbage nutrients were higher in patches of pasture that contained clover than those that did not. It is possible, then, that the positive correlation between *P. nanus* and white clover on a monthly basis was a response not only to *Pratylenchus* competition but also to increased quality of the ryegrass root resource. The negative correlations between *P. nanus* and *Poa* spp. abundance could be due as much to the plants temporal distribution as to its poor host status (i.e. *Poa* spp. are cool season grasses and are at their greatest abundance in winter and early spring).

The multiple regression models calculated here indicate that, of the factors measured in this study, total herbage dry weight accounted for 86–88% of the variation in *P. nanus* numbers on a seasonal basis. *P. nanus* responded to seasonal root resource more strongly than to other factors; no other factor could be added to the model such that significantly more variation could be accounted for. It is possible that the long period between samplings (*ca* two months) introduced variation in *P. nanus* population estimates, which could not be accounted for by the measurements conducted (other than herbage dry weight).

On a monthly basis, the *P. nanus* population at 0–10 cm depth could be described by the combination of activity index, *Pratylenchus* numbers and initial *P. nanus* numbers. This combination represents both abiotic and biotic factors, including the density-dependent nature of the *P. nanus* population itself. At 10–20 cm depth, however, a much lower proportion of the monthly variation in *P. nanus* numbers was accounted for by the factors measured, and the two significant factors both implicated other nematodes (*Helicotylenchus* and non-plant parasitic nematodes). The apparent density-independent nature of *P. nanus* at this depth coupled with the low r^2 value of the factors measured indicates that some other factor is playing a role in regulating *P. nanus* numbers at 10–20 cm depth.

Many nematode population models have been developed for use in agricultural systems (see Ferris & Noling, 1987, Trudgill *et al.*, 1996). Viscardi & Brzeski (1992) developed a simulation model for the population dynamics of *P. bukowinensis* in parsley crops in Poland. Their main parameters were P_i, P_f, logistic equilibrium (E), and constants associated with plant growth and yield in relation to nematode abundance. There is still insufficient information about the biology (i.e. predators, diseases) and plant damage potential of *P. nanus* to develop such a model for pastures, but this study indicates some areas which may be fruitfully investigated when such a model is developed.

APPENDIX 1

Seasonal abundance ($x10^3$ /m^2) (± SEM) of plant parasitic (other than *P. nanus*) and non-plant parasitic nematodes in 0–10 and 10–20 cm depths for the seasons Spring 1995 to Winter 1997. (*, **, *** denote P<0.05, 0.01, and 0.001 respectively for the difference between comparable seasons across years).

	Year 1				Year 2			
Nematode	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
Pratylenchus								
0–10 cm	290.1 (66.2)	344.5 (84.9)	235.8 (58.8)	149.3 (29.9)	327.8 (78.0)	510.7 (101.1)	272.6 (61.7)	130.6 (33.2)
10–20 cm	389.9 (74.3)	372.4 (72.0)	351.9 (87.2)	143.9 (28.0)	367.3 (75.7)	727.4 (80.3)***	466.3 (112.3)	234.6 (40.2)
Meloidogyne								
0–10 cm	83.1 (19.8)	285.7 (102.1)	50.1 (10.9)	51.2 (11.6)	52.8 (13.0)	287.1 (82.2)	118.9 (35.4)	332.1 (75.4)***
10–20 cm	52.1 (15.1)	72.0 (19.6)	67.3 (20.1)	35.2 (9.2)	20.1 (3.9)	139.5 (50.4)	77.1 (25.1)	60.3 (15.0)
Heterodera								
0–10 cm	83.1 (18.6)	46.2 (11.1)	20.0 (3.1)	40.9 (7.9)	32.3 (5.8)**	58.1 (15.1)	44.7 (11.2)	162.0 (31.9)***
10–20 cm	13.1 (1.7)	14.4 (2.5)	12.6 (1.4)	10.4 (0.8)	9.9 (0.7)	14.0 (2.0)	11.4 (1.2)	21.9 (3.5) ***
Non-plant parasites								
0–10 cm	7158.1 (410.2)	5774.0 (351.5)	6218.2 (370.9)	4012.4 (207.7)	5528.9 (459.3)**	7407.3 (568.6)*	4732.3 (332.6)***	4735.4 (293.3)
10–20 cm	2920.0 (238.4)	2976.2 (274.3)	1654.3 (153.4)	1132.0 (86.7)	2024.7 (184.3)**	3823.6 (243.4)*	2180.4 (235.7)	2383.6 (157.4)***

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Chapter 6: Damage potential of *Paratylenchus nanus* to some of its grass hosts: I. Effect on seedlings in the glasshouse

Summary – Seeds of five grasses: perennial ryegrass (cv Grasslands Nui) infected with wild-type endophyte (*Neotyphodium* sp.) (E+); a selected endophyte (AR37+); and no endophyte (E-); Italian ryegrass and cocksfoot (both without endophyte infections) were sown into tubes of field soil in a waterbath at 20°C and inoculated with one of three rates of *Paratylenchus nanus*. The rates of *P. nanus* inoculum related to field populations of *P. nanus* found in late winter (low rate), early spring or early autumn (medium rate) and summer (high rate). Nematode and plant data were collected 34, 55, 74 and 88 days post-inoculation (dpi). There was a deleterious effect of the high rate of *P. nanus* inoculum on shoot dry matter only for perennial ryegrass infected with the selected endophyte. E+ produced more shoot matter over all rates and times than both AR37+ and E-. Effects of *P. nanus* on seedling root: shoot ratio and reproductive rates of *P. nanus* are given. Implications for field sowing of these grasses in the presence of *P. nanus* populations are discussed.

Keywords: *Lolium perenne*, *Lolium multiflorum*, *Dactylis glomerata*, *Neotyphodium sp.*

In a greenhouse screening trial (Chapter 2) three pasture grass hosts of *Paratylenchus nanus* were determined: perennial ryegrass (*Lolium perenne* L.), Italian ryegrass (*L. multiflorum* L.) and cocksfoot (*Dactylis glomerata* L.). These species are all widely used in improved New Zealand pastures. Perennial ryegrass is the most commonly sown grass in the country, usually in association with white clover (*Trifolium repens* L.). Perennial ryegrass is sown as a permanent pasture due to its ease of establishment and management in medium to high fertility soils. Part of the reason for the persistence of perennial ryegrass is the association it can form with the symbiotic fungus *Neotyphodium lolii* (Latch, Christensen and Samuels) Glenn, Bacon and Hanlin (formerly *Acremonium lolii*) which produces alkaloids responsible for insect resistance (see Popay & Rowan, 1994).

Evidence for resistance to plant parasitic nematodes mediated by *Neotyphodium* spp. endophytes is equivocal. For perennial ryegrass Cook *et al.* (1991) found no effect of *N. lolii* infection on *Meloidogyne naasi* infection, whereas Stewart *et al.* (1993) and Ball *et al.* (1997a) observed fewer female *M. naasi* and *M. marylandi* respectively on

endophyte-infected than endophyte-free plants. The situation is clearer in tall fescue (*Festuca arundinacea* Schreb.) where several plant parasitic nematodes are adversely affected by grass infection with *N. coenophialum* (Morgan-Jones and Gams) Glenn, Bacon and Hanlin (Pedersen *et al.*, 1988; West *et al.*, 1988; Kimmons *et al.*, 1990).

Italian ryegrass is sown in medium to high fertility soils for its ability to provide increased production during late winter and early spring, compared to perennial ryegrass (Thom & Prestidge, 1996). It lacks infection by *Neotyphodium* sp. endophytes and is susceptible to insect attack, which can reduce its survival over summer (Prestidge *et al.*, 1994). Cocksfoot is suited to dryland pastures with medium to low soil fertility (Rumball, 1982; Barker *et al.*, 1993; Scott *et al.*, 1996) and appears to be tolerant of root feeding by the native "grass grub" (*Costelytra zealandica* Coleoptera: Scarabaeidae) (Hartley *et al.*, 1982).

The purposes of this study were to determine the effect of three field rates of *P. nanus* inoculum on the seedling growth of its pasture-grass hosts, in conjunction with the effect of *Neotyphodium* sp. endophyte-infection on *P. nanus* damage. The seasonal and monthly population dynamics of *P. nanus* described in Chapters 4 and 5 provide data on the abundance of this nematode in a field situation.

Materials and methods

Otorohanga silt loam soil (Typic Haploudand, wilting point 32%; field capacity 65%) from Tokanui Research Station was collected on 3 February 1999 by removing spade squares to 20 cm depth. This soil was sieved through a 7 mm aperture sieve to remove coarse materials and soil macrofauna. Sieved soil was placed into 235 PVC tubes (20 × 2.5 cm diameter, volume = 98 ml) which were sealed with PVC at one end. Ten soil cores (2.5 cm diameter) were also collected. Five were individually sieved and both the sieved and unsieved cores were weighed before drying at 90°C for 24 h to determine soil moisture. Mean wet weight, dry weight and percent soil moisture (by weight) of unsieved cores were 106.96 g, 69.48 g and 53.9% and for sieved cores were 105.50 g, 68.30 g and 54.5%. To attempt to approximate field soil bulk density, sieved soil equivalent to the mean wet weight of a field core (105.5 g), was added to tubes. This meant realistic soil moisture was used when watering tubes to weight.

Soil-filled tubes were placed in a freezer at –20°C to kill plant parasitic nematodes present in the soil. A total of 50 tubes, over three increasing lengths of freezing, were used in assessing nematode mortality. Nematodes in soil were extracted and counted by the variation of the Whitehead and Hemming (1965) method described in Chapter 3. After 260 h freezing the reduction in initial plant parasitic nematode populations was 80% for *P. nanus* and >90% for *Pratylenchus*, *Meloidogyne* and *Heterodera* (Table 1).

Table 1: *Effect of freezing on abundance of plant parasitic nematodes per tube in silt loam soil.*

Freezing time (h)	*P. nanus*	*Pratylenchus*	*Meloidogyne*	*Heterodera*
Unfrozen[1]	254.0	470.0	77.2	31.2
105[1]	148.8	150.4	4.4	2.0
Decrease (% unfrozen)	*41.4*	*68.0*	*94.3*	*93.6*
170[2]	85.6	67.5	0.0	0.0
Decrease (% unfrozen)	*66.3*	*85.6*	*100.0*	*100.0*
260[2]	49.3	34.4	0.0	1.1
Decrease (% unfrozen)	*80.6*	*92.7*	*100.0*	*96.6*

[1] Mean of 10 replicate tubes
[2] Mean of 15 replicate tubes

There were 195 experimental tubes. Tubes were sown with three seeds of one of five grasses: perennial ryegrass (*Lolium perenne*) cv Grasslands Nui infected with wild-type endophyte (E+), Grasslands Nui artificially infected with a selected endophyte strain (AR37+), Grasslands Nui with nil endophyte (E-), Italian ryegrass (*Lolium multiflorum*) cv Concord and cocksfoot (*Dactylis glomerata*) cv Grasslands Wana. Seeds of each of the five grasses were sown into 13 replicate pipes (20 cm high × 8.5 cm diameter with a base of 1 mm stainless steel mesh as used in Chapter 2), each containing three tubes of the same grass. Pipes were arranged in a randomised block design in a waterbath at 20°C. Seedlings were thinned to two /tube immediately after emergence.

Of the well-known endophyte produced alkaloids found in perennial ryegrass, E+ perennial ryegrass produces peramine, lolitrem B and ergovaline whereas neither AR37+ nor E- perennial ryegrass produces any of these alkaloids (Popay & Wyatt, 1995).

P. nanus were extracted from cultures maintained for *ca* six months on cocksfoot plants growing in Otorohanga silt loam. Two rates of inoculum were prepared and contained an average of 213.4 and 949.9 *P. nanus* /ml inoculum (these correspond to *ca.* 434.5 and 1935.2 x10^3 *P. nanus* /m^2).

The three tubes in each pipe received inoculum as follows: Low rate – inoculated with 1 ml distilled water (soil contained a residual mean *P. nanus* population of 49.3 individuals per tube after freezing); Medium rate – inoculated with 213.4; High rate – inoculated with 949.9 *P. nanus*. Inoculations were carried out on 4 March 1999, 10 days after sowing.

Two replicates of each grass were destructively harvested 34, 55, 74 and 88 days post-inoculation (dpi), with shoot and root components separated and processed before drying in an oven at 90°C for 24 h to determine dry matter yields. Before drying, the endophyte status of each plant in the perennial ryegrass tubes was determined. A 3 mm slice from the shoot base of plant was squashed onto nitrocellulose paper to release plant extract containing any *Neotyphodium* sp. antigens present. The slices were removed and the paper left to air dry for *ca* 1 h before being passed through the various stages of the immunoblot system described by Gwinn *et al.* (1991) to note the presence of *Neotyphodium* sp. Nematodes were extracted, counted and life stages of *P. nanus* determined from root and soil samples using the variant of the Whitehead & Hemming tray method described in Chapter 3.

Nematode data was log (n+4) transformed before analysis by ANOVA. An attempt was made to use endophyte presence as a covariate but this was abandoned as there were no significant effects of this, and a large amount of extra variation was introduced which masked otherwise significant effects.

A bias-corrected estimate (Neyman and Scott, 1960) of *P. nanus, Pratylenchus* and non-plant parasitic nematode data numbers was calculated whereby log (n+4) data was back-transformed using:

$$\text{bias-corrected mean} = 10^{\bar{X}+\left(\frac{RMS}{2}\right)}$$

where $\bar{x}$ was the mean /m^2 and RMS the residual mean squares for cultivar or depth main effects. For graphical purposes, an average back-transformed SED was calculated so that:

$$\text{back-transformed SED} = \left(\bar{x}\ \frac{10^{SEM}-10^{-SEM}}{2}\right)\sqrt{2}$$

where $\bar{x}$ was the grand mean and SEM was $\frac{SED\ of\ \log\ data}{\sqrt{2}}$.

Results

Although a larger proportion of the AR37+ perennial ryegrass plants which received the high *P. nanus* inoculum were endophyte-infected at sampling this effect was not significant (Table 2). Over all rates and times, 82.6% of E+ and 72.9% of AR37+ plants were endophyte-infected, while no E- plants were endophyte-infected.

Table 2. *Proportion of endophyte-infected perennial ryegrass plants at sampling, for pooled sampling times.*

	P. nanus inoculum rate			
Grass	Low	Medium	High	SED
E+ perennial ryegrass	81.2	93.7	75.0	14.4
AR37+ perennial ryegrass	68.7	62.5	87.5	16.1
E- perennial ryegrass	0.0	0.0	0.0	—

In E+ perennial ryegrass there was a significantly greater abundance of *P. nanus* /g root at 88 dpi in the high compared to low ($P<0.01$) and medium ($P<0.05$) rates of *P. nanus* inoculum (Fig. 1). The decline in *P. nanus* /g root for medium and low rates at 88 dpi was due to the combination of a non-significant decline in abundance of *P. nanus* and an increase in root weight (data not shown). There was no significant difference in shoot weight of E+ perennial ryegrass between any of the *P. nanus* inoculum rates at any of the sample times (Fig. 1).

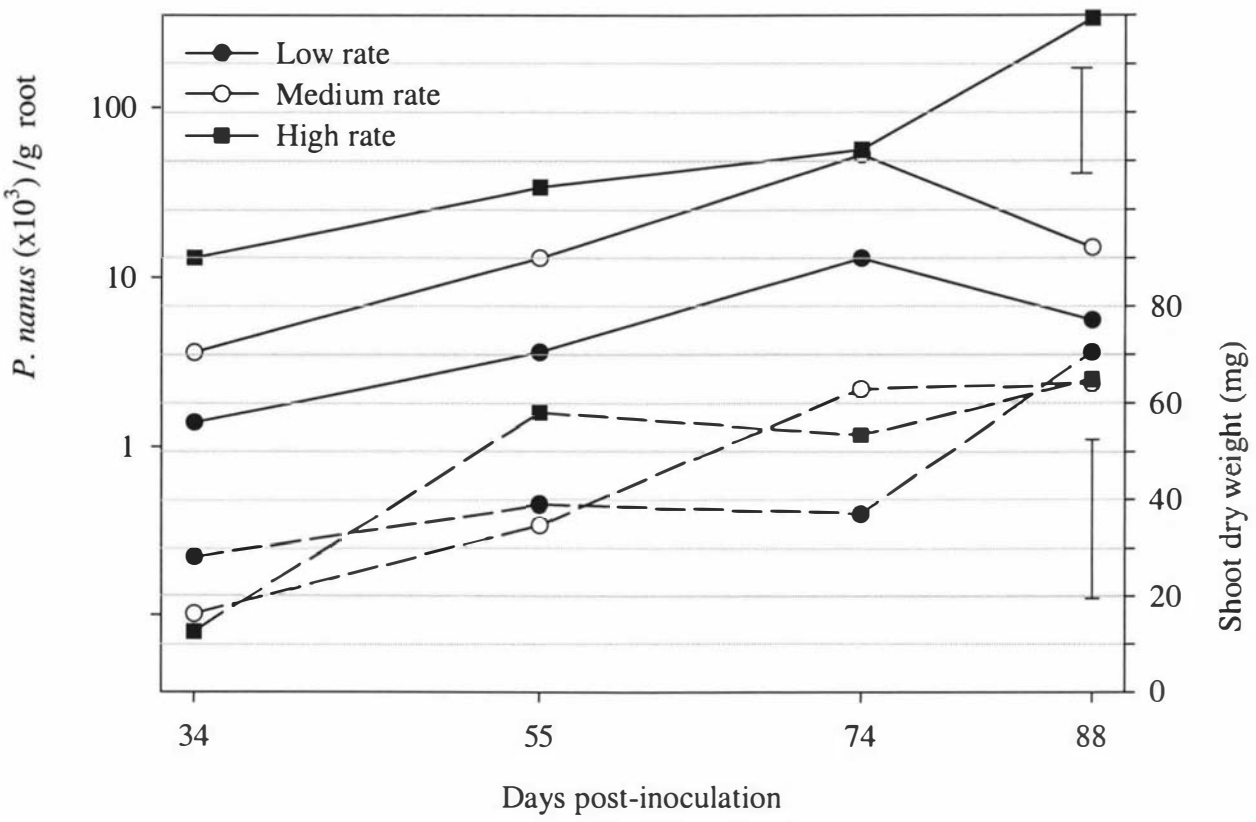

Fig. 1. *Effect of low, medium and high rates of* P. nanus *inoculum on number of* P. nanus */g root (dry weight) (solid line) and shoot weight (dashed line) of E+ perennial ryegrass at four sample times (error bars are SED between rates within sampling times).*

Abundance of *P. nanus* /g AR37+ perennial ryegrass root was significantly greater in high ($P<0.001$) and medium ($P<0.05$) inoculum rates at 55 dpi and in the high rate ($P<0.001$) at 74 dpi, compared to the low rate (Fig. 2). AR37+ plants produced significantly ($P<0.1$) less dry matter in the medium and high *P. nanus* inoculum rates compared to the low rate at 34 dpi, and significantly ($P<0.1$) less in high than medium rate at 74 dpi (Fig. 2).

