The root lesion nematode, *Pratylenchus neglectus*, in field crops in South Australia

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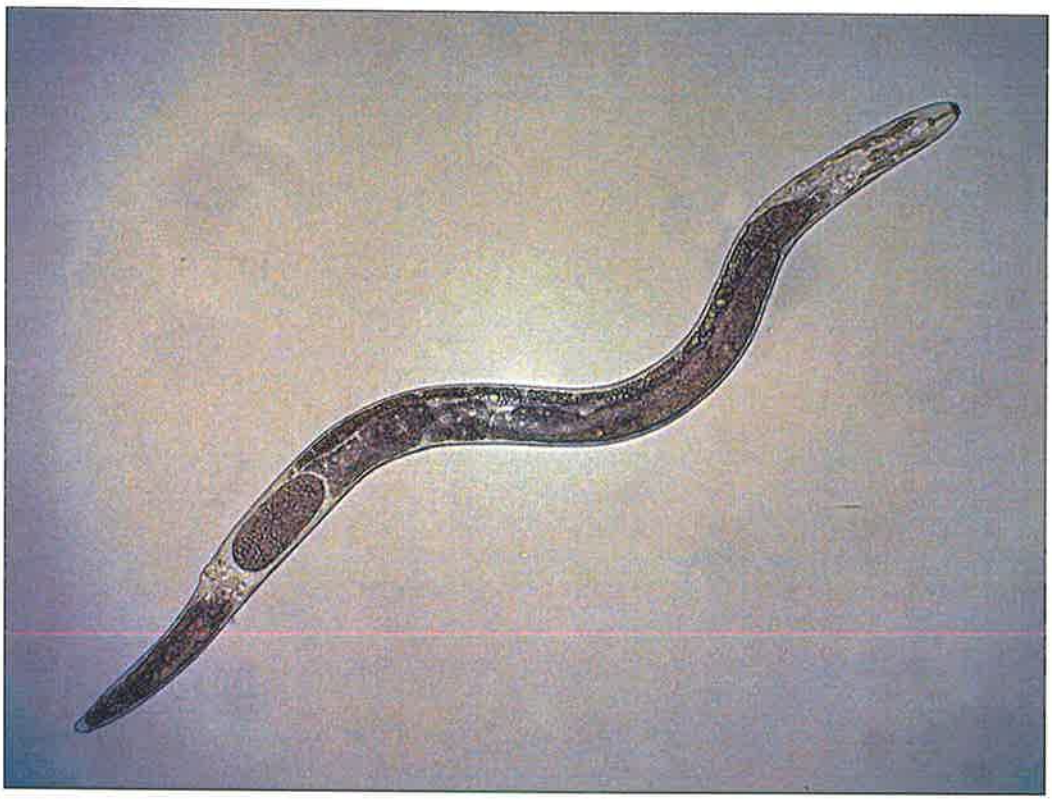

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THE ROOT LESION NEMATODE, *PRATYLENCHUS NEGLECTUS*, IN FIELD CROPS IN SOUTH AUSTRALIA

Summary

The root lesion nematodes *Pratylenchus neglectus* and *P. thornei* are common in agricultural fields in southern Australia. Prior to commencing this study, yield loss caused by *P. neglectus* in field crops was poorly defined. The major aims of this study were to evaluate sampling procedures, assess the extent and magnitude of yield loss caused by *P. neglectus*, assess the population dynamics of *P. neglectus* in cereals, determine whether resistance occurs in field crops and assess whether variation occurs between geographically isolated populations of *P. neglectus*. Research was also conducted on the closely related species, *P. thornei,* including population dynamics, variation between populations and the effects of this species on yield of cereals.

To evaluate sampling procedures for *P. neglectus*, the reliability and efficiency of sampling and extraction methods from plants and soil were assessed. For the extraction of *P. neglectus* from plant roots, the efficiency of staining and misting methods were compared, with the staining technique proving to be more labour intensive compared with misting. This was a significant disadvantage when processing large numbers of plants and misting was therefore considered a more efficient alternative. Differences were observed, however, in the rate of extraction of *P. neglectus* from roots of 6 and 16 week old wheat, chickpea, canola, faba bean and medic plants. These differences were attributed to continued multiplication of *P. neglectus* within samples in the misting chamber. Extraction methods were therefore affected by the suitability of different crop species to serve as hosts.

Because of the difficulty in comparing densities of *P. neglectus* both within and between crop species from plant roots, assessment of density of *P. neglectus* from field plots was undertaken from soil samples (containing root material) using the Whitehead Tray method. The investigation of limitations using this system showed sampling in soil with low moisture (< 2%) significantly reduced the number of *P. neglectus* recovered. This reduction was attributed to mechanical damage to anhydrobiotic *P. neglectus* in dry soil. Samples obtained from an undisturbed core in moist soil resulted in 35% greater recovery

of nematodes when compared with sampling using an earth auger in dry soil. In shallow, sandy loam soils in South Australia, the majority of *P. neglectus* were found between 0 - 10 cm, probably related to root distribution in these soils.

To gain understanding of the population dynamics of *P. neglectus*, experiments were undertaken in large pots and in field trials. For the pot study, a susceptible host (Machete wheat) was sown into infested soil (produced by the addition of infected carrot callus culture and infected wheat roots). While this experiment showed a reduction in the shoot and root dry weights of this host, inadequate differentiation was achieved between the initial densities of *P. neglectus* established in this experiment. As a result, no clear relationship was observed between plant growth and nematode density or nematode multiplication. In contrast, a range of initial densities of both *P. neglectus* and *P. thornei* were established in field experiments. At both sites, a linear relationship was observed between yield and initial nematode density for an intolerant cereal over-sown across these plots. In addition, an exponential relationship was observed between initial density and multiplication rate (Pf/Pi).

Yield loss caused by *P. neglectus* was also shown in field sites both by correlation of *P. neglectus* density with yield and by comparison between aldicarb (Temik15G®) treated and untreated plots. The addition of high rates of aldicarb (12 kg a.i./ha) did not produce a phytotoxic response although some stimulatory effect on plant growth may have occurred at low rates (< 1.5 kg a.i/ha). Using either regression analyses of initial density of *P. neglectus* with yield or treatment of plots with aldicarb, yield responses of up to 27% were demonstrated for intolerant cereals.

Over 80 varieties from 12 crop and pasture species were evaluated for resistance to *P. neglectus* at field sites. Evaluation of *P. neglectus* from soil after harvest was assumed to relate closely to resistance (where resistance is defined as the ability to reduce or inhibit multiplication of nematodes). Variation observed in final densities and multiplication rates of *P. neglectus* at different field sites were attributed both to site and seasonal conditions and also possibly variation between populations of *P. neglectus*. This was supported by genetic differences between nine *P. neglectus* and four *P. thornei*

populations, examined in allozyme studies and phenotypic differences assessed by the rate reproduction of nematodes in cereals in growth room tests.

The results reported in this study demonstrate that *P. neglectus* is a significant pathogen in agricultural cropping regions of South Australia. The broad host range of *P. neglectus* within field crops was confirmed, but resistant and/or tolerant crops and varieties were identified which can be used within cereal cropping rotations to reduce the economic impact of this species.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by any other person, except where due reference has been made in the text.

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

Signed Date 16.10.01

Publications arising from this thesis

Taylor, S. P., G. J. Hollaway, A. C. McKay and R. F. Eastwood 1997. Resistance of field crops to root lesion nematodes (*Pratylenchus neglectus* and *P. thornei*) in southern Australia. Proceedings, 11th Biennial Conference of the Australasian Plant Pathology Society, Perth, WA, September 29 – October 2, 1999, p. 118.

Taylor, S. P. and M. L. Evans 1998. Vertical and horizontal distribution of and sampling for root lesion nematodes (*Pratylenchus neglectus* and *P. thornei*) in South Australia. *Australasian Plant Pathology* **27**:90-96.

Taylor, S. P., V. A. Vanstone, A. H. Ware, A. C. McKay, D. Szot and M. H. Russ 1999. Measuring yield loss in cereals caused by root lesion nematodes (*Pratylenchus neglectus* and *P. thornei*) with and without nematicide. *Australian Journal of Agricultural Research* **50**:617-622.

Taylor, S.P., V. A. Vanstone, D. Szot and M. H. Russ 1999. Measuring yield loss in cereals caused by root lesion nematodes (*Pratylenchus neglectus* and *P. thornei*). Proceedings, 1st Australian Soilborne Disease Symposium, Gold Coast, Qld. February 10 – 12, 1999 (RC Magarey ed., Brisbane, Qld, BSES), p. 77.

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Taylor, S. P., G. J. Hollaway and C. H. Hunt 2000. Effect of field crops on density of *Pratylenchus neglectus* and *P. thornei* in southeastern Australia; Part 1: *P. neglectus*. *Journal of Nematology* **32**:591-599.

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Abbreviations used

Pi	initial nematode density
Pf	final nematode density
Pf/Pi	multiplication rate of nematodes
RO	reverse osmosis
ml	millilitre
µl	microlitre
ha	hectare
kg	kilogram
g	gram
t	tonne (1000 kg)
Rep.	replicate/replication
m.s.	mean squared
d.f.	degrees of freedom
s.e.d.	standard error of difference
s.e.	standard error
n.s.	not significant
Res.	residual
n	number
cm	centimetre
m	metre
PVC	polyvinylchloride
cv.	cultivar (= variety)
psi	pound per square inch
cvv.	cultivars
sp.	species
spp.	species (plural)
l.s.d.	least significant difference
h.s.d.	honestly significant difference
°C	degree Celsius
h	hour
rpm	revolutions per minute
DNA	deoxyribose nucleic acid
PCR	polymerase chain reaction
RFLP	restriction fragment length polymorphism
RAPD	random amplified polymorphic DNA

Chapter 1 Introduction

Agricultural production is an important component of the Australian economy, with 9.3 million hectares devoted to dryland agricultural production, of which South Australia contributes 1.5 million hectares (Figure 1.1). In South Australia, the majority of agricultural crops are sown without irrigation during the autumn - winter period between May and July, and harvested in November and December. Annual rainfall ranges from approximately 400 - 500 mm per annum in high rainfall regions to 250 - 300 mm in more marginal, low rainfall regions (Figure 1.2). Over the summer/autumn period (December - March), paddocks are left fallow and soil temperatures may reach 35°C at depths of 10 cm (unpublished data).

The major crop species grown in South Australia are wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), oat (*Avena sativa*), canola (*Brassica napus*), faba bean (*Vicia faba*), vetch (*V. sativa*) and field pea (*Pisum sativum*). Wheat is the most important crop, with 3.5 million tonnes produced in 1998. Production per hectare is generally low, however, with an average yield of only 1.7 tonnes per hectare.

While nutrient deficiency and rainfall are major factors contributing to low yields in these agricultural systems, plant disease is prevalent. The root pathogens take-all (*Gaeumannomyces graminis*), cereal cyst nematode (*Heterodera avenae*) and *Rhizoctonia solani* (Ag-2 and Ag-8) are common and, in a study assessing the economic impact of pathogens in Australia, were estimated to cause losses of $124.3 million per annum in an industry worth approximately $2.3 billion per annum (Brennan and Murray, 1998). Losses of $47.6 million per annum were attributed to the root lesion nematodes, *Pratylenchus neglectus* and *P. thornei.* This figure is believed to be an underestimate, as limited research on yield loss caused by both species was available when the information was collated.

Despite the first record of *P. neglectus* in South Australia in the 1950's, and high levels of *P. neglectus* observed in soil and most field crops in the 1970's (Kimpinski, 1972; Stynes, 1975), the impact of this nematode in South Australia was not fully recognised until the 1990's. This may have been due, in part, to changes in cropping management strategies such as an increase in cropping intensity, incorporation within rotations of varieties with resistance to pathogens other

than *Pratylenchus* spp., a reduction in fallow and the introduction of minimum tillage systems, all of which may have contributed to increased densities of *P. neglectus* in the soil.

The slow recognition of both *P. neglectus* and *P. thornei* as pathogens that may limit yield, may also have been a result of the difficulty in diagnosing damage. In general, few distinct above-ground symptoms are observed and, in South Australia, the presence of take-all, cereal cyst nematode and *Rhizoctonia*, coupled with nutrient or moisture stress, made the diagnosis of damage caused by root lesion nematodes difficult. With the development however, of varieties resistant to cereal cyst nematode and control strategies for take-all and *Rhizoctonia*, yields in many regions remained below that expected for rainfall (French and Schultz 1984) and *P. neglectus* was suspected of contributing to yield loss.

In the 1990's, post-graduate studies examining damage caused by *P. neglectus* (Vanstone 1991), interactions between fungal pathogens and *P. neglectus* (Taheri 1996), mechanisms of resistance in cereals to *P. neglectus* (Farsi 1995) and yield loss caused by *P. thornei* (Nicol 1996) greatly improved our knowledge of root lesion nematodes in cropping systems within South Australia.

This study was undertaken to further our understanding of factors which effect yield loss caused by *P. neglectus*. The main aims of this research were:

- to determine whether *P. neglectus* causes yield loss in wheat, and the subsequent extent of yield loss.
- to assess the population dynamics of *P. neglectus* on cereals.
- to identify crops and varieties with resistance to *P. neglectus*.
- to investigate sampling strategies for *P. neglectus* in field experiments.
- to assess variation between geographically isolated populations of *P. neglectus* and *P. thornei* from Australia.

Figure 1.1 **Map of Australia showing wheat growing regions (marked in blue).**

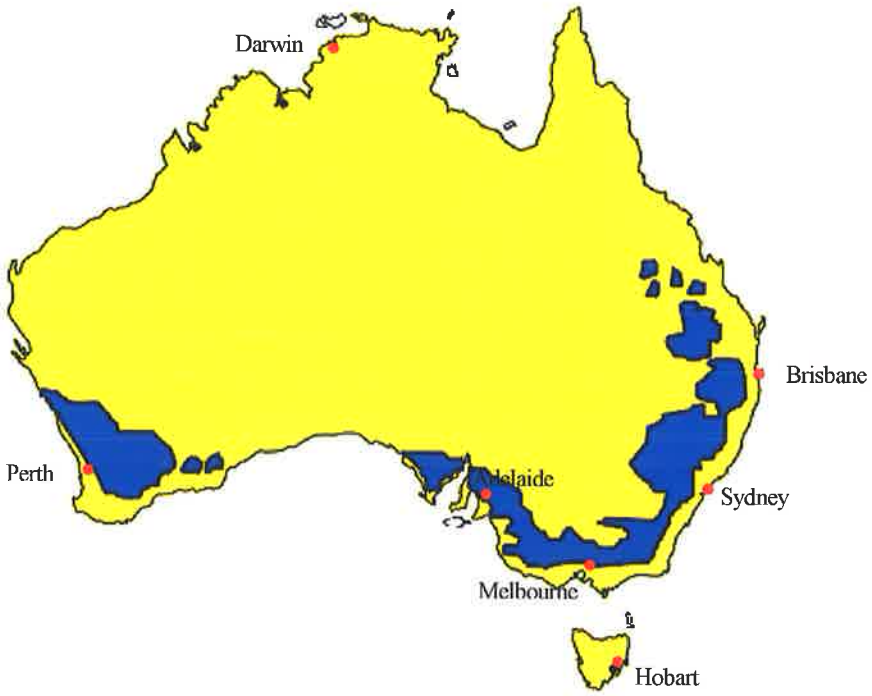
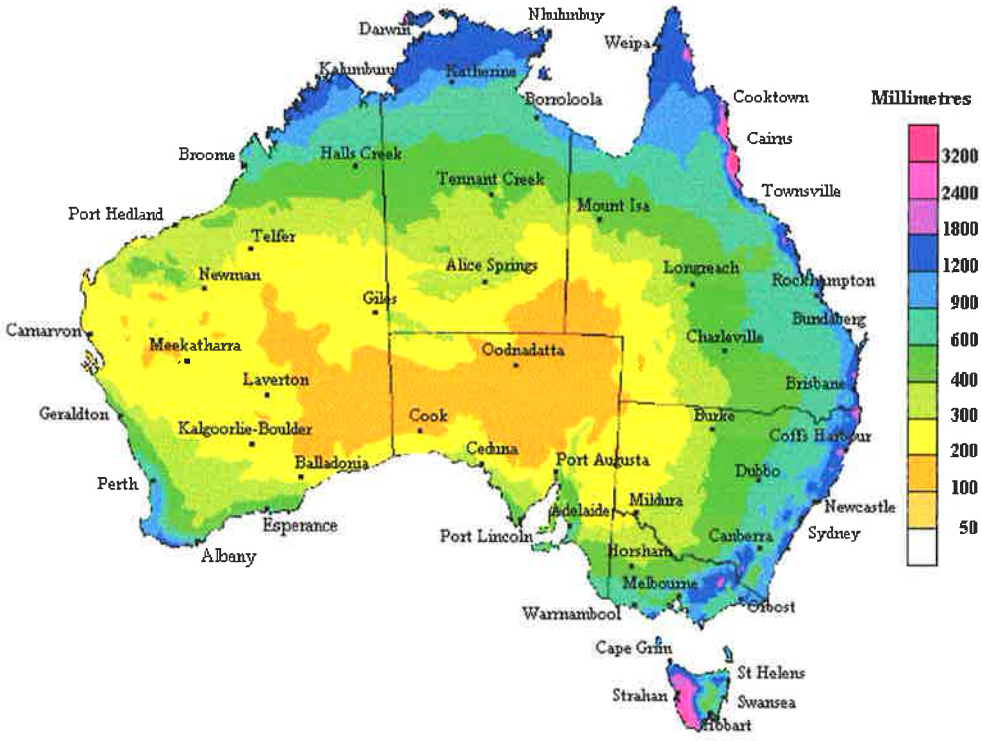


Figure 1.2 **Annual rainfall for Australia calculated from 30 year average (1961-1990). © Australian Bureau of Meteorology.**



Chapter 2 Literature review

2.0 Introduction

To assess the impact of a plant-parasitic nematode species within a cropping system, information on the life cycle (including survival strategies) and population dynamics must be obtained. This information, coupled with the development of sampling strategies, will assist in the definition of both host range (including resistant and susceptible varieties) and yield loss in field trials.

2.1 *Pratylenchus neglectus* and *Pratylenchus thornei*

Pratylenchus spp., or root lesion nematodes, are found worldwide and infect a wide range of plant species. The genus *Pratylenchus* was first described by Filipjev (1936) and has undergone several revisions (e.g. Luc 1987; Frederick and Tarjan 1989). Species within this genus are vermiform, migratory endoparasites between 0.3 and 0.8 mm in length and are morphologically very similar.

In South Australia, two species of root lesion nematodes, *Pratylenchus neglectus* and *P. thornei*, have been commonly recorded in the dryland cropping regions, and both have similar host ranges and often occur in the same geographic area. In this region, *P. neglectus* was identified in the 1950's and *P. thornei* in 1956 (Fisher pers. comm.). Yield loss caused by these nematodes was not extensively investigated until the 1990's, partly because damage was masked by disease symptoms and yield loss caused by other pathogens. In addition, changing management and cultural practices in South Australian farming systems such as a reduction in fallowing, changes in crop rotations and an increase in the use of minimal tillage may have led to increased damage by these nematodes.

2.2 Life cycle

Root lesion nematodes move freely between roots and soil and may feed either externally on root hairs and root cells or invade the cortex of roots. Once inside the root, they move intercellularly

and feed by piercing the cell wall with the stylet, eventually causing cell death (Zunke 1990). Unlike sedentary nematodes, *Pratylenchus* do not establish a fixed feeding site or syncytium.

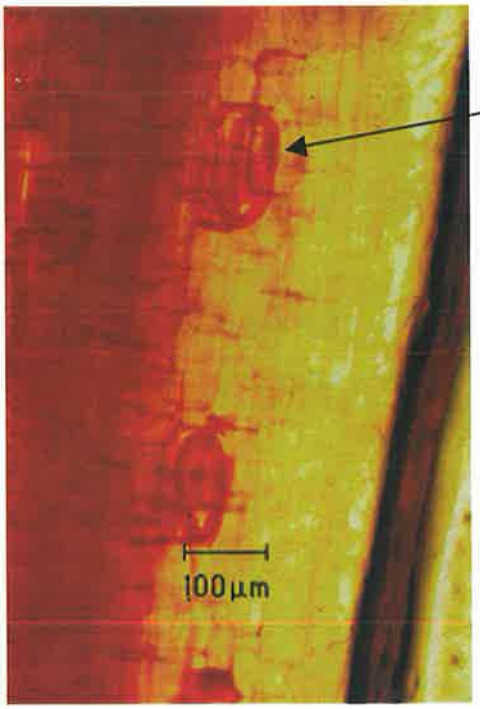
P. neglectus and *P. thornei* reproduce by mitotic parthenogenisis, with males not required for reproduction and occurring only rarely (Nicol 1996; Taheri 1996). Eggs are laid within the root, and the first moult occurs within the egg. After hatching, subsequent moults occur through second, third and fourth juvenile stages to the adult. The full cycle is completed within 45 - 65 days, depending on food source and temperature. Optimum temperature for nematode reproduction varies between *Pratylenchus* species. Siyanand *et al.* (1982) found that gravid females of *P. thornei* completed their life cycle in 25 - 29 days at 30 - 32°C and feeding within roots was necessary for post embryonic development. The length of the life cycle will differ depending on conditions, but these nematodes should complete 3 - 6 generations within plants during a growing season.

In southern Australian dryland cropping regions, the main crop, wheat (*Triticum aestivum*), is grown between May and December. Typically, less rain falls in the October - December period, and annual crops senesce. In the Northern Hemisphere, *P. thornei* survives periods without a plant host in a state of anhydrobiosis (Storey *et al.* 1982; Glazer and Orion 1983; Tobar *et al.* 1996). *P. neglectus* can survive in dry soil for over 15 months, suggesting it also enters a state of anhydrobiosis (Meagher 1970). In South Australia during the summer period, *P. neglectus* and *P. thornei* have been observed in high numbers both in soil and coiled within dry roots, a common protective mechanism associated with anhydrobiotes (Plate 2.1). While in an anhydrobiotic state, nematodes are more resistant to factors such as high temperatures (Glazer and Orion 1983) or chemical treatment (Freckman *et al.* 1980) but are susceptible to mechanical disturbance because of their fragile nature (Hinton 1968). Nonetheless, anhydrobiotes may be dispersed long distances in dust storms. Viable *Pratylenchus* spp. have been recovered from dust storms in a coiled state, suggesting this may be one mechanism for their widespread distribution (Gauer 1988; Baujard and Martiny 1994).

2.3 Symptoms

Root lesion nematodes invade the cortex of plant roots to feed and move freely between cells. This feeding behaviour causes damage to cortical cells in localised areas, resulting in lesions. These

Plate 2.1 **Coiled *P. neglectus* stained pink in wheat roots using acid fuchsin. Roots were air dried for 24 hours prior to staining.**



lesions range in colour from orange to dark brown, and as infection by nematodes increases, lesions coalesce forming large sections of discoloured root tissue. Feeding by *P. penetrans* has been shown to result in hypertrophy of root hairs (Zunke 1990). Conversely, for *P. neglectus* in South Australia, a reduction in root hairs has been observed (Plate 2.2). For *P. penetrans*, feeding has been observed on the same root hair indicating that although there are no preferential feeding sites, some roots or root hairs may be more predisposed to attack than others (Zunke 1990). Baxter and Blake (1967) observed that *P. thornei* were found in areas of localised heavy infection within wheat roots. They suggested that, while *P. thornei* may initially invade randomly, subsequent colonisation of nematodes occurs in root sections at the site of the initial invasion.

In South Australia, symptoms on the roots of field crops are generally not observed until 6 - 8 weeks after sowing, as this period of time appears to be required for nematodes to multiply within roots to sufficient levels to produce necrosis. Above ground symptoms caused by *P. neglectus* and *P. thornei* non-specific and are usually expressed as patchy areas of plant growth within a field. Within patches, plants are stunted, with reduced numbers of tillers and yellowing of older leaves (Van Gundy *et al.* 1974; Farsi *et al.* 1995).

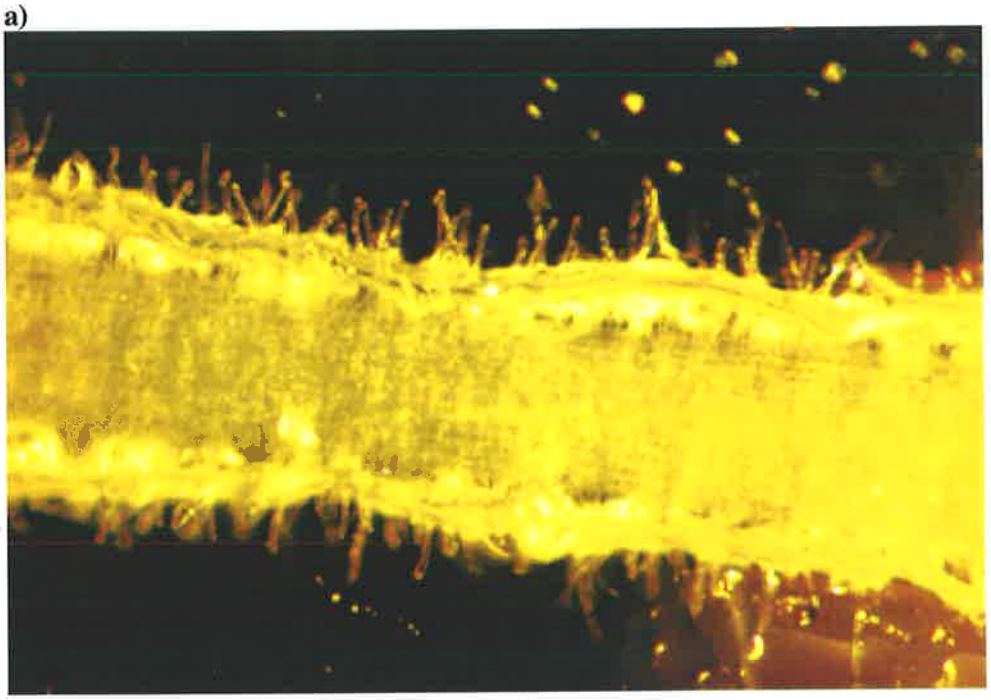
2.4 Interactions with other pathogens

In the field, there is usually a complex array of pathogenic and beneficial organisms affecting plant growth. It may be difficult to isolate the particular effects of organisms or to determine whether the effects are more or less than the sum of the effects of the individual organisms and therefore represent a true interaction (Wallace 1983).

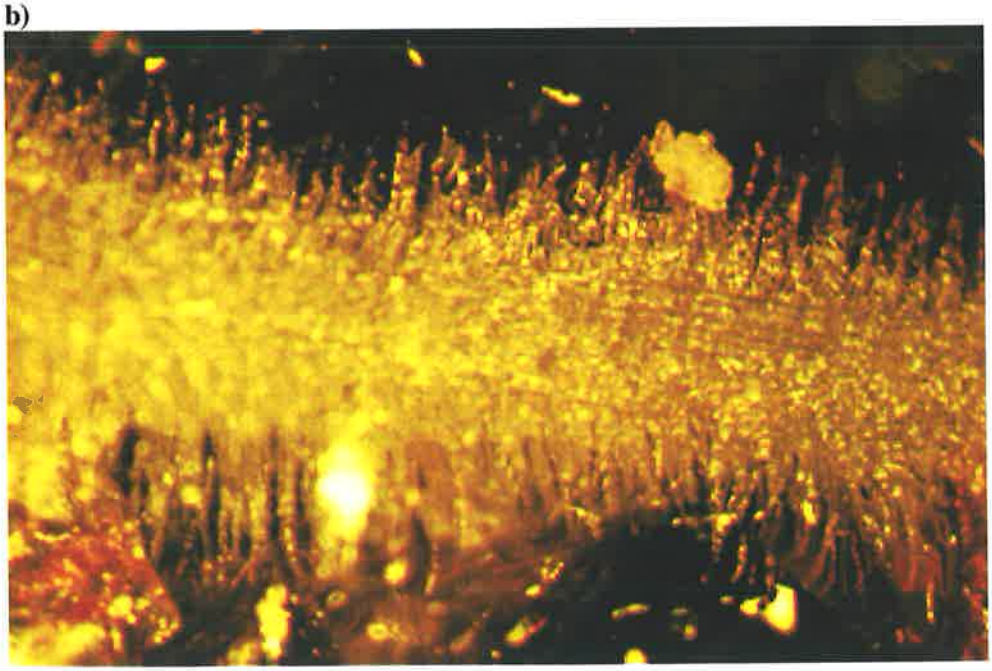
Primary pathogens are those that are capable of establishing infection and causing damage in isolation from other organisms. Combinations of primary pathogens may occur within a host and alter symptom expression or development of infection associated with individual pathogens. Infection by primary pathogens has been shown to limit multiplication of one or both pathogens, probably due to competition for feeding sites. In split root experiments on susceptible oat cultivars, *P. neglectus* levels were higher where cereal cyst nematode (*H. avenae*) was controlled, suggesting *H. avenae* has both a direct and an indirect effect on *P. neglectus* (Lasserre *et al.* 1994; Rivoal *et al.* 1995).

Plate 2.2a **Root hairs of *Medicago* spp. a) reduction in root hairs after inoculation with 3000 *P. neglectus*/plant b) root hairs without inoculation with *P. neglectus*.**

a)



b)



Secondary pathogens are those that require another organism to establish an infection before they are able to gain entry to the host. Secondary pathogens may rely on mechanical disruption of cell walls, release of chemical attractants as a result of infection, or suppression of the hosts' defence mechanisms to gain entry and establish infection.

Feeding by root lesion nematodes can allow entry of other pathogens and many interactions have been recorded with nematodes and secondary pathogens such as fungi (Evans and Haydock 1993), which may exacerbate both infection and yield loss. Taheri *et al.* (1994) showed that infection of wheat plants with the weak fungal pathogen *Microdochium bolleyi* significantly increased both numbers of *P. neglectus* within roots and lesioning on roots. Similar results were obtained with *Fusarium* spp., suggesting that physiological changes caused by nematode invasion may predispose wheat plants to damage, even by weak fungal pathogens.

2.5 Host range and geographic distribution

The genus *Pratylenchus* is one of the most widely distributed throughout cropping rotations in the world. In Australia, *Pratylenchus* is classified as the third most damaging genus after *Meloidogyne* (root knot nematodes) and *Heterodera* (cyst nematodes) (Stirling *et al.* 1992). Both *P. thornei* and *P. neglectus* have been recorded on a wide range of crops in many countries (Tables 2.1 and 2.2). This list is not exhaustive, in part because many records of nematodes and host damage are not published, but it highlights the wide host range and geographic distribution of these nematodes.