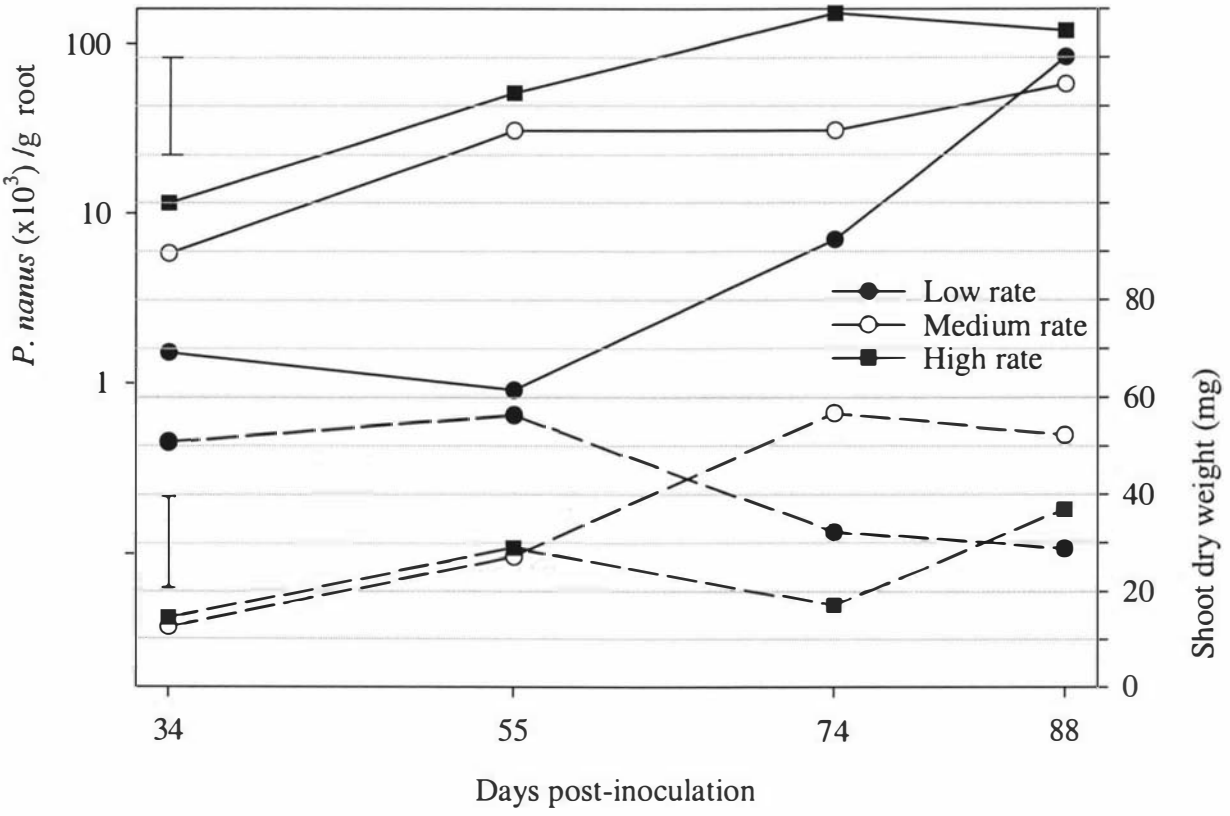

Fig. 2. *Effect of low, medium and high rates of* P. nanus *inoculum on number of* P. nanus */g root (dry weight) (solid line) and shoot weight (dashed line) of AR37+ perennial ryegrass at four sample times (error bars are SED between rates within sampling times).*

Large differences in *P. nanus* /g E- perennial ryegrass root were observed at both 74 and 88 dpi when there was significantly more at the high *P. nanus* inoculum rate compared to medium ($P<0.05$ and <0.1 for the two sample times respectively) and low rates ($P<0.01$ for both sample times) (Fig. 3). Only at 74 dpi was there a significant difference in E- perennial ryegrass shoot dry matter, when plants inoculated with the high rate of *P. nanus* produced more than the low rate ($P<0.05$) (Fig. 3).

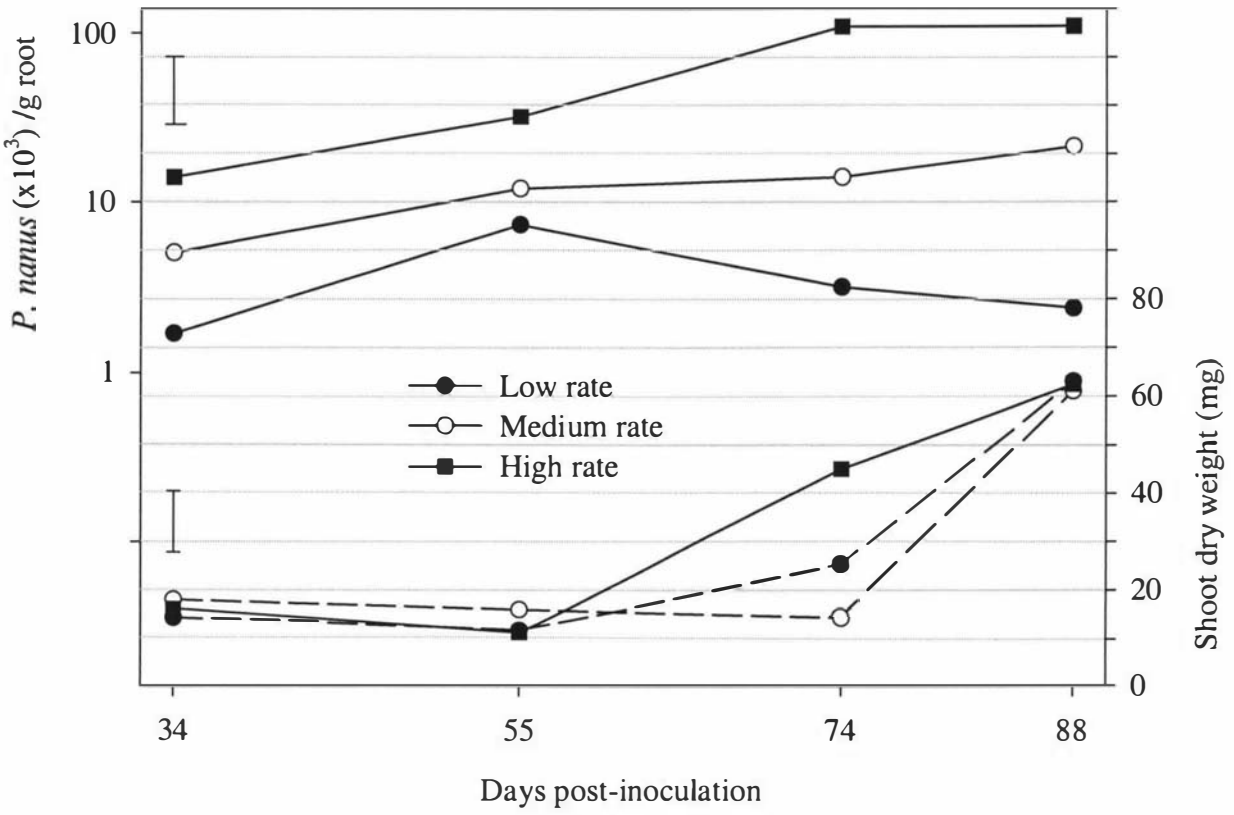

Fig. 3. *Effect of low, medium and high rates of* P. nanus *inoculum on number of* P. nanus */g root (dry weight) (solid line) and shoot weight (dashed line) of E- perennial ryegrass at four sample times (error bars are SED between rates within sampling times).*

No significant difference was observed in abundance of *P. nanus* /g Italian ryegrass root for any of the inoculum rates at any of the sample times (Fig. 4). Shoot dry matter was significantly greater at 55 dpi for plants inoculated with medium compared to low ($P<0.01$) and high ($P<0.1$) rates of *P. nanus* (Fig. 4).

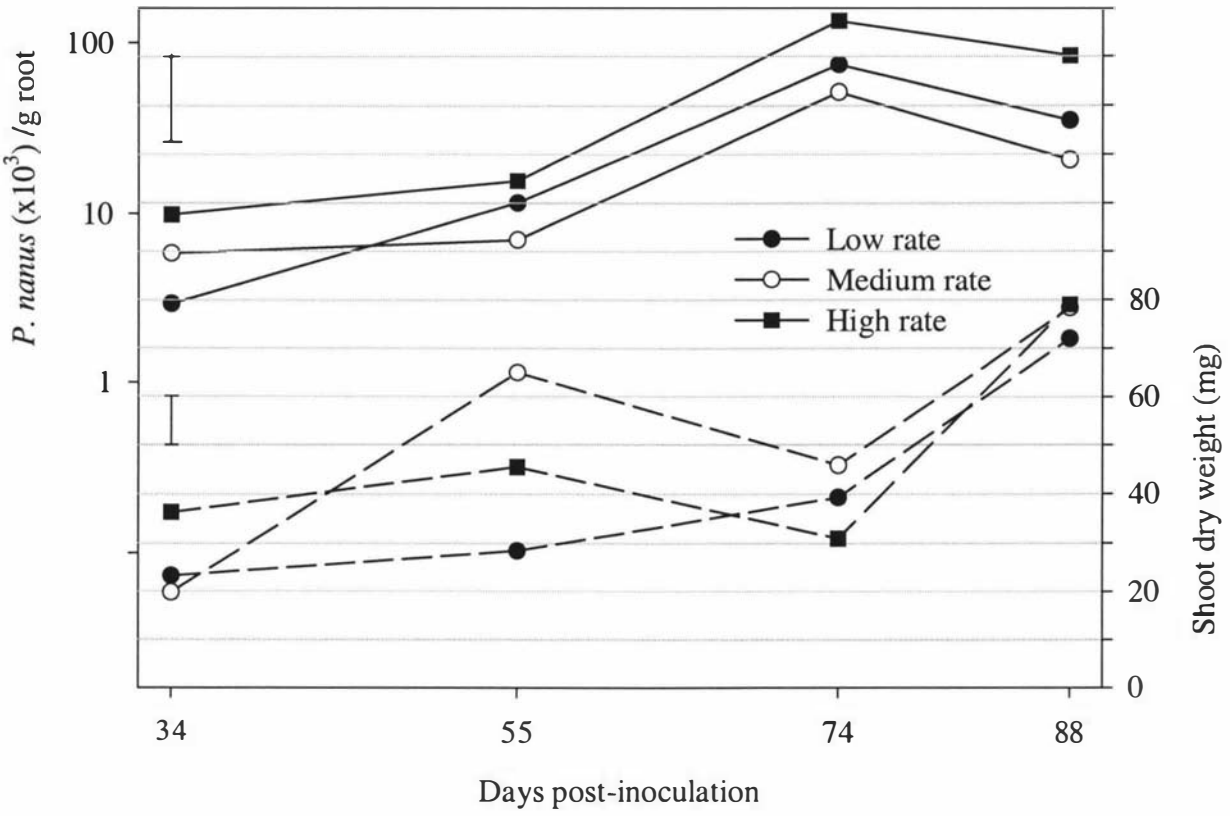

Fig. 4. *Effect of low, medium and high rates of* P. nanus *inoculum on number of* P. nanus */g root (dry weight) (solid line) and shoot weight (dashed line) of Italian ryegrass at four sample times (error bars are SED between rates within sampling times).*

Abundance of *P. nanus* /g cocksfoot root was significantly affected by inoculum rate only at 88 dpi when it was greater in the high than the low rate ($P<0.1$) (Fig. 5). Cocksfoot plants inoculated with the medium rate of *P. nanus* produced significantly ($P<0.05$) less shoot dry matter than those in the low rate at 88 dpi (Fig. 5).

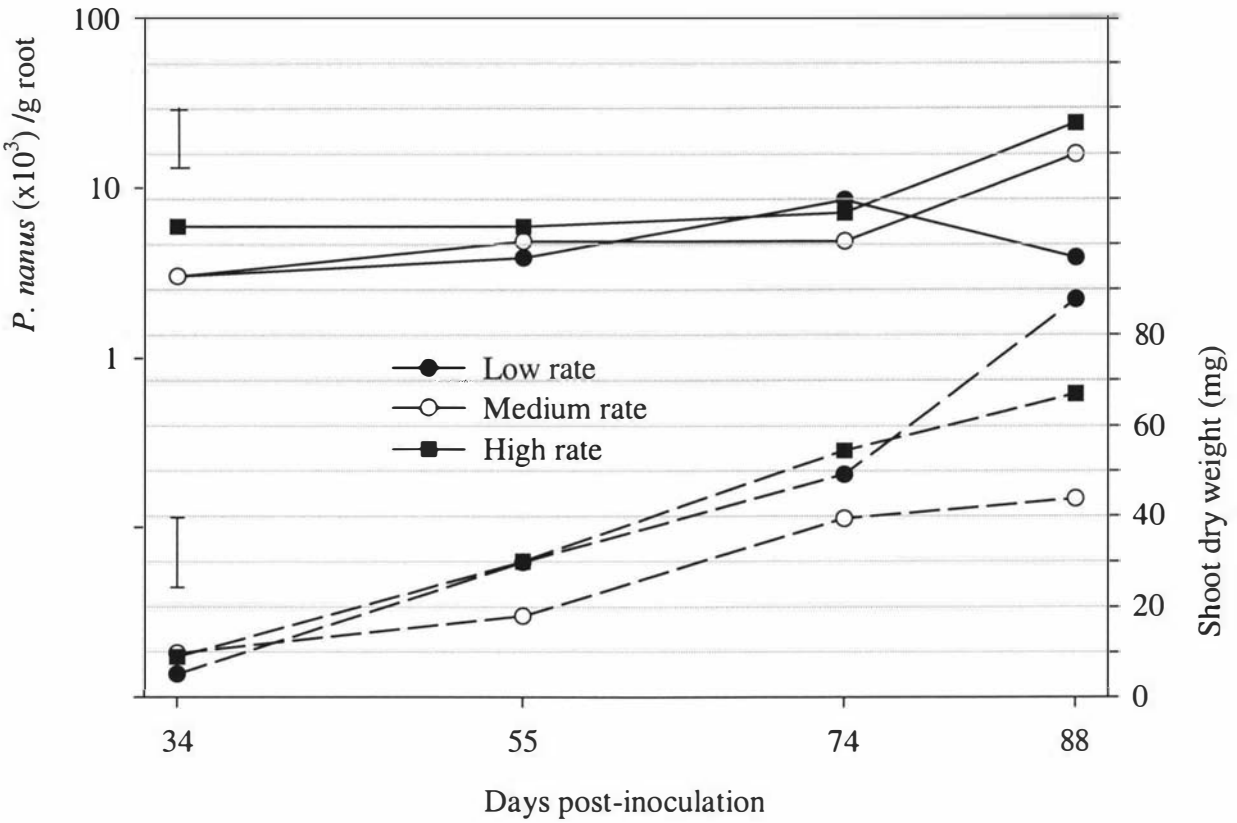

Fig. 5. *Effect of low, medium and high rates of* P. nanus *inoculum on number of* P. nanus */g root (dry weight) (solid line) and shoot weight (dashed line) of cocksfoot at four sample times (error bars are SED between rates within sampling times).*

As the effect of *P. nanus* inoculum was the same for root as shoot dry matter for all grasses (data not shown) the ratio of root to shoot weight (R:S) was investigated across time for combined rates of inoculum (Table 3). The R:S shows the amount of shoot that is produced or supported by each unit of root and can be used as a measure of root efficiency. For all the ryegrasses there was a consistent reduction in the R:S from 34 dpi so that by 88 dpi the respective R:S values were almost identical (Table 3). Cocksfoot consistently had the highest R:S and the ratio remained largely unchanged from 55 to 88 dpi. In both E+ perennial ryegrass and cocksfoot there was a marked (*ca* 40%) drop in R:S between 34 and 55 dpi.

Table 3. *Effect of days post-inoculation (dpi) of* P. nanus *on dry weight root :shoot ratio of grasses (pooled rates of inoculum).*

	Days post-inoculation			
Grass	34	55	74	88
E+ perennial ryegrass	1.25	0.75	0.59	0.56
AR37+ perennial ryegrass	1.02	0.80	0.73	0.60
E- perennial ryegrass	1.01	0.68	0.72	0.60
Italian ryegrass	1.08	0.90	0.71	0.58
Cocksfoot	1.41	0.88	0.82	0.84

Comparisons of *P. nanus* recovered per tube and expressed /g root and shoot dry matter within and between all the grasses were made by pooling data over all sample times for each rate of *P. nanus* inoculum (Tables 4 and 5). Only in the perennial ryegrasses were there significant effects of inoculum rate on abundance of *P. nanus* /g root, such that there was significantly greater abundance in high *vs* medium ($P<0.05$) and low ($P<0.01$) rates on E+ perennial ryegrass, and significant differences amongst all three rates on AR37+ ($P<0.05$) and E- perennial ryegrass ($P<0.1$) (Table 4). Despite these differences for the perennial ryegrasses, only on AR37+ was there a deleterious effect of increased *P. nanus* /g root on shoot dry weight whereby plants growing in the high inoculum rate had significantly ($P<0.1$) less shoot matter than those at the low rate (Table 5). For Italian ryegrass there was significantly greater shoot matter at medium *vs* low inoculum rates ($P<0.05$) whereas for cocksfoot this effect was reversed ($P<0.1$) (Table 5).

Table 4. *Effect of rate of* P. nanus *inoculum, pooled across all sample times (Low, Medium or High) and across all inoculum rates (All), on abundance of* P. nanus *($x10^3$) /g root (SED_1 applies across rates within a grass and SED_2 applies across grasses within each rate).*

	Rate of *P. nanus* inoculum				
Grass	Low	Medium	High	SED_1	All
E+ perennial ryegrass	4.7	14.6	53.9	8.5	16.3
AR37+ perennial ryegrass	9.3	26.7	62.8	8.3	25.2
E- perennial ryegrass	3.5	12.3	50.7	5.7	14.2
Italian ryegrass	18.4	14.3	35.8	11.6	20.6
Cocksfoot	4.9	6.6	9.7	2.3	6.6
SED_2		6.4 (54 df)			3.2 (16 df)

Significantly (P<0.05) more *P. nanus* /g root were observed for Italian ryegrass than E+, E- perennial ryegrass and cocksfoot at the low inoculum rate (Table 4). At the medium rate AR37+ perennial ryegrass had significantly (P<0.01) more *P. nanus* /g root than cocksfoot. At the high rate cocksfoot had significantly (P<0.01) less than all other grasses, and this was the case when all rates were pooled (P<0.05) (Table 4). E+ and E- perennial ryegrass supported significantly (P<0.1) less *P. nanus* /g root than AR37+ for combined rates.

For individual rates, there was no significant difference between shoot weight of the grasses at low and high inoculum rates (Table 5). At the medium rate, both E- perennial ryegrass and cocksfoot produced significantly less shoot matter than E+ perennial ryegrass (P<0.1) and Italian ryegrass (P<0.05). For combined rates E+ perennial ryegrass and Italian ryegrass produced significantly more shoot matter than AR37+ (P<0.1) and E- (P<0.01) perennial ryegrass, and Italian ryegrass produced significantly more than cocksfoot (P<0.1) (Table 5).

Table 5. *Effect of rate of* P. nanus *inoculum, pooled across all sample times (Low, Medium or High) and across all rates (All), on grass shoot dry matter (mg) (SED_1 applies across rates within a grass and SED_2 applies across grasses within each rate).*

	Rate of *P. nanus* inoculum				
Grass	Low	Medium	High	SED_1	All
E+ perennial ryegrass	43.6	44.5	47.2	16.5	45.1
AR37+ perennial ryegrass	41.9	37.0	24.2	9.4	34.4
E- perennial ryegrass	28.4	27.3	33.6	6.2	29.8
Italian ryegrass	40.7	52.2	47.9	5.0	47.0
Cocksfoot	43.0	27.7	40.1	7.7	36.9
SED_2		9.4 (54 df)			5.2 (16 df)

There was 50 to 90 % mortality of all stages of *P. nanus* in the all inoculum rates between the time of inoculation and 34 dpi (Tables 6 and 7). For all inoculum rates of *P. nanus* there was a large increase in J2+J3 stages between 34 and 55 dpi, with the largest increase occurring at the low rate (Table 6). The increase in J2+J3 abundance declined for the succeeding intervals at all rates. The J4 stage of *P. nanus* increased most between 55 and 74 dpi at the low and medium inoculum rates but not at the high rate (Table 6). From 55 dpi J2+J3 outnumbered J4 at all rates by 1.6 × (range 3.3 – 1.2 ×).

Table 6. *Proportional changes in abundance of* P. nanus *life stages J2+J3 and J4 per tube across all grasses, adjusted for interval between post-inoculation sampling days.*

	J2 + J3			J4		
Rate	Low	Medium	High	Low	Medium	High
dpi						
0	0.0	60.0	350.0	17.9	36.7	203.3
Decrease	—	*(0.2)*	*(0.1)*	*(0.4)*	*(0.5)*	*(0.2)*
34	2.3	11.9	49.1	7.6	18.8	38.2
Increase	*32.1*	*12.1*	*7.6*	*3.0*	*4.6*	*7.7*
55	74.1	142.9	371.7	22.4	86.9	292.8
Increase	*5.1*	*4.9*	*3.1*	*11.9*	*5.2*	*2.4*
74	377.4	698.3	1152.5	266.0	450.2	709.0
Increase	*1.4*	*1.1*	*2.7*	*1.5*	*1.5*	*2.5*
88	544.8	796.0	3151.2	388.8	657.6	1758.4

The proportional increase in abundance of both female and male *P. nanus* declined from 34 dpi onwards in medium and high inoculum rates, but remained similar at low inoculum rate (Table 7). From 55 dpi abundance of J4 averaged 2.3 × that of females (range 0.8 – 3.0 ×) for all inoculum rates, and the female: male ratio was 3.5:1 with no consistent effect of inoculum rate (Tables 6 and 7).

Table 7. *Proportional changes in abundance of* P. nanus *females and males per tube across all grasses, adjusted for interval between post-inoculation sampling days.*

	Female			Male		
Rate	Low	Medium	High	Low	Medium	High
dpi						
0	29.1	96.7	323.3	2.4	20.0	73.3
Decrease	*(0.4)*	*(0.2)*	*(0.1)*	*(0.4)*	*(0.1)*	*(0.1)*
34	11.5	20.1	33.3	1.0	2.3	5.3
Increase	*2.5*	*3.2*	*3.8*	*6.5*	*8.1*	*5.6*
55	29.3	63.5	126.9	6.4	18.7	29.3
Increase	*3.3*	*2.7*	*2.8*	*5.2*	*2.3*	*4.0*
74	98.0	171.9	353.4	33.0	43.7	117.0
Increase	*2.8*	*2.1*	*1.7*	*2.8*	*1.8*	*1.4*
88	272.0	358.4	584.0	92.8	80.0	159.2

The abundance of *P. nanus, Pratylenchus* and non-plant parasitic nematodes for all grasses across all sample times are given in Table 8.

Table 8: *Abundance of* P. nanus, Pratylenchus *and non-plant parasitic nematodes per tube pooled over all grasses and times.*

	Rate of *P. nanus* inoculum			
	Low	Medium	High	SED
P. nanus	99.9	297.9	804.7	60.2
Pratylenchus	19.0	18.7	14.4	2.4
Non-plant parasites	161.0	2453.0	2715.0	784.0

Discussion

The numbers of *P. nanus* used as initial inoculum on a per m^2 basis for low, medium and high rates were comparable to field populations that have been found in September (late winter), October /April (early spring /early autumn) and January (early summer) respectively (Chapter 4). The overall female: male ratio of 3.5:1 observed in the present study (Table 7) is the same as the mean ratio calculated for seasonal samples at 0–10 cm depth from the field site (Chapter 5).

Cocksfoot supported relatively few *P. nanus* /g root (Table 4) despite similar shoot and, by extension, root growth to other grasses (Table 5). Apparently some aspect of cocksfoot root physiology or structure (e.g. more 'coarse' roots) formed a poorer root resource for *P. nanus* as well as producing a higher R:S value.

Only on AR37+ perennial ryegrass seedlings was there a significant deleterious effect of high inoculum rate of *P. nanus* on shoot production (Fig. 2). The abundance of *P. nanus* /m^2 at 34 dpi, when the differences in shoot production between low and high inoculum rates were the greatest, is equivalent to field populations found in October and August (late winter) respectively (Chapter 4). The *P. nanus* field populations which would equate to the time of field sowing of grasses in New Zealand were *ca* 1200 and 500 x 10^3 (March and April respectively), i.e. at least 1.5–4.5 × the number on AR37+ at 34 dpi in the tubes inoculated with the high rate of *P. nanus*. These observations imply that the AR37+ perennial ryegrass /endophyte combination may have a lower establishment success than E+ in a field situation where there are high populations of *P. nanus* present.

For combined rates and times AR37+ produced significantly less shoot matter than E+ perennial ryegrass (Table 5), possibly because AR37+ supported a greater abundance of *P. nanus* /g root at all rates (Table 4). It could be inferred from these results that differences in alkaloid production are responsible for the increased resistance to *P. nanus* of E+, which produces the three most commonly reported *Neotyphodium* sp. associated alkaloids (peramine, lolitrem B, ergovaline) compared to AR37+, which produces none of these alkaloids (Popay & Wyatt, 1995). However, the alkaloids present in the shoots of E+ perennial ryegrass plants are not necessarily present in the roots where nematodes are feeding. Peramine is not translocated to roots and only low

concentrations (*ca* 1 μg /g) of lolitrem B have been found in roots (Ball *et al.*, 1993). Ergovaline has been found in tall fescue roots, but again only at low concentrations (*ca* 1 μg /g) (Azevedo *et al.*, 1993).

There are numerous other endophyte associated alkaloids and alkaloid precursors produced in perennial ryegrass (Rowan *et al.*, 1990; Siegel *et al.*, 1990; Rowan, 1993; Ball *et al.*, 1994; Ball *et al.*, 1997a) and it is possible that one of these may be responsible for the differences observed between AR37+ and E+ perennial ryegrass. Popay & Wyatt (1995) suggested the existence of an as yet unidentified compound produced by AR37+ after observing decreased damage by larval Argentine stem weevil (*Listronotus bonariensis* Coleoptera: Curculionidae) on this grass /endophyte association compared to E-. Ball *et al.* (1997b) were unable to demonstrate which, if any, of the three most commonly reported alkaloids were responsible for the lowered establishment of *Meloidogyne marylandi* females on grasses infected with endophytes selected for their differing alkaloid profiles. They suggested the effect on nematode establishment may have been due to increased chitinase production as shown for the tall fescue (*Festuca arundinacea*) /*Neotyphodium* sp. association (Roberts *et al.*, 1992).

It is probable that very little of the difference in abundance of *P. nanus* /g root or plant growth between AR37+ and E+ perennial ryegrass was due to purely plant growth effects as they are both Grasslands Nui-based. Eerens *et al.* (1998) found significant growth differences between the ryegrass cultivars Grasslands Nui, Grasslands Ruanui and Grasslands Pacific inoculated with *Paratylenchus* sp., but no consistent difference in either root or shoot weight within a cultivar infected with different endophyte strains. Differences in seedling growth shown in the current study could not be observed by Eerens *et al.* (1998) as their plants were 108 days old before they were planted into *Paratylenchus* sp. infested soil.

E+ produced more shoot matter than E- perennial ryegrass (33.9% more for combined *P. nanus* inoculum rates and times) (Table 5) despite E+ supporting a consistently greater abundance of *P. nanus* /g root (*ca* 2000 /g root more overall) (Table 4). The increased ability of endophyte-infected, compared to endophyte-free, perennial ryegrass to withstand stress has been well documented (see Bacon, 1993; Latch, 1993; West & Gwinn, 1993).

The large proportional increase in abundance of J2+J3 *P. nanus* once females had become established (i.e. after 34 dpi) (Tables 6 and 7) indicates the potential fecundity of females when abundance is low. That the rate of increase for all stages of *P. nanus* decreased over time suggests that there is some form of negative feedback operating, possibly in relation to root resource quantity or quality.

The density dependence observed for a field population of *P. nanus* at 0–10 cm depth (Chapter 5) was not observed here, the situation being more similar to the density independence of the *P. nanus* field population at 10–20 cm depth. It was speculated in Chapter 5 that density independence observed from a field situation was due to a lack of pathogens of nematodes at the deeper depth, because of a reduced food source for pathogens in the form of non-plant parasitic nematodes. In the current study there were more non-plant parasitic nematodes at the high inoculum rate (Table 8) (because they were introduced along with the *P. nanus*) but *P. nanus* population increase was not consistently reduced at the high inoculum rate. It may be that nematode pathogens had been killed by soil freezing as occurs with some other soil microbes (Sarathchandra *et al.*, 1995) or that the very low level of interspecific competition in the current study (Table 8) allowed density independent multiplication to continue at populations beyond those observed in the field (Chapter 5).

As the soil used in this study was from a field site it is likely that the group of nematodes referred to as non-plant parasites includes genera which are bacterial, fungal and particle feeders (see Yeates, 1975 and Yeates *et al.*, 1993). It has been shown that increased abundance of these nematodes increases plant growth through increased nutrient cycling (see Cook & Yeates, 1993). To what extent this occurred in the current study is not able to be determined as there are confounding effects such as freezing and establishment post-inoculation. If it is assumed, however, that there was greater nutrient cycling in the medium and high compared to the low inoculum rate (see Table 8) then the significant difference in shoot weight which was observed for AR37+ perennial ryegrass is strengthened.

The effects of *P. nanus* on seedlings of perennial ryegrasses with or without endophyte infection and with different endophyte strains may have implications for the agronomic performance of these plants when sown into field soils which contain

populations of *P. nanus*. A succeeding study (Chapter 7) looks at the effect of *P. nanus* on mature perennial ryegrass swards.

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Chapter 7: Damage potential of *Paratylenchus nanus* to some of its grass hosts: II. Effect on mature plants in the field

Summary – Sampling and extraction of nematodes from soil was carried out monthly for four months between two sites to determine the effect of *Paratylenchus nanus* feeding on endophyte (*Neotyphodium* sp.) -infected and -uninfected ryegrass (*Lolium perenne* cv. Grasslands Nui). Five endophyte /ryegrass combinations (treatments), along with white clover (*Trifolium repens*) /ryegrass pasture, were sampled: endophyte-free (E-); wild type endophyte-infected (E+); and three novel combinations designated AR37+, AR24+ and AR1+. For both sites and all samplings, AR37+ supported a consistently greater abundance of *P. nanus* than the other treatments. Life stage distribution of *P. nanus* was not consistently effected by any of the treatments. Neither *Pratylenchus* nor non-plant parasitic nematode abundance followed the same pattern as that for *P. nanus* suggesting that the cause of the increased abundance of *P. nanus* beneath AR37+ did not act in a similar manner on other nematodes. Dry matter production and root mass data suggest that greater comparative root production by AR37+ was at least partly responsible for the greater abundance of *P. nanus* beneath this treatment. It is suggested that AR37+ dry matter production was effected by *P. nanus* feeding but the extent to which this occurred could not be determined. Depth distributions of *P. nanus*, *Pratylenchus* and non-plant parasitic nematodes are given and discussed in terms of root distribution and possible deleterious effects of root herbivory.
Keywords: *Paratylenchus nanus, Lolium perenne*, *Neotyphodium* endophyte, *Pratylenchus*

Paratylenchus spp., in common with some other ectoparasitic nematodes, feed on root hairs (Linford *et al.*, 1949; Rhoades & Linford, 1961) and root epidermal cells (Wood, 1973). Root hairs are involved in the uptake of water and nutrients (Drew & Nye, 1969). A lack of visible pathological changes to cells that have been fed on by *Paratylenchus* spp. (Linford *et al.*, 1949; Solov'eva, 1975) has meant they are often considered weak plant pathogens. There are, however, many instances of root feeding by these nematodes having a deleterious effect on plant growth. Some of these effects may be solely due to the large numbers of *Paratylenchus* sp. encountered in soil (Chapters 4 and 5) or the additional action of secondary pathogens entering plant roots via nematode

feeding wounds (Cole *et al.*, 1973; Inagaki *et al.*, 1973; Gubina, 1975; Hirano, 1975; Savkina, 1993).

Examples of pathogenic effects of *Paratylenchus* spp. include the observations of Shesteperov (1978) who found that infestation of red clover (*Trifolium pratense*) by *P. projectus* caused a reduction in shoot weight, shoot height and an increased susceptibility to powdery mildew. Yields of birdsfoot trefoil (*Lotus corniculatus*), in addition to red clover were reduced after infestation by *P. projectus* (Townshend & Potter, 1982). Reduction of seedling establishment of lucerne (*Medicago sativa*), white clover, red clover and birdsfoot trefoil was also observed for *P. projectus* infestations (Townshend & Potter, 1982). Sunflower (*Helianthus annuus*) yields in the U.S. were significantly reduced by large numbers of *P. projectus* (Smolik, 1987). *P. bukowinensis* has been found to be pathogenic to plant species of the Umbellifera (Brzeski, 1975; Brzeski & Radzikowska, 1980). *Paratylenchus* spp. have been implicated as causing yield reductions to some ryegrass cultivars (Yeates & Barker, 1986) and ryegrass/ white clover pastures (Yeates, 1985; Watson *et al.*, 1994) in New Zealand.

The results from Chapter 6 indicated that *P. nanus* may have a deleterious effect on seedlings of the perennial ryegrass (*Lolium perenne*) /*Neotyphodium* sp. endophyte association designated AR37+. This paper reports the results of field sampling to determine the effect of *P. nanus* on mature perennial ryegrass plants grown in pure species plots, including AR37+. The seasonal and monthly population dynamics of *P. nanus* described in Chapters 4 and 5 provide data on the abundance of this nematode in a mixed pasture situation.

Materials and methods

SITE DESCRIPTION AND PLANT CHARACTERISTICS

Two sites, which form part of the National Endophyte Evaluation Trial, were sampled. They were established and maintained by a team lead by Dr J. P. J. Eerens (AgResearch, Ruakura Research Centre, Hamilton).

The two sites were located at: Tokanui Research Station (Waikato – lat. 38° 5.4' S 174° 19.3' E, ca 400 m from the site described in Chapters 4 and 5) with Otorohanga silt

loam soil (Typic Haploudand, wilting point 32%; field capacity 65%); and a private farm inland from Te Puke in the Bay of Plenty (Bay of Plenty – lat. 37° 53' S 176° 34' E) with Ohinepanea sandy soil (Typic Udivitrand, wilting point 10%; field capacity 25%). The Tokanui trial site was established in autumn 1996 after maize (*Zea mays*) had been grown for the previous two years. In autumn 1996, an area 18 × 20 m was marked out and divided into 24 plots, each 3 × 5 m. Each plot was sown with a pure sward of one of seven Grasslands Nui perennial ryegrass /*Neotyphodium* sp. endophyte combinations (herein referred to as treatments). The five treatments sampled in this study were: Grasslands Nui endophyte free (E-), Grasslands Nui infected with wild-type endophyte (E+), and Grasslands Nui artificially infected with one of three selected endophyte strains (AR1+, AR24+, and AR37+). Table 1 shows the known alkaloid profiles of each treatment (Popay & Wyatt, 1995; Ball *et al.*, 1997). It is likely that some of the selected endophyte strains produce as yet undiagnosed alkaloids (Popay & Wyatt, 1995). The site was grazed by sheep and beef cattle in common with the rest of the field until spring 1996 after which it was fenced and grazed separately.

The Bay of Plenty site was also established in autumn 1996 as for the Tokanui site. The site was grazed in common with the rest of the field, by dairy cattle.

Table 1. *Alkaloid profiles of Grasslands Nui perennial ryegrass plants infected with* Neotyphodium sp. *endophytes which were sown at Tokanui and Bay of Plenty sites (+, – denote presence and absence respectively).*

Endophyte status	Peramine	Lolitrem B	Ergovaline
E-	–	–	–
E+	+	+	+
AR1+	+	–	–
AR24+	+	–	–
AR37+	–	–	–

NEMATODE AND HERBAGE SAMPLING

Nematode sampling of the Tokanui site was carried out on the 21 November 1997, 11 February and 16 March 1998. Fifteen perennial ryegrass plots were sampled to give the following treatment × replicate combinations: E- × 3, E+ × 3, AR37+ × 2, AR24+ ×

4 and AR1+ × 3. Three samples from grazed pasture adjacent to the ryegrass trial site were also taken on each date.