Table 2.1 Host range and worldwide geographic distribution of *P. thornei.*

Crop	Country and citation
Wheat (*Triticum aestivum*)	Australia (Doyle *et al.* 1987; Thompson 1990, Nicol et al. 1999; Hollaway *et al.* in press) Mexico (VanGundy *et al.* 1974; Lawn and Sayre 1992) Israel (Orion et al. 1984) Spain (Castillo *et al.* 1996; Nombela *et al.* 1998) France (Esmenjaud *et al.* 1990)
Chickpea (*Cicer arietinum*)	Syria (Greco *et al.*1984; DiVito *et al.* 1991; DiVito *et al.* 1992) Spain (Castillo *et al.* 1995) Africa (DiVito *et al.* 1994) India (Ali 1993)
Vetch (*Vicia sativa*)	Spain (Nombela *et al.* 1998) Australia (Hollaway *et al.* in press) Portugal (Abrantes *et al.* 1987)
Barley (*Hordeum vulgare*)	Spain (Nombela *et al.* 1998) Australia (Thompson *et al.* 1995; Hollaway *et al.* in press)
Oat (*Avena sativa*)	Australia (Hollaway *et al.* in press)
Clover (*Trifolium subterraneum*)	Australia (Hollaway *et al.* in press)
Alfalfa (*Medicago sativa*)	Israel (Orion *et al.* 1979) Syria (Greco *et al.* 1988)
Sorghum (*Sorghum vulgare*)	Egypt (Sharma and McDonald 1990) India (Sharma and McDonald 1990)
Pea (*Pisum sativum*)	England (Green and Dennis 1981)
Faba bean (*Vicia faba*)	Syria (Greco *et al.* 1992) North Africa (DiVito *et al.* 1994)
Lentil (*Lens culinaris*)	North Africa (DiVito *et al.* 1994) Syria (Greco *et al.* 1992)
Maize (*Zea mays*)	India (Siyanand *et al.* 1982)
Chilli (*Capsicum annuum*)	Pakistan (Shaukat and Khan 1993)
Potato (*Solanum tuberosum*)	Israel (Orion *et al.* 1979)
Peppemint (*Mentha citrata*)	India (Haseeb and Shukla 1995)
Canola (*Brassica napus*)	United Kingdom (Webb 1995)
Annual medic (*Medicago rigidula*)	Syria (Greco *et al.* 1988)
Medicago nigra	Portugal (Abrantes *et al.* 1987)

Table 2.2 **Host range and worldwide geographic distribution of *P. neglectus*.**

Crop	Country and citation
Wheat (*Triticum aestivum*)	Australia (Kimpinski *et al.* 1976; Vanstone *et al.* 1998; Chapter 7) North America (Mojtahedi and Santo 1992) South Africa (Jordaan *et al.* 1992) Spain (Lamberti 1981) Italy (Lamberti 1981) France (Lamberti 1981; Lasserre *et al.* 1994)
Chickpea (*Cicer arietinum*)	Australia (J. Thompson pers. comm.; Chapter 7)
Vetch (*Vicia sativa*)	Australia (Chapter 7)
Barley (*Hordeum vulgare*)	Italy (Lamberti 1981) France (Lamberti 1981) North America (Umesh and Ferris 1992; Ferris *et al.* 1994) Australia (Chapter 7)
Oat (*Avena sativa*)	Italy (Lamberti 1981) France (Rivoal *et al.* 1995) Australia (Chapter 7)
Alfalfa (*Medicago sativa*)	North America (Griffin and Gray 1990; Griffin 1991) Canada (Townshend 1984)
Sorghum (*Sorghum vulgare*)	Sudan (Sharma and McDonald 1990)
Maize (*Zea mays*)	South Africa (Jordaan *et al.* 1989) North America (Todd and Oakley 1996)
Potato (*Solanum tuberosum*)	North America (Ferris *et al.* 1994) Canada (Olthof and Wolynetz 1991)
Peach (*Prunus persica*)	Spain (Pinochet *et al.* 1991)
Pear (*Pyrus communis*)	Spain (Pinochet *et al.* 1991)
Apple (*Malus silvestris*)	Spain (Pinochet *et al.* 1991)
Canola (*Brassica napus*)	Australia (Potter *et al.* 1999; Chapter 7)
Annual medic (*Medicago* spp)	Australia (Chapter 7)
Wheat grass (*Agropyron* spp.and *Elymus* sp.)	North America (Griffin 1996)

2.6 Economic importance

The economic importance of plant-parasitic nematodes is often difficult to determine as damage to hosts may be affected by environmental factors and the degree of tolerance of the host. Stirling *et al.* (1992) estimated that plant-parasitic nematodes cause more than $AUS300 million p.a. damage to field, horticultural and vegetable crops in Australia. They did not however, include damage caused by root lesion nematodes, as little information was known about yield loss,

distribution and frequency of these nematodes. In a more recent study detailing the economic impact of plant disease in Australia, losses due to *P. neglectus* and *P. thornei* were estimated at $AUS44.3 million and $AUS80.5 million respectively (Brennan and Murray 1998). This study did not consider the widespread distribution of *P. neglectus* in eastern Australia, Victoria and Western Australia or the occurrence of *P. thornei* in South Australia and Western Australia. Both studies will therefore have underestimated the economic losses caused by these nematodes.

2.6.1 *Pratylenchus thornei*

Yield loss caused by *P. thornei* has been observed on a wide range of crops in many countries, although as with all plant-parasitic nematodes, the magnitude of loss varies under different environmental and site conditions. In Queensland, losses of 42% were recorded for intolerant wheat varieties where water stored within the soil profile was limited (Thompson *et al.* 1995). Yield losses of up to 80% has been observed in the intolerant variety, Gatcher, and high yield losses attributed to *P. thornei* may occur in this region because of the low incidence of other cereal root pathogens (Thompson pers. comm.). Similarly, studies in northern New South Wales have demonstrated that *P. thornei* is a major factor limiting the yield of wheat. In this region, the application of high rates of phosphorus, nitrogen and zinc caused only minimal yield increases compared with nematicide treated plots where an increase of 37% was observed (Doyle *et al.* 1987).

In South Australia, yield losses of 20% and 33% were recorded in separate studies by correlating nematode numbers with yield in resistant and susceptible varieties (Vanstone *et al.* 1998; Nicol *et al.* 1999). Damage also occurs on other crops grown in rotation with wheat, with losses of up to 38% recorded on chickpea and vetch varieties in Victoria (Hollaway pers. comm.).

In the Northern Hemisphere, *P. thornei* has been shown to cause yield loss on various hosts including wheat in Mexico and Israel (Van Gundy *et al.* 1974; Orion *et al.* 1984), chickpeas in Syria (DiVito *et al.* 1992) and *Mentha citrata* in India (Haseeb and Shukla 1995). *P. thornei* has been recognised as a major pathogen in these areas and appears most damaging where intolerant cereals and pulses are grown in more arid environments.

2.6.2 *Pratylenchus neglectus*

Information on yield loss caused by *P. neglectus* is more limited. In South Australia, yield loss caused by this species has been shown in wheat (22%, Taheri 1996; 22%, Vanstone *et al.* 1998; 23%, Taylor *et al.* 1999). The evaluation of the magnitude of yield loss caused by *P. neglectus* in South Australia is discussed in Chapters 6 and 8.

In North America, *P. neglectus* has been shown to cause reduction in shoot and root growth in wheat grass (Griffin 1992), alfalfa (Griffin and Gray 1990) and barley (Umesh and Ferris 1992), although this nematode was considered only a minor pathogen on these crops. Yield loss caused by *P. neglectus* on wheat has also been observed in interaction with fungal pathogens in microplot and field plot studies. Taheri (1996) demonstrated that in wheat, where *P. neglectus* and the fungus *Fusarium acuminatum* were present in combination, nematode reproduction was increased by 48%, and root dry weight was decreased by 36%.

2.7 Effects of edaphic factors on nematode density and yield loss

Often the economic importance of plant-parasitic nematodes cannot be determined in isolation from environmental conditions encountered at a field site, as yield loss may be dependent on the host, tillage, plant nutrition and/or water availability (Wallace 1987). For this reason, it is sometimes not possible to observe damage caused by nematodes unless host plants are compromised by one or more of these factors (Barker and Noe 1987).

In addition, the tolerance of a host may be affected by its efficiency in extracting nutrients from water and soil and therefore tolerance in a crop/variety may be conferred for more than one nematode species. For example, tolerance in the soybean variety Wright to *Heterodera glycines* was linked with increased efficiency of root systems in the uptake of phosphorus (Price *et al.* 1995) and this variety was also rated as tolerant to chloride toxicity (Parker *et al.* 1985). In addition, this variety was tolerant to *Hoplolaimus columbus* (Boerma and Hussey 1984) which suggests that the physiological nature of a tolerance mechanism may be applicable for other nematode species or factors that adversely affect plant growth.

2.7.1 Tillage

Nematode species may be affected by tillage. In South Australia, levels of cyst nematodes were higher in mechanically cultivated compared with minimum till plots. While this was thought to be caused exclusively by higher densities of hosts in the minimum till systems (Roget and Rovira 1985, Neate 1988), it may also be due to the increased distribution of cysts as a result of cultivation.

For nematodes without the protective covering offered by cysts or galls, many studies have reported levels of nematodes highest under no till or minimum till systems compared with mechanically cultivated plots. Possible reasons include mechanical disturbance (Boag *et al.* 1992), warmer temperatures, and increased soil water retention and root growth in minimum till systems (Thomas 1978). Tillage has been found to have greater effects on numbers of *P. scribneri* than crop rotation, with lower nematode densities under cultivated treatments (McSorley and Gallaher 1993). The effects of tillage are not always consistent, as other studies have shown no significant difference in nematode levels between minimum till and mechanical cultivation (Kunelius *et al.* 1988; Thompson *et al.* 1995).

2.7.2 Nutrition

Macro-nutrients such as nitrogen, phosphorus and zinc will have direct effects on plant growth that may change the level and expression of damage caused by plant-parasitic nematodes. Increased application of these nutrients can reduce yield loss caused by many diseases as this may compensate where root systems are damaged (Stirling 1989).

In addition to these affects, nutrients such as nitrogen compounds can affect plant-parasitic nematodes. Nitrogen may act indirectly by increasing the incidence of nematophagous fungi (Cooke 1962). Application of products that release ammonium ions has also been shown to have nematicidal properties. A chitin urea amendment was demonstrated to reduce nematode populations in potato, walnut and tomato, although there was some indication of phytotoxicity by this compound (Westerdahl *et al.* 1992).

In South Australia, most agricultural soils are deficient in nutrients (particularly zinc and phosphorus) and interactions between nutrient deficiency and plant disease occur. For example, in South Australia, the symptoms caused by *Rhizoctonia solani* on *Medicago* spp. grown in zinc deficient soil were reduced following application of zinc. Zinc deficiency reduces membrane integrity (which can lead to the leaking of potassium, amino acids and sugars into the rhizosphere [Catmak and Marschner 1998]), possibly resulting in weakened cell walls and the increased attraction of pathogens to root exudates.

2.7.3 Water availability

Water availability can affect both multiplication of, and yield loss caused by, plant-parasitic nematodes. McDonald and van den Berg (1993) showed that, in corn, low water regimes supported the fewest *P. zeae*. Invasion and multiplication of *P. penetrans* in alfalfa was also lowest under lower moisture regimes (Kable and Mai 1968) and similar responses have been observed with *P. neglectus* in wheat (Kimpinski *et al.* 1976).

Conversely, higher multiplication of *P. neglectus* in both resistant and susceptible cereal varieties has been observed under conditions of moisture stress in laboratory experiments conducted in South Australia (V. Vanstone pers. comm.). In Queensland, Thompson *et al.* (1995) showed that susceptible wheat varieties suffered higher yield loss than resistant barley varieties as wheat was unable to use stored soil moisture due to root damage caused by *P. thornei*. In Israel, both multiplication of *P. thornei* and resulting yield loss in wheat were exacerbated by dry conditions (Orion *et al.* 1984).

2.8 Measuring yield loss

As previously described, the magnitude of yield loss caused by *Pratylenchus* spp. is affected by a variety of factors including other pathogens, moisture availability, temperature and plant nutrition. These factors may be difficult to simulate in the laboratory as conditions may not adequately reflect the stresses on crop growth and nematode multiplication seen in field sites. It is therefore important when measuring economic damage to conduct experiments under field conditions (Barker and Noe 1987).

In field experiments, yield loss caused by root lesion nematodes can be difficult to identify and quantify as symptoms may be indistinct or affected by biotic and abiotic factors. Root symptoms such as discolouration and a reduction in lateral roots and root hairs may be misleading due to concomitant infection with fungi (Taheri 1996). Above ground symptoms such as yellow leaves, stunting or reduced tillering can be affected by, or confused with, water or nutrient stresses (Farsi 1995). As a result, nematicides have been used extensively in experiments to identify yield loss (Doyle *et al.* 1987; McDonald *et al.* 1987; Badra and Adesiyan 1990; Thompson *et al.* 1999).

In many studies, the nematicide aldicarb has been shown to be very effective in controlling nematodes (Brown 1972; Anyango 1991). It does, however, have a range of effects on nematodes and plant growth. Barker and Powell (1988) showed that response to the nematicide, aldicarb, in soybean varieties was affected by rainfall, application rate of the chemical and soil type. At low rates of aldicarb, a stimulatory response was observed as maximum soybean yields occurred even though the aldicarb treatment resulted in only the third highest nematode control compared with other nematicide treatments. Barker *et al.* (1988) showed that aldicarb increased soybean growth (although not necessarily yield), with the largest growth increases seen in soils containing higher organic matter. Water has also been shown to effect the efficacy of nematicides controlling *Pratylenchus* spp. in maize, with lower moisture regimes having poorest nematode control (McDonald *et al.* 1987). In contrast, no effect of pH on plant response to nematicide was observed in field trials assessing damage caused by *Belonolaimus longicaudatus* on soybean (Schmitt 1989).

For these reasons, yield loss caused by plant-parasitic nematodes has also been measured by determining the relationship between nematode density and yield by correlation analyses (Stynes and Veitch 1983; Marshall 1998). In field trials, Vanstone *et al.* (1998) demonstrated a significant negative correlation between final density of *P. neglectus* and yield, with 56-74% of the observed varietal yield differences attributed to nematode damage (Vanstone *et al.* 1998). Assessment of yield loss caused by *P. neglectus* on cereals is a major component of this thesis, and the use of both aldicarb and correlation analyses to measure yield loss is further discussed in Chapter 8.

2.9 Population dynamics

In order to estimate yield loss caused by plant-parasitic nematodes, an understanding of the population dynamics of each species is required. Ferris and Wilson (1987) defined population dynamics as "changes in numbers, age class distribution, sex ratio, and behaviour of a population through time and space.....mediated by environmental conditions, food resources, and interacting biotic agents". For plant-parasitic nematodes, densities in cropping systems are rarely stable, i.e. the birth rate rarely equals the death rate (Nusbaum and Barker 1971) and where intensive monoculture occurs, factors including host, temperature, moisture and cultural practices will effect densities of nematodes. When considering management strategies to control plant-parasitic nematodes, thereby maximising yield and available crop choices, knowledge of nematode population dynamics is essential. Studies on population dynamics assess changes in nematode densities and must also evaluate factors that influence these changes. The population dynamics of *P. neglectus* in a trial and of both *P. neglectus* and *P. thornei* in field experiments are discussed in Chapters 6 and 8.

2.9.1 Evaluation of population dynamics

Changes in nematode populations are commonly determined by assessing either final nematode density (Pf) (at or near plant harvest/senescence) or multiplication rate, which is determined by dividing final nematode density by initial density (Pf/Pi). Multiplication rate is often used to assess changes in nematode populations as this can allow comparison between different crops, sites and seasons and also nematode species. Multiplication rate has also been defined as the "maintenance level concept" (Nusbaum and Barker 1971), i.e. where Pf = Pi (or multiplication rate = 1) no population growth has occurred. An important consideration in using multiplication rate as the sole determinant of hosting status is the possibility that magnitude of multiplication may be dependent on the initial nematode density (Seinhorst 1970).

One of the most important factors affecting nematode population growth is the resistance and/or tolerance of the host. When food source or feeding sites are limited, the nematode population becomes density-dependent and the growth of the population is termed logistic, i.e. the population is limited by a maximum threshold. This threshold is determined by the equilibrium density,

defined by Seinhorst (1966) as the "density (at which) the available food is just sufficient to maintain the population". The rate at which populations grow, and the maximum density they reach, is dependent on both the equilibrium density and the intrinsic rate of population growth, and is defined by the model:

$$DP/dt = rP[(E-P)/E] \text{ (Equation 1)}$$

Where dP/dt = rate of population growth; r = constant (intrinsic rate of increase); and E = equilibrium density.

For logistic growth, it is assumed that population growth occurs until the equilibrium density is reached, and can be derived from the equation (Seinhorst 1966):

$$P = E\,(1 + e^{k-r\,t})^{-1} \text{ where } P < E \text{ (Equation 2)}$$

$$Pf = aEPi/(a-1)Pi + E \text{ (Equation 3)}$$

which are represented in Figures 2.3 and 2.4 respectively. In Equation 2: P = population density; E = equilibrium density; t = time; e = base of natural logarithm; r and k are constants. In Equation 3: a = maximum rate of multiplication; E = equilibrium density. Using these models, the highest population growth (or multiplication rate) is recorded at the lowest initial nematode density and, for very good hosts, both a and E will be large (Seinhorst 1967b).

Factors such as nutrition (Price *et al.* 1995), plant density, soil type and texture (Seinhorst 1967b; Ferris and Bernard 1971; Wallace *et al.* 1993), temperature (Nicol 1996), moisture (Kimpinski *et al.* 1976; Noe and Barker 1983) and interactions with other pathogens will also affect the multiplication rate and/or tolerance of the host.

Plant-parasitic nematode populations can exhibit exponential growth (i.e. density-independent growth) in situations where food source is not limiting. This situation may occur where initial densities are low or where there is little competition for feeding sites. McSorley and Dickson (1989) found exponential growth in populations of several nematode species and believe this

occurred because low initial nematode densities were present and an equilibrium density was never reached.

Figure 2.3 **Theoretical relationship between rate of population increase for plant-parasitic nematodes against time ($P = E\ (1 + e^{k-r\ t})^{-1}$ where P (population density) < E (equilibrium density). From Seinhorst (1970).**

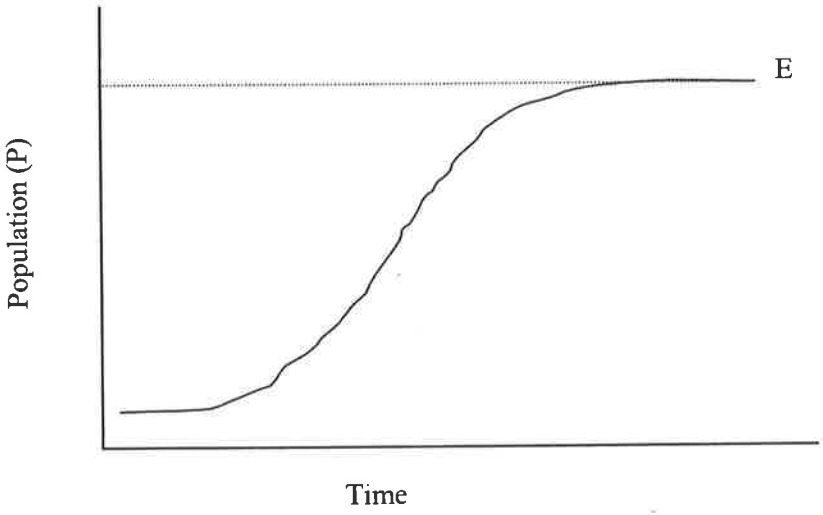
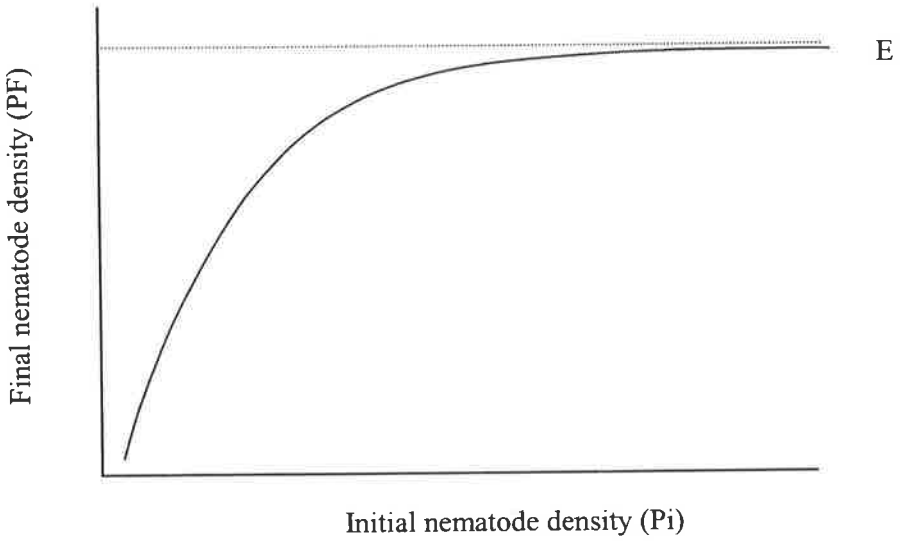


Figure 2.4 **Theoretical relationship between final nematode density and initial nematode density. E = Equilibrium density. Pf = aEPi/(a-1)Pi + E. From Seinhorst (1970).**



One of the most important factors influencing multiplication rate is the host itself. McSorley and Gallaher (1992) showed that even where initial levels of *Meloidogyne incognita* were very high under corn, sorghum and soybean, multiplication rate was also high. An equilibrium density did not appear to be reached, but these plants were the preferred hosts.

The relationship between final and initial nematode densities will differ between different hosts and thus resistant and/or tolerance (discussed in the following sections) will be critical in determining population growth.

2.9.2 Resistance

Resistance is defined as "the capacity of the plant to prevent/decrease nematode multiplication" (Trudgill *et al.* 1998). Crops and cultivars that are resistant to plant-parasitic nematodes are desirable in agricultural systems as there is often no requirement for specialised application or equipment, there is usually no additional cost when compared to using susceptible cultivars and the use of resistant cultivars may allow rotations to be shortened (Cook and Evans 1987). In addition, nematicides may be eliminated, a factor necessary in broad acre agriculture in Australia where the use of these chemicals is either not sanctioned or the cost is prohibitive.

Cook and Evans (1987) suggest that cultivars may be "completely resistant" (allowing no nematode multiplication) or "partially resistant" (allowing intermediate levels of multiplication). Complete resistance is most likely to be obtained against nematodes that have a close relationship with their host, such as *Meloidogyne* or *Heterodera* spp. (Cook and Evans 1987). Examples of complete resistance include resistance to *Meloidogyne* spp. in *Prunus* root stocks (Fernández *et al.* 1994) and resistance to *Globodera* spp. in potato cultivars (Franco and Evans 1978). In these instances, it is known that the parasite must activate the host response throughout the association, and specific resistance genes that in some way disturb the plant-nematode interaction have been identified within hosts for cyst and gall forming nematodes. Examples are the *Cre3* gene (from *Triticum tauschii*) which confers resistance to *Heterodera avenae* (Eastwood *et al.* 1994) and the H_1, H_2 and H_3 genes which confers resistance to *Globodera* spp. in potatoes (Franco and Evans 1978). In addition to complete resistance, a gene for partial resistance to *H. glycines* has been identified in

soybean (Concibido *et al.* 1996). Partial resistance of potato to *G. pallida* has also been recorded in field and pot experiments (Phillips and Trudgill 1985), indicating that reduced levels of resistance can be defined and mapped for sedentary nematodes.

Partial resistance appears especially common for migratory endoparasites with a less specialised host/parasite association than the sedentary endoparasites with wide host ranges. Examples of partial resistance have been demonstrated with *P. vulnus* on *Prunus* spp. (Alcañiz *et al.* 1996), *Rotylenchus reniformis* on soybean (Robbins *et al.* 1994), *P. penetrans* on alfalfa (Thies *et al.* 1994) and strawberry (Potter and Dale 1994). Differing levels of reproduction have been recorded for a number of *Pratylenchus* species in different crops and cultivars. For *P. neglectus* in both wheatgrass and alfalfa, reproduction was strongly affected by both cultivar and temperature but no resistance was found (Griffin 1991, 1992). In both studies, only limited numbers of cultivars were evaluated and resistance in these crops may yet be identified.

In order to select for resistance, a large number of lines/selections must usually be evaluated. Nelson *et al.* (1984) found that even though alfalfa is generally susceptible to *P. penetrans*, 9 selections (from a total of 70) had reduced numbers of nematodes and increased root and shoot weights when tested for resistance and tolerance to this species. Conversely, Greco *et al.* (1988) found no resistance to *P. thornei* in 97 chickpea lines, indicating that a larger number of cultivars or those with a wider range of pedigrees may be required to identify resistance in this crop. The resistance of South Australian field crop and pasture species to *P. neglectus* is discussed in Chapter 7.

The stability of plant resistance may be affected by the mechanism conferring resistance. In many cases, nematodes invade resistant and susceptible plants at the same rate but fail to reproduce in resistant plants (Creech *et al.* 1995). As a result, changes in environmental conditions may change the resistance status of plants. For example, at higher temperatures, tomato, bean and sweet potato cultivars have been shown to be more susceptible to *Meloidogyne* species (Holtzmann 1965; Dropkin 1969; Jatala and Russell 1972). Interactions between nematode species can also affect resistance. In South Australia, resistance to stem nematode (*Ditylenchus dipsaci*) in the oat cultivar Echidna will break down with concurrent infection with *H. avenae* (Scurrah pers. comm.).

Eisenback (1983) found that resistance to *M. incognita* in tobacco may break down when plants were also infected with either *M. arenaria* or *M. hapla*.

Resistance breaking populations of plant-parasitic nematodes have resulted in the characterisation of different races or pathotypes in genera such as *Meloidogyne* and *Heterodera* (see Section 2.9.2). Lassere *et al.* (1996) utilised both reproductive fitness on the oat cv. Panema and enzyme polymorphisms between populations to identify a resistance breaking pathotype of *H. avenae*. Anand *et al.* (1995) suggested that levels of resistance to *H. glycines* in soybean declined in response to selection pressure with continuous monocultures of resistant cultivars. Similar findings were obtained by Young and Hartwig (1992) who proposed a blend of 25% susceptible: 75% resistant cultivars to delay shifts from Race 3 to Race 1 of *H. glycines*.

2.9.3 Tolerance

Tolerance is defined as the ability of a plant to yield well despite infection with high numbers of nematodes, and thus tolerance is a function of yield loss (Jones 1956). Tolerance will be affected by the initial nematode density, suitability of the crop as a host and environmental conditions. Tolerance may be independent of resistance, i.e. a low initial nematode density may cause damage within an intolerant, resistant host. In South Australia, Vanstone *et al.* (1998) found that resistance and tolerance to *P. neglectus* often concur in wheat. In contrast, varieties have been produced in Queensland, with superior tolerance to *P. thornei* but conferring no resistance (Thompson *et al.* 1999).

Potter and Dale (1994) identified variation in tolerance in strawberry cultivars to *P. penetrans* and suggested tolerance may be due to a number of factors including increased sugar production and more vigorous root growth. Further examples include tolerance to: *Globodera* spp. in potato cultivars (Evans and Haydock 1990); *H. glycines* in soybean cultivars (Boerma and Hussey 1984); and *H. schactii* in sugarbeet (Heijbroek *et al.* 1977). In cropping rotations, tolerant plants may have benefits both by directly minimising yield loss and also in assisting to reduce the selection pressure on nematode populations that occurs using consecutive resistant varieties (Evans and Haydock

1990). An obvious disadvantage of crops that are tolerant but susceptible, however, is that yield loss may occur if intolerant cultivars are grown in the following year, as high levels of nematodes generally remain.

2.10 Virulence, pathogenicity and aggressiveness

When assessing the reaction of a nematode population in relation to plant resistance, definition of the terms virulence and aggressiveness is useful. Trudgill (1991) defined virulence as the capacity of the nematode to "overcome/circumvent/suppress resistance genes". The virulence of a nematode population is therefore related to changes in nematode reproduction/multiplication within plants. Cook and Rivoal (1998) used the term "virulence phenotype" to define the phenotypic response of a population to virulence genes (Cook and Rivoal 1998). This is a useful term when defining field or geographically isolated populations of nematodes as it describes differences in multiplication of nematodes within plants without the need for information on the genetics of either the nematode or host. Aggressiveness relates to the reproductive fitness of a nematode population and has been defined as the capacity of a population to reproduce well on all host genotypes (Vanderplank 1975).

In contrast to virulence and aggressiveness, the term pathogenicity is related to yield loss in plants, and is defined as "the capacity (of a nematode population) to cause disease or damage" (Trudgill 1991).

In Chapter 9, the virulence phenotypes of populations of *P. neglectus* or *P. thornei* are characterised by assessing differences in multiplication within cereal hosts.

2.11 Population variability

Differences in the virulence phenotypes of geographically isolated populations of plant-parasitic nematodes may have implications for research, prediction of yield loss and screening for resistance and aggressiveness.

Population variability has been extensively studied for some species of plant-parasitic nematodes, with differences often sufficiently characterised for the various groupings to be termed races. For

example, four races have been defined within *M. incognita* based on differential reactions in six hosts (Hartman and Sasser 1985). The presence of races appears to be more easily identified for sedentary plant-parasitic nematodes with a specialised relationship with their host, possibly because resistance or susceptibility is well characterised. For migratory nematodes, the identification of races is often less clear. Within stem nematode (*D. dipsaci*) 30 races have been identified, but the host range of each is not completely known (Sturhan and Brzeski 1991). For *Pratylenchus* species, partial resistance is more common and the presence of races does not appear to be as clearly established. Differences between Australian populations of *P. neglectus* and *P. thornei* identified using both allozyme electrophoresis and differential host range studies are discussed in Chapter 9.

Several techniques can be used for determining population variability, including assessment of nematode morphology, variation within proteins using allozyme electrophoresis, variation within the genome using RAPD or RFLP and reproduction on different hosts.

2.11.1 Morphology

Traditional morphological techniques have been used to differentiate between populations/isolates of plant-parasitic nematodes. For example, in a study examining 23 populations of *Xiphenema americanum* from North America, 4 discrete populations were identified based on morphological measurements (Cho and Robbins 1991). Prasada *et al.* (1998) found that for six geographic isolates of the reniform nematode, *Rotylenchulus reniformis*, although variation was observed within isolates in characters such as body length, body width and the length of the hyaline part of the tail, these characters were stable between isolates. Based upon this, one isolate was concluded to be a morphological variant. Morphological characters were also used to differentiate races of *P. coffeae,* and differences were further supported by host range studies (Kumar and Viswanathan 1972).