Twelve perennial ryegrass plots at the Bay of Plenty site were sampled on 2 April 1998 to give the following cultivar × replicate combinations: E- × 2, E+ × 2, AR37+ × 2, AR24+ × 3 and AR1+ × 3.

Each sample consisted of three 2.5 cm diameter cores to 20 cm depth, divided into 0–10 and 10–20 cm depths. The pasture samples consisted of transects adjacent to three sides of the trial, along which three 2.5 cm diameter cores were taken, and were included for comparison between ryegrass plots and normal pasture which contained at least 40% white clover, along with ryegrass, *Poa* sp., and a small percentage of broadleaf weeds.

Nematodes were extracted from the three bulked cores using a variant of the tray method (Chapter 3), and counted in a Doncaster dish under a stereomicroscope at 80× magnification. All plant parasitic nematode genera were identified and counted separately, all other nematodes were counted collectively. Life stages of *P. nanus* were determined during counting.

Four soil moisture samples were taken at the time of nematode sampling: two from within the ryegrass plots and two from the outside plots. Samples consisted of three 2.5 cm diameter cores, divided as for nematode samples then dried in an oven at 90°C for 48 h. Soil moisture is expressed as percent dry soil.

At the March 1998 sampling, root samples were taken from all ryegrass plots at Tokanui. Each sample consisted of three 5 cm diameter cores to 20 cm depth, divided into 0–10 and 10–20 cm depth. Roots were washed from cores, oven dried at 90°C and weighed.

Herbage was mown to 4 cm height to coincide with grazing, and herbage dissections were taken prior to each mowing. Herbage mowing and dissection was carried out by a team lead by Dr J. P. J. Eerens and the data presented here is part of their data set.

All statistical analyses were as reported in Chapter 6.

Results

The soil moisture data are presented in Table 2. At both sites, soil moisture content was greater in 10–20 than 0–10 cm depth on all sampling dates. Soil moisture was lower under pasture than under ryegrass.

Table 2. *Soil moisture as percent dry soil beneath ryegrass and pasture from Tokanui and Bay of Plenty sites.*

	Ryegrass		Pasture	
Site/ Date	0–10 cm	10–20 cm	0–10 cm	10–20 cm
Tokanui				
November 1997	70.9	71.5	69.9	70.0
February 1998	36.0	45.0	33.7	39.4
March 1998	65.3	68.3	62.9	65.4
Bay of Plenty				
April 1998	26.1	30.1	—	—

At Tokanui there were more *P. nanus* /m^2 in soil under AR37+ than any of the other ryegrass treatments at all sample times (Fig. 1). This difference was significant ($P<0.05$) for the comparison with all plots in November, for AR24+ and E- in February and for AR1+, AR24+, and E+ in March (Fig. 1). Abundance of *P. nanus* in pasture samples were significantly ($P<0.05$) less than under AR37+ in November and significantly ($P<0.05$) less compared to AR37+, AR1+ and E+ in February, and AR37+, AR1+, AR24+ and E- in March (Fig. 1). Aside from AR37+, the other ryegrass plots did not have significantly different abundance of *P. nanus* except that E+ plots had significantly ($P<0.05$) more *P. nanus* than AR1+ in November (Fig. 1). At the Bay of Plenty site there was significantly ($P<0.1$) more *P. nanus* in AR37+ than in AR24+ and E+ plots (Fig. 1). At all sample times for both sites, the differences in numbers of *P. nanus* life stages between ryegrass treatments followed those of the population as a whole (data not shown).

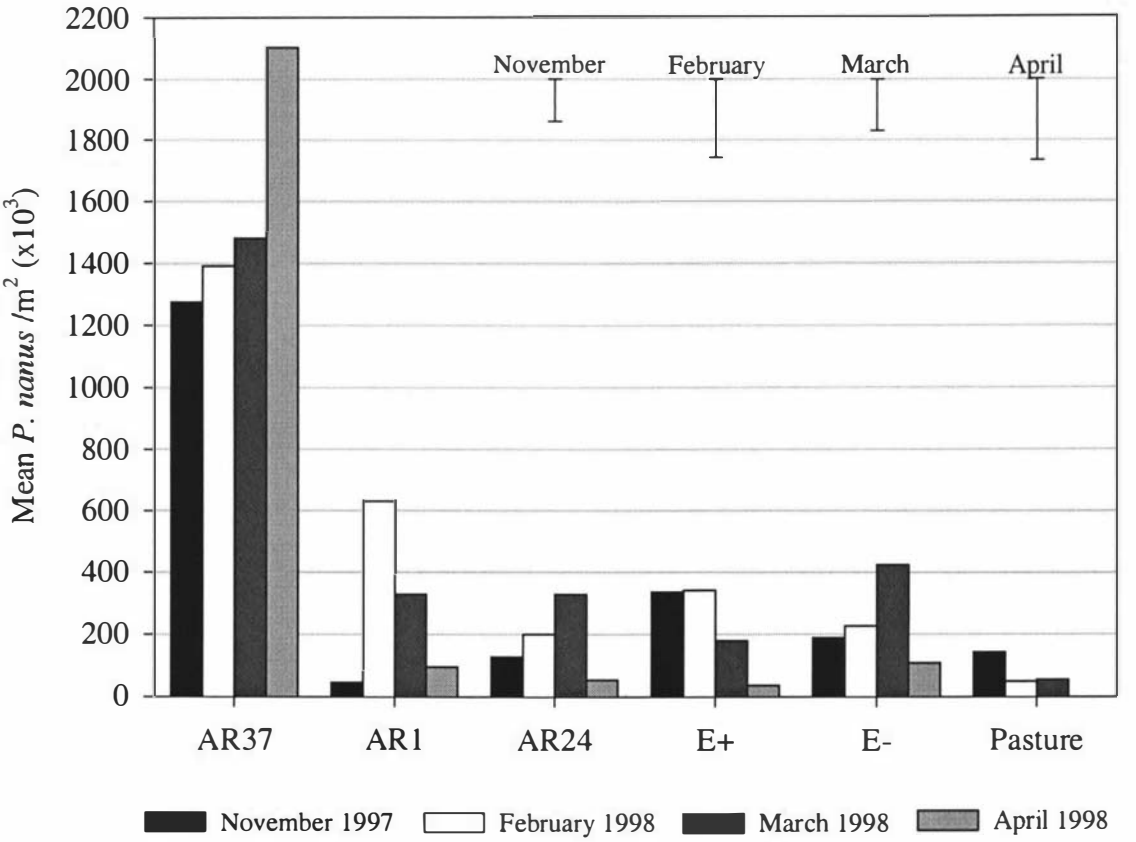
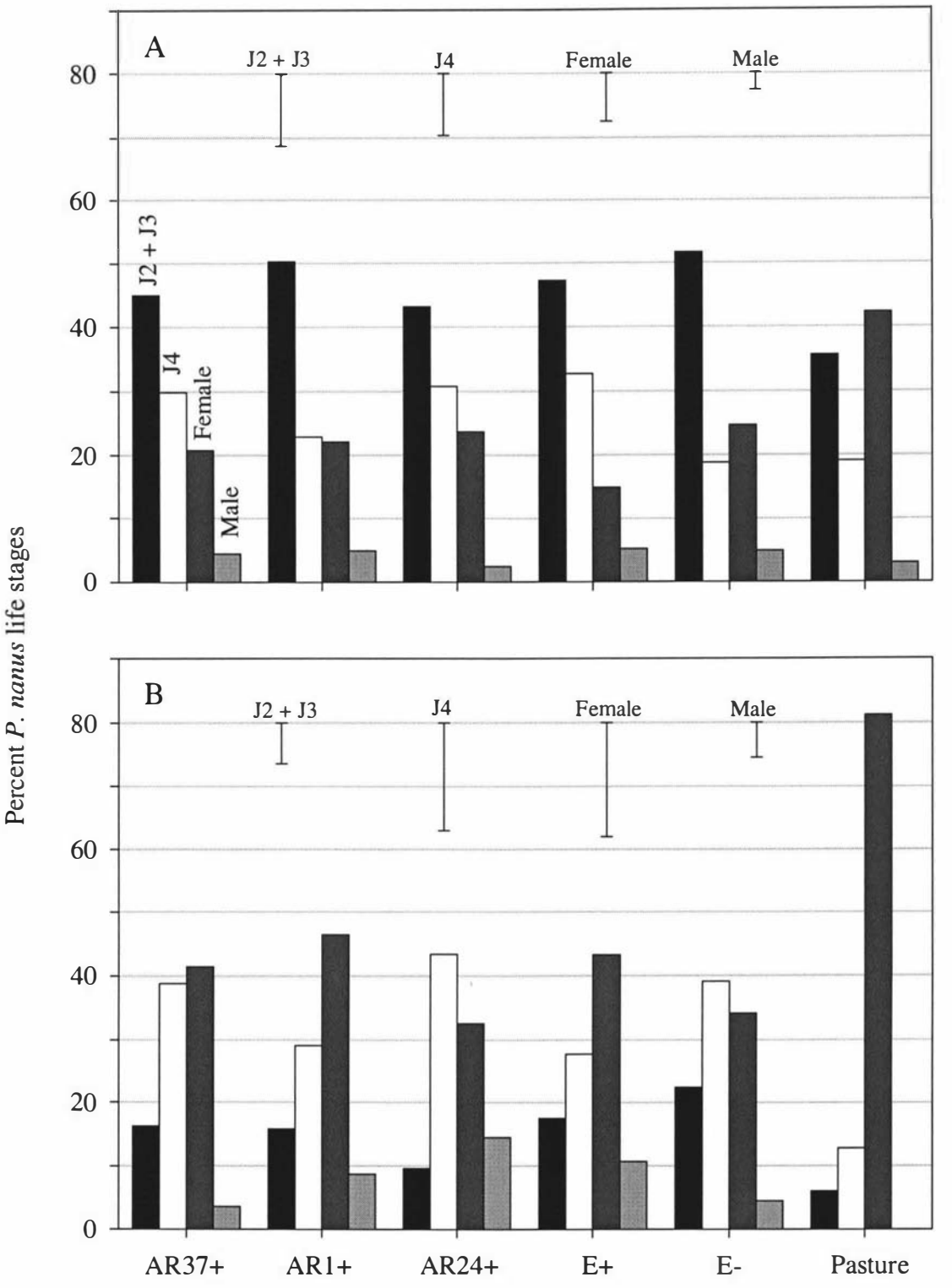


Fig. 1. *Mean* P. nanus */m² in 0–20 cm soil under five ryegrass treatments and an adjacent pasture in November 1997, February and March 1998 at Tokanui and April 1998 at Bay of Plenty (error bars are average SED).*

The population structure of *P. nanus* under pasture differed from that under the various ryegrass treatments. Pasture soil contained a significantly ($P<0.05$) higher proportion of females compared to: all treatments in November (Fig. 2A); AR24+, E+ and E- plots in February (Fig. 2B) and AR37+ in March (Fig. 2C). The large proportion of females under pasture was reflected in a significantly ($P<0.05$) lower proportion of: J2 + J3 stages than AR24+ and E- plots in February; males than AR24+ plots in February (Fig. 2B) and J4 than AR37+ and E- plots in March (Fig. 2C). Of the ryegrass plots there was a significant ($P<0.05$) difference in proportion of J2 + J3 between E- and AR24+ treatments in February (Fig. 2B) and in proportion of females between AR24+ and AR37+ plots in March ($P<0.05$) (Fig. 2C). At the Bay of Plenty site, there was a significant ($P<0.05$) difference only in the J2 + J3 stages, with a higher proportion under E- than AR1+, AR24+ and E+ (Fig. 2D).


A
B
J2 + J3
J4
Female
Male
Percent P. nanus life stages
0
20
40
60
80
AR37+
AR1+
AR24+
E+
E-
Pasture

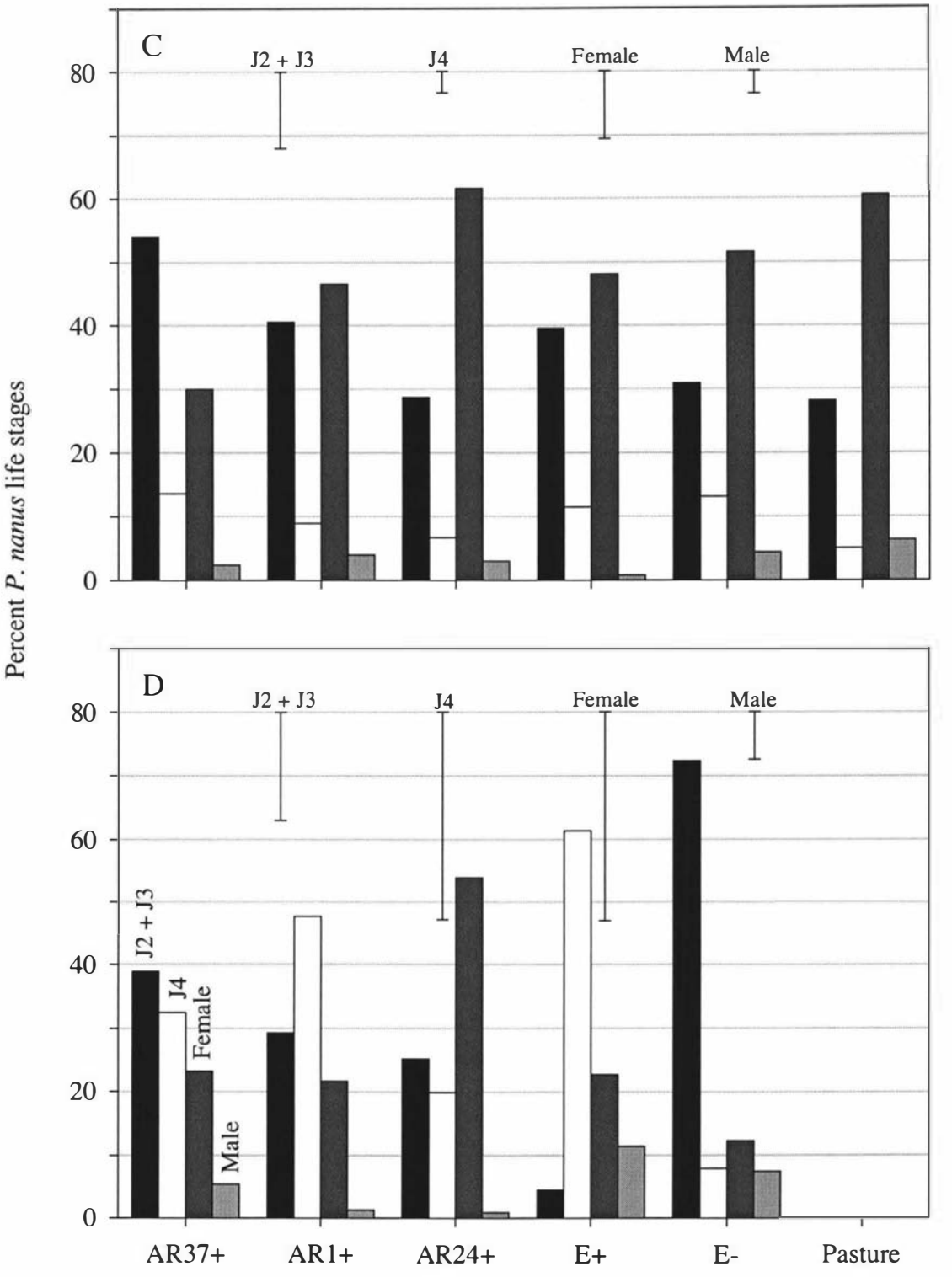


Fig. 2. *Proportion of* P. nanus *life stages in 0–20 cm soil under five ryegrass treatments and an adjacent pasture in A) November 1997, B) February 1998, C) March 1998 at Tokanui, and in D) April 1998 at Bay of Plenty (error bars are average SED).*

For ryegrass plots at Tokanui there were no significant differences in mean *P. nanus* /m^2 between 0–10 and 10–20 cm depths for any of the sample times (Table 3). There was a significant difference in the proportion of J2 + J3 stages between depths at all sample times with a greater proportion in 10–20 cm than 0–10 cm depth in

November and February while in March the difference was reversed (Table 3). Differences in the proportion of female and male *P. nanus* between 0–10 and 10–20 cm depths were always the reverse of that observed for J2 + J3 *P. nanus*, while J4 stages were evenly distributed between the depths at all sample times (Table 3). At the Bay of Plenty site there was no significant difference in number of *P. nanus* or proportions of any of the life stages between 0–10 and 10–20 cm depth (Table 3).

Table 3. *Mean* P. nanus *total numbers ($x10^3$ /m^2) and percentage of each life stage as a proportion of the total* P. nanus *population for combined ryegrass plots at Tokanui (November 1997, February and March 1998) and Bay of Plenty (April 1998) sites (values in brackets are $log_{10}(n+4)$). Symbols ns, †, *, **, *** denote non-significance, significance at P<0.1, 0.05, 0.01 and 0.001 respectively.*

Depth	Total	J2 + J3	J4	Female	Male
Nov. 1997					
0–10 cm	232.4 (2.32)	30.8	28.9	32.6	7.0
10–20 cm	216.9 (2.29)	63.9	25.2	10.2	0.9
SED	(0.24) ns	2.9 ***	3.0 ns	2.8 ***	1.9 **
Feb. 1998					
0–10 cm	322.0 (2.59)	10.4	36.6	41.9	11.2
10–20 cm	250.5 (2.48)	21.3	35.2	36.0	7.0
SED	(0.15) ns	4.5 *	4.8 ns	7.1 ns	3.7 ns
Mar. 1998					
0–10 cm	341.4 (2.61)	43.3	10.1	44.9	1.8
10–20 cm	415.3 (2.69)	30.9	10.5	54.5	4.0
SED	(0.18) ns	4.3 *	2.5 ns	5.2 †	1.0 *
Apr. 1998					
0–10 cm	79.1 (1.78)	39.8	22.7	29.9	7.6
10–20 cm	30.1 (1.36)	26.1	45.0	27.3	1.6
SED	(0.31) ns	8.1 ns	6.5 ns	7.4 ns	2.2 ns

In November and February there was no significant difference in the number of *Pratylenchus* /m^2 beneath any of the treatments (Fig. 3). In March, the number of *Pratylenchus* was lowest in soil beneath E+ and was significantly (P<0.05) less than occurred beneath E-, AR1+, AR24+ and pasture plots (Fig. 3). There was also

significantly (P<0.05) less *Pratylenchus* in AR37+ than AR24+ plots. At Bay of Plenty site there was no significant difference in numbers of *Pratylenchus* nematodes between treatments for 0–20 cm depth (Fig. 3).

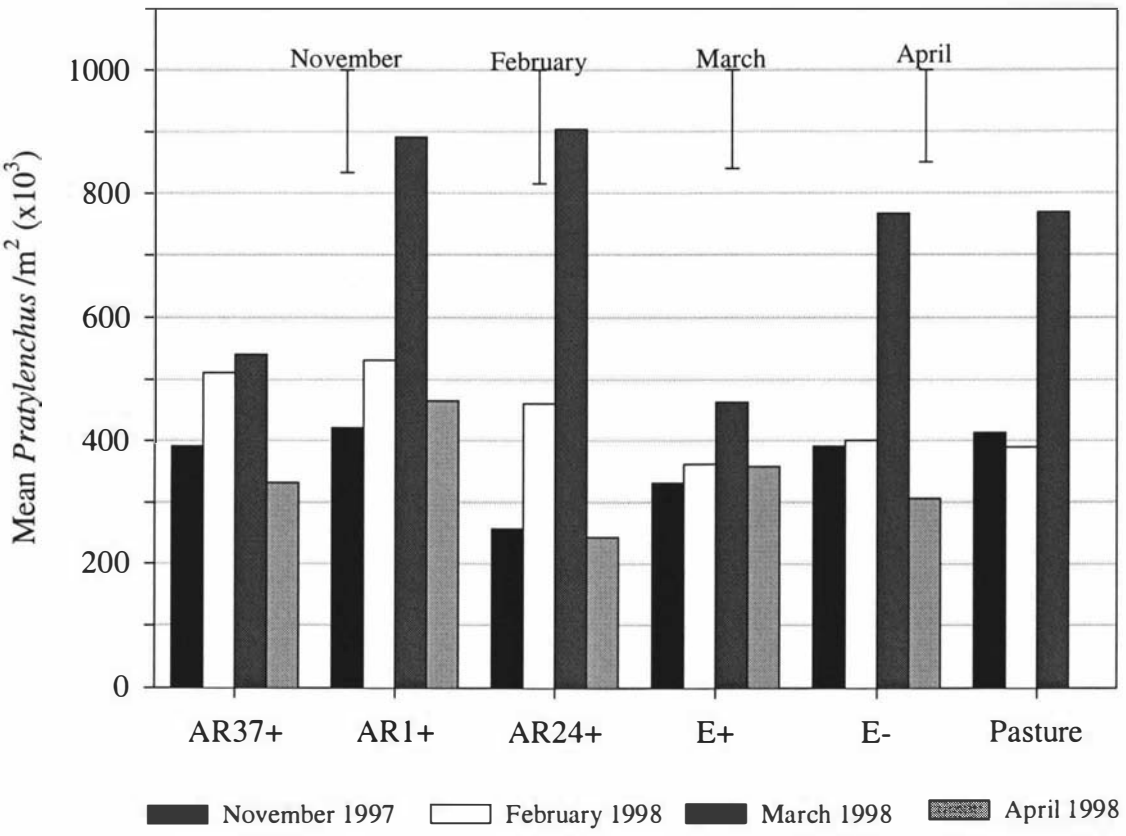


Fig. 3. *Mean* Pratylenchus */m² in 0–20 cm soil under five ryegrass treatments and an adjacent pasture in November 1997, February and March 1998 at Tokanui, and in April 1998 at Bay of Plenty (error bars are average SED).*

Soil beneath pasture had significantly (P<0.05) lower abundance of non-plant parasitic nematodes in February than in all the other treatments (Fig. 4). At the other sample times, there was no significant difference in numbers of non-plant parasitic nematodes amongst any of the treatments (Fig. 4). At the Bay of Plenty site there was no significant difference in numbers of non-plant parasitic nematodes between treatments for 0–20 cm soil (Fig. 4).

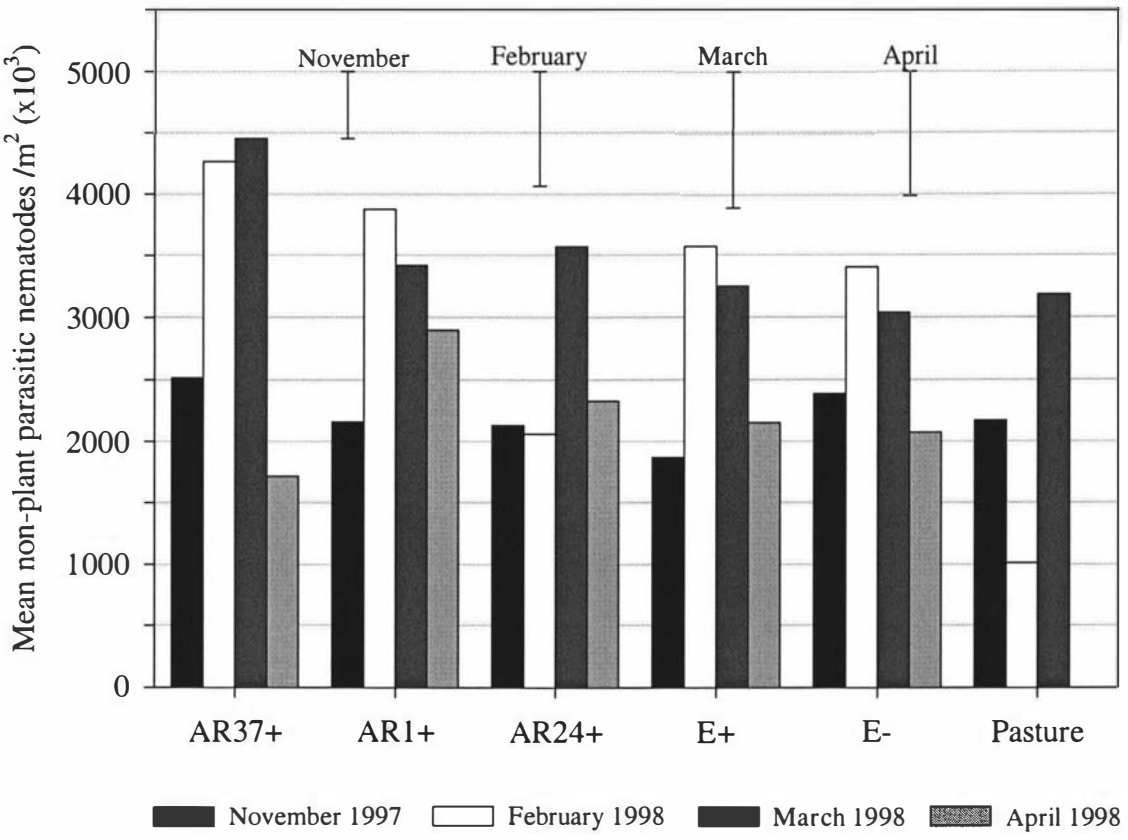


Fig. 4. *Mean non-plant parasitic nematodes /m^2 in 0–20 cm soil under five ryegrass treatments and an adjacent pasture in November 1997, February and March 1998 at Tokanui, and in April 1988 at Bay of Plenty (error bars are average SED).*

Within ryegrass plots significantly more *Pratylenchus* and non-plant parasitic nematodes were observed in 10–20 than 0–10 cm depth for both the February and March samplings (Table 4). In pasture samples there was significantly more *Pratylenchus* at 10–20 cm depth at all samplings, but consistently more non-plant parasites at 0–10 cm depth (Table 4). At Bay of Plenty there was significantly more *Pratylenchus* and significantly less non-plant parasitic nematodes in 10–20 than 0–10 cm depth (Table 4).

Table 4. *Mean* Pratylenchus, *non-plant parasitic nematodes ($x10^3$ /m^2) for ryegrass plots and adjacent pasture at Tokanui (November 1997, February and March 1998) and Bay of Plenty (April 1988) sites (values in brackets are log_{10} (n+4)). See Table 3 for explanation of symbols.*

	Pratylenchus		Non-plant parasites	
Depth	Ryegrass	Pasture	Ryegrass	Pasture
Nov. 1997				
0-10 cm	289.6 (2.62)	231.1 (2.52)	2881.4 (3.62)	2584.5 (3.57)
10-20 cm	329.4 (2.68)	602.2 (2.94)	1612.9 (3.36)	1784.0 (3.41)
SED	(0.05)ns	(0.12)***	(0.06)ns	(0.13)†
Feb. 1998				
0-10 cm	310.5 (2.65)	151.1 (2.34)	650.6 (2.96)	1373.9 (3.29)
10-20 cm	548.3 (2.90)	865.5 (3.09)	934.0 (3.12)	709.5 (3.00)
SED	(0.05)***	(0.13)***	(0.08)†	(0.17)*
Mar. 1998				
0-10 cm	493.5 (2.85)	341.1 (2.69)	865.8 (3.09)	3201.9 (3.66)
10-20 cm	1031.1 (3.17)	1745.5 (3.40)	1586.4 (3.35)	2904.4 (3.63)
SED	(0.06)***	(0.14)***	(0.07)**	(0.13)ns
Apr. 1998				
0–10 cm	170.0 (2.37)	—	2756.9 (3.59)	—
10–20 cm	586.9 (2.90)	—	1470.4 (3.32)	—
SED	(0.11) ***	—	(0.07) **	—

At Tokanui there was no significant difference in ryegrass growth rates between treatments in October and November 1997 (Fig. 5). In January 1998, AR37+ had a significantly ($P<0.05$) higher growth rate than both AR1+ and E+. Both AR37+ and E+ had significantly ($P<0.05$) higher growth rates than: E- in March and; AR1+, AR24+ and E- in May 1998 (Fig. 5). Across all treatments, ryegrass as a proportion of total herbage was 60.5, 83.8, 32.1, 50.9 and 75.7% for the five herbage samplings. The majority of the remaining herbage consisting of other grasses in October and November, and dead material in January, March and May.

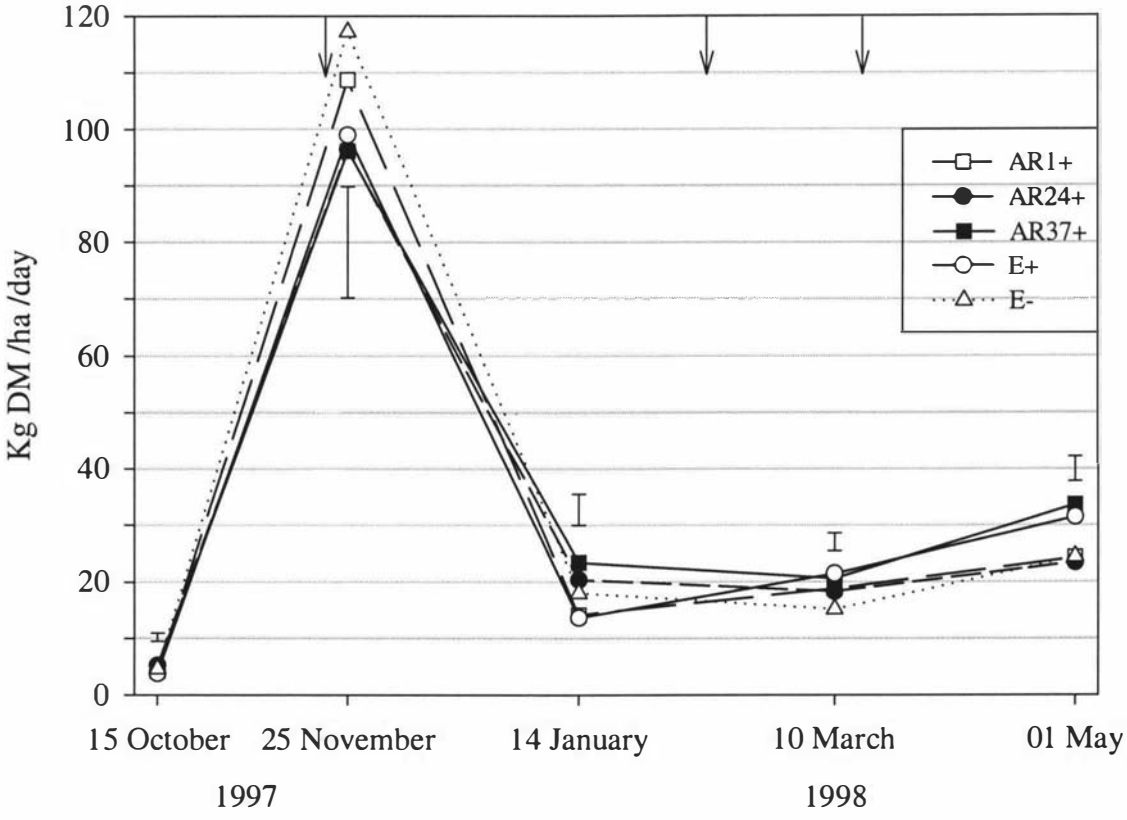


Fig. 5. *Mean growth rate for five ryegrass treatments at Tokanui. Arrows indicate nematode sampling times. Error bars are average SED. (From the data set of Dr J. P. J. Eerens).*

At Bay of Plenty, ryegrass growth for AR37+ (1016.3 kg DM /ha) and E+ (932.7 kg DM /ha) treatments was significantly ($P<0.05$) higher than E-, AR24+ and AR1+ treatments (662.7, 582.7 and 588.4 kg DM /ha) in March. Over all treatments, ryegrass made up 63.2% of the total herbage sampled with other grasses making up the majority of the remainder.

Compared to E- (with the lowest root mass), AR37+ had 81.1% more root mass and the other treatments had between 5.9% (AR1+) and 22.4% (E+) more root mass in 0–20 cm depth at Tokanui (Table 5). For all treatments combined, there was significantly greater root mass in 0–10 than 10–20 cm depth (Table 5). There was no significant treatment × depth interaction (data not shown).

Table 5. *Mean dry root mass /sample and root mass /m² for 0–20 cm soil and for combined ryegrass plots, from March 1998 sampling at Tokanui. See Table 3 for explanation of symbols.*

	Root mass (g /sample)	Root mass (g /m²)
E-	0.392	66.5
E+	0.480	81.5
AR 37	0.710	120.5
AR 24	0.435	73.8
AR 1	0.415	70.5
SED	0.170 ns	—
0–10 cm	0.761	129.2
10–20 cm	0.175	29.7
SED	0.100 ***	—

The abundance of *P. nanus* /g root was significantly (P<0.01) greater under AR37+ than E+ treatments, and at 10–20 cm than 0–10 cm depth (Table 6). For *Pratylenchus* in soil, expressed as number /g root, AR37+ and E+ treatments had significantly (P<0.05) fewer than AR24+ and, as for *P. nanus*, there was significantly (P<0.001) more at 10–20 cm then 0–10 cm depth (Table 6).

Table 6. *Mean* P. nanus */g root and* Pratylenchus *in soil on a /g root basis for 0–20 cm soil and for combined ryegrass plots, from March 1998 sampling at Tokanui (values in parentheses are log (n+4)). See Table 3 for explanation of symbols.*

	P. nanus /g root	*Pratylenchus* in soil /g root
E-	1963.6 (3.21)	3788.8 (3.55)
E+	704.8 (2.77)	1961.1 (3.26)
AR 37	4943.7 (3.61)	1920.9 (3.25)
AR 24	1459.0 (3.08)	4330.2 (3.61)
AR 1	1399.7 (3.06)	4050.5 (3.58)
SED	(0.25) **	(0.15) *
0–10 cm	765.1 (2.76)	1048.1 (3.00)
10–20 cm	3905.7 (3.47)	9234.5 (3.95)
SED	(0.18) **	(0.07) ***

There was a significant correlation between root mass and abundance of *P. nanus* in 0–10 cm depth, and between all stages of *P. nanus*, except the J4 stage (Table 7). There was no significant correlation of either *Pratylenchus* or non-plant parasites with root mass.

Table 7. *Pearson's correlation coefficient between number* P. nanus, Pratylenchus *or non-plant parasites /m^2 and dry root mass (g /m^2) for combined ryegrass plots (n=15), from March 1998 sampling at Tokanui. See Table 3 for explanation of symbols.*

	Root mass	
	0–10 cm	10–20 cm
P. nanus		
Total	0.742 **	0.143
J2 + J3	0.784 **	0.184
J4	0.299	0.293
Female	0.572 *	–0.023
Male	0.638 *	–0.169
Pratylenchus	–0.039	0.164
Non-plant parasites	0.348	–0.151

Discussion

At both Tokanui and Bay of Plenty sites AR37+ consistently supported the greatest abundance of *P. nanus* (Fig. 1). The other treatments were similar to each other in abundance of *P. nanus*. The adjacent pasture at Tokanui supported relatively low *P. nanus* populations. The root sampling at Tokanui (Table 5) suggests that a larger root mass could have been part of the reason for the abundance of *P. nanus* under AR37+, and the significant correlation of *P. nanus* with root mass (Table 7) supports this idea. Eerens *et al.* (1998) observed a significant correlation between *Paratylenchus* sp. and ryegrass root weight in a pot experiment with E+, E- and ryegrass infected with another endophyte (AR6+). In the results given here the non-feeding J4 stage of *P. nanus* was the only life stage not correlated with root mass.

Similar to the results from mature plants, a seedling experiment (Chapter 6) showed AR37+ supported a greater abundance of *P. nanus* /g root than either E+ or E- when results were pooled across *P. nanus* inoculum rates. Compared to the high

inoculum rate used in a seedling experiment (Chapter 6) the abundance of *P. nanus* /g root was lower in mature plants by at least an order of magnitude. This could partly be due to the larger proportion of coarser roots, with proportionally fewer root hairs, in mature plants. It is difficult, therefore, to estimate the relative effects of *P. nanus* on seedling and mature plants although it is expected that seedlings are more susceptible to root feeding damage than mature plants.

The similarity of treatment effects across the two field sites, with AR37+ supporting the largest population of *P. nanus,* shows that the results may be applicable to other sites where *P. nanus* is present. At both sites, *P. nanus* abundance was similar at 0–10 cm and 10–20 cm depths.

The increased abundance of J2 + J3 *P. nanus* under E- ryegrass at Bay of Plenty (Fig. 2) suggests that the E- plants were able to produce more new roots, or roots of higher quality, than other treatments in late autumn, once drought and pest pressures were reduced. While ryegrass production was lower overall at Bay of Plenty than Tokanui, the relative growth between treatments was similar, with AR37+ and E+ producing more ryegrass than other treatments. In a seedling experiment (Chapter 6) E+ produced more shoot dry matter than AR37+ and E- across three rates of *P. nanus* inoculum.