There is often a degree of variability between morphological characters used, and it may be difficult to quantify differences between populations as variation may occur depending on the nutrition of the nematode or host. Singh and Khan (1981) attempted to use morphometric measurements to determine variation between *P. thornei* populations and concluded that values such as body length, lip dimension, body length/spear length showed large variation even within a single population.

Variation in body length of *P. coffeae* was found to be affected by the concentration of starch in citrus roots (Duncan *et al.* 1998 and 1999).

In addition to variation in morphological characters, the time and level of expertise required make identification of populations based on morphological studies difficult. After assessment of 12 populations of *Nacobbus aberrans* from Central and South America, Manzanilla-Lopez *et al.* (1999) suggested that a large number of characters must be used and that at least three to four populations of *N. aberrans* from each region should be assessed before conclusions regarding populations can be reached.

For these reasons, while morphological techniques are essential for determining nematode species, traditional taxonomy has more limited application when assessing differences within species.

2.11.2 Host range studies

Differences in multiplication rates (host range tests) are often used as an indication of variability in virulence between populations as this allows assessment of measurable differences within species.

France and Brodie (1995) showed differences in the reproduction of two isolates of *P. penetrans* in potato which explained discrepancies previously observed in the hosting status of the cultivar Hudson. Differential reproduction in *P. neglectus* populations in wheat grass has been reported (Griffin and Jensen 1997). Griffin (1991) also established differences between populations of *P. neglectus* on alfalfa with one found to be highly pathogenic when compared with three others, but no races were defined in any of these studies. Some regional differences between *Pratylenchus* populations have therefore been recorded and suggest the need to assess differences for both resistance and/or tolerance screening and for research purposes.

2.11.3 Molecular techniques

Use of morphological studies and host range tests can prove difficult and confusing when differentiating between species or pathotypes, especially where species are morphologically very

similar. The introduction of techniques for the differentiation of species/pathotypes based on differences in proteins and DNA polymorphism has allowed further characterisation of plant-parasitic nematodes.

One such tool, allozyme electrophoresis, compares differences between proteins and has proven useful for assessing genetic diversity within and between species. While proteins are a reflection of nucleic acids, assessment of differences between proteins can offer a broader comparison between populations and individuals than comparisons at the nucleic acid level. Variability observed using electrophoretic studies may therefore be useful in determining differences, such as those in reproductive fitness, within or between species. Electrophoresis has been used in assessing interspecific differences within *Meloidogyne, Heterodera* and *Ditylenchus* (Dickson *et al.* 1970; Hussey *et al.* 1972; Hinch *et al.* 1998).

2.12 Distribution, sampling and extraction

Understanding the limitations and advantages of the various sampling and extraction techniques for the estimation of plant-parasitic nematodes in soil and/or plants is necessary if the assessment of population dynamics, yield loss or resistance is undertaken in field experiments. In the work described here, sampling strategies and extraction procedures for *P. neglectus* in South Australia were evaluated prior to the assessment of population dynamics and yield loss in field experiments and are discussed in Chapters 4 and 5.

2.12.1 Sampling

Although reliable sampling procedures are an essential tool for determining densities of the invertebrate populations under consideration, sampling has been referred to as a combination of "art, science and drudgery" (Morris 1960). The intensity and level of sampling required will depend upon the purpose of the study, which could be detection, survey, diagnosis or research. Factors to be considered when deciding upon a sampling procedure will depend largely upon the spatial distribution of nematodes and the host type.

The nematode species will affect the sampling methodology used. Species such as cyst nematodes are well protected from mechanical damage and many small sub-samples can be taken and bulked with no loss in nematode recovery (Boag *et al.* 1992). For large, virus vector nematodes such as *Longidorus elongatus* and *Trichodorus* spp., however, that the minimum sample size that can be used is a 2.5 cm diameter coring tube, as anything smaller will result in unacceptable levels of mechanical damage (Boag and Brown 1985). The sampling equipment used may also affect precision of results because samples taken with unequal core sizes can increase error estimates (McSorley and Parrado 1982).

The time of the year (i.e. the growth stage of plants), at which sampling is undertaken can also affect recovery of nematodes from plants and or soil. Sedentary nematodes will only be present in the soil as juveniles for short periods before invading roots and forming fixed feeding sites. Migratory nematodes may be present in plants when hosts are present but are also found in soil surrounding roots. Soil moisture also affects nematode recovery and is discussed as a factor causing reduced recovery of *P. neglectus* and *P. thornei* in Chapter 4. It is therefore necessary to devise sampling strategies appropriate for the purpose of the study and nematode species under consideration.

2.12.2 Field experiments - design and sampling patterns

Much work has focussed on the number of samples and sampling patterns required for precision (Ferris 1984a and b; Francl 1986a and b; Ferris *et al.* 1990). This is important either for determining the presence or absence of nematode pathogens or for determining densities of nematodes within plots to allow correlation with yield loss or assessment of resistance in hosts.

Physical characteristics within a field trial also affect sampling protocols given that factors such as soil type or water drainage alter nematode distribution. Percentage sand/silt/clay content, pH and percentage organic matter can affect nematode aggregation. At a site with mixed nematode populations in Western Australia, *Radopholus* sp. was found only in low pH (< 6.5) soils while *P. neglectus* was found in areas of pH > 7.0 (I. Riley pers. comm.).

When sampling for nematodes, uneven distribution of most species often limits the accuracy of data and, to maximise sampling accuracy and efficiency and determine the optimum plot size, information on spatial distribution and patch size should be collected prior to commencement of a study (Shaukat and Khan 1993). Extreme variability in the density of nematodes can be observed, and many studies recommend randomisation, replication and blocking of field experiments (McSorley 1987). The randomised block design appears to be the most applicable and is commonly used in field experiments where a field site may have a predictable gradient (such as soil type), although the aim of the experiment must be considered (McSorley 1987). To increase the precision and accuracy of sampling within field plots, larger numbers of small cores should be taken and bulked (Francl 1986b).

2.12.3 Horizontal distribution

The measurement of variation in the horizontal distribution of plant-parasitic nematodes allows the development of sampling strategies that, in turn, allow measurement of factors such as the relationship between yield loss and nematode density or population decline/increase throughout the season. Horizontal distribution of plant-parasitic nematodes is rarely random and instead tends more towards an aggregated distribution. This can be represented as a negative binomial distribution (Goodell and Ferris 1980) or described by Taylor's Power Law (Taylor 1961). The horizontal distribution of nematodes is often aggregated as a result of distribution of the host's root system (Kimpinski and Welch 1971) and plant-parasitic nematodes may therefore tend to occur in patterns based on the previous season's plant rows (Ferris and McKenry 1974; Francl 1986a). This is especially true for hosts such as soybean with widely spaced rows and widely spaced plants within rows. In this crop, distribution of *Pratylenchus*, *Meloidogyne* and *Belonolaimus* spp. was concentrated around rows and this effect became more obvious as the season progressed (McSorley and Frederick 1996).

The root type of the host can also affect distribution. *P. neglectus* was found to be ten times more concentrated in the seminal roots compared to the crown roots of wheat (Kimpinski *et al.* 1976). Hosts with a fibrous root system such as cereals may therefore have higher nematode densities and/or a different distribution than hosts with a single tap root such as legumes.

Wallace *et al.* (1993) assessed edaphic factors which affect horizontal distribution, and found five out of eight nematode taxa were significantly correlated with sand particle size. In another study, high levels of *P. brachyurus* in pineapple roots were correlated with low pH but this effect diminished as the season progressed (Sarah *et al.* 1991).

Levels of nutrition in the soil will also alter nematode distribution. Delaville *et al.* (1996) showed that *P. zeae* only aggregated around sugarcane rows in areas where nitrogen, potassium and carbon levels were poorest as this was where root densities were highest.

Differences in the horizontal spatial variation of *P. neglectus* within plots are investigated in Chapter 5.

2.12.4 Vertical distribution

As with horizontal distribution, the vertical distribution of nematodes in the soil will also depend on a variety of factors. The distribution of the host's root system may determine the vertical distribution of nematodes as plant parasites are most likely to be found at depths where maximum root densities occur. In bentgrass putting greens, *Criconomella, Tylenchorhynchus* and *Helicotylenchus* spp. were more abundant in the upper 2.5 cm of the soil profile (Davis *et al.* 1994). Other nematode species occurring on different hosts are found deeper in the soil profile (Barker and Nusbaum 1971; Yeates *et al.* 1983). In the black clay vertisols of Queensland and the grey cracking clays in Victoria, the majority of *P. thornei* are found between 15 and 60 cm but nematodes are still found to depths of 90 cm (Thompson 1990; G. Hollaway pers. comm.). Most studies report maximum nematode densities in the top 15 - 20 cm of the soil profile and this appears to be the most common sampling depth (McSorley and Dickson 1990). Sohlenius and Sandor (1987) suggest that a decrease in nematode density below this depth is most likely the result of decreased food source rather than factors such as waterlogging or oxygen deficiency.

Changes in the vertical distribution of nematodes may occur throughout the year, possibly because of nematode migration or environmental conditions which favour reproduction at various depths

(Ferris and McKenry 1974). For example, the density of *P. scribneri* at different depths was found to change depending on crop and time of season sampled (MacGuidwin and Stanger 1991).

Different nematode species may vary in vertical distribution (Yeates *et al.* 1983; Sohlenius and Sandor 1987; Wick and Vittum 1988; Davis *et al.* 1994). Even for a single nematode species, the proportion of the population found at different depths may change between sites, suggesting that abiotic factors play a major role in determining vertical distribution (Yeates *et al.* 1983).

The vertical distribution of *P. neglectus* in South Australian clay, clay loam and calcareous sands is examined in Chapter 5.

2.12.5 Extraction and storage

In addition to the development of sampling strategies for determining densities of nematodes, once sampled, accurate quantification from plants and soil is required. Ferris (1987) defined two terms for the extraction efficiency of nematodes from plants and soil: "catch efficiency" and "separation efficiency". Catch efficiency is the proportion of the nematode population that can be recovered using the tools and methodology available. Separation efficiency is the efficiency of the technique used to separate nematodes from plant or soil and can be determined by measuring the proportion of recovery from repeated extractions from the same sample.

Common methods used for extraction of nematodes from plants include the misting method (Seinhorst 1950) and the Baermann funnel method (Baermann 1917) which are used for recovery of motile nematodes. Extraction of nematodes from plants can also be achieved using maceration (e.g. Dunn 1973; McClure *et al.* 1973) or centrifugal sugar flotation (Greco and D'Addabo 1990). The latter methods do not rely on nematode motility and are therefore extremely useful for the extraction of eggs or sedentary nematode species.

Extraction from soil can be achieved by centrifugal sugar flotation or by elutriation (e.g. Persmark *et al.* 1992), which do not rely on nematode motility, or by the use of Whitehead trays (Whitehead

and Hemming 1965), which does. Factors affecting both the catch and separation efficiency of *P. neglectus* from soil are investigated in Chapters 4 and 5.

2.13 Importance of this research

Prior to the commencement of this study, little was known about the biology of *P. neglectus* in South Australian dryland cropping rotations. To determine the impact of *P. neglectus* in this region, information is required on the tolerance and resistance of different crops and cultivars, levels of yield loss caused by this species, sampling strategies in plants and soil and variability within and between Australian populations of *P. neglectus*.

In order to establish yield loss caused by plant-parasitic nematodes, techniques for both manipulating nematode density and accurately determining nematode density must be assessed. This study evaluated the use of both nematicide or correlation analyses for determining yield loss caused by *P. neglectus* or *P. thornei* in field plots and some of the limitations that may arise using these methods. Sampling and extraction techniques for estimating the density of these nematodes from roots or soil were also assessed, with particular reference to the limitations imposed by dryland agricultural systems in South Australia. Differences in the virulence of populations of *P. neglectus* and *P. thornei* are important when considering evaluation of yield loss, and especially for the development of resistant and/or tolerant varieties, as measurable differences may determine the usefulness and longevity of each variety produced.

This research was conducted to contribute to practical management systems for reducing the impact of both *P. neglectus* and *P. thornei* in broad acre cropping systems in southern Australia.

Chapter 3 General techniques

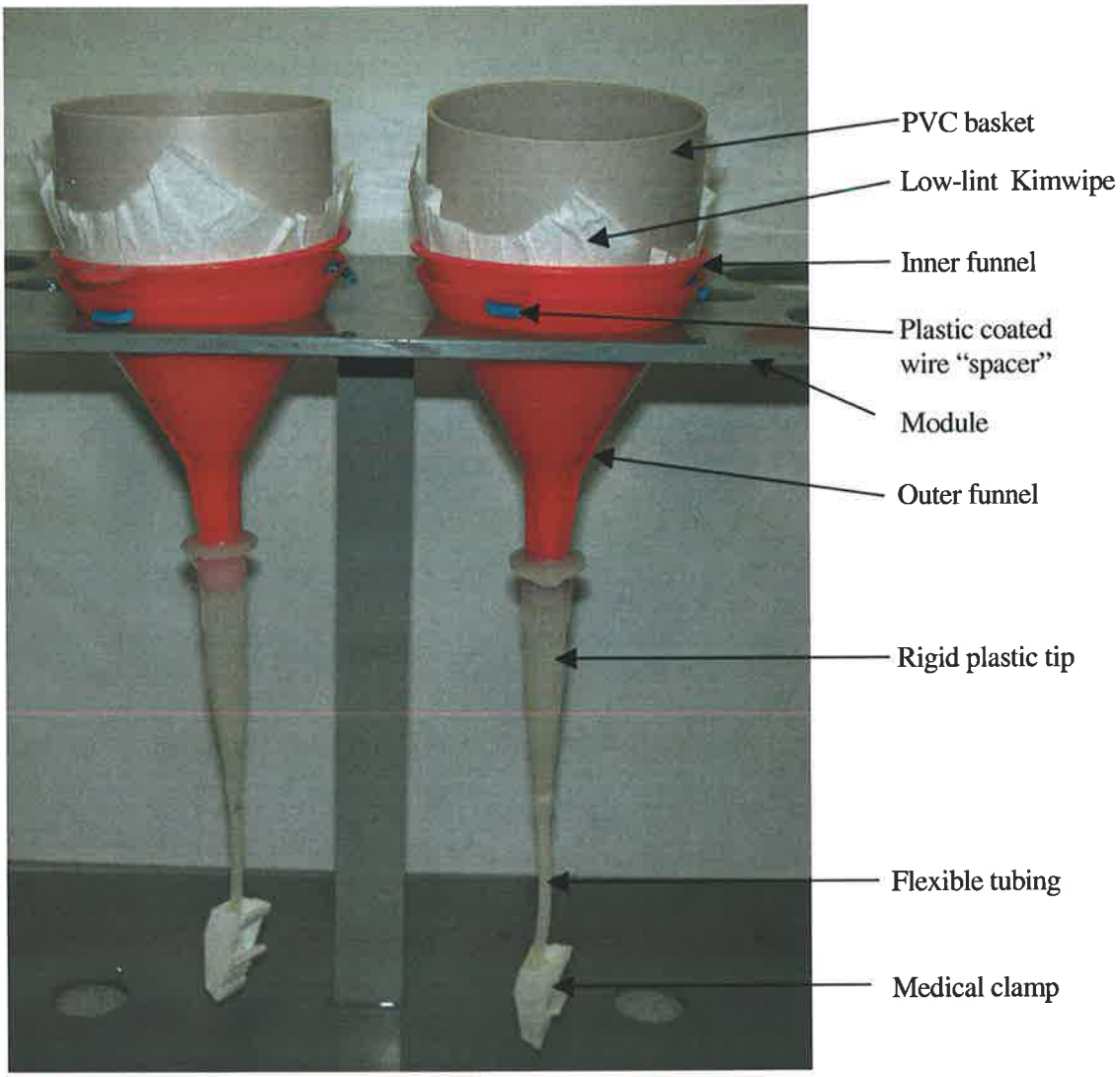
3.1 Extraction of nematodes from plant material

3.1.1 Construction of the misting chamber – the double funnel method

A purpose-built misting chamber to extract nematodes from plant or soil material was constructed based on the modified Double Baermann Funnel technique of Bohmer and Weil (1978). Samples were held in baskets which rested on the double funnel (Plate 3.1). The nematode suspension from each sample settled in the clamped tubing at the base of the outer funnel and, at the end of the extraction period, only a small volume (approximately 5 – 10 ml) was required to obtain all nematodes in the extracted sample. Excess water flowed away in the gap between inner and outer funnels. This eliminated the need to concentrate the suspension prior to counting, which occurs when nematode suspensions are collected in larger test tubes or flasks.

Misting chamber and equipment: To hold plant samples, baskets (9 cm diameter by 7 cm high) were constructed of PVC drain pipe covered at one end with plastic fly wire gauze (attached with PVC tubing glue) (Plate 3.1). The outside of each basket was lined with low-linting tissue (Kimwipe®) and baskets rested on the inner funnel. Inner funnels were modified by having the neck removed and rested on the outer funnel. Outer funnels (10 cm diameter) were modified by attaching a rigid plastic Selley® glue tip to the base of the funnel to which flexible tubing (6 mm outer diameter by 11 cm long) was attached. The tubing was clamped with a quick release, Urocare® medical thumb clamp. To provide a gap between the inner and outer funnels, three holes were punched at even intervals in the top of the outer funnel through which plastic coated copper wire (3 mm diameter) was threaded and folded over.

Plate 3.1 **Double funnel method used for the extraction of *Pratylenchus* from root material. Modified from Bohmer and Weil (1978).**



Funnels were supported in the misting chamber (Plate 3.2) in removable plastic "modules" 760 cm long by 21.5 cm wide by 30 cm high. Each module held 12 funnels in a 6 by 2 arrangement (Plate 3.3).

The misting chamber was set up with brass spray cone jets 15 cm above the funnels. One jet was located over every four funnels (in a square pattern), with the centre of each funnel 12 cm apart. There was minimal overlap between spray from each jet. Water temperature was regulated to be 22°C at the surface of the sample. The misting chamber was set up as four independent compartments, each capable of holding 108 samples (9 modules).

3.1.2 Calibration of the misting chamber

3.1.2.1 Materials and methods

To identify a misting protocol that maximised the number of nematodes recovered from each sample, reduced the number of nematodes in the overflow, and produced the cleanest samples, methods were assessed using oat straw infected with stem nematode, *Ditylenchus dipsaci*. *D. dipsaci* was chosen as it is rapidly extracted (24 hours) from oat straw and inoculum was readily available. One gram of oat straw infected with approximately 400 *D. dipsaci* was placed in baskets and misted for 24 hours. To catch the overflow from each funnel, large jars (1 litre capacity) were placed the funnel (and tubing). In this experiment, wire supports provided a gap between the funnel and the module to allow the overflow to run directly into the jar. Five replicates were included per misting time and the following periods of misting spray were assessed: 10 seconds on, 10 minutes off; 15 seconds on, 10 minutes off; 20 seconds on, 10 minutes off; 30 seconds on, 10 minutes off.

3.1.2.2 Results and discussion

There was no significant difference between the number of *D. dipsaci* recovered from any of the misting regimes tested or between number of nematodes recovered in the overflow (Table 3.1).

Plate 3.2 **Misting chamber showing individual modules for the extraction of *Pratylenchus* from root material.**

Plate 3.3 **Module used for supporting funnels within the misting chamber for the extraction of *Pratylenchus* from root material.**

Visually, less tissue lint was recovered in samples with longer spray times. A reduction in tissue lint resulted in clearer samples thereby increasing the efficiency with which samples were counted (Section 3.3). Minimal variation was observed between the rate at which funnels filled (i.e. the volume of water in different funnels).

Table 3.1 **Recovery of *Ditylenchus dipsaci* from infected oat straw in the nematode suspension and overflow from each sample. Nematodes were extracted using the double funnel method in a misting chamber.**

Misting time (seconds)	*D. dipsaci* recovered from sample (± s.e.d.)	*D. dipsaci* recovered from overflow (± s.e.d.)
10	466 ± 290	2 ± 2
15	598 ± 475	7 ± 11
20	392 ± 222	4 ± 5
30	551 ± 249	8 ± 12

No significant difference was observed between the number of *D. dipsaci* in the extracted sample at any misting regime or the number recovered from the overflow suggesting that, in this misting chamber, nematodes were efficiently extracted using a variety of spraying times. Although spraying for intervals of only 10 seconds resulted in the lowest number of nematodes in the overflow, misting for intervals of 15 seconds resulted in visibly less tissue lint and was therefore chosen for routine extraction of nematodes from plant material.

3.1.3 Extraction of nematodes from plant roots - misting

The outside of baskets within the misting chamber were lined with two layers of Kimwipe® tissue which produce low levels of lint. Plant root systems were washed thoroughly under running tap water and cut into 1 cm lengths. A maximum of 4 g wet weight of root was placed in each basket. Over 96 hours, samples were misted (with tap water) for 15 seconds (30 psi) every 10 minutes.

Misting jets were turned off 30 minutes prior to collection of nematode suspension to allow nematodes to settle in the base of the tubing. A small volume (10 – 15 ml) of water containing nematodes was then released from the tubing and stored in 50 ml sample jars at 4°C before counting (Section 3.3).

3.1.4 Extraction of nematodes from plant roots – staining then maceration

Plant roots were stained using the method developed by Taheri (1996). Washed roots were soaked in 30 - 50 ml sodium hypochlorite (1% NaOCl) for 10 minutes, then washed under running tap water. Roots were stained by heating until boiling in 30 - 50 ml acid fuchsin (3.5%). Stained roots were cooled to room temperature, washed thoroughly in tap water, blotted dry then heated in 30 – 50 ml glycerol (50%) until boiling to destain the root material. Roots were stored in glycerol solution. To assess numbers of nematodes within stained roots, roots were blended using a Pro200® "stab" blender, and two, 1 ml sub-samples were removed and nematodes counted.

3.2 Extraction of nematodes from soil

3.2.1 The modified Whitehead tray method

Methods assessing the optimisation of extraction of *P. neglectus* from soil using the Whitehead tray method are presented in Chapter 4, Section 4.1.1. In summary, nematodes were extracted from 200 g soil samples using the modified Whitehead tray method (Whitehead and Hemming 1965) shown in Plate 3.4. Seedling trays (29 cm wide by 36 cm long by 5.5 cm high) were lined with one sheet of low-linting, heavy duty Kimwipe® (bottom layer) and one sheet of facial tissue (top layer) and placed in photographic trays (31 cm wide by 45 cm long by 6.5 cm high). Large soil clumps were broken by hand and soil was evenly distributed over the tissue. Tap water was added to the photographic tray to wet (although not cover) the soil sample. Samples were extracted for 72 hours and the nematode suspension was concentrated by passing water through a

Plate 3.4 **Whitehead tray method for the extraction of *Pratylenchus* from soil (modified from Whitehead and Hemming 1965).**

20 µm sieve. Nematodes were washed off the sieve in 5 - 10 ml water and stored at 4°C in sample jars before counting (Section 3.3).

3.2.2 Assessment of soil moisture

A 50 g sub-sample from each soil sample was placed into a paper bag, dried at 60°C for 72 hours. Soil was re-weighed after drying and soil moisture determined.

3.3 Counting and identification of nematodes

Samples were counted in modified Doncaster (1962) dishes using a dissecting light microscope at either 20x or 40x magnification. For higher nematode densities (> 200 nematodes/sample), samples were weighed to determine volume, then two, 1 ml sub-samples were taken and nematodes per ml counted. For lower nematode densities, the entire sample was counted. *Pratylenchus* spp. were identified based on the position of the vulva in adult females (*P. neglectus* V = 81 - 86%; *P. thornei* V = 73 - 80% [Loof 1991]) and also by using allozyme electrophoresis (Chapter 9).

Unless otherwise stated, all results were expressed as nematodes/g of dry soil (Section 3.10.2).

3.4 Preparation of nematode inocula

3.4.1 Carrot culture inoculum

Pratylenchus neglectus or *P. thornei* were produced on carrot callus cultures using the protocol described by Nicol (1996) and Nicol and Vanstone (1993) and modified from Moody *et al.* (1973). Carrot cultures were established either with up to 10 adult females (termed "populations") or with individual females (termed "clonal lines") extracted from soil or plants. *P. neglectus* inoculum for determining the efficiency of separation from plants (Chapter 4) and population dynamics experiments in microplots (Chapter 7) came from a population established

in 1991 by V. Vanstone (University of Adelaide), originally sourced from Palmer, South Australia.

To establish carrot cultures, nematodes were surface sterilised in a solution of 1% streptomycin / 1% penicillin for 3 hours then rinsed 3 times in sterile RO water. Surface sterilised nematodes were added to callus using a flamed needle to transfer individual nematodes to a 2 µl drop of sterile RO water on the callus. After inoculation, cultures were maintained in dark incubators at 20 - 22°C and checked weekly for the presence of nematodes. Nematodes were seen either clumping on walls of tubs or on the carrot callus as fine "cotton wool" (Plate 3.5). Carrot cultures took approximately 6 months (*P. thornei*) to 18 months (*P. neglectus*) to produce up to 1,000,000 nematodes per culture. Nematodes were extracted from cultures by chopping carrots into 1 cm sections which were then misted (see Section 3.1.3). The nematode suspension was collected from the misting chamber every 24 hours and nematodes were collected for up to 2 weeks. Nematode inoculum was prepared by subsequent dilution of the resulting suspension.

3.4.2 Soil inoculum

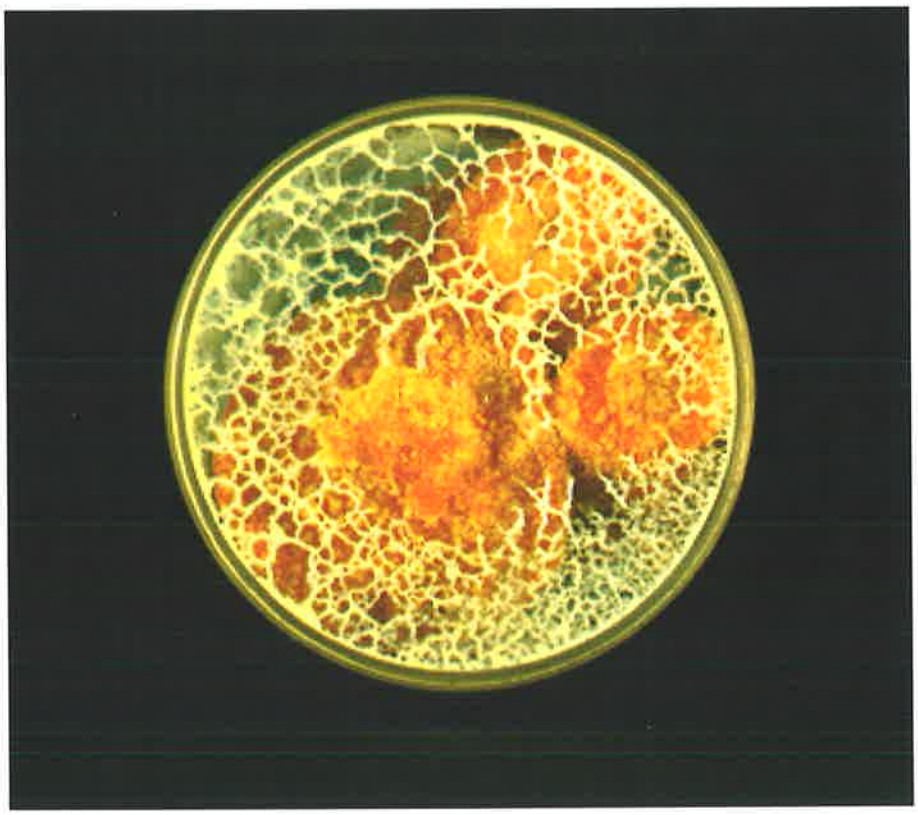
Soil inoculum containing *P. neglectus* was prepared by filling 25 cm diameter pots with washed Tailem Bend sand (99% sand, 1% clay; soil analysis presented in Appendix 1) which had been steam pasteurised at 65°C for 45 minutes. Ten ungerminated wheat seeds (cv. Spear) were sown per pot. After emergence, pots were inoculated with *P. neglectus* by adding approximately 5 g of carrot culture infested with *P. neglectus*, chopped into 0.5 cm pieces, to the soil surface. Plants were grown in the glasshouse (average temperature 25°C), and watered as required. After 12 weeks, tops of plants were removed and watering was stopped. The resulting soil inoculum was stored in dry soil in pots at 22°C for up to 12 months.

When required, soil in pots was moistened with 300 – 500 ml tap water, then removed, bulked, and mixed on sheets of black plastic. Root systems were roughly chopped and mixed evenly through the soil. Soil was kept moist and, after 2 - 3 days, sub-samples of 200 g were removed and nematodes extracted using Whitehead trays for determination of nematodes per gram of soil.

Plate 3.5 **Carrot culture for the production of asceptic *Pratylenchus* inoculum. a) Culture on the left shows *Pratylenchus* clumping on the tub wall; culture on the right shows early stages of development (no nematodes visible). b) Large numbers of *Pratylenchus* clumping on the base of tub.**

a)

b)



The required soil inoculum density was prepared by dilution with steam pasteurised Tailem Bend sand.

For experiments in Chapter 6 measuring the population dynamics of *P. neglectus* in pots, soil inoculum was prepared by adding infected carrot culture directly to steam sterilised Tailem Bend sand and mixing thoroughly.

3.5 Growth room experiments

3.5.1 Germination of seed

Seed was placed in a 50 ml sample jar, covered with 1 cm of water and incubated at 4°C in the dark for 24 h. Water was removed, and seed was placed in unlined baskets within the misting chamber (3.1.1) for 24 h. No clamps were used and water was allowed to drain continuously. For each experiment, germinated seed of the same approximate size and root length was selected.

3.5.2 Tube tests

Plants were grown in open-ended electrical conduit tubes (2.5 cm diameter by 12.5 cm high) supported in a wire, square mesh frame (5 cm by 5 cm mesh size) and placed on a tray (82 cm long by 62 cm wide by 6 cm deep) filled with University of California soil mix (specifications presented in Appendix 2). Tubes were packed with Tailem Bend soil which had been steam pasteurised at 65°C for 45 minutes. A single pre-germinated seed was sown per tube. Tubes were kept in growth rooms maintained at a 12 hour light (metal halide, 400 watt lamps), 12 hour dark photoperiod, at temperatures of 20°C (light) and 15°C (dark) (Plate 3.6). Plants were watered as required and harvested 6 weeks after inoculation with nematodes.