The increased abundance of *P. nanus* under AR37+ ryegrass is particularly interesting in terms of *P. nanus* damage thresholds to ryegrass growth. In the March sampling, for instance, AR37+ had a large root mass compared to other treatments (Table 7). This root mass did not result in increased aboveground growth compared to, especially, E+ (Fig. 5). Indeed, at no time did AR37+ have a higher growth rate than all other treatments. It may be that *P. nanus* feeding had a sufficiently large effect on AR37+ root efficiency (e.g. by compromising root hair function) to limit herbage growth to a similar level as the other treatments. To confirm this it would be necessary to examine the performance of the ryegrass treatments in the presence and absence of *P. nanus* in the field (possibly using nematicides). This would confirm the need to include *P. nanus* in the plant breeding component of perennial ryegrass improvement.

Despite large differences in *P. nanus* populations between AR37+ and other ryegrass treatments (Fig. 1), there was very little difference in life stage proportions (Fig. 2). This tends to support the idea that root mass was a determining factor in *P. nanus* abundance, as indicated by AR37+, and weakens the proposition that endophyte-produced alkaloids are directly limiting *P. nanus* abundance (see also Chapter 6).

In this study there was no significant difference in abundance of *P. nanus* between E- and E+ at any sample time (Fig. 1). In a similar experiment, (Eerens *et al.*, 1998) sampled *Paratylenchus* sp. from beneath grazed E- and E+ in southern New Zealand and found that in spring (but not in summer or autumn) there was significantly more *Paratylenchus* sp. in 0–10 cm depth under E- than E+. On occasions when significant differences were observed in the current study, E+ had a higher dry matter yield than E- (Fig. 5). In contrast Eerens *et al.* (1998) measured higher dry matter yields for E- than E+ swards. The difference between the two studies is probably explained by climatic (summer dry) and pest (in particular Argentine stem weevil – *Listronotus bonariensis*) stresses that were present at this site but not in southern New Zealand.

All ryegrass plots had greater root masses at 0–10 cm than 10–20 cm depth (Table 7), yet *P. nanus* was found at equivalent population densities across the two depths (Table 3) and *Pratylenchus* was more abundant at 10–20 cm depth (Table 4). This suggests that more actively growing roots were produced by the ryegrass plants at 10–20 cm depth (where soil moisture was greatest) which supported larger populations of plant parasitic nematodes, resulting in higher parasite levels per gram of root. The greater nematode pressure on ryegrass roots in the deeper soil may exacerbate plant stress, especially during times of low soil moisture. It was reported in Chapter 5 that *P. nanus* has a population peak in January and this may be partly responsible for decreased ryegrass performance at this time of year (Fig. 5). The loss of plant roots is often associated with dry summer conditions and perennial ryegrass plants are susceptible to "pulling" by stock before new roots are produced in the autumn which are able to re-anchor the plants.

Relative ryegrass growth rates (Fig. 5) did not appear to reflect relative abundance of *P. nanus* (Fig. 1). Only in January did AR37+ support both the greatest number of *P. nanus* and the greatest ryegrass growth rate. AR37+ and E+ treatments had the highest

ryegrass growth rates in March when these treatments had the lowest soil populations of *Pratylenchus* (Fig. 3). There was no significant difference in abundance of non-plant parasitic nematodes between any of the ryegrass treatments at either site (Fig. 4). This is unexpected if increased root mass was causing the increase in *P. nanus* abundance in AR37+ plots. It would be expected that a large root mass, with increased organic matter reserves, would result in increased abundance and activity of rhizosphere organisms that could act as a resource for microphagous nematodes (see Yeates, 1987).

The soil from beneath pasture at Tokanui contained only a small population of *P. nanus* (Fig. 1), this was probably due to a lower proportion of ryegrass, and higher proportion of clover compared to ryegrass plots. Eerens *et al.* (1998) observed significantly less *Paratylenchus* sp. from field plots of E- or E+ when clover was present. In addition, Matthew *et al.* (1995) showed that in clover patches both ryegrass tiller density and tiller size are reduced, and it is plausible that root mass would be similarly affected. *P. nanus*, with its limited range of host plants in pasture, would be especially vulnerable to the effect of plant competition on host root abundance.

The similarity in abundance of *P. nanus* between depths observed here (Table 3) is at variance to that shown in Chapter 5 where many more *P. nanus* were recovered from 10–20 cm than 0–10 cm depth. It is possible that in pure species ryegrass plots produced more roots at 0–10 cm depth in the absence of competition from other pasture species. Sward age differences between the two studies may also have contributed to differences in vertical root distribution. In common with observations in Chapter 5, the proportion of females in the Tokanui population increased in 0–20 cm depth from *ca* 20% in November to *ca* 50% in March (Table 3) compared to *ca* 20 and 48% in November and May as recorded in Chapter 5. This tends to support the idea put forward in Chapter 5 that *P. nanus* populations 'age', so that by autumn the population largely consists of adults.

The only occasion where a difference in *Pratylenchus* abundance between treatments occurred was in the March 1998 sampling at Tokanui (Fig. 3). This suggests that factors which enable the consistently large populations of the ectoparasite *P. nanus* to be supported beneath AR37+, are not acting in a similar manner on the migratory endoparasite *Pratylenchus*. There was a trend across all treatments for *Pratylenchus*

abundance in soil to increase from November to March. At both the Tokanui and Bay of Plenty sites there was more *Pratylenchus* at 10–20 cm than 0–10 cm depth (Table 4). It should of course be noted that *Pratylenchus* also occur within roots so results from soil only should be treated with caution. MacGuidwin (1989) estimated that only *ca* 20% of the *Pratylenchus scribneri* population in a potato crop occurred in the soil during the growing season, rising to 50% between seasons.

Populations of *Pratylenchus* in soil /g root recorded here (Table 6) are higher than observed by Yeates *et al.* (1985) at Tokanui for *Pratylenchus* within grass roots (maximum *Pratylenchus* population 1713 /g root). Yeates *et al.* (1985) observed peaks of *Pratylenchus* abundance in grass root in mid December and early August at Tokanui.

The only sample time at which significant treatment differences in populations of non-plant parasites were observed was in February at Tokanui when they were least abundant in pasture soil (Fig. 4) and also when soil moisture beneath pasture was at its maximum variance to ryegrass plots (Table 2). The difference in soil moisture almost certainly reflected differences in amount of plant cover between the ryegrass plots and the more heavily grazed pasture plots. Large differences in soil surface temperatures have been observed when grazing of pasture was deferred over the summer period and this translates to differences in soil moisture (Watson *et al.*, 1995, Harris *et al.*, 1999). There were more non-plant parasitic nematodes in 0–10 cm than 10–20 cm depth in April at Bay of Plenty (Table 4) and this was similar to the situation at Tokanui in November, suggesting that the increased abundance at the shallower depth was temperature mediated.

Yeates *et al.* (1985) considered the plant parasitic nematodes *Pratylenchus*, *Meloidogyne* and *Heterodera* to be complementary in their seasonal timing of clover root invasion and suggested that any attempt to improve clover production by breeding for *Meloidogyne* and /or *Heterodera* resistance could be thwarted through utilisation of unused resource by *Pratylenchus*. It may be that an analogous situation exists between *P. nanus* and *Pratylenchus* in grasses, so that *P. nanus* utilises any grass root resource not occupied by *Pratylenchus*, as appears to be the case here for AR37+ Nui.

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Chapter 8: Dynamics of sympatric *Paratylenchus nanus* and *Paratrichodorus minor* populations in soil under pasture

Summary – Sampling was carried out over six successive seasons in a grazed pasture in Waikato, New Zealand which contained populations of both *Paratrichodorus minor* and *Paratylenchus nanus*. Neither seasonal periodicity nor a consistent vertical distribution pattern was observed in the *P. minor* population. The *P. nanus* population had distinct spring and summer peaks, and abundance was greater at 0–10 than 10–20 cm depth. *P. minor*, but not *P. nanus*, abundance was significantly correlated with some abiotic factors, namely rainfall and an activity index combining rainfall and soil temperature for the week before sampling. It is suggested that significant correlations between *P. minor*, *P. nanus* and some soil nutrients were reflections of interactions with other, biotic factors such as plant growth. There was no evidence for competition occurring between these two nematodes and possible reasons for this are discussed.

Keywords: *Paratrichodorus minor*, *Paratylenchus nanus*, population dynamics, sympatric, rainfall, temperature, activity index, competition.

Plant parasitic nematode species do not exist in monoculture in soil but form part of a community that utilises plants as a resource for their growth and development. Host range differences may limit interaction between some members of the community, but where host ranges overlap competition for resources may ensue. Competition between plant parasitic nematodes has been recorded for all combinations of ectoparasitic, migratory, and sedentary forms (see Eisenback, 1985; Eisenback & Griffin, 1987). Closely related species may coexist under some hosts while under other hosts one species may dominate, as shown for two trichodorid species by Alphey (1985). Antagonistic competition has been shown between the ectoparasites *P. minor* and *Tylenchorynchus* spp. and the migratory endoparasite *Pratylenchus brachyurus* (Johnson & Nusbaum, 1968), and the sedentary endoparasite *Meloidogyne naasi* (Sikora *et al.*, 1979). *P. nanus* populations have been observed to dramatically increase after the removal of competing plant parasites (Boag & Alphey, 1988; Yeates & van der Meulen, 1996).

The dynamics of sympatric populations of *P. minor* and *P. nanus* have not previously been studied in detail. Sympatric populations of *P. minor* and *Paratylenchus*

have previously been recorded in New Zealand pastures by Yeates & Prestidge (1986). In the present study population dynamics of these two species were investigated in relation to abiotic and biotic factors.

Materials and methods

SITE CONDITIONS

The populations of *P. minor* and *P. nanus* were from Hamilton clay loam soil (Typic Haplohumult, wilting point 20%; field capacity 36%) at Ruakura Agricultural Centre, Hamilton, New Zealand as described in Chapter 2. The site consisted of a *ca* 0.3 ha paddock gently sloping east-west. Climate data (daily grass minimum temperature, 10 cm and 20 cm earth temperatures and rainfall) were obtained from the nearest New Zealand Meteorological Service climate station (Ruakura station C75731, altitude 40 m above mean sea level), *ca* 1 km from the trial site.

SAMPLING

The site was divided into a 40-plot grid, with each plot being 8 × 8 m. A preliminary sampling of 30 plots (one core /plot) was undertaken on 6 July 1995 and the sampling precision (standard error to mean ratio, see McSorley, 1987) for *P. minor* and *P. nanus* was 29.9 and 40.8% respectively; mean populations in 0–20 cm depth were 54.8 and 56.6 x 10^3 /m^2 respectively. The same 30 plots were sampled on a further six occasions: 29 November 1995, 26 February, 27 May, 27 August 1996, (Year 1) 28 November 1996 and 26 February 1997 (Year 2); these occasions are taken to represent spring, summer, autumn, winter, spring and summer respectively.

Details of nematode, soil moisture and herbage sampling methods are given in Chapter 4. *P. nanus* life stages were discriminated at each sampling whereas *P. minor* stages were discriminated only from autumn 1996.

Soil nutrient analyses (phosphorus (P), sulphur (S), magnesium (Mg), potassium (K), calcium (Ca), sodium (Na), nitrogen (N) and pH measurements were carried out on oven-dried core samples taken for soil moisture determinations for the November 1996, February and May 1997 monthly samplings. P was determined by a modification of Olsen *et al* (1954), SO_4-S by chromatography (Watkinson & Kear, 1991), pH by the water slurry method (Blakemore *et al.*, 1987), percent total N by the Kjedahl method

(Bradstreet, 1965) and the extractable cations Mg, K, Ca and Na using a flame spectrophotometer (Davies, 1952). Values for soil nutrient levels except for N (expressed as percent dry soil) were converted from 'MAF Quicktest' units to ppm (μg /g soil) in soil.

STATISTICS

Details of nematode data transformation and calculations of spatial distribution (k), Taylor's Power law, Pearson's correlation coefficients and Activity Index (a combination of accumulated temperature and rainfall) are given in Chapter 4.

The chi-squared test was used to determine independence between *P. minor* and *P. nanus* populations by scoring the presence or absence of each species in each soil core to give one of four combinations: *P. minor*-only, *P. nanus*-only, both *P. minor* and *P. nanus* or neither.

Results

SITE CONDITIONS

Total rainfall during Year 1 (October to September) was 1522.4 mm, 351.2 mm above the 20 year average (1977 to 1997; National Climate Centre, National Institute of Water and Atmospheric Research Ltd, Wellington, New Zealand), whereas the 318.6 mm during Year 2 (October to February) was 115 mm below the 20 year average for those five months (Fig. 1A). Earth temperature differences were similar to those for rainfall, with slightly higher (0.09°C) and lower (–0.84°C) than the 20 year average temperature for Years 1 and 2 respectively (Fig. 1B). Maximum daily rainfall was 64.6 mm on 21 May 1996. Maximum and minimum earth temperatures were 22.5°C on 18 February 1997 at 20 cm and 4.6°C on 31 July 1996 at 10 cm respectively.

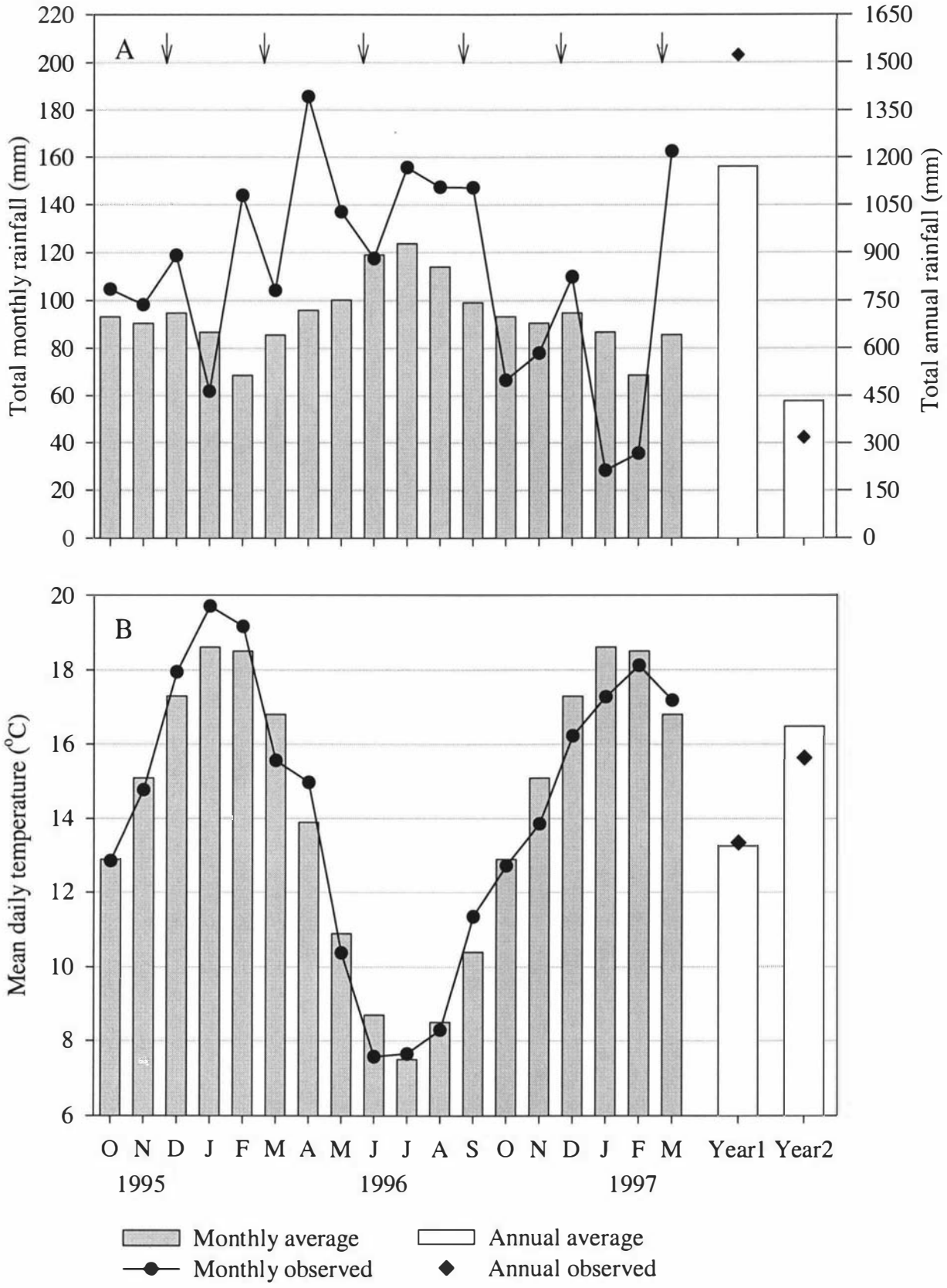

Fig. 1. *Observed and 20 year average A) rainfall and B) daily 10 cm earth temperature for: the monthly periods beginning 1 October 1995; and A) total rainfall B) daily 10 cm earth temperature for sampling years 1 (1 October 1995 to 30 Sept 1996) and 2 (1 October 1996 to 28 February 1997). Arrows indicate sampling times.*

In Year 1, soil moisture levels were similar in spring and summer then higher in autumn and winter before declining sharply to spring and summer of Year 2 (Fig. 2). Soil moisture was significantly higher in the spring of Year 1 at 10–20 cm depth than in Year 2, and at both depths in summer. Soil moisture dropped markedly below field capacity (36% dry weight) only in summer of Year 2 (Fig. 2).