3.6 Pot trials on outdoor terraces

Outdoor pot/microplot experiments were grown on irrigated plant beds, called terraces, shown in Plate 3.7. Each terrace was constructed using wooden railway sleepers covered with shade-cloth

Plate 3.6 **Controlled environment growth room test for the assessment of pathogenicity of *Pratylenchus* in cereals. Individual plants were grown in tubes and supported by wire mesh frame.**

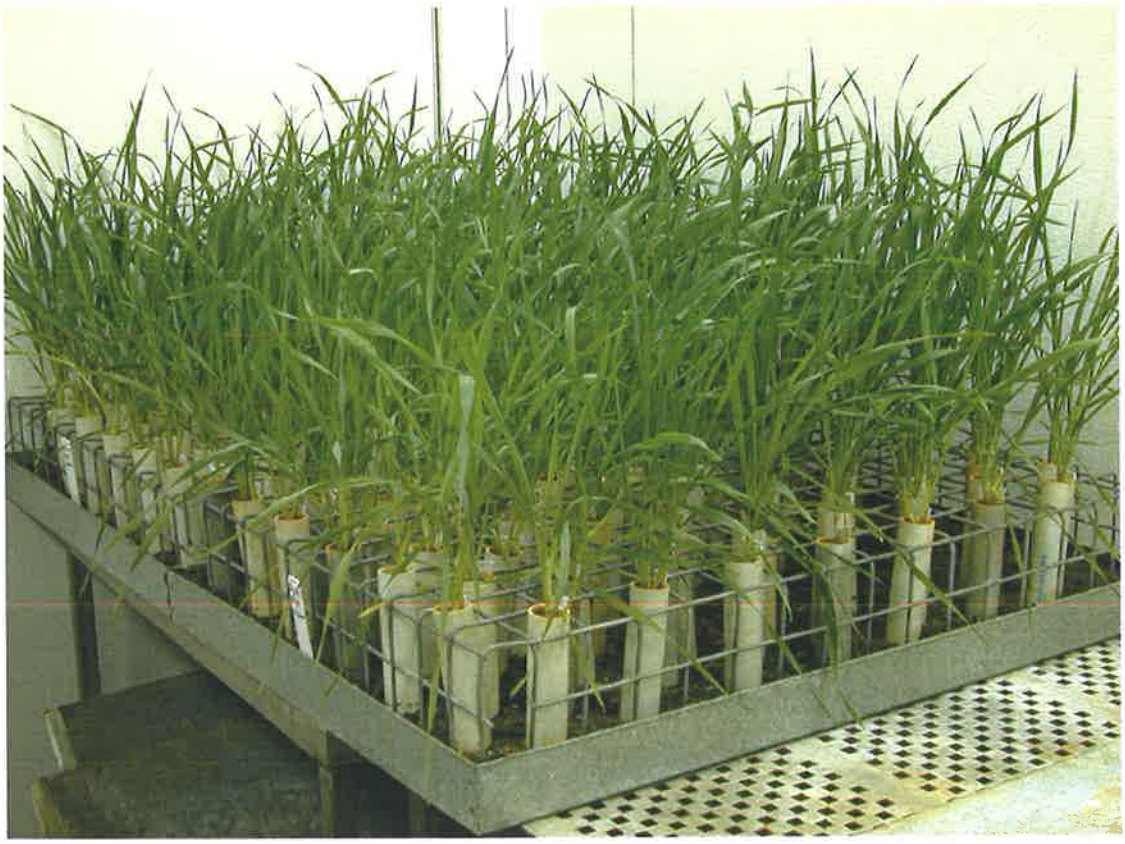
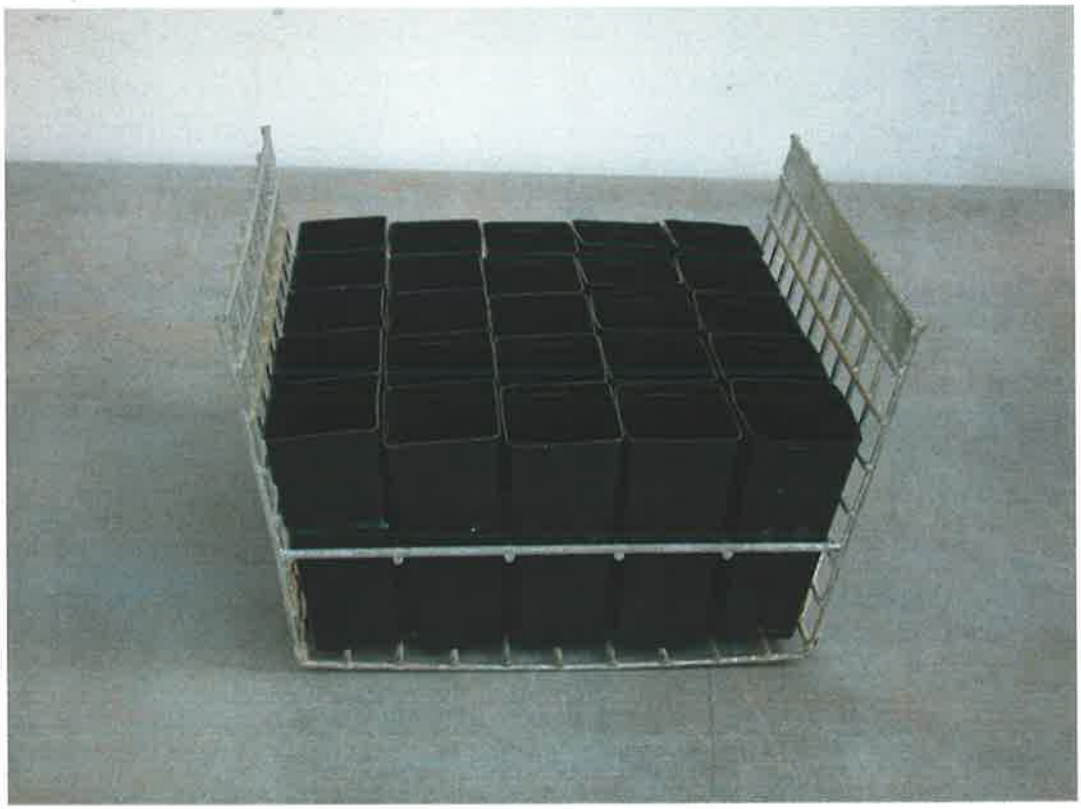


Plate 3.7 **Outdoor plant beds (terraces) for the assessment of multiplication of *Pratylenchus* in cereals.**

Plate 3.8 **Crates (and seedling tubes) used in the outdoor terrace test.**



and filled with washed sand to create a plant bed. Plants were watered daily for 10 minutes using overhead sprinklers. Terraces were kept free of weeds and pathogens by soil solarisation for 2 weeks at the commencement of each experiment.

Plants on terraces were grown in seedling tubes held in purpose-built wire mesh crates constructed from galvanised steel mesh grid (mesh size 5 cm by 5 cm) in either a 5 by 10 or 5 by 5 arrangement (Plate 3.8). One seed was planted per tube. Crates were partially buried into the plant beds allowing plants to grow through the open bottomed tubes into the sand-bed below. Plants tillered and reached maturity in approximately twelve weeks.

Tubes were packed with Tailem Bend sand which had been steam pasteurised at 65°C for 45 minutes. A granular, slow release fertiliser treatment, OsmocotePlus® (16% N, 3.5% P, 10% K, 1.2% S, 1.2% Mg, 0.15% Fe, 0.06% Mn, 0.05% Cu, 0.02% Mo, 0.02% B, 0.02% Zn, 2% Ca: 8 – 9 months release at 21°C) was added at a rate of 4 g per kg of soil and mixed evenly throughout the soil.

3.7 Field sites

3.7.1 Site selection

To select sites for field trial experiments, five samples (each comprised of 20 bulked sub-samples) were taken in a diamond pattern across an area of approximately one hectare to evaluate *Pratylenchus* numbers and/or the presence of other pathogens. Areas in the field that were not uniform e.g. headlands (strips of land where double applications of fertiliser and seed had been applied in the previous year), sheep camps or gateways, were avoided. Field sites with mixed populations of *P. neglectus* or *P. thornei* or with cereal cyst nematode (*Heterodera avenae*) or take-all (*Gaemannomyces graminis*) (determined by bioassay) were not selected.

3.7.2 Field site establishment and maintenance

All field trials were sown using a cone seeder. Delineation between plots in each column was achieved by spraying or slashing 1 m from both ends of each plot before harvest (Plate 3.9). Fertiliser was applied at sowing (see Materials and methods for each trial site). Weeds were managed using district practice at each site. The location of field sites used in Chapters 5, 7 and 8 is shown in Figure 3.1, and the location of sites used in population variability studies in Chapter 9 is shown in Figure 3.2.

3.7.3 Application of nematicide

The nematicide aldicarb (Temik15G®) was applied at sowing to a subplot within the main plot using a cone seeder. A rate of 2.5 a.i./ha was used unless otherwise specified. A measured dose of nematicide was delivered to the cone using a gun powder dispenser (Plate 3.10).

3.7.4 Soil sampling

After collection, soil samples were kept cool in ice boxes while transported to the laboratory and stored at 4°C prior to processing. Soil was sampled using either an undisturbed core, soil sampler or earth auger described in the following sections (Plate 3.11).

3.7.4.1 Undisturbed soil core

PVC cylinders (15 cm long by 7 cm diameter) were placed firmly on the soil surface, hammered into the soil to a depth of 10 cm and then pulled free and sealed with a PVC cap. If wet soil cores were required from dry field soil, water was added to the cylinder prior to hammering into the soil and allowed to infiltrate for 30 minutes to 3 hours prior to sample collection.

Plate 3.9 **An example of a field trial for the assessment of resistance to *P. neglectus* (Chapter 7), or yield loss caused by *P. neglectus* or *P. thornei* (Chapter 8). Plots were delineated within columns by either slashing or the application of herbicide.**

Plate 3.10 **Dispenser used for delivering a measured application of nematicide (aldicarb) in field sites. The dispenser was mounted on the cone seeder and aldicarb was applied with seed.**

Plate 3.11 **Equipment used for sampling soil for *Pratylenchus*. The soil sampler (Arborline soil corer) is shown on the left and the earth auger on the right.**

Figure 3.1 **Field trial site locations used within South Australia for:**

a) **the assessment of sampling strategies (Condada, Miltaburra, Kimba, Balaklava, Tanunda, Cleve, Palmer, Cungena; Chapter 5)**

b) **assessment of resistance in field crops to *P. neglectus* (Paskeville, Sandilands and Condada; Chapter 7)**

c) **yield loss and population dynamics (Condada and Minnipa; Chapter 8).**

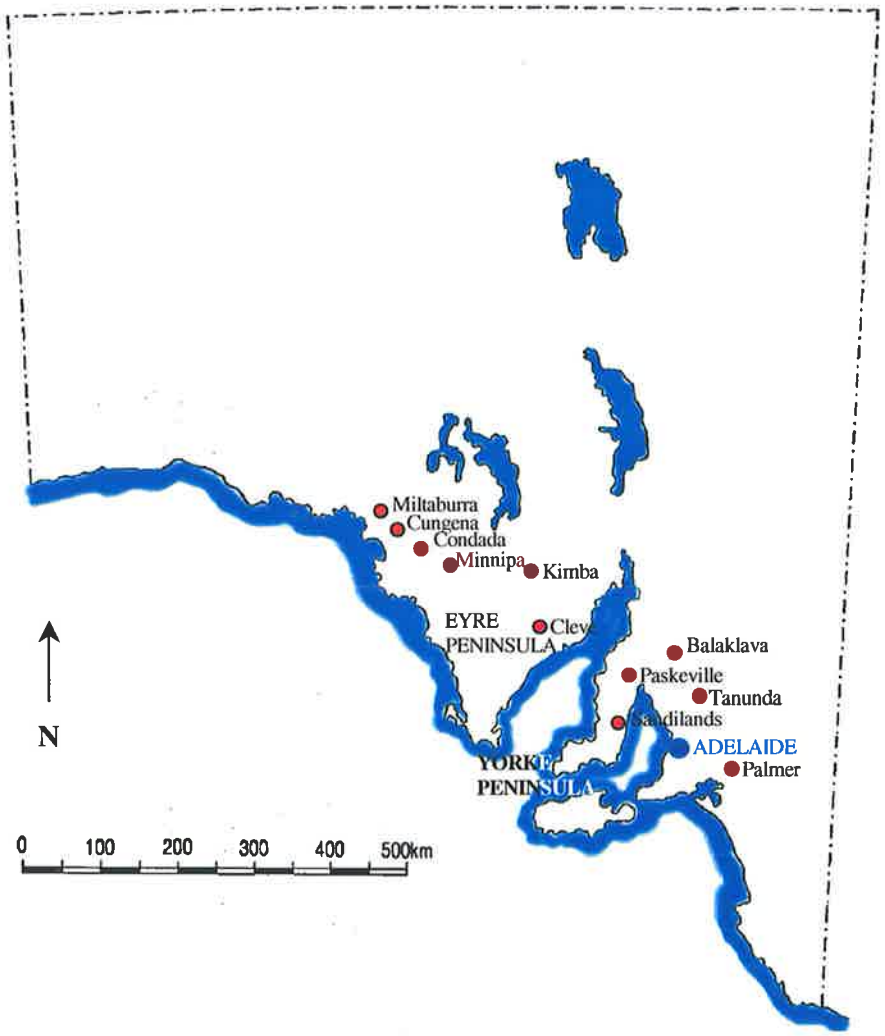
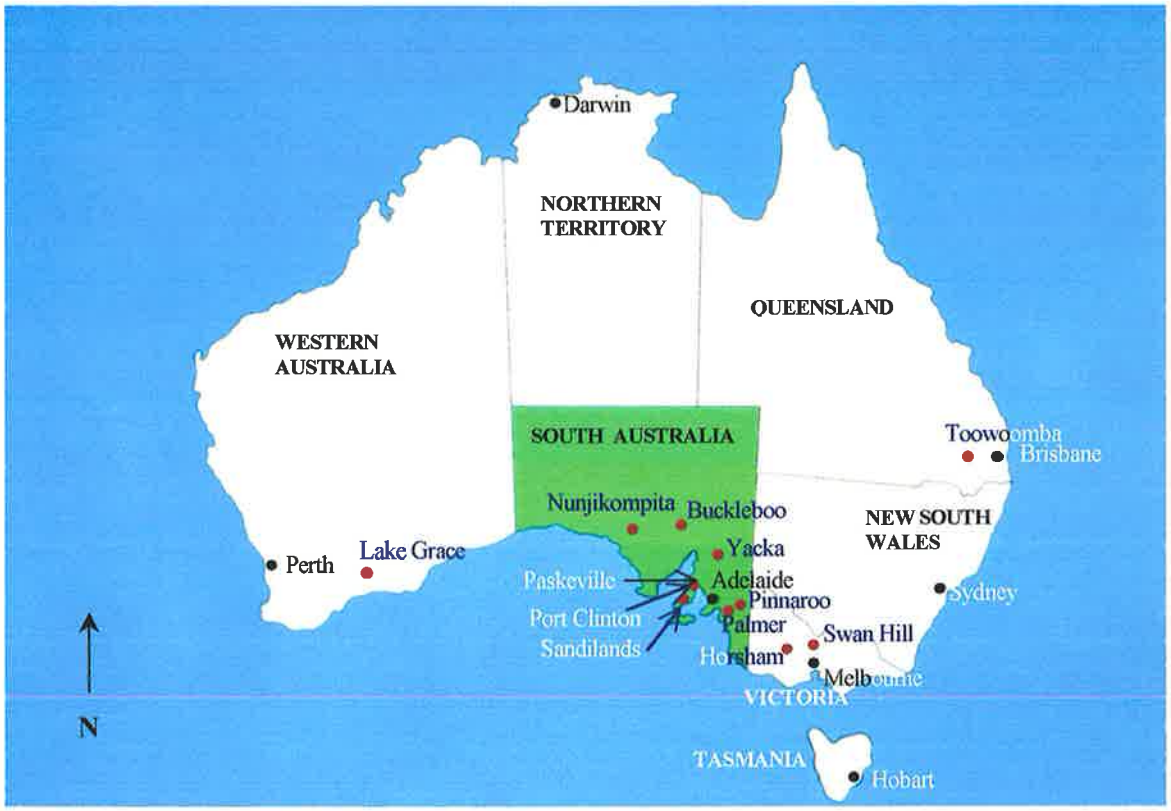


Figure 3.2 **Sites for collections of *Pratylenchus* populations used in population variability studies (Port Clinton, Swan Hill, Pinnaroo, Sandilands, Buckleboo, Palmer, Paskeville, Yacka, Lake Grace, Nunjikompita, Waite Campus [Adelaide], Toowoomba, Horsham; Chapter 9).**



3.7.4.2 Soil sampler

Soil was sampled using a commercially available Arborline® corer (coring tube 2.5 cm by 10 cm) or a soil auger (5 cm diameter) (Plate 3.11). For the assessment of nematodes in field plots, 12 - 15 sub-samples were taken using the Arborline® corer in moist soil to a depth of 10 cm and bulked to produce a 500 g composite sample. Samples were taken from each plot within one week following seeding (initial) and within 4 weeks either pre or post-harvest (final).

3.8 Harvesting and measurements

For field trials, grain yield was recorded. The density of root lesion nematodes was assessed in moist soil at planting (initial density) and within 4 weeks of harvest (final density). For pot trials, dry shoot weight, dry root weight and nematodes per plant were evaluated.

Nematode multiplication rate was determined by dividing final nematode density by initial nematode density (Pf/Pi).

3.9 Allozyme electrophoresis

Nematodes were extracted from carrot cultures using the method described in Section 3.4.1, washed 3 times in sterile RO water and spun in 10 ml centrifuge tubes for 3 minutes at 4000 rpm to obtain a nematode pellet. Pellets were collected, transferred to 2 ml microcentrifuge tubes, spun for 3 minutes at 6000 rpm to further pelletise nematodes, and kept on ice until used. For the carrot culture control, pieces of sterile uninoculated carrot culture (approximately 4 weeks old) were cut into 2 mm pieces and suspended in sterile RO water. The carrot culture suspension was then transferred to 2 ml microcentrifuge tubes and spun for 3 minutes at 6000 rpm and kept on ice until used.

To disrupt cells, pellets of either uninoculated carrot or nematodes were sonicated in a 1:1 volume of pellet:lysing solution (0.2% β-mercaptoethanol and 0.02% NADP). Aliquots of sonicated nematodes or uninoculated carrot culture were either used immediately or stored in glass capillary tubes at –20°C. Electrophoresis was run on cellulose acetate gels (Cellogel,

Chemetron; Milan) at 200 volts for 1.5 hours in a 4°C cold room. Gels were then stained for twenty seven loci and the resulting bands on gels were scored alphabetically, with the slowest migrating band designated 'a', the next slowest 'b', etc. A summary of loci, specific staining buffers and substrate solutions required are presented in Appendices 3 and 4.

3.10 Experimental design and statistical analyses

3.10.1 Experimental design

The experimental design chosen depended on the aim or nature of each experiment and included:

Complete randomised block design: used in field trials and some pot experiments to assess varietal differences. This was used where smaller numbers of treatments were being compared and eliminated sources of variation between varieties, especially if blocks were kept as square as possible (Gomez and Gomez 1984).

Split plot design: Used in field trials comparing nematicide treated with untreated plots. Varieties were designated as main plots and nematicide or untreated plots were designated as sub-plots. Each nematicide treatment was randomly assigned within the main plot and main plots were arranged in a complete randomised block design. Split plot designs allow greater precision in determining affects of the sub-plots compared with main plots (Gomez and Gomez 1984).

Completely randomised design: Used for some pot experiments, applicable because small areas were used and all experimental units (pots) were considered equal (Gomez and Gomez 1984).

3.10.2 Data analysis and transformation

Where necessary (i.e. if residuals plotted against fitted values produced skewed data, indicating large variation within replicates), data were transformed to normalise the data sets prior to

analysis. $Log_e(x)$ or $(x + 1)$ transformation was used where the standard deviation was proportional to the mean. Square root ($\sqrt{x+0.5}$) transformation was used where the variance was proportional to the mean. Square root transformation is considered appropriate where data consist of small whole numbers (such as number of nematodes recovered from samples). Statistical significance of differences between treatments was determined by conducting a general analysis of variance (ANOVA). Differences between treatment means were separated using either Fisher's protected least significant difference (l.s.d.) or Tukey's honestly significant difference test (h.s.d.) from a general analysis of variance (ANOVA; $P < 0.05$) (Gomez and Gomez, 1984).

Unless otherwise stated, nematode densities were presented as nematodes per of dry soil and expressed as $\log_e(x)$, $\log_e (x + 1)$ or as the detransformed mean.

Relationships between plant growth and nematode numbers were determined by simple linear correlation analyses (Gomez and Gomez 1984). Models describing the horizontal distribution of *P. neglectus* in field plots or determining the relationship between final density and initial density of *P. neglectus* in field trials are described in Chapters 5 and 8 respectively.

Chapter 4 Efficiency of extraction and separation of *Pratylenchus neglectus* from plant roots and soil

4.0 Introduction

The extraction of nematodes from plants and soil is measured by both catch and separation efficiency, previously defined in Section 2.10.5. Catch efficiency will be determined both by the purpose of the study and, probably most importantly, by the nematode species under consideration. Each nematode species will differ in temporal and spatial distribution (e.g. Barker and Campbell 1981; Goodell and Ferris 1981; McSorley *et al.* 1985), motility (Flegg 1967) and survival strategy (e.g. the ability to undergo anhydrobiosis or the percentage of life cycle spent in a vermiform stage [Barker *et al.* 1969a; Boag and Brown 1985]), all of which have implications for sampling strategies. Methods for sampling *P. neglectus* and *P. thornei* in South Australian soils and their effect on catch efficiency are evaluated in Chapter 5.

Separation efficiency can be determined by measuring the proportion of recovery from repeated extractions from the same sample until zero nematodes are recovered (Ferris 1987). As with catch efficiency, separation efficiency from plants and soil will be affected by the tools and methodology available as well as the nematode species. Considerable research has been undertaken comparing extraction techniques for different nematode species from both plants and soil.

For the extraction of plant parasitic nematodes from plant samples, factors such as sample size, carbon dioxide or oxygen level and type of plant host all affect the efficiency of recovery. Chapman (1957) observed a sharp decrease in the number of *Pratylenchus* recovered as sample size increased. He also found superior recovery in aerated samples and suggested that reduced recovery in larger samples was due to reduced oxygen levels. Prot *et al.* (1993) found that while maceration-filtration gave greatest recovery of *P. zeae* from rice roots, after extraction for 72 hours over 90% of nematodes were recovered using either maceration-filtration or mister techniques. Moore *et al.* (1992) also demonstrated that the mister method was adequate for extraction of *Pratylenchus* from root samples, although sample size and plant type affected recovery. The efficiency of the mister method for extraction of endoparasitic nematodes has

been questioned, however, as nematodes continue to emerge over time as a result of egg hatch (Viglierchо and Schmitt 1983). In contrast, if plant material is stained, nematodes are killed and fixed, eliminating the possibility of multiplication during the extraction process and therefore allowing a direct assessment of numbers within roots at the time of harvest.

Sample storage has been shown to affect recovery of nematodes. In general, transportation or storage of samples at high temperatures (i.e. > 30°C) results in decreased recovery of nematodes (Goodell and Ferris 1980; Barker and Campbell 1981). Storage of soil at temperatures of 15 - 20°C for 1 - 2 weeks prior to extraction led to increased recovery of *Rotylenchulus* and *Meloidogyne* spp., probably because of increased egg hatch (Mishra and Gauer 1987). Rough handling of soil samples may result in decreased recovery, possibly because of physical disturbance of soil (Brown and Boag 1985). The effect of soil disturbance on recovery of *P. neglectus* is further discussed in Chapter 5.

The efficiency of extraction methods for recovery of nematodes from soil has been studied extensively (e.g. Barker *et al.* 1969b, Brown and Boag 1988, Persmark *et al.*1992, Prot *et al.* 1993). Using the Whitehead tray method (Whitehead and Hemming 1965), nematode recovery and the clarity of the resulting nematode suspension will depend on both the soil type and the tissue layer used to retain the sample (McSorley and Parrado 1982, Viglierchо and Schmitt 1983).

The determination of the number of nematodes from plants (or soil) is essential for the assessment of plant resistance. While the extraction of motile species such as *P. neglectus* and *P. thornei* using techniques that rely on migration of living nematodes from roots (e.g. the mister method) or soil (e.g. the Whitehead tray method) appear adequate, limitations within these methods must be assessed. In this study, the Whitehead tray method for the recovery of *P. neglectus* from soil was optimised to increase the efficiency of nematode extraction and the clarity of the extracted sample and recovery of *P. neglectus* from stored soil was assessed. Comparison of two extraction methods for the quantification of *P. neglectus* from wheat roots and the efficiency of separation of this species from different crops and varieties was also considered.

4.1 Materials and methods

4.1.1 Elimination of lint and soil contamination from samples

A modified Whitehead tray method (Section 3.2.1) was evaluated using different tissue layers to minimise both dirt and tissue lint from extracted nematode suspensions. *P. neglectus* were extracted from a naturally infested sandy loam soil from Condada (Figure 3.1), with fifteen replicates included per treatment. Soil was sampled after the autumn rain with samples collected randomly across a 500 m^2 area using a hand trowel. Each sample was split into three 250 g samples and *P. neglectus* was extracted using the following tissue layers:

Treatment 1 - Black seedling trays lined with two tissue (Kleenex®, 31 cm by 31 cm) layers.
Treatment 2 - Trays lined with two layers of low linting tissue (Kimwipe®, 42 cm by 31 cm).
Treatment 3 - Trays lined with one layer of Kimwipe® (bottom layer) and one layer of Kleenex® tissue (top layer).

4.1.1.1 Data analysis

Nematode densities were expressed as *P. neglectus*/g of dry soil. To assess the effect of different Whitehead tray methods, data were $\log_e$ transformed and means were separated using ANOVA ($P < 0.05$). Differences between treatments were determined using Tukey's honestly significant difference (h.s.d.).

4.1.2 Separation efficiency of nematodes using a modified Whitehead tray method

To determine the separation efficiency of *P. neglectus* recovered from sandy loam soil (i.e. the percentage recovery after different time periods), nematodes were extracted from soil collected from five sites naturally infested with *P. neglectus* (five replicates per site) using the modified Whitehead tray method described in Section 3.2.1. Soil samples were collected after autumn rain (prior to seeding) using a hand trowel, and contained organic matter and root material from the previous crop. The crops present in the previous season were: barley (cv. Galleon), field pea (variety not known), wheat (cv. Spear), barley (cv. Galleon) and oat (cv. Wallaroo) for Sites 1 to 5 respectively. Nematode suspensions were collected from each sample after 72, 96, 120 and 144 hours. Water was added to the sample after the suspension was taken at each extraction time.

4.1.2.1 Data analysis

Nematodes were expressed as *P. neglectus*/g of dry soil. To assess the efficiency of extraction using the Whitehead tray method, within each site differences in number of *P. neglectus* between extraction times were assessed using standard error of difference.

4.1.3 Recovery of *P. neglectus* from stored soil

4.1.3.1 Experiment 1

Soil (15 kg) was collected following autumn rain from a black clay soil at Tanunda and a sandy loam soil at Condada. Nematodes were assessed in samples from soil stored at 4°C for 1, 3 or 7 days after collection. At each storage time, fifteen 250 g replicates were removed from the bulk stored soil. From each replicate, the number of nematodes determined from a 200 g sub-sample processed on a Whitehead tray (as described in Section 3.2.1).

In transit from the collection site, samples were kept in a cold box on ice, then stored in plastic bags in a controlled temperature room (4°C) for the required time period. Soil collected from Condada took 24 hours to reach the laboratory and this period was included in the experiment i.e. samples for the 24 hour storage time were processed on the day they arrived at the laboratory. Samples from Tanunda reached the laboratory on the same day as sampling and were immediately transferred to the cold room. Prior to processing, stored soil was removed from the cold room and kept at room temperature for a minimum of 5 hours before being processed.

4.1.3.2 Experiment 2

Soil (90 kg) was collected from a sandy loam (Palmer) and a clay loam (Sandilands) after autumn rains (27 mm and 34 mm, respectively). In transit from the collection site, samples were kept in a cold box on ice. At the laboratory, soil from each site was separated into two batches and stored in plastic bags either at room temperature (18 - 22°C) or in a controlled temperature cold room (4°C). When required, soil stored at 4°C was removed and kept at room temperature for 5 hours before being processed. Soil was processed using the modified Whitehead tray method and soil moisture determined (Section 3.2). Six 250 g replicates were taken from the bulk stored soil at each storage temperature and nematodes extracted at the following times after soil collection: same day, 1 day, 3 days, 1 week, 2 weeks, 3 weeks, 4 weeks, 6 weeks, 8 weeks, 10 weeks, 12 weeks, 16 weeks.

4.1.3.3 Data analysis

For Experiments 1 and 2, numbers of nematodes were expressed as *P. neglectus*/g of dry soil. For Experiment, 1 data were $\log_e$ transformed and for Experiment 2, $\log_e + 1$ transformed to normalise data sets. Means were separated using ANOVA ($P < 0.05$). Differences between recovery times were assessed using Tukey's h.s.d. For Experiment 2, soil moisture was expressed as percentage soil moisture/sample and differences between extraction times were assessed using standard error of difference.

4.1.4 Comparison of two methods for extraction of *P. neglectus* from wheat roots

To assess the effect of two different *P. neglectus* extraction techniques on wheat roots of varying age, a comparison of misting and staining techniques (Sections 3.1.3 and 3.1.4) was made using plants grown in microplots for the Population Dynamics experiment described in Chapter 6. Three nematode soil inoculum levels were used to inoculate wheat plants: low (1.2 *P. neglectus*/g of soil); medium (9.4 *P. neglectus*/g of soil); and high (36.5 *P. neglectus*/g of soil). Plants were grown outdoors in pots and harvested every 4 weeks for 20 weeks (see Chapter 6 for detailed methods). Each harvest time was comprised of 10 replicates, of which five plants were randomly chosen for nematode extraction by misting, and five for extraction by staining then blending.

4.1.4.1 Data analysis

Nematodes were expressed as *P. neglectus*/plant and data were $\log_e$ transformed to normalise data sets. Means were separated using ANOVA ($P < 0.05$), and treatments were compared using Fisher's protected l.s.d.

4.1.5 Efficiency of separation of *P. neglectus* from different crops

The separation efficiency of *P. neglectus* was assessed from single cultivars of wheat (*T. aestivum*), chickpea (*C. arietinum*), canola (*B. napus*), faba bean (*V. faba*) and annual medic (*Medicago* sp.) by evaluating repeated extractions from the same sample over a 21 day period in a misting chamber.