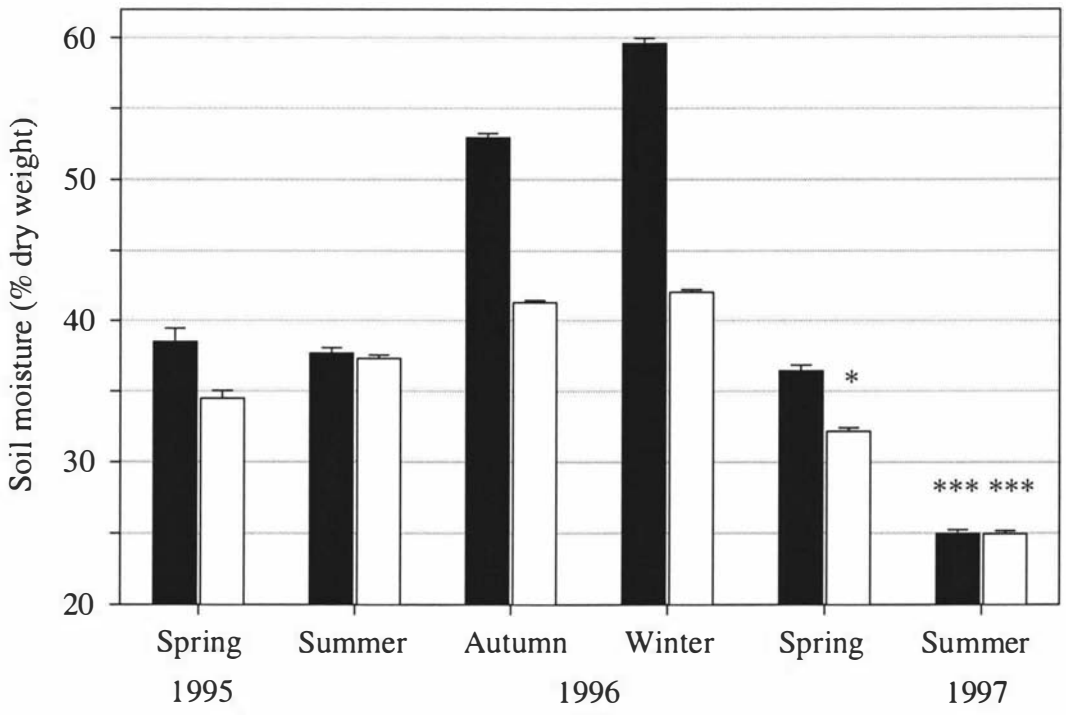

Fig. 2. *Seasonal soil moisture (error bars are standard error) in 0–10 and 10–20 cm depths during the six seasons sampled. Significant differences in soil moisture between seasons in successive years is indicated by * and *** which denote P<0.05 and P<0.001 respectively.*

The pasture sward at this site was dominated by ryegrass (*Lolium perenne* L.) (>60%) and broadleaf weeds (e.g. *Plantago lanceolata* L., *Taraxacum officinale* Weber and *Rumex obtusifolius* L.) (Fig. 3). There was a significantly higher proportion of white clover (*Trifolium repens* L.) and other grass (98.7% *Poa annua* L.) in spring of Year 2 than in spring of Year 1, with a consequent decrease in the proportion of weeds (Fig. 3).

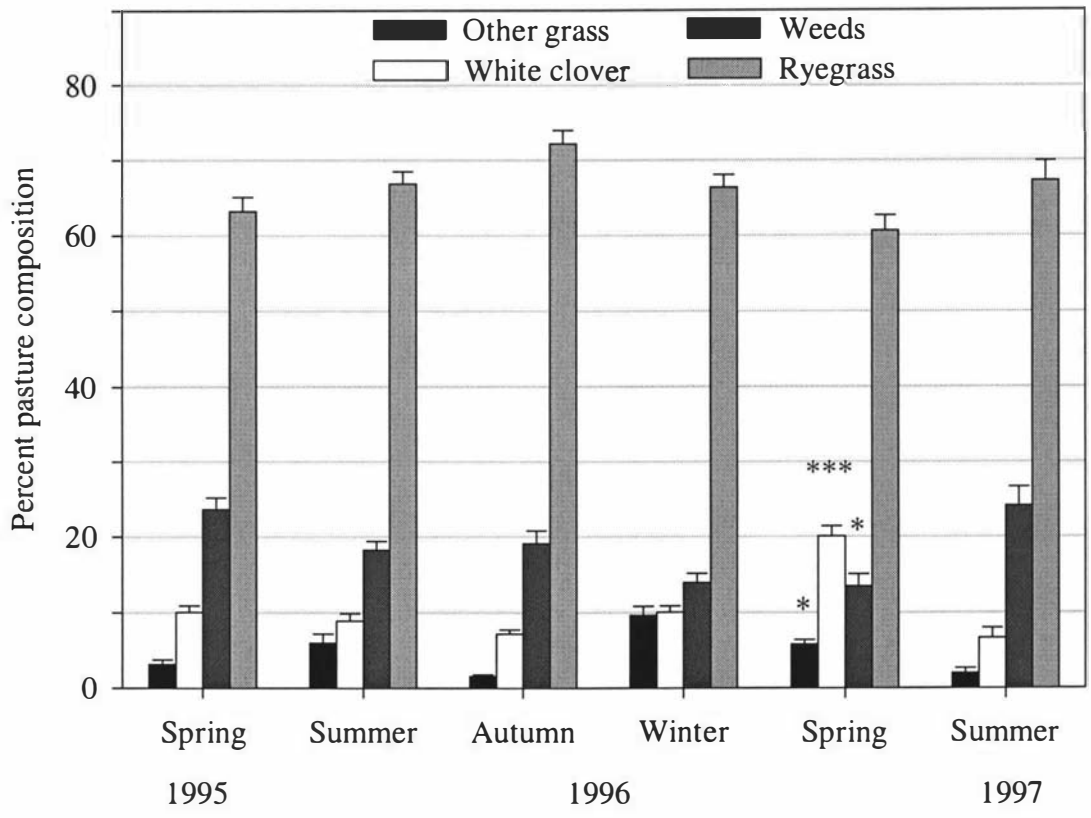

Fig. 3. *Seasonal pasture herbage composition (% dry mass) (error bars are standard error) from spring 1995 to summer 1997. See Fig. 2 for explanation of symbols.*

NEMATODE POPULATIONS

The overall population of *P. minor* in 0–20 cm soil declined steadily from a peak of 71.0 x 10^3 /m^2 in spring 1995 to 26.4 x 10^3 /m^2 in winter 1996 after which the population stayed almost constant. Populations at each depth are given in Fig. 4. The abundance of *P. minor* at both 0–10 and 10–20 cm depths was significantly greater in spring and summer of Year 1 than comparative samplings in Year 2.

P. nanus abundance was highest in the spring and summer samplings at 0–10 cm depth, with no apparent seasonality at 10–20 cm depth (Fig. 4B). At all sample times there were more *P. nanus* in 0–10 cm than 10–20 cm depth (Fig. 4). The seasonal abundance of other plant parasitic nematodes and total non-plant parasites is presented in Appendix 1.

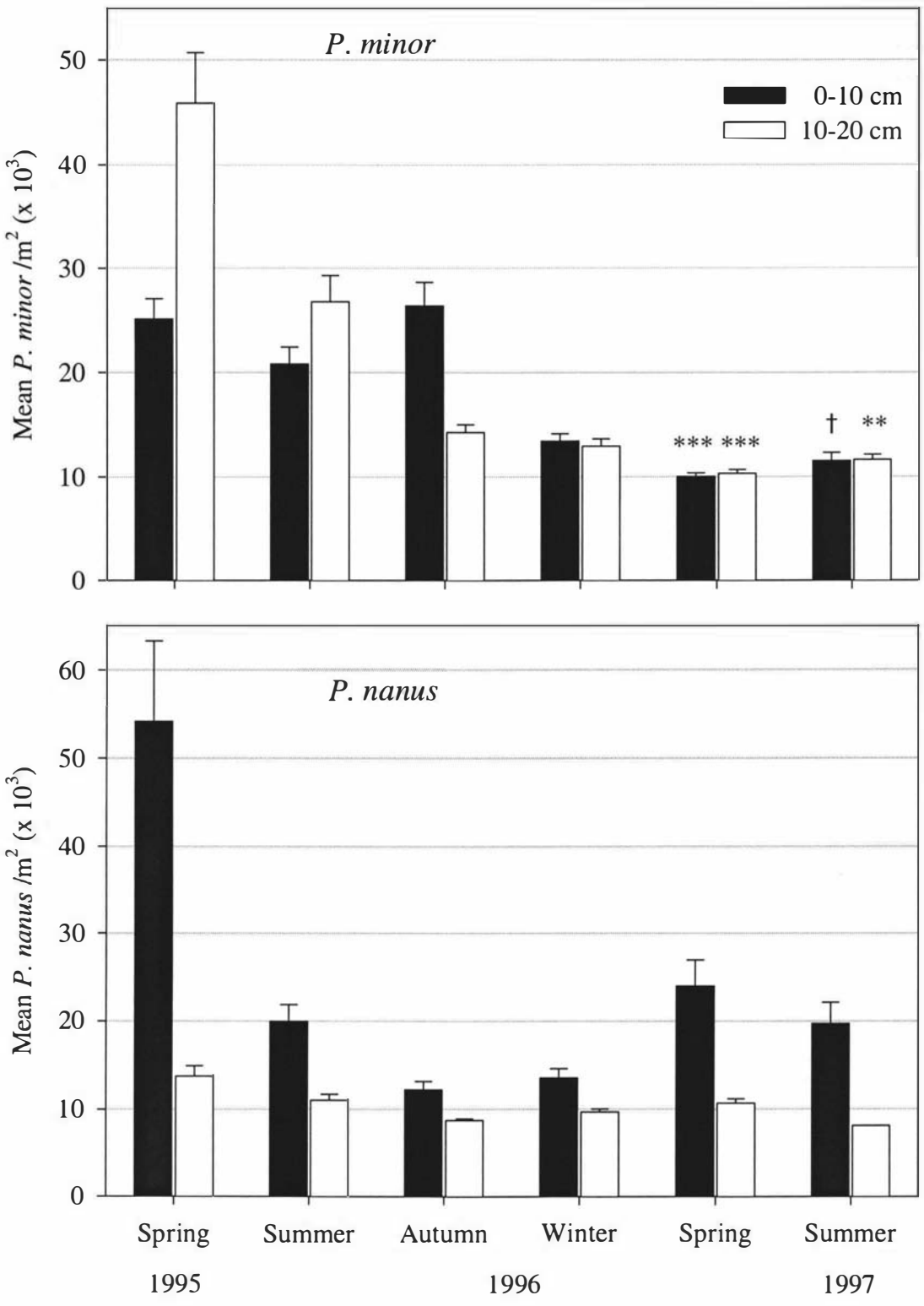

Fig. 4. *Seasonal abundance of* P. minor *and* P. nanus */m^2 (error bars are standard error) in 0–10 and 10–20 cm depths for the six seasons sampled. † denotes P<0.1, see Fig. 2 for explanation of other symbols.*

The proportion of juvenile *P. minor* increased consistently from autumn to summer with a consequent decline in proportion of females (Fig. 5). During the course of sampling, only one male *P. minor* was found, in summer 1996 (see Chapter 2).

Female *P. nanus* had the most consistent seasonal pattern of any of this species life stages (Fig. 5). The proportion of females increased from spring to winter of Year 1, with a repeated increase from spring to summer in Year 2. In the summer of Year 1, the proportion of J2 + J3 was high with a small proportion of J4 but this pattern was reversed in the summer of Year 2 (Fig. 5). Due to the high proportion of samples that did not contain *P. nanus*, no *t*-test was performed on the comparisons in life stage proportions between seasons.

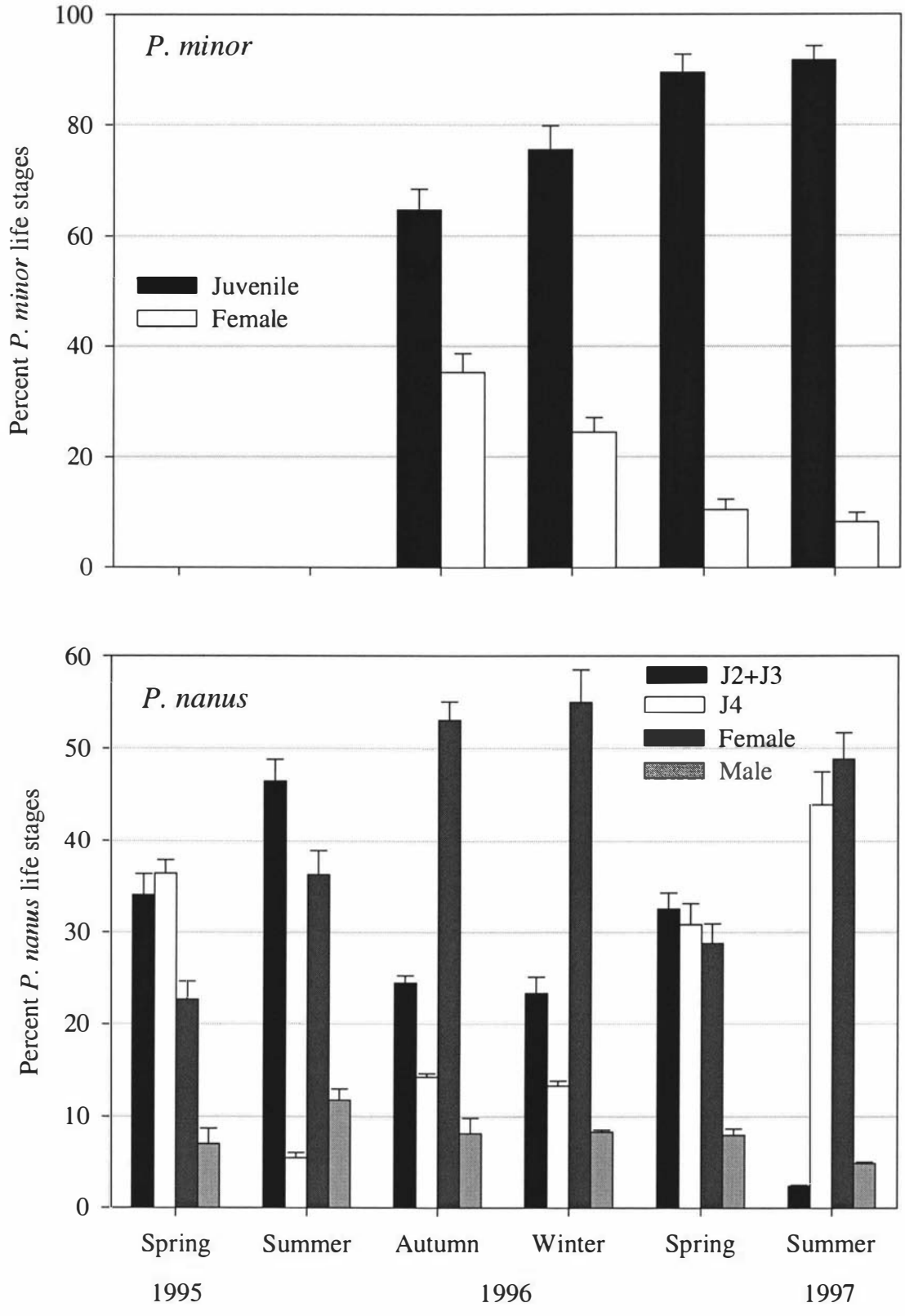

Fig. 5. *Seasonal occurrence of life stages of* P. minor *and* P. nanus *as a proportion of the total population (error bars are standard error) from spring 1995 to summer 1997.*

Consistently low k values demonstrate spatial aggregation of *P. minor* and *P. nanus* populations (i.e. $k \geq 8$ equates to a Poisson distribution and $k = 0$ equates to a logarithmic distribution), with *P. nanus* being more strongly aggregated than *P. minor* (Table 1). There was an increased degree of *P. minor* aggregation in 0–10 cm soil in spring and summer of Year 2 (Table 1). A k value was not calculated for the Year 2 summer population of *P. nanus* in 10–20 cm depth due to the large proportion of samples which contained no individuals of the species.

Table 1. *Values of the aggregation index* k *in the negative binomial for* P. minor and P. nanus *from spring 1995 to summer 1997. (nd = not determined).*

	P. minor		*P. nanus*	
Year : Season	0–10 cm	10–20 cm	0–10 cm	10–20 cm
Year 1: Spring	0.49	0.97	0.08	0.05
Summer	0.34	0.25	0.22	0.04
Autumn	0.57	0.50	0.08	0.07
Winter	0.48	0.35	0.07	0.15
Year 2: Spring	0.22	0.31	0.12	0.17
Summer	0.08	0.40	0.07	nd

The population specific index of aggregation (b) derived from Taylor's Power Law (where distribution is uniform as b approaches zero, random when $b = 1$ and highly aggregated as b approaches infinity) was 1.95 and 2.00 for *P. minor* and *P. nanus* respectively, for 0–20 cm depth over all sampling times. These values indicate aggregated populations. The value for the sampling factor a, also from Taylor's Power Law, was 28.36 and 9.97 for the two nematodes respectively.

The seasonal abundance of *P. minor* at 0–10 cm depth was significantly correlated with cumulative rainfall and Activity Index (basal temperature = 10°C) only when these variables were calculated for the week preceding sampling (Table 2). Longer time lags resulted in non-significant correlations. Abundance of *P. minor* at 0–10 and 10–20 cm was not correlated with either soil moisture or grass minimum temperature at these time intervals (data not shown).

Table 2. *Values of environmental variables and their correlations* (r) *with log (n+4)* P. minor */m*^{2} *(n = 6). († and * denote significant correlations at P<0.1 and 0.05 respectively.*

Environmental variable	Year 1				Year 2		Correlation coefficient	
	Spring	Summer	Autumn	Winter	Spring	Summer	0–10 cm	10–20 cm
Cumulative Rainfall (mm)								
1 week lag	38.8	45.3	91.9	54.8	3.2	2.8	0.771 †	0.216
2 week lag	47.9	67.6	122.4	82.7	19.0	25.4	0.670	0.073
4 week lag	98.2	155.2	156.2	143.9	76.4	35.2	0.642	0.246
Earth temperature °C (10 cm)								
1 week lag	16.3	20.1	13.3	10.1	16.1	20.5	–0.119	0.201
2 week lag	16.4	20.8	13.2	10.0	15.9	20.4	–0.084	0.239
4 week lag	16.1	20.9	13.1	10.0	15.7	20.1	–0.066	0.246
Activity Index (10 cm)								
1 week lag	20.6	12.6	23.8	–2.1	4.2	2.8	0.891*	0.613
2 week lag	26.8	64.9	29.3	–4.0	11.7	23.8	0.493	0.494
4 week lag	52.1	156.3	43.4	–16.5	26.5	31.8	0.433	0.506

There was no significant correlation between *P. nanus* seasonal abundance and any of the environmental variables, for any of the time lags (data not shown). Neither were there any significant correlations between any of the life stages of *P. nanus* and any of the environmental variables.

Of the interactions between seasonal abundance of other nematodes (both plant and non-plant parasitic), *P. minor* was significantly positively correlated with only the feeding stages (J2+J3, females) and males of *P. nanus* in 10–20 cm soil depth (Table 3).