4.1.5.1 *Pratylenchus neglectus* inoculum and plant establishment

P. neglectus soil inoculum was prepared from a mixture of infected carrot culture and Tailem Bend sand, as described in Section 3.4. No nil treatment was included as no comparisons were made between plant growth and the density of *P. neglectus*. Seedling tubes (300 ml) were filled with soil inoculum and packed into mesh crates (Section 3.6). A single un-germinated seed from one of five crop species was sown into each tube: wheat (cv. Machete); chickpea (cv. Dooen); faba bean (cv. Fiord); canola (cv. Barossa); and annual medic (cv. Harbinger). The chickpea, medic and faba bean cultivars were inoculated at seeding with commercially available *Rhizobia* appropriate for each crop species. After seeding, crates were maintained at $20^{\circ}C \pm 3^{\circ}C$ in a glasshouse for 10 days and watered daily, then transferred to outdoor terraces and partially buried in sand beds (Plate 3.7). Plants on terraces were irrigated using an overhead sprinkler system.

4.1.5.2 Experimental design and assessment

Plants were assessed at two harvest times, 6 or 16 weeks after sowing. Seedling tubes were arranged as a Complete Randomised Block Design with five replicates and replicates within each harvest time comprised four separate plants (one plant/tube) arranged in a block. For example, in replicate 1, crate 1:

6 week harvest	W	W	C	C	Ch	Ch	M	M	F	F
	W	W	C	C	Ch	Ch	M	M	F	F
16 week harvest	Ch	Ch	W	W	F	F	C	C	M	M
	Ch	Ch	W	W	F	F	C	C	M	M

Each square represents a separate seedling tube. W = Machete wheat; C = Barossa canola; Ch = Dooen chickpea; M = Harbinger medic; F = Fiord faba bean.

At each harvest time, roots growing through the bottom of the seedling tubes were removed and the root system within each tube was washed, cut and placed in separate baskets within the misting chamber. The four plants comprising each replicate were misted in separate baskets but

remained adjacent and so all received spray from the same jet (Section 3.1). The nematode suspension was collected after 4, 7, 10, 13 and 21 days, and each sample remained in the misting chamber for a total of 21 days. At collection, the suspension from the four samples comprising each replicate was bulked (all replicates remained separate). For harvest time 2 only, the number of adults was recorded separately to juveniles (stages 2 – 4).

4.1.5.3 Data analysis

Results were expressed as number of *P. neglectus*/plant or as percentage recovery of *P. neglectus*. The percentage recovery of *P. neglectus* at each extraction period was calculated by dividing the number recovered at each extraction time by the cumulative total recorded after 21 days. Total number of *P. neglectus* recovered per replicate was divided by four (to achieve number per plant) and $\log_e$ transformed. Differences in number of *P. neglectus* between harvest times were separated using ANOVA ($P < 0.05$). Differences between the number of adults and juveniles at harvest time 2 were assessed using a Chi-squared analysis ($P < 0.05$) (Gomez and Gomez 1984).

4.2 Results

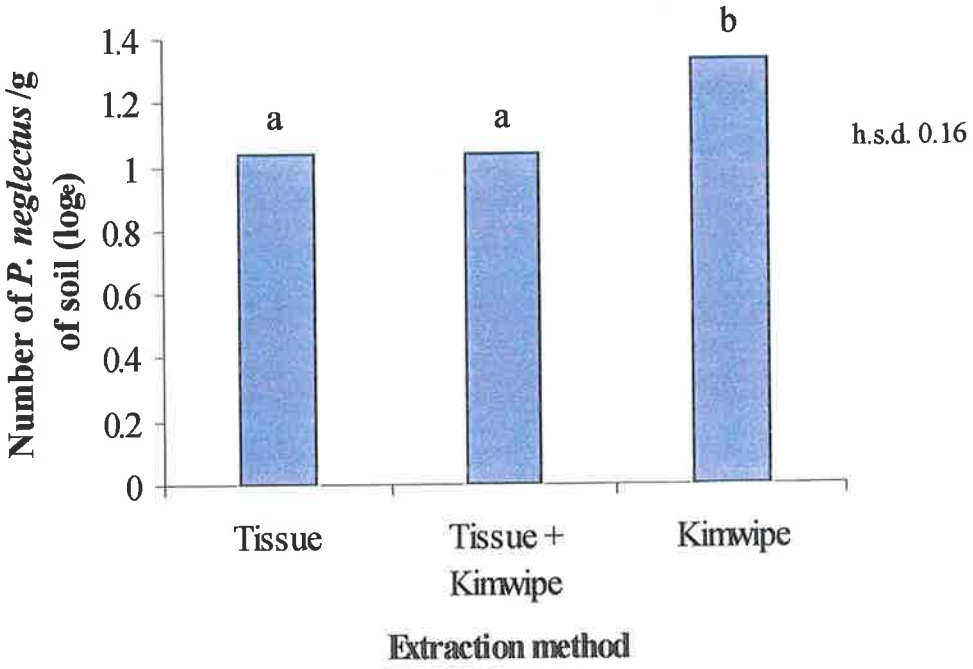
4.2.1 Elimination of lint and soil contamination from samples

The ANOVA of the results assessing differences between tissue layer treatments in modified Whitehad trays is presented in Table 4.1. There was a significantly higher number of *P. neglectus* extracted using trays lined with Kimwipe® alone compared to those lined with tissue alone (Figure 4.1). However, there was also a higher proportion of fine silt and sand particles in Kimwipe® samples when compared with samples extracted using tissue alone or Kimwipe® + tissue. Visually, there was a higher proportion of tissue lint in samples extracted using tissue alone compared with either low-linting Kimwipe® or Kimwipe® + tissue.

Table 4.1 The ANOVA of the results comparing number of *P. neglectus* recovered from soil using different tissue layers in the Whitehead tray.

	d.f.	m.s.	P
Rep.	14	0.063	0.0581
Tissue layer treatment	2	0.419	0.0001
Rep.*Tissue	21	0.030	

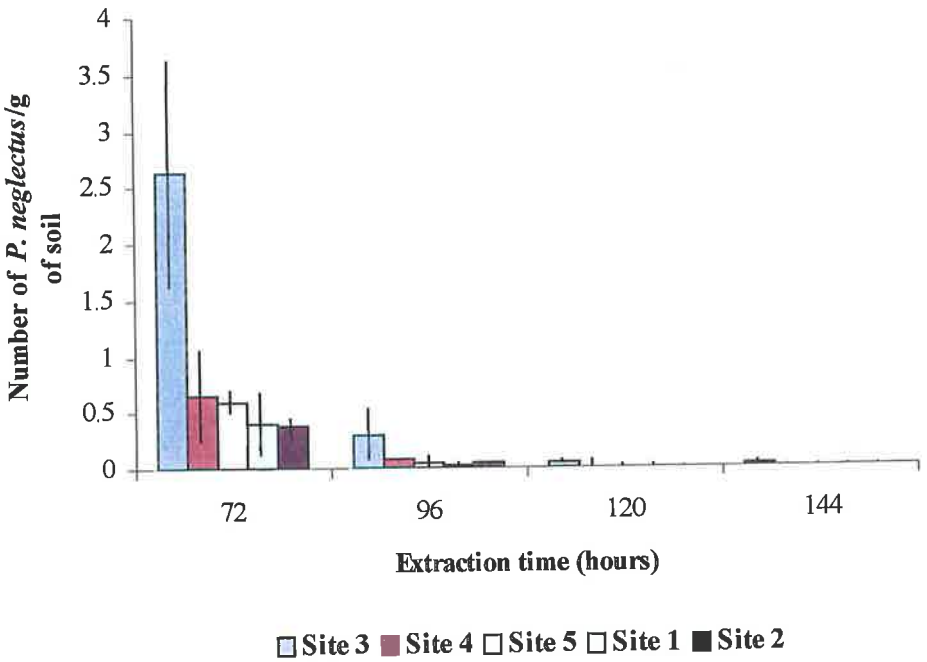
Figure 4.1 **Density of *P. neglectus* in samples extracted using a modified Whitehead tray. Trays supporting soil were lined with either two layers of Kleenex® tissue (Tissue), one layer of Kleenex® tissue and one layer of Kimwipe® (Tissue + Kimwipe), or two layers of Kimwipe® (Kimwipe) (n = 15). Treatments with the same letters do not differ significantly (P < 0.05).**



4.2.2 Separation efficiency of nematodes using a modified Whitehead tray method

The number of *P. neglectus* recovered from soil from 5 sites in South Australia after extraction in Whitehead trays for 72, 96, 120 and 168 hours is shown in Figure 4.2. Highest numbers of *P. neglectus* were recovered from Site 3 after 72 hours (2.6 *P. neglectus*/g of soil). This represented 85% of the total number of *P. neglectus* recovered. At sites 1, 2, 4, and 5 lower total *P. neglectus* densities were obtained, and a greater percentage recovery occurred after 72 hours (95%, 95%, 93% and 96%, respectively).

Figure 4.2 **Recovery of *P. neglectus* extracted using a modified Whitehead tray from sandy loam soil collected from five sites in South Australia. Bars within columns indicate standard deviation from the mean.**



4.2.3 Recovery of *Pratylenchus* from stored soil

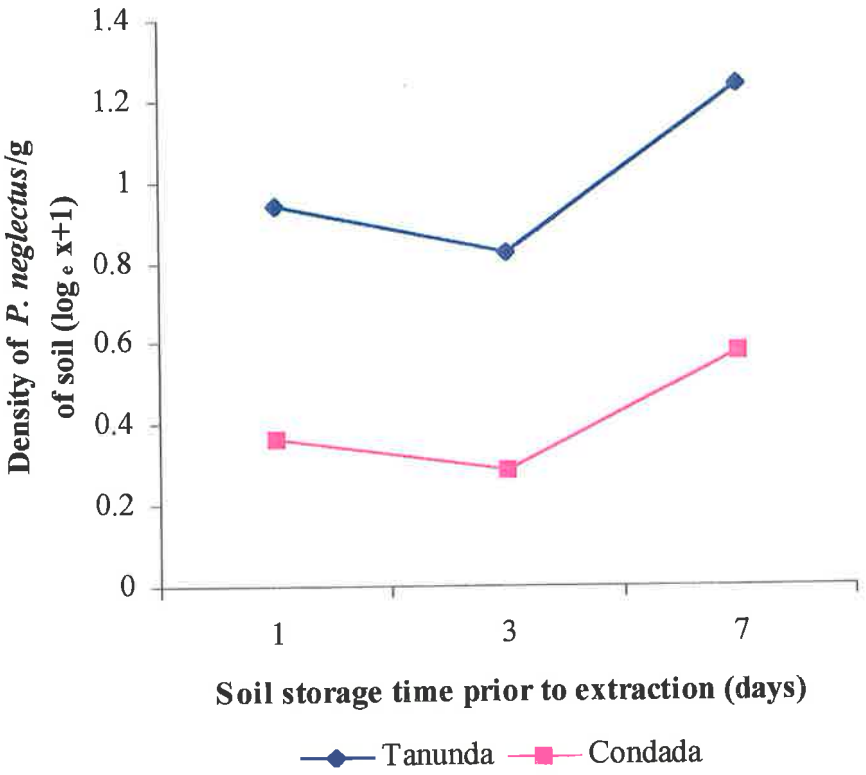
4.2.3.1 Experiment 1

P. neglectus was identified at Condada and *P. thornei* at Tanunda. The ANOVA of the results for differences in numbers of *Pratylenchus* from stored soil is shown in Table 4.2. The number of *P. neglectus* and *P. thornei* recovered from each soil type and percentage soil moisture is presented in Figure 4.3. In soil from Condada, the density of *P. neglectus* recovered was significantly higher after storage for one week at 4°C compared with storage for 1 or 3 days. The percentage soil moisture in Condada soil after 1, 3 or 7 days was 11%, 11% and 19%, respectively. At Tanunda, the number of *P. thornei* recovered after storage of soil for 7 days was significantly higher than after soil storage for 3 days, but not from soil stored for 1 day at 4°C. The percentage soil moisture from samples at this site after 1, 3 and 7 days was 27%, 28% and 39%, respectively.

Table 4.2 **The ANOVA of the results comparing the difference between number of *P. neglectus* (Condada) or *P. thornei* (Tanunda) from soil stored at 4°C for one, three or seven days prior to extraction using the modified Whitehead tray.**

		d.f.	m.s.	P
Condada	Rep.	14	0.204	0.0009
	Storage time	2	0.338	0.0038
	Rep.*Storage	24	0.048	
Tanunda	Rep.	14	1.326	0.0000
	Storage time	2	0.693	0.0486
	Rep.*Storage	27	0.205	

Figure 4.3 **Density of *P. thornei* recovered from a clay soil (Tanunda) and *P. neglectus* from a sandy loam soil (Condada) using a modified Whitehead tray. Samples were stored at 4°C for 1 day, 3 days or 7 days prior to extraction (n = 15).**



4.2.3.2 Experiment 2

The number of *P. neglectus* recovered from soil stored at 4°C or 18°C from the Palmer and Sandilands sites is presented in Figure 4.4. There was a significant effect of storage time on recovery of *P. neglectus*, but only for samples from the Palmer site and only at a storage temperature of 18°C. The ANOVA of the effect of storage time and temperature is shown in Table 4.3. In samples from the Palmer site, there was a general decrease in *P. neglectus* recovered from soil stored over time. After 16 weeks storage at 4°C or 18°C, there was an approximately 30% reduction in *P. neglectus* extracted, compared with nematode recovery from the same day as sampling. In soil stored at 18°C, moisture generally decreased over the storage period, from 8% soil moisture on the day of collection to 4% after 16 weeks of storage (Figure 4.5a).

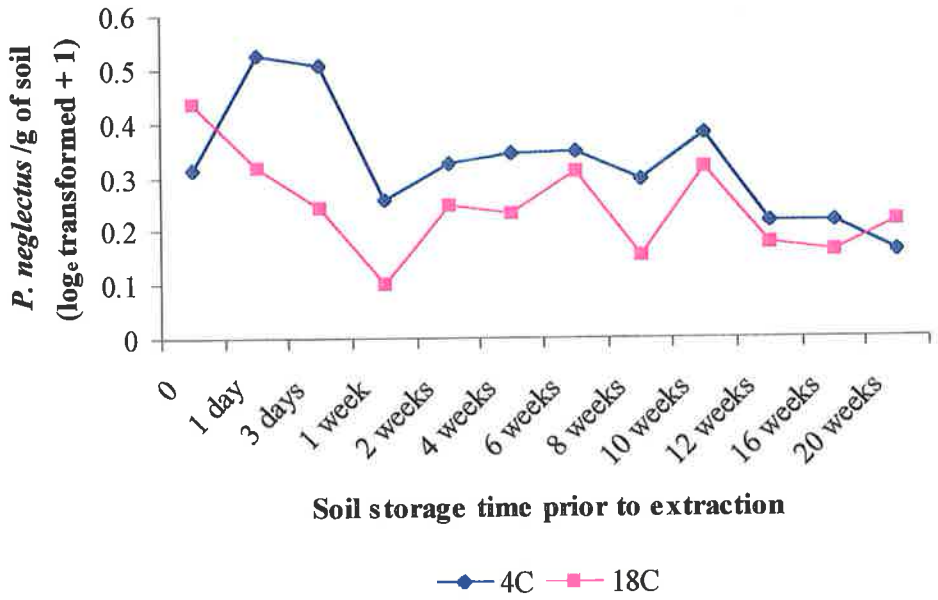
In comparison, there was no significant difference in the recovery of *P. neglectus* from the clay loam soil from Sandilands over the 20 week storage period at either 4°C or 18°C (Figure 4.4b). Although fluctuations were observed in soil moisture during storage of the Sandilands samples, there were consistently lower moisture levels in samples stored at 18°C compared with those stored at 4°C (Figure 4.5b). At 18°C, soil moisture was 13% on the day of collection, falling to 9% after 16 weeks of storage.

Table 4.3 The ANOVA of the effect of storage period and temperature of soil (from Palmer and Sandilands) on the recovery of *P. neglectus*.

	d.f.	m.s.	P
Palmer 4°C storage			
Rep.	5	0.129	0.0162
Storage time	11	0.073	0.0912
Rep*Storage time	52	0.042	
Palmer 18°C storage			
Rep.	5	0.048	0.0036
Storage time	11	0.051	0.0001
Rep*Storage time	54	0.012	
Sandilands 4°C storage			
Rep.	5	0.014	0.4637
Storage time	11	0.025	0.0953
Rep*Storage time	55	0.015	
Sandilands 18°C storage			
Rep.	5	0.039	0.2063
Storage time	11	0.022	0.5796
Rep*Storage time	55	0.026	

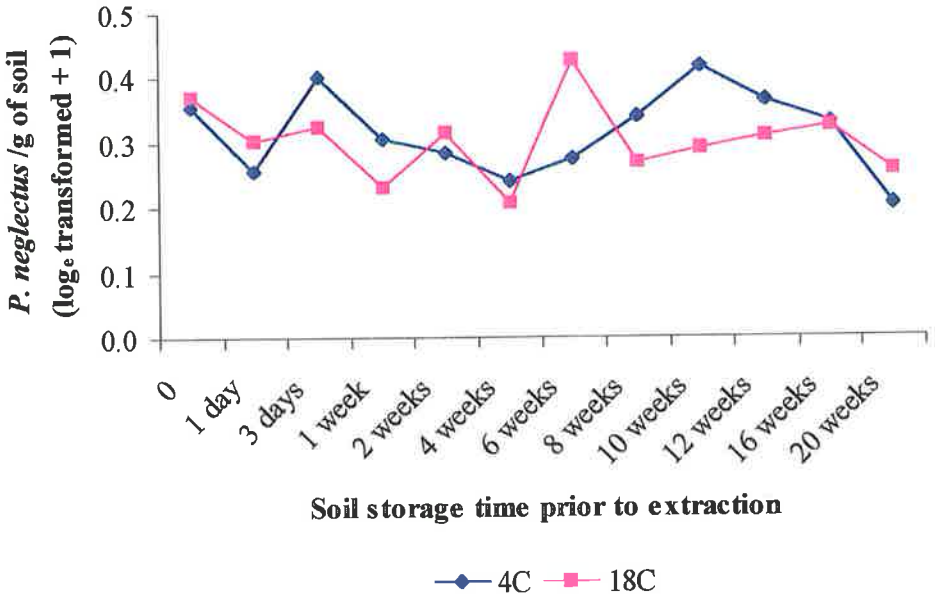
Figure 4.4 **Density of *P. neglectus* extracted from a) Palmer (sandy loam soil) and b) Sandilands (sandy clay loam soil). Soils were stored at 4°C or 18°C for differing time periods prior to extraction using a modified Whitehead tray. Same day = processed on the same day as sampling.**

a) Palmer



h.s.d.	
4°C	0.40
18°C	0.22

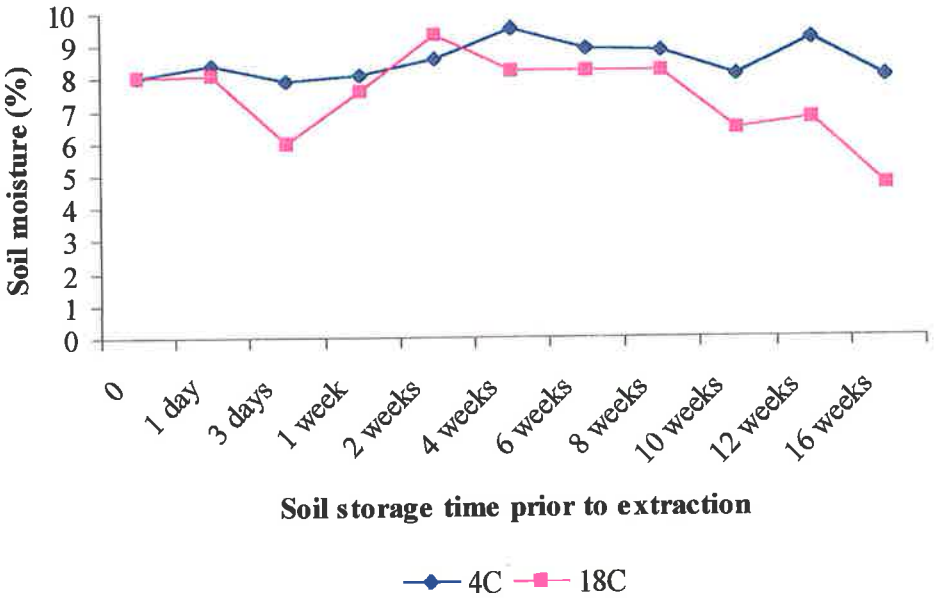
b) Sandilands



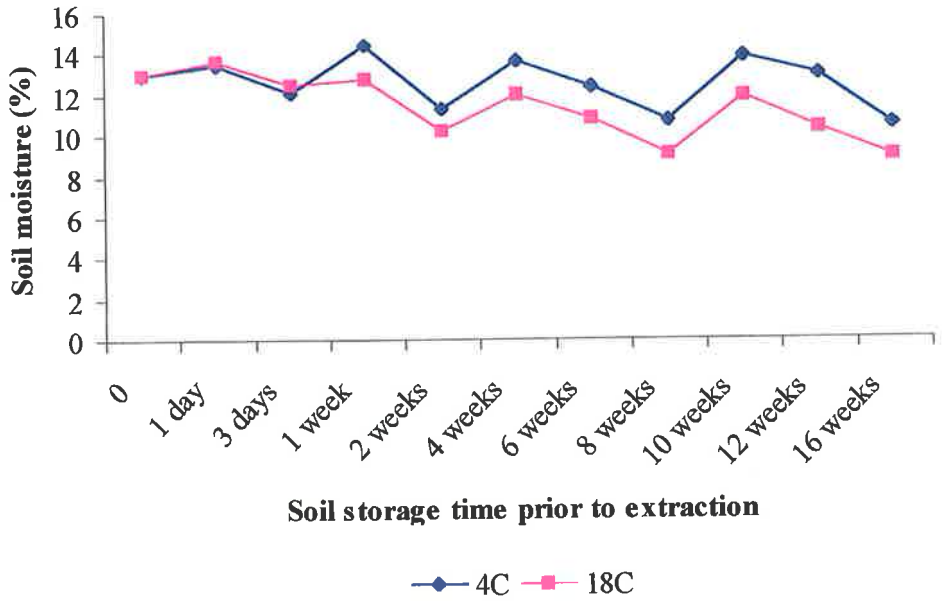
h.s.d.	
4°C	0.24
18°C	0.32

Figure 4.5 **Percentage soil moisture from a) Palmer (sandy loam soil) and b) Sandilands (sandy clay loam soil) stored at 4°C or 18°C and for differing time periods. Same day = processed on the same day as sampling.**

a) Palmer



b) Sandilands



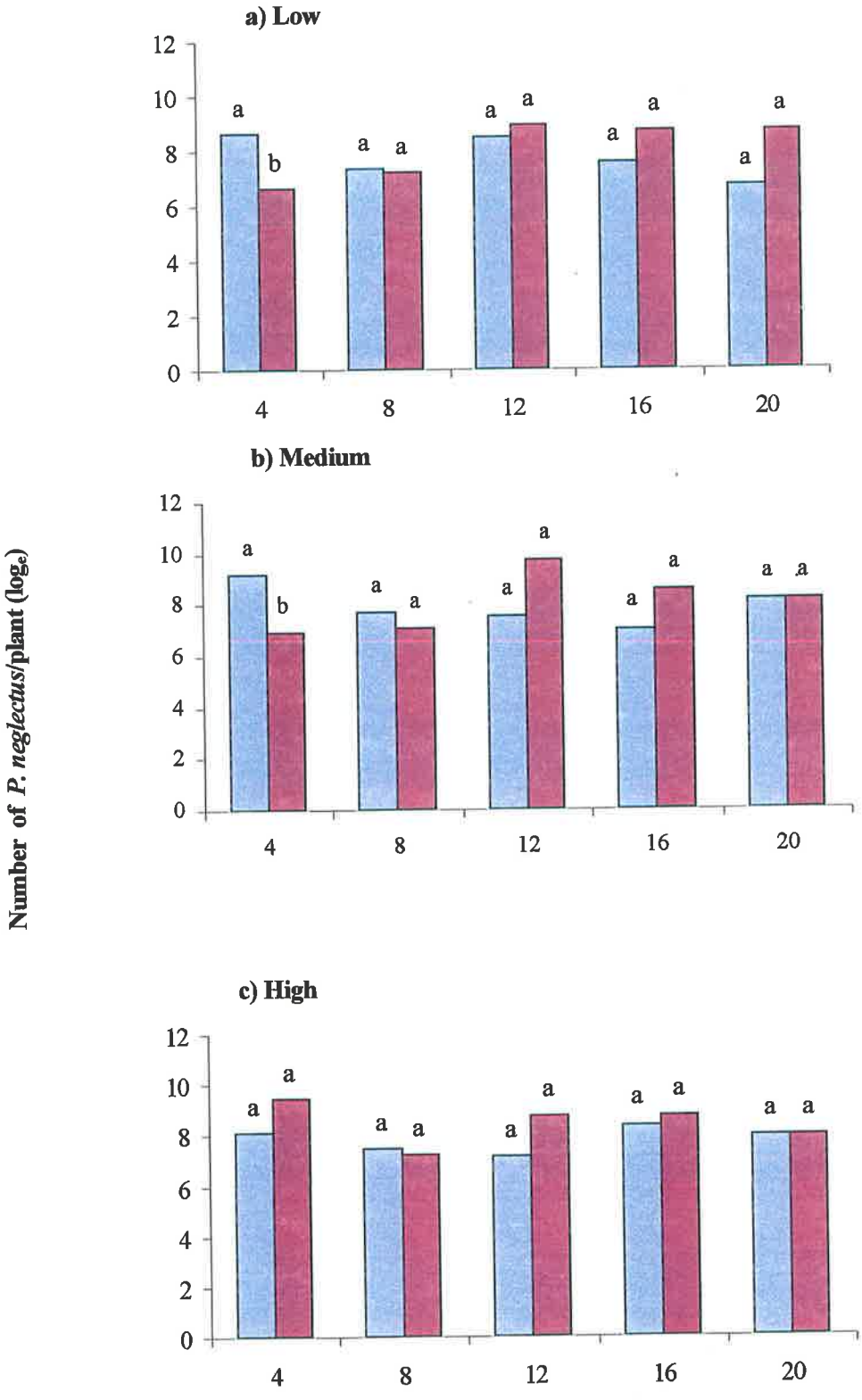
4.2.4 Comparison of two methods for extraction of *P. neglectus* from wheat roots

The ANOVA of the results comparing number of *P. neglectus* estimated from wheat roots using staining and misting techniques is presented in Table 4.4. The number of *P. neglectus* recovered using either staining or misting at low, medium or high inoculum levels is presented in Figure 4.6. For the combined data from all harvest times, there was no significant differences between the number of *P. neglectus* recovered using either misting or staining methods. Within individual harvest times, there was a significant difference between extraction methods only at the 4 week harvest. At this time, a higher number of *P. neglectus* was estimated for the low and medium inoculation rates using the staining method.

Table 4.4 **The ANOVA of results comparing numbers of *P. neglectus* recovered at different harvest times (4, 8, 12, 16 or 20 weeks after sowing) using staining or misting extraction techniques for plants inoculated with low (1.2/g soil), medium (9.4/g soil) or high (36.5/g soil) rates (n = 5). * significant (P < 0.05).**

		Low			Medium			High	
		d.f	m.s	P	d.f.	m.s.	P	d.f.	m.s	P
4 weeks	Rep.	4	0.317	0.551	3	0.407	0.170	3	0.652	0.386
	Extraction technique	1	10.48	0.029*	1	10.17	0.008*	1	3.453	0.152
	Rep.*Extraction	2	0.313		2	0.081		1	0.206	
8 weeks	Rep.	4	0.550	0.708	4	0.671	0.389	4	1.203	0.515
	Extraction technique	1	0.049	0.834	1	1.038	0.227	1	0.185	0.718
	Rep.*Extraction	4	0.989		3	0.452		3	1.182	
12 weeks	Rep.	4	1.161	0.361	4	0.906	0.326	4	3.802	0.264
	Extraction technique	1	0.447	0.488	1	11.86	0.079	1	2.136	0.353
	Rep.*Extraction	3	0.719		1	0.186		4	1.934	
16 weeks	Rep.	4	3.800	0.227	4	5.268	0.415	4	2.071	0.177
	Extraction technique	1	3.341	0.238	1	5.958	0.299	1	0.698	0.369
	Rep.*Extraction	4	1.693		4	4.187		3	0.628	
20 weeks	Rep.	4	3.780	0.095	3	0.426	0.090	4	0.412	0.779
	Extraction technique	1	6.887	0.059	1	0.003	0.817	1	0.007	0.982
	Rep.*Extraction	1	0.061		2	0.042		3	0.939	

Figure 4.6 **Number of *P. neglectus* recovered from wheat roots using staining or misting (96 hours) at three inoculation levels a) Low = 1.2 *P. neglectus*/g soil; b) Medium = 9.4 *P. neglectus*/g soil; and c) High = 36.5 *P. neglectus*/g soil (n = 5). Within each harvest time, columns with the same letters do not differ significantly between treatments ($P < 0.05$).**



4.2.5 Efficiency of separation of *P. neglectus* from different crops

4.2.5.1 Harvest time 1 (6 weeks)

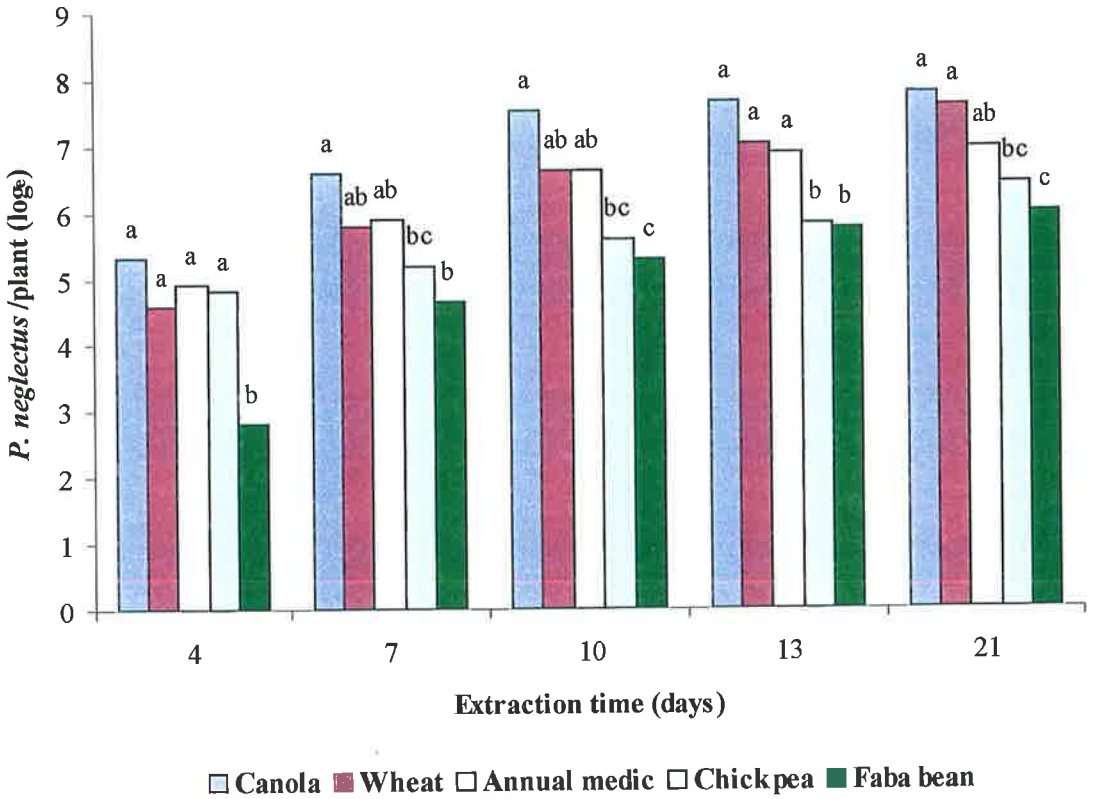
The ANOVA of the results of the effect of period of extraction in the misting chamber on the recovery of *P. neglectus* for each crop is shown in Table 4.5. There was a significant effect of time of extraction on recovery of *P. neglectus* for Barossa canola, Machete wheat and Dooen chickpea, while for Harbinger medic and Fiord faba bean there was no significant difference between nematode numbers recovered at each extraction time.

Table 4.5 The ANOVA of the results for effect of differing extraction periods on numbers of *P. neglectus*/plant recovered from five crop species (6 week old plants).

Variety		d.f.	m.s.	P
Barossa canola	Rep.	4	0.932	0.537
	Extraction period	4	3.753	0.039
	Rep.*Extraction period	16	1.149	
	Total	24		
Machete wheat	Rep.	4	0.926	0.367
	Extraction period	4	2.577	0.041
	Rep.*Extraction period	16	0.802	
	Total	24		
Dooen chickpea	Rep.	4	11.174	0.003
	Extraction period	4	3.429	0.046
	Rep.*Extraction period	16	1.110	
	Total	24		
Harbinger medic	Rep.	4	1.527	0.112
	Extraction period	4	1.998	0.055
	Rep.*Extraction period	16	0.686	
	Total	24		
Fiord faba bean	Rep.	4	1.885	0.306
	Extraction period	4	2.069	0.265
	Rep.*Extraction period	16	1.432	
	Total	24		

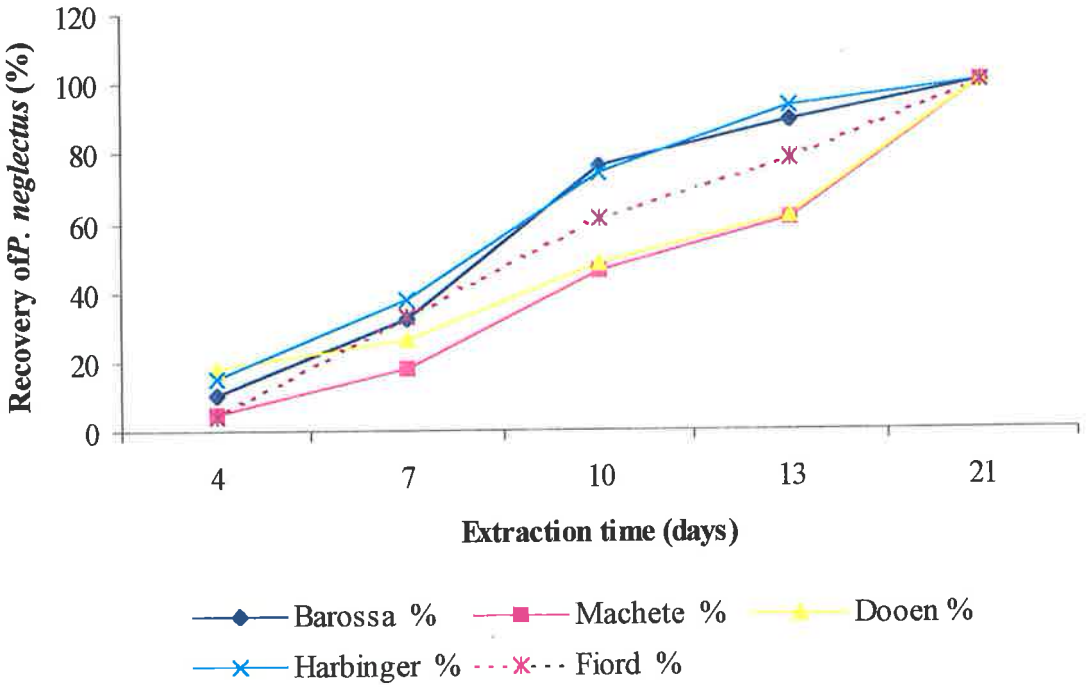
The cumulative number of *P. neglectus* recovered ($\log_e$ transformed) after different times from each crop species is given in Figure 4.7. After 21 days, significantly higher total numbers of *P. neglectus* were recovered from Barossa canola, Machete wheat and Harbinger medic compared with Fiord faba bean. Total numbers recovered from Dooen chickpea were not significantly different from either Harbinger or Fiord. High levels of *P. neglectus* were still being recovered at the 21 day extraction, with the most obtained from Machete wheat.

Figure 4.7 **Cumulative numbers of *P. neglectus* recovered per plant (log_e transformed) after extraction in a misting chamber for 4 (SED = 0.52), 7 (SED = 0.48), 10 (SED = 0.54), 13 (SED= 0.50) and 21 (SED = 0.44) days from 6 week old plants of canola (Barossa), wheat (Machete), annual medic (Harbinger), chickpea (Dooen) and faba bean (Fiord) (n = 5). For each extraction time, columns with the same letters do not differ significantly (P < 0.05).**



The percentage of *P. neglectus* recovered after each extraction time from plants grown for 6 weeks is shown in Figure 4.8. Low percentages of *P. neglectus* were recovered after 4 days from all crops, ranging from 4% (Machete wheat) to 18% (Dooen chickpea). After 10 days, almost 50% of the total number was recovered from Machete and Dooen and 60% and 75% from Barossa canola and Harbinger medic, respectively.

Figure 4.8 **Percentage of *P. neglectus* (number recovered at each harvest time divided by the cumulative total after 21 days) after extraction in a misting chamber for 4, 7, 10, 13 and 21 days from 6 week old plants of canola (Barossa), wheat (Machete), chickpea (Dooen), annual medic (Harbinger) and faba bean (Fiord) (n = 5).**



4.2.5.2 Harvest time 2 (16 weeks)

The results from the Chi-Squared analysis assessing differences in number of adults and juveniles are presented in Table 4.6. Within each variety, there was no significant difference between the number of adults and juveniles recovered, so the total number of adults and juveniles was used.

Table 4.6 **Chi-squared analysis of numbers of adult and juvenile *P. neglectus* recovered after different extraction periods from faba bean (Fiord), wheat (Machete), chickpea (Dooen), canola (Barossa) and medic (Harbinger) 16 weeks after planting. Overall Chi-Square = 3.26; P value = 0.5152 (P < 0.05).**

		Fiord	Machete	Dooen	Barossa	Harbinger	Total
Juveniles	Observed	31	198	149	178	163	
	Expected	26.9	197.2	153.2	170.4	171.4	719
	Cell Chi-Sq	0.64	0.00	0.11	0.34	0.41	
Adults	Observed	19	169	136	139	156	
	Expected	23.1	169.8	131.9	146.7	147.6	619
	Cell Chi-Sq	0.74	0.00	0.13	0.40	0.48	
	Total	50	367	285	317	319	1338

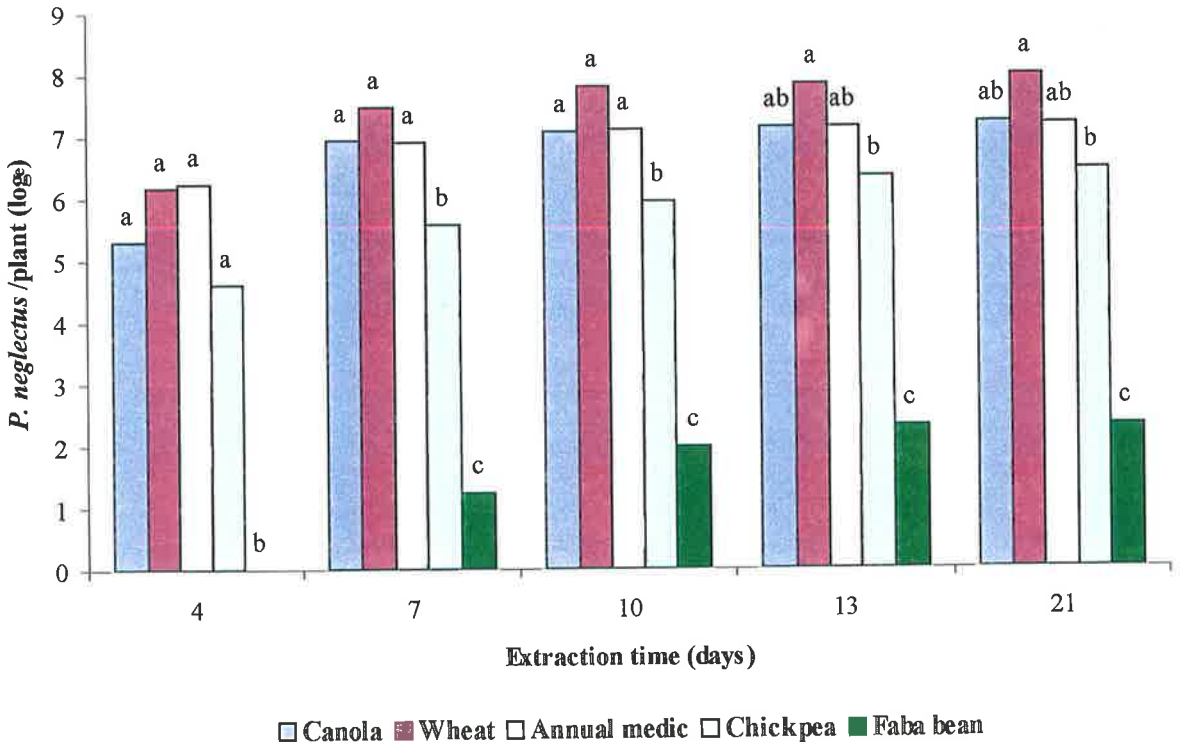
The ANOVA of the results for the effect of extraction period from wheat, canola, chickpea, medic and faba bean is given in Table 4.7. In 16 week old plants, there was a significant effect of time of extraction on recovery of *P. neglectus* from Barossa canola, Machete wheat and Harbinger medic, while for Dooen chickpea and Fiord faba bean there was no significant difference between nematode number recovered at each extraction time.

Table 4.7 **The ANOVA of the results for effect of differing extraction periods on number of *P. neglectus* recovered from five crop species (16 week old plants).**

Variety		d.f.	m.s	P
Barossa canola	Rep.	4	1.331	0.283
	Extraction period	4	5.959	0.004
	Rep.*Extraction period	15	0.953	
	Total	23		
Machete wheat	Rep.	4	1.59	0.18
	Extraction period	4	6.040	0.002
	Rep.*Extraction period	16	0.886	
	Total	24		
Dooen chickpea	Rep.	4	2.363	0.139
	Extraction period	4	0.762	0.628
	Rep.*Extraction period	15	1.152	
	Total	23		
Harbinger medic	Rep.	4	2.645	0.02
	Extraction period	4	8.466	0.001
	Rep.*Extraction period	16	0.664	
	Total	24		
Fiord faba bean	Rep.	4	0.474	0.849
	Extraction period	4	0.969	0.61
	Rep.*Extraction period	16	1.404	
	Total	24		

The cumulative number (i.e. total number) of *P. neglectus* recovered after each extraction time from the different crops is shown in Figure 4.9. Although results were similar to those obtained for 6 weeks old plants (see Figure 4.7), in 16 week old plants significantly fewer nematodes were recovered from Fiord faba bean compared with all other crops tested. In addition, in contrast to results from 6 week old plants, very few *P. neglectus* were still being recovered from 16 week old plants after 21 days.

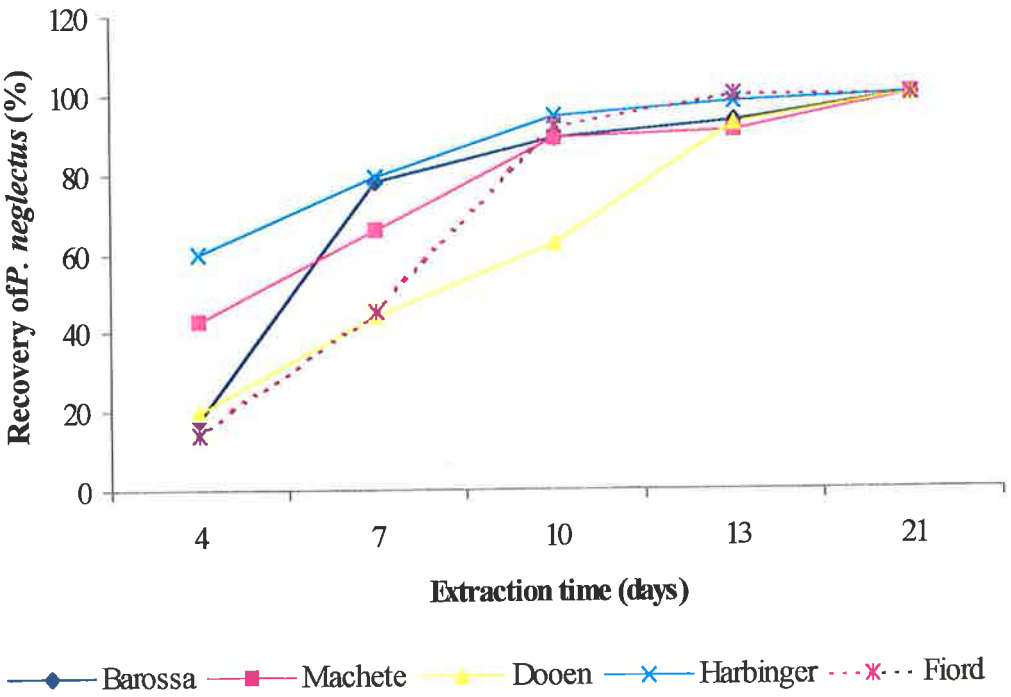
Figure 4.9 **Cumulative number of *P. neglectus* recovered per plant (log_e transformed) after extraction in a misting chamber for 4 (SED = 0.83), 7 (SE = 0.55), 10 (SE = 0.52), 13 (SE = 0.47) and 21 (SE = 0.47) days from 16 week old plants of canola (Barossa), wheat (Machete), chickpea (Dooen), annual medic (Harbinger) and faba bean (Fiord) (n = 5). For each extraction time, columns with the same letters do not differ significantly (P < 0.05).**



The percentage of *P. neglectus* recovered after different extraction times from plants grown for 16 weeks is shown in Figure 4.10. In contrast to results obtained for harvest time 1 (6 weeks), there were much higher rates of recovery after 4 days from all crops except Dooen chickpea. Recovery ranged from 20% in Dooen to 60% in Harbinger medic. After 10 days, over 90% of

the total number of *P. neglectus* was recovered from Barossa canola, Machete wheat, Harbinger medic and Fiord faba bean.

Figure 4.10 **Percentage of *P. neglectus* (number recovered at each harvest time divided by the cumulative total after 21 days) after extraction in a misting chamber for 4, 7, 10, 13 and 21 days from 16 week old plants of canola (Barossa), wheat (Machete), chickpea (Dooen), annual medic (Harbinger), and faba bean (Fiord) (n = 5).**



For both 6 and 16 week old plants, significantly fewer *P. neglectus* were extracted from Fiord faba bean than from the other cultivars and this difference was more pronounced at harvest time 2. For 6 and 16 week old plants, there was no significant difference in the number of *P. neglectus* recovered from Barossa canola, Machete wheat, Harbinger medic and Dooen chickpea after extraction for 4 days. In comparison, using the cumulative total after extraction for 21 days, differences were observed between cultivars. At both 6 and 16 weeks after 21 days extraction, there was no significant difference between Barossa canola, Machete wheat and Harbinger medic but in 6 week old plants, significantly higher number of *P. neglectus* were recovered from Machete and Barossa than Dooen. For 16 week old plants, there was no significant difference between numbers recovered from Barossa canola and Dooen chickpea.

4.3 Discussion

4.3.1 Extraction of *P. neglectus* from soil

While the use of two layers of low-linting Kimwipes® to line trays in a modified Whitehead Tray extraction resulted in both higher recovery of *P. neglectus* and reduced tissue lint contamination, samples had higher amounts of sand particle and silt contamination, making the assessment of numbers of nematodes from samples difficult. Contamination of samples by soil particles has been observed in extraction from soils with a high clay content, where fine soil particles pass through the tissue layer and clog sieves when the nematode suspension is being concentrated (McSorley and Parrado 1982). The tissue layer can affect both the clarity of the nematode suspension obtained by influencing the number of nematodes extracted and the level of both soil and tissue lint contamination within the sample (Viglierchio and Schmitt 1983).

In this study, the importance of the tissue layer in determining both efficiency of nematode extraction and sample clarity was confirmed. Of the techniques tested, a combination of Kimwipe® + tissue was chosen for routine samples as the bottom layer of low-linting Kimwipe® (in direct contact with the water) and the top layer of tissue (in contact with the soil sample) reduced both soil particles and tissue lint in the nematode suspension, while allowing motile nematodes to move through both layers.

In experiments assessing the efficiency of separation of *P. neglectus* from soil using the modified Whitehead tray method, over 85% of nematodes were recovered after 72 hours. Where there were lower total numbers of *P. neglectus*, a higher rate of extraction occurred (up to 96% after 72 hours). The quantity of organic matter (specifically root material) may affect extraction rates from different sites, as nematodes may emerge more slowly if present in root material. Tobar *et al.* (1995) observed that by using elutriation over 4 days, 95% of *P. thornei* were recovered from soil following a poorer host (sunflower), but it took 8 days to recover the same proportion following a wheat crop. The slower rate of recovery was thought to be due to the release of *P. thornei* from very small pieces of wheat roots within the soil sample. In this study, higher densities of *P. neglectus* at Site 3 may also have been associated with a higher proportion of nematodes within organic material (remaining from the previous wheat crop), resulting in a slower rate of extraction on Whitehead trays.

While differences in the proportions of *P. neglectus* extracted from soil following different hosts occurred, the 72 hour extraction time was chosen as the routine extraction period for nematodes from soil using the Whitehead tray. This time produced a high proportion of nematode recovery. Little additional benefit was observed by increasing the extraction time to 96 hours and the shorter extraction time allowed a larger number of samples to be processed.

Differences were observed in the recovery of *P. neglectus* from soil stored at different temperatures. In other studies, the temperature at which soil is stored has been shown to affect recovery of nematodes, either as a result of nematode mortality or egg hatch. Mishra and Gaur (1987) observed increased recovery of both *Meloidogyne* and *Rotylenchulus* after soil was stored at 15 - 25°C for 7 days, probably due to increased egg hatch. At higher temperature, increased recovery of *Pratylenchus* spp. from soil and roots may also occur as a result of increased hatching and migration from root fragments (Barker and Campbell 1981). Gnanapragasam and Sivapalan (1991) assessed the effect of soil storage for up to 3 weeks on the recovery of *P. loosi* and found that significantly higher numbers of this species were recovered from samples stored for 7 days at room temperature compared with samples processed on the same day as collection. In soil stored for up to 7 days in the present study (Experiment 1, Section 4.2.3), a similar finding was observed, with a significant increase in the recovery of *P. neglectus* from both black clay (Tanunda) and sandy loam soils (Condada) after 7 days storage compared with storage for 3 days at 4°C. A similar trend was observed in sandy loam soil collected from Palmer when stored at room temperature. In all cases, this increase was associated with higher soil moisture at the 7 day extraction period compared with the 3 day period, and may be a result of differences in the sub-samples taken from the bulk soil at these extraction times. Soil moisture has been shown to have a large impact on recovery of *Macroposthonia xenoplax,* with higher numbers recovered at the highest soil moisture (Lawrence and Zehr 1978) and the addition of moisture may revive anhydrobiotic nematodes (Ferris 1987). The effect of soil moisture on recovery of *P. neglectus* from soil is further discussed in Chapter 5.

In soil stored for an extended period of time (Experiment 2, Section 4.2.3), differences were observed between soil types/sites in the recovery of *P. neglectus*. Storage for up to 12 weeks at either 4°C or 18°C had no significant effect on recovery of *P. neglectus* from the clay loam (Sandilands) samples. Less consistent results were obtained for recovery of *P. neglectus* from stored soil samples from Palmer, with significant reductions in the number of *P. neglectus* recovered from samples stored at 18°C after only 1 week and at 4°C after 2 weeks storage.

Numbers of *P. neglectus* recovered at different storage times from these samples fluctuated, and no significant difference was observed between the assessment on the day of sample collection and numbers recovered after 6 or 10 weeks of storage. The data for 6 and 10 weeks of storage may have been aberrant results produced by insufficient replication. The trend in this soil was for a reduction in *P. neglectus* recovered after only 2 weeks storage. Soil moisture in the Palmer samples was lower and more variable than in those from the Sandilands site, and this may have contributed to reduced or more inconsistent nematode recovery. Barker *et al.* (1969b) found that storage of soil at 13°C for up to 16 weeks had little effect on the recovery of *P. zeae.* Storage at 36°C however, resulted in significantly lower recovery of *P. zeae.* This may reflect both a loss in soil moisture and increased activity of nematodes at the higher temperature, resulting in more rapid use of lipid reserves and thus a reduction in the ability of nematodes to migrate from soil in the Whitehead tray.

In this study, there was greater consistency in extraction of *P. neglectus* from samples stored at 4°C compared with 18°C. It was concluded that while samples could be stored at 4°C for up to 1 week with no significant loss in numbers of *P. neglectus*, recovery of nematodes from soil stored for longer periods will depend on soil moisture.

4.3.2 Extraction of *P. neglectus* from root samples

When staining and misting were compared for the estimation of *P. neglectus* in plant roots, there were significantly higher numbers of *P. neglectus* recovered using staining at the 4 week harvest time only. It was assumed that staining would give a more reliable indication of *P. neglectus* present within roots as it kills and preserves all nematodes at the time of harvest. In a study by Potter *et al.* (1999), no significant difference was observed between staining compared with misting (120 hour extraction time) for the estimation of *P. neglectus* in 6 week old wheat and canola roots, and it was concluded that both methods had an equal extraction efficiency. The results from this experiment confirm these findings, even with the shorter extraction time of 96 hours in the misting chamber. However, the staining technique is more labour intensive than misting, which is a significant disadvantage when processing large numbers of plants.

Assessment of the separation efficiency of *P. neglectus* from crop species of different ages using the misting chamber showed higher rates of extraction for 16 week old compared with 6 week old plants (assuming the cumulative total after 21 days of repeated extractions was taken as the total number of nematodes within plant roots at harvest). If this was the case, only 15% of

P. neglectus were recovered from 6 week old medic plants after extraction for 4 days, compared with 60% from 16 week medic plants. It appears more likely (based on the assumption that estimates of numbers *P. neglectus* from stained roots are similar to those recovered from roots misted for 96 hours) that differences observed between rates of extraction largely reflected the ability of the crop species to act as hosts of the nematodes (i.e. multiplication of *P. neglectus* during the extraction period) rather than the separation efficiency using the misting chamber.

For example, in 6 week old plants the lowest number of *P. neglectus* recovered after extraction for 4 days was from Machete wheat, reflected by the high numbers still being extracted after 21 days. In both field (Chapters 7 and 8) and pot (Chapter 6; Farsi 1995) experiments, this variety has been identified as susceptible, and the low percentage of extraction observed was therefore probably due to continued multiplication of the nematode within the misting chamber. Continued multiplication has been identified as a problem associated with extraction using this system (Barker and Nusbaum 1971; McSorley *et al.* 1984; Moore *et al.* 1992) as the misting chamber offers an ideal environment (i.e. warm, moist and oxygenated) for nematode multiplication while the food source remains intact. Hatching of eggs laid during plant growth would also have occurred during the extraction period.

To further confound the problem of assessing efficiency of separation from plant roots, it has been observed that nematodes will exit more resistant plants at a higher rate than susceptible plants, especially if assessed soon after nematode invasion (France and Brodie 1995). This may have occurred within this experiment, as very low rates of extraction were observed from 6 week old plants of susceptible Machete wheat. The more resistant cultivar Fiord (see Chapter 7), had a very low rate of extraction after 4 days (4%) but the rate increased to 33% after 7 days (compared with 18% from Machete), and may suggest faster egression from more resistant than from susceptible plants.

There was greater efficiency of separation from 16 week old plants, as *P. neglectus* were recovered from the roots at a faster rate for all crops except chickpea. Where whole plant senescence occurs (e.g. in wheat), nutrients are mobilised to the developing seeds rather than in the roots (Peñarraubia and Moreno 1995). A 10-fold increase in the number of *P. penetrans* in actively growing plants compared with dead or senescing plants was recorded by Seinhorst (1970), a response supported by these data. When placed in the misting chamber, it may be that limited multiplication occurs in 16 week old plants over the course of 21 days incubation

compared with 6 week old plants (harvested closer to an active growing period for root cells) and, as a result, *P. neglectus* exited roots of older plants at a faster rate.

As nematode extraction is often done for the purpose of defining host status (i.e. resistance) of varieties, it is interesting to assess the rating for each variety based on the cumulative total of nematodes compared with a shorter extraction period. The overall ranking for susceptibility of cultivars based on total extraction at the end of the 21 day period was: wheat > canola > medic > chickpea > faba bean. This ranking changed for plants of different age and with different extraction periods. For example, no significant difference was observed between plants of 6 week old Fiord faba bean and Machete wheat at the 7 day extraction period. At 16 weeks however, large differences were observed between faba bean and all other crop species, regardless of extraction time.

It was difficult to separate differences in the relative resistance ratings of Machete wheat, Barossa canola, Dooen chickpea and Harbinger medic based on 4 day extraction from 6 or 16 week old plants, as a different ranking was produced following assessment of the numbers of *P. neglectus* extracted after 4 days compared with the cumulative total after 21 days. Inconsistencies also occurred between the varietal ranking obtained after 4 days extraction in both 6 and 16 week old plants, and those produced following extraction of *P. neglectus* from soil in field trials (see Chapter 7). In field trials at two sites, Harbinger medic had significantly fewer *P. neglectus* than Machete wheat or Dooen chickpea compared with relatively high numbers recovered in this microplot experiment. Medic varieties have previously been observed to be more susceptible in pot trials compared with field experiments (V. Vanstone pers. comm.; Neal 1996) and these differences are discussed further in Chapter 7.

Chapter 5 Spatial distribution of, and sampling for, *Pratylenchus neglectus* and *P. thornei* in South Australia

5.0 Introduction

A reliable sampling technique is essential for diagnosing damage caused by plant-parasitic nematodes, correlating yield loss with nematode density and assessing management strategies. Factors such as the horizontal and vertical distribution of nematode species and survival mechanisms of nematodes in host plants and soil will differ for a variety of reasons even for a single species.

The distribution of plant rows and/or plants within rows may affect the horizontal distribution of plant-parasitic nematodes. McSorley and Frederick (1996) showed *Pratylenchus*, *Meloidogyne* and *Belonolaimus* spp. were aggregated around soybean plants and the concentration of these species around soybeans became more pronounced as the season progressed. Root type may also affect distribution of plant-parasitic nematodes, as quantity, depth and distribution of roots differs between plant species and environments.

The measurement of variation in the horizontal distribution of plant-parasitic nematodes allows the development of sampling strategies that, in turn, enable measurement of factors including the relationship between yield loss and nematode density or population decline/increase throughout the season. The vertical distribution of plant-parasitic nematodes is similarly affected by host root type, root distribution and environmental factors. Host root development is important in determining nematode distribution within the soil profile. For hosts with shallow roots such as bentgrass, distribution of *Criconomella*, *Tylenchorhynchus* and *Helicotylenchus* spp. was predominantly in the upper 2.5 cm of the soil profile (Davis *et al.* 1994). High densities of most plant-parasitic nematodes are not generally found below 60 cm, probably as a result of decreased food source rather than factors such as waterlogging or oxygen deficiency (Sohlenius and Sandor 1987). Where hosts with deep root systems are present, such as tree crops, nematodes are found deeper e.g. high densities of *Xiphenema index* were found to a depth of 90 cm on grapevine roots (Esmenjaud *et al.* 1992). In agricultural soils, most studies report maximum nematode densities in the top 15 - 20 cm of the profile (McSorley and Dickson 1990).

Although the purpose of the study, the tools available and the crop type all govern the sampling method chosen, the nematode species under consideration is perhaps the most important factor when determining sampling methods. Plant-parasitic nematode species have a variety of different life cycle strategies which will affect their ability to survive prolonged periods without a host and cope with factors such as soil disturbance or extremes in temperature and moisture levels. The motility of different life cycle stages of nematodes may also change throughout the year. For example, when sampling after host plant senescence in dry soil, species with a tough integument such as cyst nematodes are well protected from both desiccation and mechanical disturbance and many small sub-samples can be taken and bulked with no loss in nematode recovery (Boag *et al.* 1992). While other nematodes such as *Pratylenchus* and *Trichodorus* spp. can enter an anhydrobiotic state and are therefore more resistant to factors such as increases in temperature or chemical treatment (Glazer and Orion 1983, Freckman *et al.* 1980), they are sensitive to mechanical disturbance because of their brittle nature (Hinton 1968). Soil disturbance both during sampling and when transporting samples can therefore result in decreased recovery.

This study examines sampling techniques for, and spatial distribution of, *P. neglectus*. Prior to this study, no information was available on the horizontal or vertical distribution of either *P. neglectus* or *P. thornei* in broad-acre crops in South Australia, although both are known to be common in this region. Implications for devising sampling strategies are also discussed.