Table 3. *Pearson's correlation coefficients* (r) *between log (n+4)* P. minor *and* P. nanus *life stages from spring 1995 to summer 1997 (n = 6).*

	P. minor	
	0–10 cm	10–20 cm
Total *P. nanus*	0.231	0.722
P. nanus life stages		
J2 + J3	0.397	0.799 †
J4	–0.005	0.319
Female	0.121	0.834 *
Male	0.201	0.968 **

Soil P, K, Mg, Ca and N contents in this clay loam soil were higher in the 0–10 cm depth, only S being at a greater level in 10–20 cm depth. Soil pH and Na were similar across depths (Table 4).

Table 4. *Mean values (± standard error) (n = 30) of nutrient and pH analyses for 0–10 and 10–20 cm depth soil sampled on 29 November 1995.*

	Depth	Mean value		Depth	Mean value
pH	0–10 cm	5.8 (0.02)	Mg (ppm)	0–10 cm	97.2 (3.8)
	10–20 cm	5.7 (0.04)		10–20 cm	40.0 (1.8)
P (ppm)	0–10 cm	26.9 (1.5)	Ca (ppm)	0–10 cm	783.3 (29.9)
	10–20 cm	11.4 (1.5)		10–20 cm	429.2 (28.5)
S (ppm)	0–10 cm	42.2 (1.3)	Na (ppm)	0–10 cm	31.2 (1.2)
	10–20 cm	77.5 (6.2)		10–20 cm	30.3 (1.6)
K (ppm)	0–10 cm	422.0 (30.1)	N (%)	0–10 cm	0.520 (0.011)
	10–20 cm	323.3 (32.8)		10–20 cm	0.242 (0.007)

At the time of nutrient sampling *P. minor* abundance at 0–10 cm depth was significantly and positively correlated with potassium, which itself was significantly negatively correlated to clover dry weight – a correlation which is in the same direction as the *P. minor* /clover dry weight relationship (Table 5). At 10–20 cm depth, where *P. minor* was significantly correlated with the level of a nutrient, except for potassium, the nutrient was also significantly correlated (but in the opposite direction) to either non-plant parasitic nematodes or *P. nanus* (Table 5).

Table 5. *Pearson's correlation coefficients (r) between log (n+4)* P. minor */m*^{2}, P. nanus */m*^{2}, *and nutrients for the November 1995 sampling (n=30). ns = not significant.*

0–10 cm	*P. minor*	K (ppm)	
K (ppm)	0.361*	—	
Clover dry weight (g)	–0.215	–0.384*	
10–20 cm	*P. minor*	Non-plant parasites	*P. nanus*
S (ppm)	0.362*	–0.422*	ns
K (ppm)	0.476**	ns	ns
Mg (ppm)	–0.526**	0.414*	ns
Ca (ppm)	–0.550**	0.429*	ns
Na (ppm)	–0.449*	ns	0.525**

A chi-square analysis of the possible combinations of *P. minor* and *P. nanus* extracted from each soil core over all sample dates indicated that the two populations were independent of each other (chi-square P>0.1) at both 0–10 and 10–20 cm (0–20 cm given in Table 6). The proportion of cores containing *P. minor*-only was greater than those containing *P. nanus*-only at five of the six samplings, and the proportion containing neither *P. minor* nor *P. nanus* increased over the sampling time (Table 6), in parallel with the declining population of *P. minor* (Fig. 5).

Table 6. *Proportion of cores, 0–20 cm depth, (n = 30) at each sampling from which* P. minor-*only,* P. nanus-*only, both* P. minor *and* P. nanus *or neither were extracted.*

	Spring 1995	Summer 1996	Autumn 1996	Winter 1996	Spring 1996	Summer 1997
P. minor-only	50.0	40.0	60.0	46.7	16.7	26.7
P. nanus-only	10.0	6.7	0.0	10.0	26.7	6.7
Both	33.3	33.3	13.3	20.0	13.3	13.3
Neither	6.7	20.0	26.7	23.3	43.3	53.3

Discussion

Rainfall and temperature data show that, at this site, Year 1 overall was wetter than normal with close to normal mean temperature, whereas the seasons sampled in Year 2 were drier and cooler than normal (Fig. 1). Soil moisture determinations reflected this trend with higher contents in Year 1 spring and summer than in Year 2 (Fig. 2). A number of trichodorid nematode populations seem sensitive to low soil moisture. This may explain the much lower populations of *P. minor* in Year 2 of the current study (Fig. 4). Bird & Mai (1967) found, in a clay loam soil, that soil moisture close to field capacity supported a significantly greater *P. minor* population increase than lower soil moistures. Baujard & Martiny (1995) and Mojtahedi *et al.* (1997) observed similar soil moisture effects with *P. minor* and *P. allius* respectively. Jones *et al.* (1969) pointed out the effect of low soil moisture on decreased incidence of sugar beet stunting by *Trichodorus* spp. Boag & Alphey (1988), however, observed that low soil moisture had no effect on *P. pachydermus* abundance.

Soil texture may exacerbate the effects of soil moisture, so that at the same moisture content silty clay loam supports lower multiplication of *P. minor* than do loam and sandy loam soils (Thomason, 1959; Schilt & Cohn, 1975). Jones *et al.* (1969) found *Trichodorus* spp. nematodes were most abundant in coarse soils (>30% coarse sand) and related this to pore size within the soil which would allow lateral migration of nematodes of their diameter (*ca* 33 µm average adult diameter, cf *ca* 30 µm average female diameter for *P. minor* from the current study).

Of the nutrients measured here (Table 4), P (0–10 cm) and K (10–20 cm) were at higher levels and N at lower levels than in Otorohanga silt loam soil at Tokanui

Research Station (Chapter 4). Other nutrients did not differ significantly. Most significant correlations between *P. minor*, *P. nanus* and nutrients coincided with correlations between these nematodes and other, biotic factors (e.g. white clover and non-plant parasitic nematodes) (Table 5). This suggests that factors other than the nutrients themselves were effecting abundance (e.g. nutrients levels related to nematode abundance through plant growth). A significant correlation between a low density *Paratylenchus* sp. population and Na has been reported by Wallace *et al.* (1994). For *Criconemella* sp. from the same site, the positive correlation with Na was attributed to the nutrients positive influence on plant growth.

The population density of *P. nanus* in this clay loam soil (Fig. 4) was at least an order of magnitude lower than observed in Otorohanga silt loam (see Chapter 4) and this may be the reason no significant correlations could be found between this nematode and environmental variables. That is, the effects of sampling error and aggregation may have been such that relationships were masked.

The vertical distribution of *P. nanus* at this site, with consistently low populations at 10–20 cm (Fig. 4), differs from that observed in Otorohanga silt loam soil (see Chapter 4). The Hamilton clay which forms the subsoil (below 10–15 cm depth) of the present site may be a barrier to *P. nanus* population development through the physical nature of the soil itself, through an effect on rooting depth of it's host plants, or through an effect on soil moisture regime. It is plausible that soil texture in the current study was the predominant factor controlling abundance of *P. nanus*.

The vertical distribution of *P. minor* (Fig. 4) does not seem to be affected by the subsoil texture in the same way as *P. nanus*, suggesting either that it could overcome physical properties of the soil which may hinder *P. nanus,* or that some of it's host plants (e.g. white clover) were deeper rooting than the grasses favoured by *P. nanus* (see Chapter 2). Lower soil N content at this site compared to a silt loam soil, especially at 10–20 cm depth (0.52 *vs* 0.97% and 0.24 *vs* 0.54% for 0–10 cm and 10–20 cm depths respectively), supports the possibility of a shallower rooting depth of *P. nanus* than *P. minor* hosts (i.e. grasses are limited by nitrogen availability more than nitrogen-fixing clovers).

The change in the relative proportions of J2+J3 and J4 *P. nanus* between summer 1996 and summer 1997 (Fig. 5) reflects population responses to some "stress" by ceasing development at the non-feeding J4 stage in the summer of 1997. It is possible the "stress" was a combination of decreased soil moisture (low rainfall and high temperatures) (Fig. 2), and the resulting slow plant root growth. Rhoades & Linford (1961) observed that the *P. projectus* J4 stage was able to survive desiccation and became the dominant life stage when plant hosts became root-bound in pots (i.e. limiting fresh root growth). Moulting of J4 *Paratylenchus* spp. individuals occurs in response to host root diffusates (Rhoades and Linford, 1959) i.e. once plant root growth has recommenced.

There was no periodicity in the *P. minor* population on the timescale used here (Fig 4), a similar situation to that observed by Boag & Alphey (1988) for *P. pachydermus*. A significant increase in white clover levels in spring Year 2 (Fig. 3) did not result in an increase in *P. minor* abundance, perhaps due to increased *Meloidogyne* root invasion in the clover (which was followed by a significant increase in Year 2 summer soil populations – Appendix 1). Complementary utilisation of white clover roots has been observed for *Meloidogyne*, *Heterodera* and *Pratylenchus* in New Zealand (Yeates *et al.*, 1985). Seasonal changes in the *P. nanus* population, at 0–10 cm depth (Fig 4), were similar to that observed in Otorohanga silt loam soil (Chapter 4), with an increase in abundance over spring and summer. The lack of this seasonal pattern at 10–20 cm depth again suggests that the clay subsoil was directly or indirectly unfavourable for *P. nanus* population growth.

The apparent lack of competition between the *P. minor* and *P. nanus* populations observed here (Table 6) differs from that observed by Boag & Alphey (1988) between *P. pachydermus, P. nanus* and *Rotylenchus robustus* after nematicide application. Boag & Alphey (1988) considered *P. nanus* and *P. pachydermus* to be r and K selected, respectively, and that once competition was removed the more rapid population growth of *P. nanus* allowed it to dominate. Similarly, Yeates & van der Meulen (1996) observed a replacement of *Helicotylenchus* by *Paratylenchus* after methyl bromide treatment of pastoral soil.

Competition may have been expected between these two sympatric ectoparasites with similar stylet (or onchiostyle) lengths (27.3 and 27.0 μm mean female stylet length

in *P. minor* and *P. nanus* respectively) and overlapping host ranges (see Chapter 2). It should be noted, however, that the *P. minor* stylet has a different form and mode of action to that found in *P. nanus*, so that in this case a simple comparison of lengths may not be valid. Body length and width dimensions of the two nematodes are quite different (*ca* 600 and 330 µm mean female length and *ca* 30 and 15 µm mean female width for *P. minor* and *P. nanus* respectively – see Chapter 2) and may be important in determining the habitable space available to the nematodes (Elliott *et al.*, 1980). Yeates (1986) suggested that the relationship between female stylet and body length of plant parasitic nematode genera could be used to indicate niche similarities. From his calculations it seems unlikely that *Paratylenchus* and *Paratrichodorus* would share a niche even if stylet lengths were functionally comparable; the different feeding sites of the two nematodes tends to support this idea. For example, *P. minor*, like other trichodorid nematodes, feeds in the elongation zone of plant roots (Pitcher, 1967; Hogger, 1973; Schilt & Cohn, 1975; Wyss, 1982) while *P. projectus* has been reported to feed on root hairs and the root epidermis in the J2 stage and on the "young mature" region of roots in later stages (Rhoades & Linford, 1961).

The present results indicate that, at 10–20 cm depth, the abundance of *P. minor* and feeding stages of *P. nanus* are linked (Table 3) and some of the differential characters of the two species allow them to co-exist without competing. However, competition would only be observed when a resource that both species can utilise is limiting. In this soil with low numbers of both *P. nanus* and *P. minor* present the available root resource may have been sufficient to allow these two nematodes, and the other plant parasites, to coexist. This agrees with Eisenback & Griffin's (1987) conclusion that competition between nematodes is generally weak and that ectoparasites have less competitive advantage than endoparasites.

APPENDIX 1

*Seasonal abundance ($x10^3$ /m^2) (± SEM) of other plant parasitic and non-plant parasitic nematodes in 0–10 cm and 10–20 cm depth from Spring 1995 to Summer 1997. (†, *, **, *** denote P<0.1, 0.05, 0.01 and 0.001 respectively for the difference between comparable seasons across years)*

	Year 1				Year 2	
Nematode	Spring	Summer	Autumn	Winter	Spring	Summer
Pratylenchus						
0–10 cm	184.1 (37.0)	248.9 (48.7)	55.4 (12.4)	89.6 (20.6)	71.7 (12.0) ***	99.3 (26.5) *
10–20 cm	122.2 (24.6)	265.7 (70.8)	86.6 (16.7)	117.0 (28.6)	88.6 (13.6)	135.6 (29.2)
Meloidogyne						
0–10 cm	36.0 (9.4)	18.5 (4.2)	15.3 (2.0)	18.1 (3.6)	66.9 (22.4)	184.1 (59.8) **
10–20 cm	12.9 (1.8)	13.3 (1.9)	15.8 (2.6)	9.3 (0.4)	9.2 (0.7)	22.4 (5.3)
Heterodera						
0–10 cm	62.9 (18.4)	40.2 (11.1)	18.1 (2.6)	26.6 (4.4)	47.0 (11.6)	80.9 (25.7)
10–20 cm	14.4 (1.9)	15.2 (2.7)	14.4 (2.4)	13.3 (2.0)	12.3 (1.4)	30.0 (7.7)
Non-plant parasites						
0–10 cm	6327.7 (411.3)	3799.8 (301.4)	2655.9 (213.7)	1999.8 (194.6)	5570.6 (421.0)	6072.5 (335.9) *
10–20 cm	1588.1 (132.8)	863.4 (87.2)	738.0 (65.8)	728.3 (69.0)	1326.7 (143.9) †	2280.5 (174.8) ***

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Chapter 9: General Discussion

This thesis elucidates for the first time some properties of *Paratylenchus nanus* and *Paratrichodorus minor* populations in pastoral ecosystems. Specific identifications and host range determinations (Chapter 2) provide the basis for determining their status and management in pasture. Optimising extraction procedures (Chapter 3) facilitated ecological studies (Chapter 4, 5 and 8) which indicate the spatial and temporal distribution of the nematodes, and those factors important in determining their population dynamics. The damage potential of *P. nanus* to both seedlings and mature plants (Chapters 6 and 7) highlights the potential of this nematode to influence pasture productive potential. There is a need for this nematode to be considered in plant yield experiments especially when *Neotyphodium* sp. endophytes are being evaluated for their agronomic impact.

In terms of damage potential it appears that *P. nanus* is a comparatively weak pathogen as evidenced by a lack of consistent damage to pasture plants in conditions suitable for good plant growth. However, the root hair feeding by this nematode combined with its potentially great abundance and a summer population peak suggests that it would exacerbate poor plant performance during a period usually associated with moisture stress. This becomes more probable when it is considered that *P. nanus* populations extend throughout the soil depths in which grass roots are most likely to occur.

The wide host range of *P. minor* would make it an intractable pest problem if serious pathogenicity were demonstrated. The localised distribution that *P. minor* occupies currently lessens the extent of any agronomic impact by this nematode. Yeates *et al.* (1998) investigated the effect of a 2°C global temperature rise on *P. minor* distribution in Australia and Africa. They showed that *P. minor* distribution would shift southwards in Australia to include Tasmania and coastal areas of the Great Australian Bight. Twenty six potential new distribution points for *P. minor* were predicted (Yeates *et al.*, 1998), a situation that may occur in New Zealand if a similar rise in temperature were to occur. The impact of *P. minor* on agricultural production therefore, may be more serious in the future if global warming predictions become reality.

As mentioned in the General Introduction, the clover component of New Zealand pastures has received much attention in relation to plant parasitic nematodes. The grass component deserves increased attention, not only in terms of the nematodes studied here but also in terms of other taxa such as *Pratylenchus* and *Helicotylenchus*. *P. nanus* and *Pratylenchus* appear to have complementary grass root utilisation, analogous to the temporal situation that exists for *Pratylenchus*, *Meloidogyne* and *Heterodera* in white clover (Yeates *et al.*, 1985). Whether this complementarity means plant damage is additive, or that competition leads to either no increase (Yeates *et al.*, 1977) or even a decrease in damage is not clear and requires investigation under a range of soil and fertility conditions.

Although New Zealand pastures are often sown as a legume /grass biculture the composition of long-term pastures is more complex due to non-sown weed and grass species. Pastures, therefore, are a complex of plant hosts being affected by a complex of invertebrate pests. The relatively recent development of nematology in New Zealand means that only some of the components of the plant parasitic nematode fauna are understood. For example, the species and distribution of *Helicotylenchus* have been elucidated from soils under pasture in New Zealand (Yeates & Wouts, 1992) but their host ranges and damage potential have not. Interactions between plant parasitic nematodes are poorly understood and can realistically only begin to be elucidated after components of the root feeding biota have received much more study.

Detailed knowledge of the plant parasitic nematode fauna of New Zealand has the potential to play a vital role in internal and external biosecurity. There is a need to know the presence and distribution of plant parasitic nematode species presently in New Zealand so that border control authorities can be appraised of the sort of material which should be closely examined before it is allowed entry to this country. Identification and subsequent study needs to be carried out at the species level, which will not only benefit external biosecurity but will inevitably lead to new species and distribution records (e.g. this study; Mercer, 1986; Mercer *et al.*, 1997a). Nematodes have to be physically moved, usually by man's intervention, to establish new infestation sites. It is important for internal biosecurity to understand the damage potential of several nematode genera (e.g. *Xiphinema*, *Longidorus* and *Tylenchorhynchus*) which currently have restricted distributions in New Zealand pastures. A greater knowledge of the capacity of the plant

parasitic nematode fauna to reduce agricultural production will have impacts in the areas of sustainability and maintaining international competitiveness in New Zealands pasture based industries.

Approaches to minimise plant parasitic nematode damage to pastures currently being assessed in New Zealand include: breeding white clover for resistance (Mercer *et al.*, 1997b) and tolerance (Mercer *et al.*, 1999); and evaluating alternatives to white clover as a pasture legume [e.g. Caucasian clover (Watson *et al.*, 1996, 1998)]. Attempts at microbial control of nematode pests (especially *Heterodera trifolii*) have been made (Hay & Skipp, 1993; Hay *et al.*, 1996) and further work is required in this area. Soil amendments have also been investigated for their efficacy in controlling plant parasitic nematodes. Chitin amendment resulted in reduced populations of *Heterodera trifolii* and *Pratylenchus* spp. (Sarathchandra *et al.*, 1996) and plant-derived chitinases have been shown to increase egg mortality in *Meloidogyne hapla* (Mercer *et al.*, 1992). All of these approaches require a detailed understanding of the dynamics and interactions of not only the target nematodes but of the whole root-associated fauna (both arthropod and nematode) if they are to be successful in field situations. Population and damage modelling of a type that is useful to pastoral farmers or farm consultants is some way off, but ecologically based studies of the kind carried out in this study are essential in reaching that goal.

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