5.1 Materials and methods

5.1.1 Horizontal distribution – plant row effects

To obtain information on the effects of plant row on the distribution of *P. neglectus* or *P. thornei* following cereal crops, wet soil cores were collected as described in Section 3.7.4.1. At each site, stubble was retained, allowing location of the previous season's plant rows. Samples were collected prior to cultivation and planting (March - June, 1993 and 1994). Site, sampling details and soil type (as described by Northcote *et al.* 1975) are presented in Table 5.1 and site locations in Figure 3.1. Twenty paired core samples were collected. Each pair consisted of a sample taken within the plant row and a sample from between the plant rows. At the Condada and Miltaburra sites, oat stubble (cv. Wallaroo) was present. At Kimba, Balaklava and Tanunda,

wheat stubbles (cvv. Machete, Janz and Janz, respectively) were present. *Pratylenchus* was extracted from soil cores using Whitehead trays (Section 3.2.1) and counted (Section 3.3).

5.1.1.1 Data analysis

Nematodes were expressed as *P. neglectus* or *P. thornei*/g of dry soil. Comparison of treatment means within and between plant rows at each site were compared using paired t-tests ($P < 0.05$).

5.1.2 Vertical distribution

All samples were collected prior to cultivation and planting (March - June, 1993 and 1995). Site, sampling details and soil type (as described by Northcote *et al.* 1975) are presented in Table 5.1 and site locations in Figure 3.1. Samples were collected with a 5 cm diameter auger at 10 cm depth increments, i.e. 0 - 10 cm, 10 - 20 cm, etc. At two sites (Condada, June 1994 and Miltaburra, June 1993), samples were also collected at 0 - 5 cm and 5 - 10 cm and thereafter at 10 cm increments. At all sites, ten replicates were collected evenly over a 50 m^2 area. At Cungena and Miltaburra in 1993, samples were taken before and after opening rain. In South Australian broad-acre cropping, opening rain is generally considered the first major rainfall event (> 25 mm) occurring after March. Samples were taken to within 15 m of the previous sampling points. In March 1994, samples were taken after opening rain, to the maximum possible depth at each site (Table 5.1). All soil samples were stored at 4°C until processed using the Whitehead tray method (Section 3.2.1) and counted (Section 3.3).

5.1.2.1 Data analysis

The number of nematodes within each increment of the soil profile was expressed both as *P. neglectus* or *P. thornei*/g of dry soil and as a percentage of total nematode numbers recovered from the soil profile. Differences in the percentage recovery between sites at the 0 – 10 cm increment were compared using Scheffe's F statistic for the multiple comparison of means ($P < 0.05$) (Gomez and Gomez 1984).

5.1.3 Comparison of sampling methods

All samples were collected prior to cultivation and planting (March - May, 1994 and 1995). Site, sampling details and soil type (as described by Northcote *et al.* 1975) are presented in

Table 5.1 and site locations in Figure 3.1. At each site, three sampling procedures were assessed in both wet and dry soil. For each sampling method, ten replicates were taken and, within a replicate, samples were taken as close together as possible. Plant residue was scraped away from the soil surface prior to sampling. Up to 500 g of soil was collected per sample from which a 200 g sub-sample was removed and nematodes extracted (Section 3.2.1) and counted (Section 3.3).

The following sampling procedures were compared in wet and dry soil:
Undisturbed core: PVC cylinders (15 cm long x 7 cm diameter) were placed firmly on the soil surface, hammered in to a depth of 10 cm, then pulled up and sealed at the base. To collect wet samples in dry soil, 200 – 500 ml of water (depending on soil type) was poured into the cylinder after it was placed on the soil surface and allowed to infiltrate for 30 minutes to 3 hours prior to hammering the cylinder into the soil.

Auger: Soil was sampled to a depth of 10 cm with a 5 cm diameter auger (Plate 3.11). To collect wet samples in dry soil, a steel ring (25 cm diameter by 15 cm deep) was placed firmly on the soil surface and 500 - 1500 ml of water was added (depending on soil type). Water was allowed to infiltrate for 30 minutes to 3 hours prior to the sample being augered from within the area of the steel ring.

Soil sampler: A commercial Arborline corer (Plate 3.11) was used to collect soil samples. For collection of wet samples in dry soil, soil was wet as described for auger samples. An additional treatment was taken at Palmer, Condada and Tanunda, where 200 - 500 ml of water was added to dry soil in plastic bags immediately after collection.

5.1.3.1 Data analysis

Numbers of nematodes were expressed as *P. neglectus* or *P. thornei*/g of dry soil. Data were $\log_e$ transformed to normalise data sets. Within sites, an ANOVA ($P < 0.05$) was used to determine the significance of differences.

Table 5.1 **Site and sampling details for evaluation of spatial distribution and comparison of sampling methods for *Pratylenchus neglectus* and *P. thornei* in South Australia. Soil type was defined as described by Northcote *et al.* 1976.**

			Site details time of sampling and maximum sampling depth		
Site	Nematode species	Soil type	Horizontal distribution	Vertical distribution	Comparison of sampling methods
Balaklava	*P. neglectus*	Hard, alkaline, red, duplex earth	Stubble, March 1994	Before rain, March 1994, depth 60 cm	Before rain, March 1995
Cleve	*P. neglectus/P. thornei*	Hard, alkaline, red, duplex earth	-	-	Before rain, March 1995
Condada	*P. neglectus*	Grey-brown, calcareous, loamy earth	Stubble, June 1994	After rain, June 1994, depth 60 cm	Before rain, January 1995
Cungena	*P. neglectus*	Shallow, red-brown, sandy soil	-	Before rain, March 1993, depth 40 cm	After rain, May 1994
			-	After rain, June 1993, depth 60 cm	-
Kimba	*P. neglectus*	Grey-brown, calcareous, loamy earth	Stubble, March 1994	Before rain, March 1994, depth 60 cm	-
Miltaburra	*P. neglectus*	Brown, calcareous, earth	Stubble, June 1993	Before rain, March 1993, depth 40 cm	Before rain, March 1995
			Within crop, August 1993.	After rain, June 1993, depth 60 cm	Before rain, March 1994
Palmer	*P. neglectus*	Brown, calcareous, earth	-	-	Before rain, March 1995
			-	-	After rain, April 1995
Tanunda	*P. thornei*	Black earth	March 1994	Before rain, March 1994, depth 90 cm	After rain, May 1995

5.2 Results

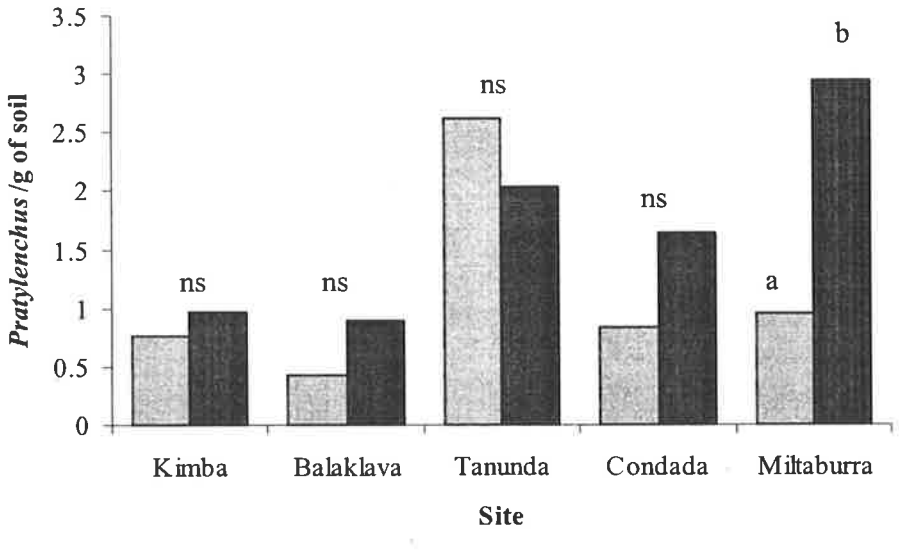
5.2.1 Horizontal distribution – plant row effects

Pratylenchus spp. present and site details are shown in Table 5.1. Results from the paired t-test comparing *P. neglectus* recovered from within and between stubble rows are presented in Table 5.2. There were significantly higher levels of nematodes recovered from soil sampled within plant rows compared with between rows at Miltaburra (Table 5.2; Figure 5.1). A similar trend occurred at Kimba and Condada where *P. neglectus* was recorded, but results were not significant (Figure 5.1). Tanunda (*P. thornei*) was the only site were there was a trend for higher densities of *Pratylenchus* between rows compared to within plant rows.

Table 5.2 **Results from the paired t-test comparing number of *Pratylenchus* recovered within or between stubble rows at five sites.**

	Kimba	Balaklava	Tanunda	Condada	Miltaburra
T	1.39	2.72	-0.96	1.61	3.28
d.f.	19	19	19	19	18
P	0.182	0.140	0.348	0.124	0.004

Figure 5.1 **Density of *P. neglectus* (Kimba, Balaklava, Condada and Miltaburra) and *P. thornei* (Tanunda)/g of dry soil sampled within and between stubble rows of a previous cereal crop. ns not significant. Columns with different letters differ significantly (P < 0.05).**



5.2.2 Vertical distribution

Nematode species and site details are presented in Table 5.1. At all sites assessed, maximum numbers of *Pratylenchus* were recovered from the 0 - 10 cm layer of the soil profile. The percentage of *Pratylenchus* recovered from this soil layer was significantly lower in the deeper, red duplex soil and black earth sites (Kimba, Balaklava and Tanunda) compared with the shallower sands and loams (Miltaburra and Cungena) (Table 5.3). Proportions recovered from the 0 - 20 cm soil layer did not differ significantly between sites (Table 5.4). At Condada and Miltaburra, where samples were collected at the 0 - 5 and 5 - 10 cm increments, the majority of *P. neglectus* were found at 5 - 10 cm (52 and 55% of the total sample at these sites) compared with 14 - 12% recovered from the 0 - 5 cm layer.

The percentage of nematodes recovered from the top 10 cm at the Miltaburra and Cungena sites after rain was significantly higher than percentages recovered before rain (Table 5.3), as were total nematode numbers recovered (Figure 5.2). For sites sampled after rain, soil moisture increased in deeper soil layers compared with either the 0 - 10 cm or 10 - 20 cm layers (Table 5.5).

Table 5.3 **The ANOVA of the results comparing proportion of *Pratylenchus* in the top 10 cm of the soil profile and contrasts between sandy (Cungena and Miltaburra before and after rain) and clay (Kimba, Balaklava and Tanunda) sites.**

ANOVA for proportion in top 10 cm			
	d.f.	m.s.	P
Rep.	9	0.02	0.486
Site	6	0.275	0.000
Rep.*Site	51	0.021	
Contrasts for proportion in top 10 cm of sandy (before and after rain) vs. clay sites			
Contrast		3.311	
Scheffe's F statistic		8.74	
SE (contrast)		0.457	
P (Scheffe's F)		0.000	

Table 5.4 **Vertical distribution of *Pratylenchus* spp. expressed as a percentage of total nematodes recovered from soil profile at six sites in South Australia. Sites were sampled before or after rain as indicated (percent soil moisture presented in Table 5.5). Percent recovery at the 0 - 10 cm depth followed by different letters indicate sites differ significantly (Scheffe's F statistic; P < 0.05). # Cumulative figures for 0 - 5 and 5 - 10 cm increments (Condada and Miltaburra before rain only). Dash indicates not sampled. Values in brackets are *Pratylenchus* numbers/g of dry soil.**

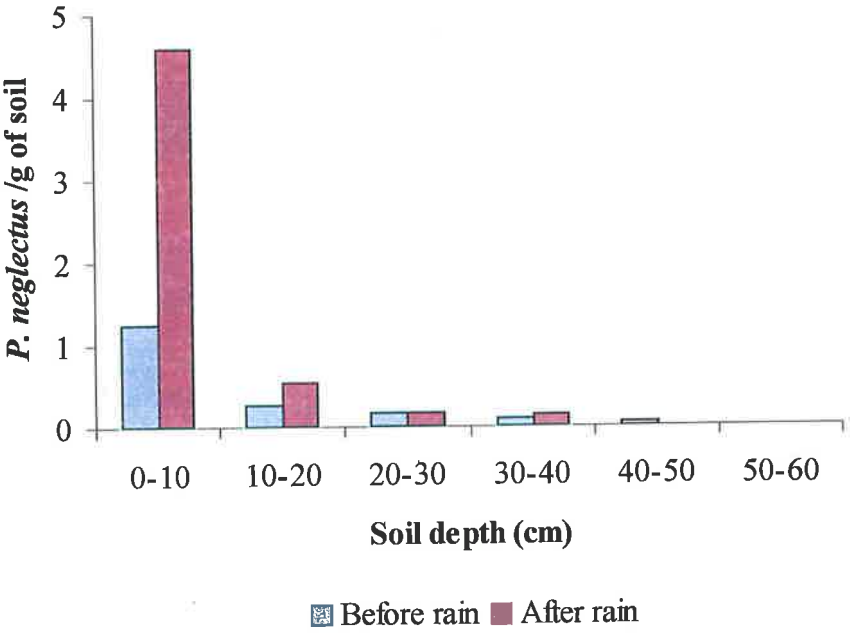
	Pratylenchus population (%)							
Depth (cm)	Kimba Before rain	Balaklava Before rain	Tanunda Before rain	Cungena Before rain	Cungena After rain	Condada Before rain	Miltaburra Before rain	Miltaburra After rain
0 - 5	-	-	-	-	-	14 (0.9)	12 (0..2)	-
5 - 10	-	-	-	-	-	52 (3.5)	48 (0.6)	-
0 - 10	47a (0.4)	39a (0.8)	43a (0.9)	67b (1.3)	84c (4.6)	66b# (4.4)	60b# (0.8)	67b (8.7)
10 - 20	35 (0.3)	25 (0.5)	28 (0.1)	15 (0.3)	10 (0.5)	21 (1.3)	25 (0.3)	22 (2.9)
20 - 30	8 (0.1)	16 (0.3)	13 (0.3)	9 (0.2)	3 (0.2)	10 (0.5)	9 (0.1)	6 (0.9)
30 - 40	5 (0.1)	8 (0.2)	6 (0.1)	5 (0.1)	3 (0.2)	2 (0.8)	5 (0.1)	5 (0.6)
40 - 50	2 (0.02)	6 (0.1)	3 (0.1)	3 (0.1)	-	1 (0.1)	1 (0.02)	-
50 - 60	2 (0.02)	5 (0.1)	2 (0.04)	1 (0.01)	-	0 (0.1)	0 (0)	-
60 - 70	-	1 (0.03)	2 (0.04)	-	-	-	-	-
70 - 80	-	-	2 (0.04)	-	-	-	-	-
80 - 90	-	-	1 (0.02)	-	-	-	-	-

Table 5.5 **Percent soil moisture at different depths before and after rain at six sites in South Australia. Densities of *Pratylenchus* recovered at each depth increment is presented in Table 5.4.**

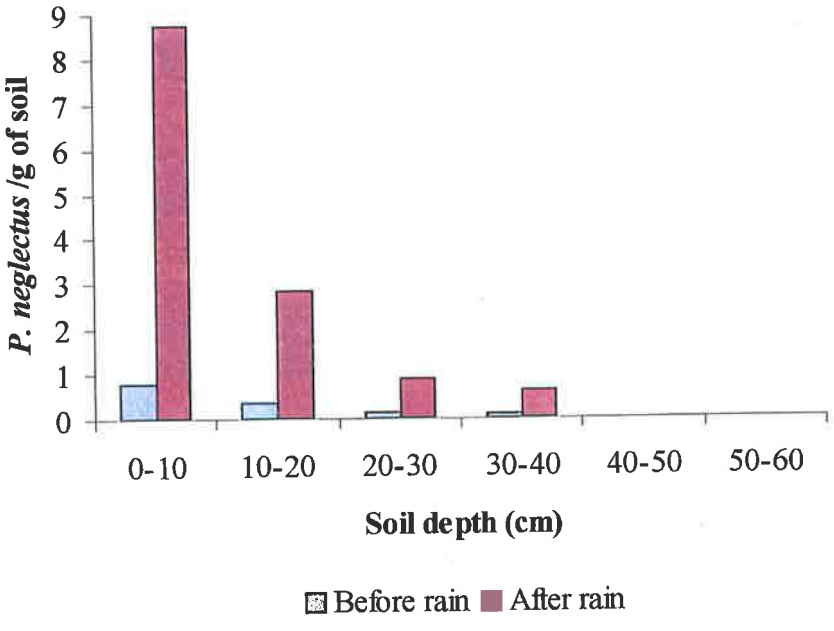
	Soil moisture (%)							
Depth (cm)	Kimba Before rain	Balaklava Before rain	Tanunda Before rain	Cungena Before rain	Cungena After rain	Condada Before rain	Miltaburra Before rain	Miltaburra After rain
0 - 5	-	-	-	-	-	<1	<1	-
5 - 10	-	-	-	-	-	<1	<1	-
0 - 10	<1	2	1	<1	7	-	-	12
10 - 20	<1	2	1	<1	6	1	1	13
20 - 30	<1	1	3	<1	7	<1	1	12
30 - 40	1	2	3	<1	7	1	1	12
40 - 50	1	2	2	1	-	1	1	-
50 - 60	1	3	3	1	-	1	1	-
60 - 70	-	-	4	-	-	-	-	-
70 - 80	-	-	9	-	-	-	-	-
80 - 90	-	-	12	-	-	-	-	-

Figure 5.2 **Density of *P. neglectus* recovered from different soil depths at a) Cungena and b) Miltaburra, South Australia, before and after rain.**

a) Cungena



b) Miltaburra



5.2.3 Comparison of sampling methods

The ANOVA of the results showing significant effects of sampling treatments at each site is presented in Table 5.6. Differences between sampling treatments are shown in Table 5.7 and the percentage increase in the number of *Pratylenchus* recovered using each sampling method compared with an auger in dry soil is shown in Table 5.8. Significantly higher levels of nematodes were recovered with undisturbed cores (wet) than with auger samples (dry) at five of the seven sites. At Condada and Palmer (after rain), there was no significant difference between numbers obtained using the undisturbed cores and either the soil sampler or the auger. At all sites except Kimba, there was no significant difference between nematode levels recovered using the wet and dry undisturbed core methods. Percent soil moisture for wet samples or after rains ranged from 9% - 21% compared with percent moisture in dry soils of 1% - < 7% (Table 5.9).

Addition of water to soil samples immediately after collection did not improve recovery of nematodes and, at Palmer (after rain), recovery was significantly lower than from samples taken in soil with water added just prior to sampling. Difficulties were encountered in sampling the heavy black earth site at Tanunda when water was added. The soil sampler could not be used at this site as wet clay would not pass through the coring tube into the collection bucket (even after lining the collection tube with talc).

Table 5.6 **The ANOVA of results comparing sampling treatments for the recovery of *Pratylenchus* at seven sites in South Australia.**

Site		d.f.	m.s.	P
Kimba	Rep.	9	2.944	0.001
	Sampling treatment	3	4.275	0.000
	Rep.*treatment	27	0.484	
Cleve	Rep.	9	12.48	0.003
	Sampling treatment	3	9.34	0.05
	Rep.*treatment	26	3.25	
Miltaburra	Rep.	9	0.746	0.352
	Sampling treatment	3	3.071	0.008
	Rep.*treatment	27	0.637	
Balaklava	Rep.	9	0.818	0.793
	Sampling treatment	3	4.737	0.031
	Rep.*treatment	27	1.383	
Palmer before rain	Rep.	9	0.762	0.054
	Sampling treatment	5	1.341	0.005
	Rep.*treatment	54	0.377	
Palmer after rain	Rep.	9	0.401	0.077
	Sampling treatment	3	1.102	0.006
	Rep.*treatment	27	0.199	
Condada	Rep.	9	0.435	0.173
	Sampling treatment	6	0.973	0.007
	Rep.*treatment	50	0.29	
Tanunda	Rep.	9	2.052	0.001
	Sampling treatment	5	1.809	0.012
	Rep.*treatment	44	0.546	

Table 5.7 **Levels of *P. neglectus* and *P. thornei* obtained with different soil sampling methods in South Australia. Samples were taken either before or after rain (soil moisture presented in Table 5.9). Values in brackets are back-transformed means (*Pratylenchus*/g of dry soil). Values in columns followed by the same letters do not differ significantly ($P < 0.05$). Soil was sampled: dry (no additional moisture added); + water (water added to soil just prior to sampling); or bag + water (water added to plastic bags immediately after sample collection). Dash represents no sample taken.**

Sampling Method	*Pratylenchus*/g of soil ($\log_e$ transformed)							
	Kimba Before	Cleve Before	Miltaburra Before	Balaklava Before	Palmer Before	Palmer After	Condada After	Tanunda After
Core+water	4.87a (0.7)	6.18ab (2.4)	4.69a (0.5)	4.59a (0.5)	6.12a (2.3)	5.64a (1.4)	5.76a (1.6)	7.43a (8.4)
Core dry	4.03b (0.3)	7.23a (6.9)	4.37a (0.4)	3.59ab (0.2)	6.12a (2.3)	-	5.55ab (1.3)	7.04ab (5.7)
Auger+water	4.86a (0.6)	7.48a (8.9)	3.99ab (0.3)	3.33b (0.1)	5.57b (1.3)	4.39b (0.7)	5.19bc (0.9)	6.88bc (4.9)
Auger dry	3.55b (0.2)	5.39b (1.1)	3.4b (0.2)	2.99b (0.1)	5.49ab (1.2)	-	4.81c (0.7)	6.27c (2.6)
Soil sampler+water	-	-	-	-	5.14b (0.9)	5.56a (1.3)	5.46ab (0.9)	-
Soil sampler bag+water	-	-	-	-	5.37b (1.1)	5.28b (1.0)	5.14bc (1.2)	6.44bc (3.2)
Soil sampler dry	-	-	-	-	5.64ab (1.4)	-	5.29ab (1.1)	6.62bc (3.8)

Table 5.8 **Percentage difference in recovery of *Pratylenchus* spp. between sampling methods compared with sample collection using an auger in dry soil (soil moisture presented in Table 5.9). ns not significantly different from auger in dry soil, * significantly different (ANOVA $P < 0.05$). Soil was sampled: dry (no additional moisture added); + water (water added to soil just prior to sampling); or bag + water (water added to plastic bags immediately after sample collection). Dash represents no sample taken. na not applicable.**

Sampling Method	Percentage difference in nematode recovery (compared with using an auger in dry soil)							
	Kimba Before	Cleve Before	Miltaburra Before	Balaklava Before	Palmer Before	Palmer After	Condada After	Tanunda After
Core+water	+27 *	+13 ns	+28 *	+35 *	+10 *	na	+10 *	+16 *
Core dry	+12 ns	+26 *	+22 *	+17 ns	+10 *	-	+12 *	+11 *
Auger+water	+27 *	+28*	+15 ns	+10 ns	+1 ns	na	+6 ns	+9 ns
Auger dry	na	na	na	na	na	-	na	na
Soil sampler+water	-	-	-	-	-6 ns	na	+11 *	-
Soil sampler bag+water	-	-	-	-	-2 ns	na	+ 11 ns	+3 ns
Soil sampler dry	-	-	-	-	3 ns	-	+9 *	+4 ns

Table 5.9 **Percent soil moisture from samples assessing methods for recovery of *Pratylenchus* before and after rain at seven sites in South Australia. Soil was sampled: dry (no additional moisture added); +water (water added to soil just prior to sampling); bag+water (water added to plastic bags immediately after sample collection).**

Sampling Method	Soil moisture (%)							
	Kimba Before	Cleve Before	Miltaburra Before	Balaklava Before	Palmer Before	Palmer After	Condada After	Tanunda After
Core+water	10	12	17	14	20	8	13	21
Core dry	<1	<1	<1	<1	2	-	4	6
Auger+water	10	10	15	15	15	7	9	19
Auger dry	<1	<1	<1	<1	3	-	3	6
Soil sampler+water	-	-	-	-	15	9	11	-
Soil sampler, bag+water	-	-	-	-	18		15	26
Soil sampler dry	-	-	-	-	3	-	3	7

5.3 Discussion

The absence of statistically significant differences between number of nematodes from samples collected within the stubble rows from the previous season (compared with samples taken between plant rows) at four of the five sites is probably a result of the relative proximity of rows (18 cm row spacing) in broad-acre cereal crops and the wide distribution of the fibrous root systems. In addition, the migratory behaviour of nematodes such as *Pratylenchus* spp. allow dispersal throughout the season, providing sufficient moisture is available for movement between soil pore spaces (Wallace 1971).

Edaphic factors may affect horizontal distribution of plant-parasitic nematodes. Wallace *et al.* (1993) assessed the horizontal distribution of eight nematode taxa and found five were significantly correlated with sand particle size. Sarah *et al.* (1991) found that levels of *P. brachyurus* in pineapple crops were affected by soil pH. The significantly higher density of *P. neglectus* observed in samples taken within plant rows at the Miltaburra site may have been a result of site conditions. This site was classified as nutrient poor compared with the other sites sampled, and *P. neglectus* was aggregated where these nutrients were applied at seeding and therefore where root growth was greatest. A similar effect was observed in soils where levels of carbon, potassium and nitrogen were low, with the highest density of *P. zeae* occurring within sugarcane rows where additional nutrients had been applied and therefore root growth was highest (Delaville *et al.* 1996). In contrast, Tanunda was the only site where there was a trend (although non-significant) for a higher number of *Pratylenchus* to be found between, compared to within plant rows. Tanunda was a more fertile, self-mulching black clay, and the maximum density of nematodes was also found deeper in the soil profile than the shallower sandy loam soils, suggesting root growth and distribution (between rows) was greater than in the nutrient poor sites.

In this study, the shallower the soil, the higher the proportion of *Pratylenchus* found in the top 10 cm. In the self mulching black clay at Tanunda, the majority of *Pratylenchus* were lower in the soil profile (i.e. 20 cm), although *P. thornei* were still not found at depths seen in the deeper soils of Queensland and Victoria, where high proportions are found between 20 and 30 cm (Thompson *et al.* 1999).

In South Australia, of the proportion of *P. neglectus* found in the top 10 cm, over half of the total number was found within the 5 - 10 cm soil layer compared with < 15% at 0 - 5 cm. This may be a result of greater root concentration in the 5 - 10 cm layer or the more hostile environment in the top 5 cm where soil temperatures can reach 35°C in summer (Ware pers. comm.).

Greater recovery of *P. neglectus* was observed from soil following rains compared with samples taken before rain. This did not appear to be a result of migration of *Pratylenchus* through the soil profile as total number of *P. neglectus* recovered was significantly higher at all depth increments following rain. The greater number of *P. neglectus* recovered was therefore most likely due to differences in resulting from the sampling conditions before and after rain. The addition of moisture to the soil resulted in greater recovery and this was due to factors associated with anhydrobiosis of *P. neglectus* such as immediate release of juveniles from females or the re-activation of nematodes from anhydrobiosis. These findings further illustrate the importance of assessment of depth distribution of plant-parasitic nematodes within soil types and cropping areas in determining the most appropriate sampling strategy for each environment.

Sampling technique has been shown to affect recovery of nematodes from soil (Boag *et al.* 1987). This study confirmed that sampling technique affects recovery of *Pratylenchus* spp. in South Australian agricultural soils, and that soil moisture is important in determining the recovery of both *P. neglectus* and *P. thornei* using the Whitehead tray extraction method. Soil disturbance has been shown to reduce the recovery of nematodes. Repeated dropping of samples reduced recovery of both trichodorid and longidorid nematodes (Brown and Boag 1988). In addition, Gnanapragasam and Sivapalan (1991) demonstrated that significantly fewer *P. loosi* were recovered from sieved soil compared with unsieved soil.

In all types of dry soil, increased soil disturbance resulted in reduced recovery of *Pratylenchus*. *Pratylenchus* can survive long periods in dry soil in an anhydrobiotic state unless mechanical damage disrupts the cuticle. The Whitehead tray extraction method used in this study relies on nematode motility so only living nematodes will be recovered . The addition of water to dry soil prior to sampling using undisturbed cores increased nematode recovery by up to 35%

compared with sampling using an auger in dry soil. This increase in nematode recovery occurred with water added immediately prior to sampling at all sites, suggesting a limited revival time is required to prevent mechanical damage of anhydrobiotic nematodes. There was a less pronounced effect of addition of water at sites where rain had occurred prior to sampling. This suggests that nematodes at these sites were not in a state of anhydrobiosis when sampled and therefore the buffering effect of adding moisture prior to sampling was not as significant as that seen in very dry soils (< 1% soil moisture).

The addition of water to dry soil in plastic bags after collection did not result in increased nematode recovery compared with sampling using an auger at any of the sites where this comparison was made, suggesting that damage to nematodes is caused by sampling rather than transport or processing.

This study demonstrates the need to investigate responses of nematodes to site characteristics. While no sampling method will eliminate all errors associated with sampling techniques or nematode distribution, a better understanding of the limitations imposed by sampling strategies can be achieved.

Chapter 6 Population dynamics of *Pratylenchus neglectus* in wheat in a pot study

6.0 Introduction

The population dynamics of plant-parasitic nematodes have been studied extensively, with many models proposed to predict both nematode reproduction rates and damage as a result of nematode infection (see Section 2.9).

In most situations, the initial nematode density (Pi) is critical in determining both the multiplication rate (related to plant susceptibility or nematode reproduction within a plant host) or yield loss (related to plant intolerance or reaction of the host to nematode attack) (Ferris and Noling 1987). With plant-parasitic nematodes, initial nematode density is of particular importance because of the inability of nematodes to move large distances and their relatively low reproduction rate (Barker and Olthof 1976). Initial density will also effect both final nematode density (Pf) and crop damage, as plants extensively damaged early in growth will have reduced ability to recover from nematode attack and may therefore support fewer nematodes. Often, the degree of damage caused by nematodes will depend on the ability of the plant to tolerate infection, and the density of plant-parasitic nematodes at which no yield loss occurs is defined as the tolerance limit (Barker and Olthof 1976). Plants already compromised by abiotic stresses such as high temperature, moisture or nutrient stress will have reduced capacity to withstand nematode damage and will therefore be more intolerant (Wallace 1969; Colhoun 1973).

Prediction of population changes over time for nematodes with several generations per season (i.e the relationship between Pi and Pf) can be represented by the equation:

$$Pf = aEPi/[(a-1) + E]$$

where a = maximum multiplication rate and E = the equilibrium density (i.e. where food source is sufficient to replace nematodes that die) (Seinhorst 1970). The equilibrium density will change over time and, with particular environments, as conditions affecting the host (and therefore multiplication rate and nematode density) change.

Studies of the population dynamics of *P. thornei* on susceptible and intolerant wheat cultivars under South Australian conditions showed that, at high initial nematode densities, both root and shoot weight were reduced and nematode multiplication rate was lowered (Nicol 1996; Nicol *et al.* 1999).

Little information is currently available on the population dynamics of *P. neglectus* in wheat, or in crops grown in rotation with wheat. The relationship between the density of *P. neglectus* and population changes of *P. neglectus* under different cultivars and yield loss in field trials are considered later in this thesis (Chapters 7 and 8, respectively).

In this study, the effect of initial density on the multiplication rate of *P. neglectus* in a susceptible, intolerant wheat variety (cv. Machete) was assessed in pots designed to simulate conditions that occur in the autumn, winter and spring periods in southern Australia. Attempts were made to evaluate the subsequent yield loss caused by *P. neglectus* in the absence of other pathogens.

6.1 Materials and methods

6.1.1 *Pratylenchus neglectus* inoculum

Pratylenchus neglectus soil inoculum was prepared from a mixture of carrot culture infested with *P. neglectus* (Section 3.2.2). The nil treatment was produced by adding uninoculated carrot culture to sterile, Tailem Bend sand. To determine the nematode level in the soil inoculum, eight, 50 g sub-samples were assessed from the bulk soil inoculum using the mister method (Vanstone *et al.* 1998).

Medium and low nematode densities were produced from the initial soil inoculum by dilution with sterile, Tailem Bend sand. The initial soil inoculum was used as the high nematode treatment. To assess nematode densities in the low and medium nematode treatments produced after dilution with sterile sand, four 50 g sub-samples were extracted using the mister method. Prior to use, the soil inoculum was stored at 4°C for 5 days before being mixed with Osmocote® fertiliser (4 g/kg; see Section 3.6 for specifications) and used to fill 7 kg pots (25 cm diameter).

6.1.2 Experimental design and assessment

The experiment was set up as a randomised complete block design. Five ungerminated seeds of Machete wheat (identified in field and pot trials as susceptible and intolerant [Vanstone *et al.* 1998; Chapters 7 and 8]) were sown per pot with two pots per harvest time (i.e. n = 10 plants per nematode treatment). Pots were immediately transferred to outdoor terraces (Plate 3.7) and buried into sand beds. The experiment was watered as needed using an above ground sprinkler system. Rainfall (including additional irrigation) was recorded each day. Air and soil (at a depth of 10 cm) temperatures were recorded every 4 hours using separate data loggers (Tiny Tag®).

Four *P. neglectus* levels (presented in Table 6.1) and five harvest times (4, 8, 12, 16 and 20 weeks after plants were sown) were included in the experiment. At each harvest, nematodes were assessed from both soil and plant roots. Nematodes were extracted from soil using the modified Whitehead tray method (Section 3.2.1). For assessment of *P. neglectus* from plant roots, soil was washed from root systems and each plant was assessed separately. Five root systems per treatment (selected randomly from the two pots) were misted, and five were stained then blended (Section 3.1.4). Numbers of nematodes were counted as described in Section 3.3. Plant top dry weight was recorded for each plant. Root dry weight was taken for misted samples only.

6.1.3 Data analysis

Data for both top and root weights were square root transformed. The top and root weights for the 4 and 8 week harvest times were analysed separately as large differences were seen between these data and those from other harvest times. Top and root weight data from the 12, 16 and 20 week harvests were more similar and were analysed as a group. The number of *P. neglectus* was expressed as *P. neglectus*/plant. Multiplication rates of *P. neglectus* were determined by dividing initial nematode density in plant roots (as determined at the 4 week plant assessment) by number of nematodes recovered from roots at the 8, 12, 16 and 20 week assessments, respectively.

6.2 Results

6.2.1 *Pratylenchus neglectus* inoculum

Initial nematode density in the bulk soil inoculum was 36.5 *P. neglectus*/g of soil (n = 8). The low and medium inoculum densities produced by dilution with Tailem Bend sand are presented in Table 6.1.

Table 6.1. Density of *P. neglectus* in soil inoculum prepared by dilution of original soil inoculum (produced by adding infested carrot culture to Tailem Bend sand [Pi = 36.5 *P. neglectus*/g of soil]) with Tailem Bend sand.

Inoculum rate	Average *P. neglectus*/g (dry soil) ± Standard deviation
Low	1.2 ± 0.4
Medium	9.4 ± 3.5
High	36.5 ± 19.7

6.2.2 Nematode densities and multiplication rates

The comparison of misting and staining techniques for the assessment of *P. neglectus* from plant roots was examined as a separate experiment to determine which method produced the most efficient separation of *P. neglectus* from plant roots and detailed results were presented in Section 4.2.4. In summary, there was a significant difference in the number of nematodes recovered from root samples using either misting or staining only at the 4 week harvest. Data for each harvest time are therefore presented as the average of 10 samples per treatment. There were significantly more nematodes recovered from the low, medium and high treatments compared with the nil treatment.

The ANOVA of the results for the effect of *P. neglectus* inoculum density on *P. neglectus* density at each harvest time is shown in Table 6.2. No significant difference was seen in density of *P. neglectus* (recovered at 8, 12, 16 and 20 week harvest times) between the low, medium and high nematode inoculum treatments. A significant difference was observed between the nil and all other nematode inoculum treatments. Changes in the density of *P. neglectus* over the 20

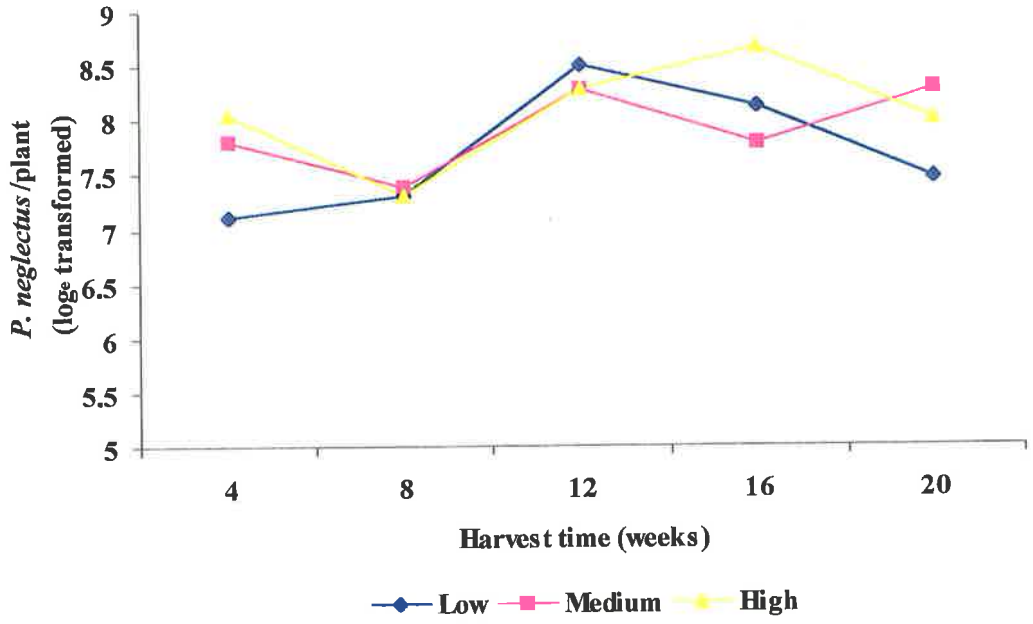
week assessment period are shown in Figure 6.1 and the effect of initial density (as measured at the 4 week assessment) on the density of *P. neglectus*/plant recorded at 8, 12, 16 and 20 weeks is presented in Figure 6.2. Significantly higher numbers of *P. neglectus* were recovered at the 12 and 16 week compared with 8 and 20 week assessment periods. Both log transformed and untransformed data for nematode density are presented, as relationships may be seen more clearly with the latter.

Table 6.2 The ANOVA of results for the effect of *P. neglectus* inoculum on *P. neglectus* density at different harvest times (4, 8, 12, 16 and 20 weeks after sowing).

	Log_e transformed *P. neglectus*/plant			Untransformed *P. neglectus*/plant		
	d.f.	m.s.	P	d.f.	m.s.	P
Rep.	9	1.973	0.068	9	3.41+E07	0.379
Nematode inoculum	3	783.53	0.000	3	4.59E+08	0.000
Harvest time	4	4.68	0.002	4	1.99+E08	0.000
Nem.*Harvest	12	1.44	0.207	12	2.93E+07	0.516
Res.	148	1.082		148	3.14E+07	

Figure 6.1 **Density of *P. neglectus*/plant at 4, 8, 12, 16 and 20 weeks a) $\log_e$ transformed b) untransformed data. Low = 1.2 *P. neglectus*/g of soil; medium = 9.4 *P. neglectus*/g of soil; high = 36.5 *P. neglectus*/g of soil.**

a) $\log_e$ transformed data



b) untransformed data

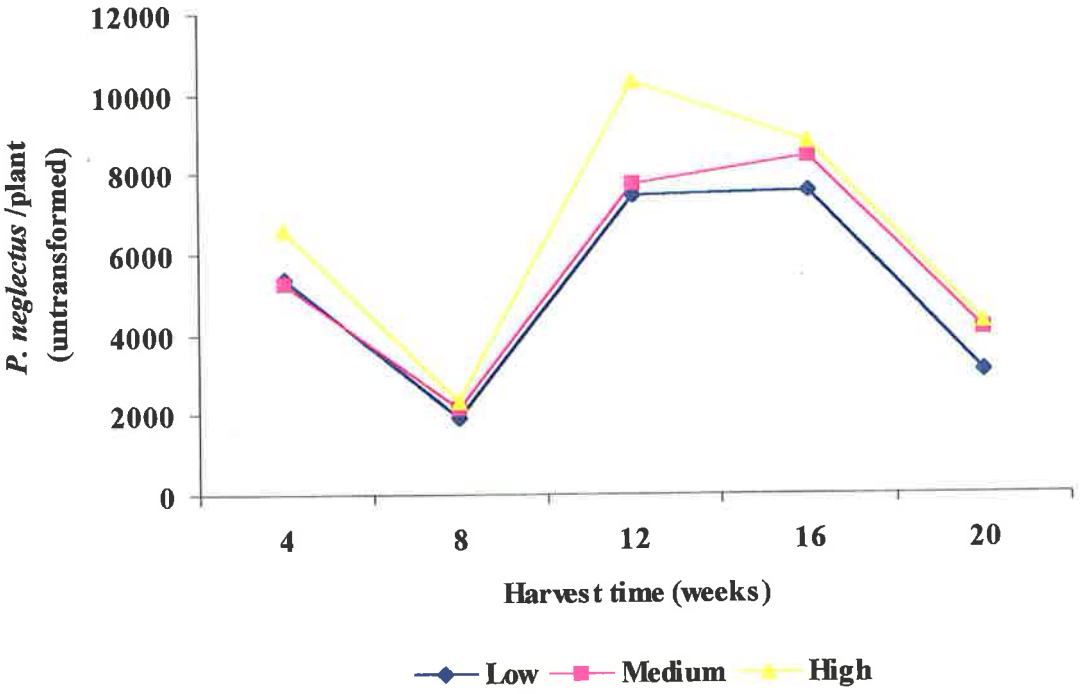
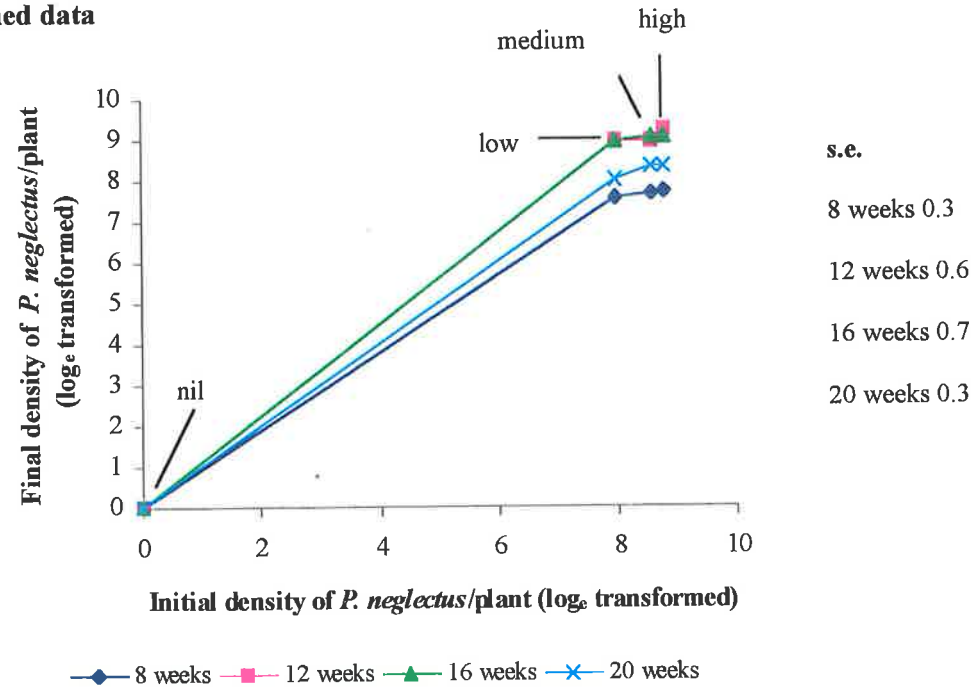


Figure 6.2 **Effect of initial density (as assessed at the 4 week harvest) of *P. neglectus* in wheat (cv. Machete) on final density per plant, a) $\log_e$ transformed b) untransformed. Initial densities obtained from numbers/plant harvested at 4 weeks: Nil = 0 *P. neglectus*/plant; low = 2970 *P. neglectus*/plant; medium = 5260 *P. neglectus*/plant; high = 6554 *P. neglectus*/plant. Plants were harvested at 8 weeks, 12 weeks, 16 weeks and 20 weeks.**

a) $\log_e$ transformed data



s.e.

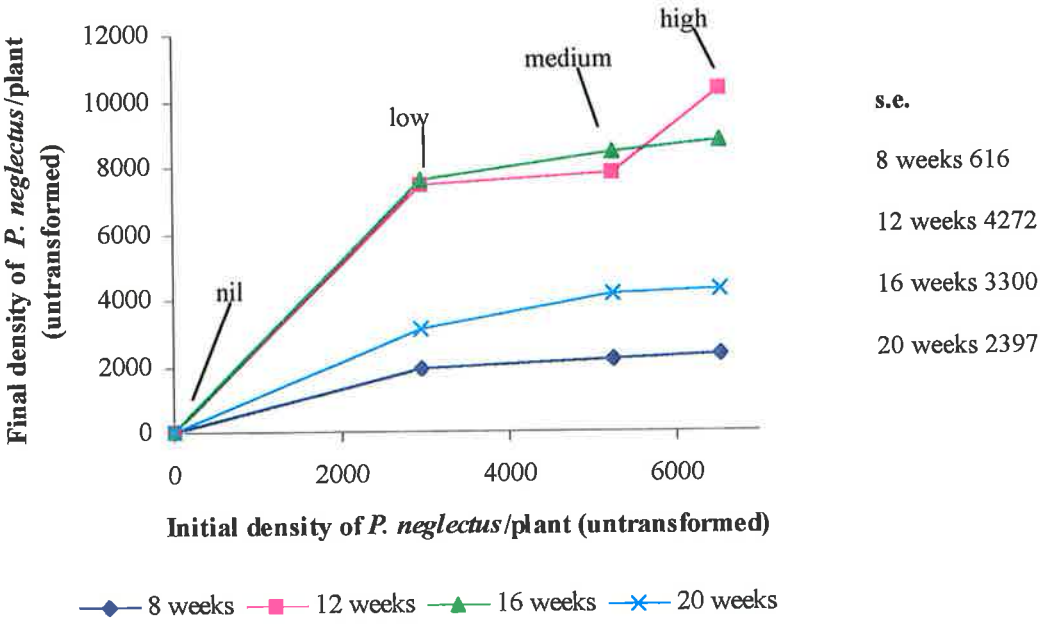
8 weeks 0.3

12 weeks 0.6

16 weeks 0.7

20 weeks 0.3

b) untransformed data



s.e.

8 weeks 616

12 weeks 4272

16 weeks 3300

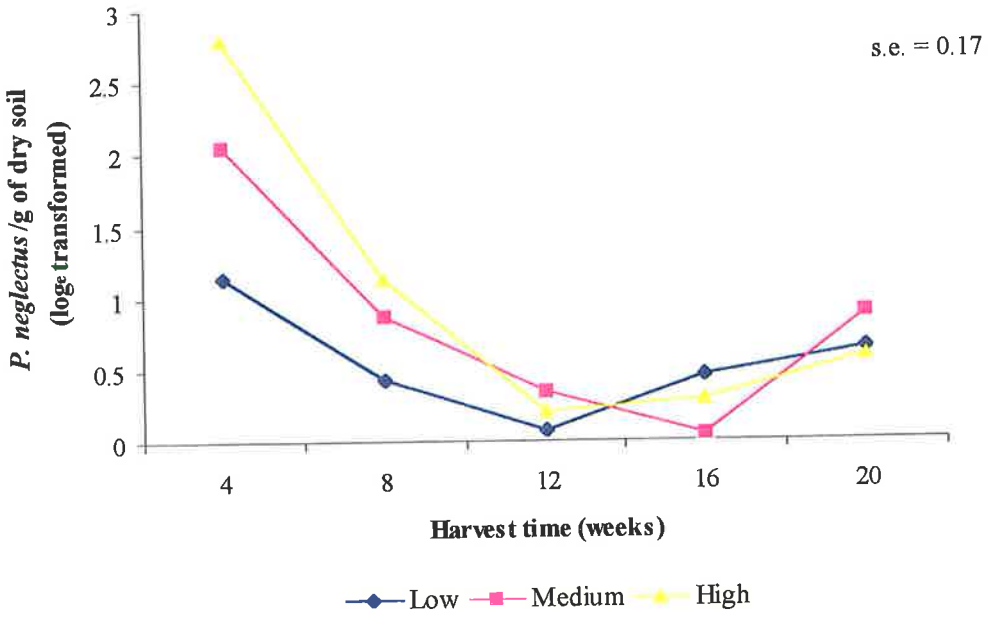
20 weeks 2397

Numbers of *P. neglectus* recovered from soil around wheat plants is shown in Figure 6.3 and the ANOVA of the results of *P. neglectus*/g of soil at each harvest time in Table 6.3. The highest densities of *P. neglectus* in soil were observed at 4 weeks and, at this time, densities in the low and medium treatments were comparable to initial soil inoculum (detransformed means of 3.1 and 7.7 *P. neglectus*/g of soil respectively). However, only about half of the initial *P. neglectus* inoculum for the high treatment was recovered (detransformed mean of 16.2 *P. neglectus*/g of dry soil). Soil nematode densities declined significantly after 4 weeks, only gradually increasing at the 20 week harvest (detransformed means of 1.9, 2.4 and 1.8 *P. neglectus*/g of soil in low, medium and high inoculum rates, respectively).

Table 6.3 **The ANOVA of results for the effect of *P. neglectus* inoculum rate and harvest time on density of *P. neglectus* in soil surrounding Machete wheat roots.**

	Log_e transformed *P. neglectus*/g of dry soil		
	d.f.	m.s.	P
Rep.	3	0.052	0.819
Nematode inoculum	2	1.058	0.004
Harvest time	4	6.239	0.000
Nem.*Harvest	8	0.620	0.003
Rep.*Nem.*Harvest	38	0.168	

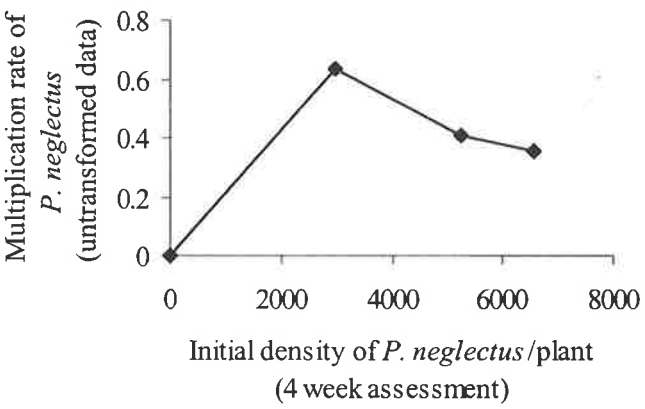
Figure 6.3 **Density of *P. neglectus*/g dry soil recovered from soil surrounding wheat plants (cv. Machete) harvested every 4 weeks, July-December, 1997. Initial density of soil inoculum (*P. neglectus*/g of dry soil): Low = 1.2; medium = 9.4; high = 36.5.**



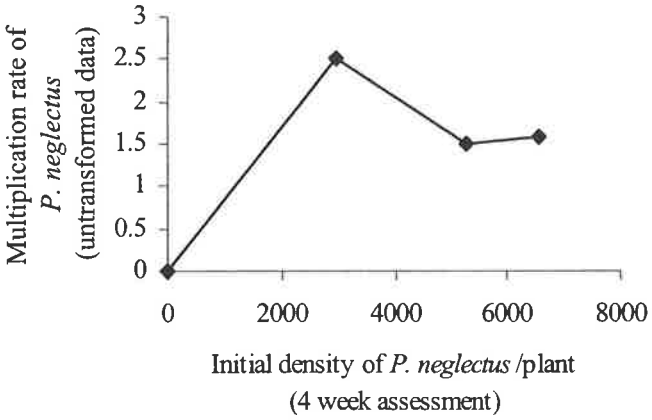
Changes in multiplication rate of *P. neglectus* (Pf/Pi) for each inoculum density are shown in Figure 6.4. Multiplication rates were highest for the low nematode treatment (Pf/Pi = 2.5 at both 12 and 16 weeks). Lower multiplication rates (< 1) were observed at both 8 and 20 week assessments.

Figure 6.4 **The effect of initial density (measured at 4 weeks) on multiplication rate of *P. neglectus* at a) 8 week, b) 12 week, c) 16 week and d) 20 week assessment periods of wheat (cv. Machete).**

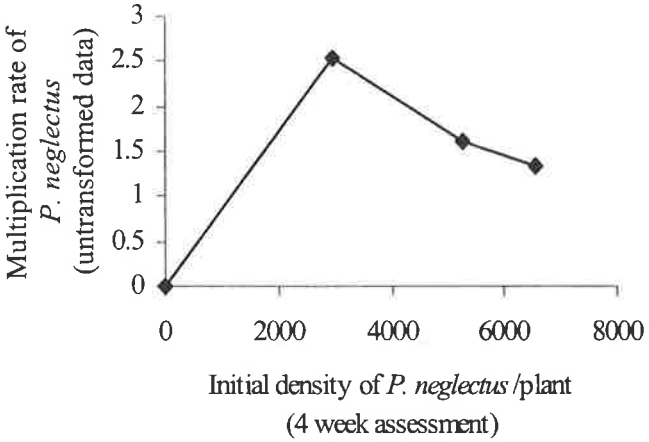
a) 8 week



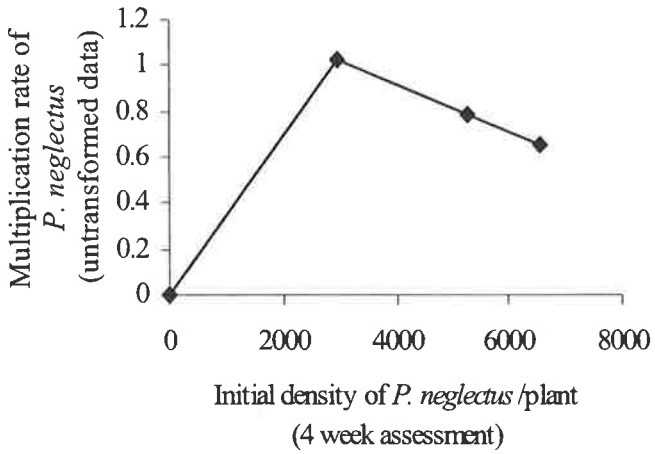
b) 12 week



c) 16 week



d) 20 week



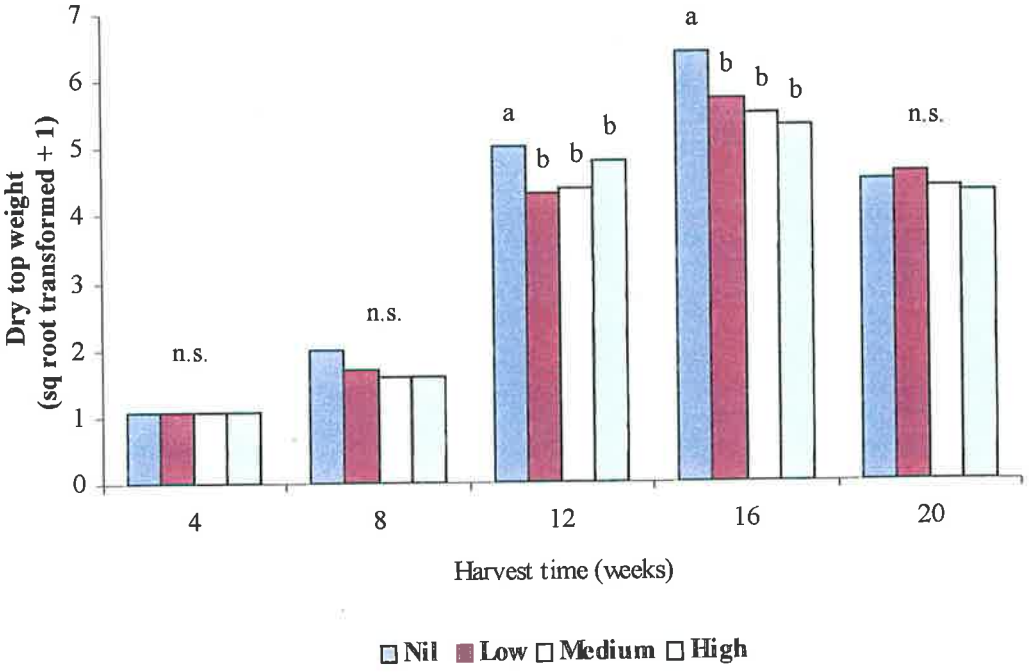
6.2.3 Plant dry matter responses

There were no significant responses to nematode density in either top or root weight at 4 or 8 weeks after planting (Figure 6.5). At 12 and 16 weeks, there were significant reductions in top weight for the nil nematode density compared to the inoculated treatments, although there were no differences between low, medium or high treatments (Figure 6.5a). At the final harvest (20 weeks), there were no significant differences in top weights between treatments. Grain yield was not recorded due to damage to plants by birds.

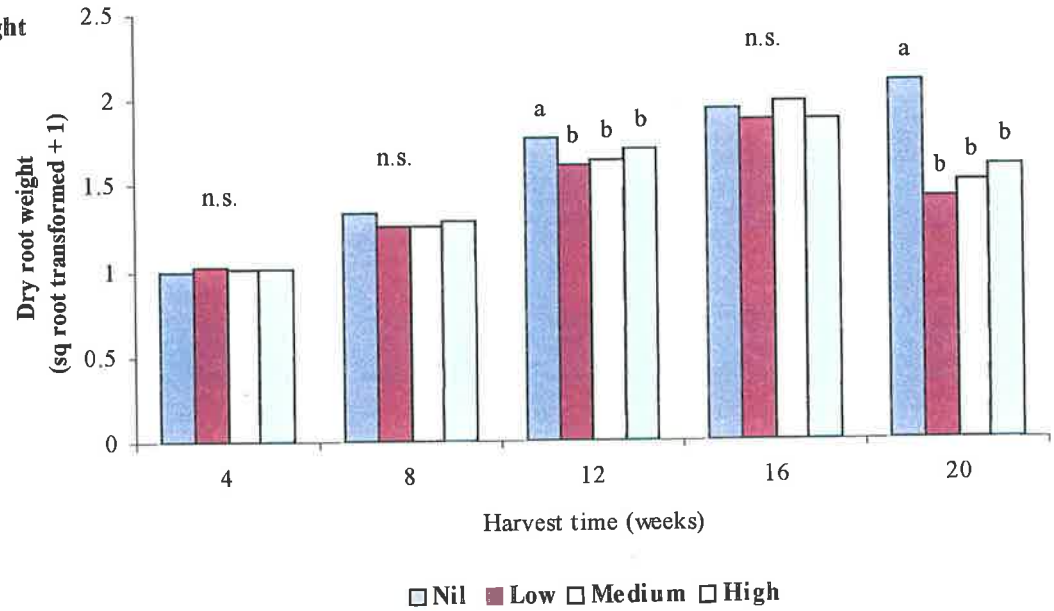
At 12 and 20 weeks, root weight was significantly reduced in the treatments with *P. neglectus* compared with the nil treatment (Figure 6.5b). At other harvest times, no significant differences in root weights were observed.

Figure 6.5 **Changes in wheat a) dry top weight and b) dry root weight (cv. Machete) inoculated with 4 levels of *P. neglectus* (nil = 0 nematodes/g; low = 1.2 nematodes/g; medium = 9.4 nematodes/ g; high = 36.5 initial soil inoculum) harvested every 4 weeks, July - December, 1997. Columns with same letters do not differ significantly within harvest times (P < 0.05); n.s. not significant.**

a) Dry top weight



b) Dry root weight



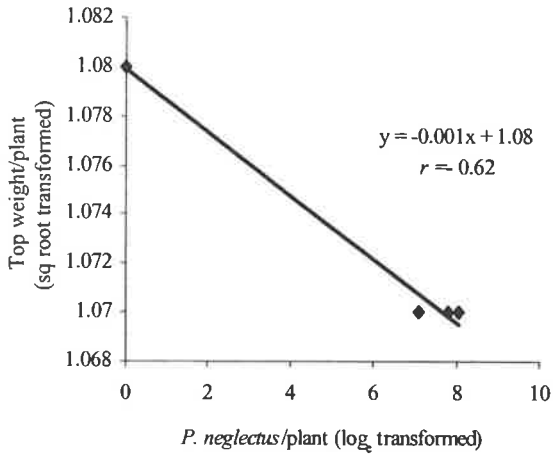
6.2.4 Regression analyses

A significant, negative relationship was observed between top dry weight and nematode density only at the 16 week harvest time. Data for nematode inoculum treatments were clumped i.e. all values for nematode inoculum treatments were clustered at one end of the regression ([4 weeks r = -0.39, 8 weeks r = -0.37, 12 weeks r = -0.76, 16 weeks r = -0.97, 20 weeks r = -0.21] as derived by calculating r value against tabular r value of 0.95 (n-2 = 2 d.f.; $P < 0.05$) (Gomez and Gomez 1984). Graphs of the regression of plant top weight against density of *P. neglectus* are presented in Figure 6.6.

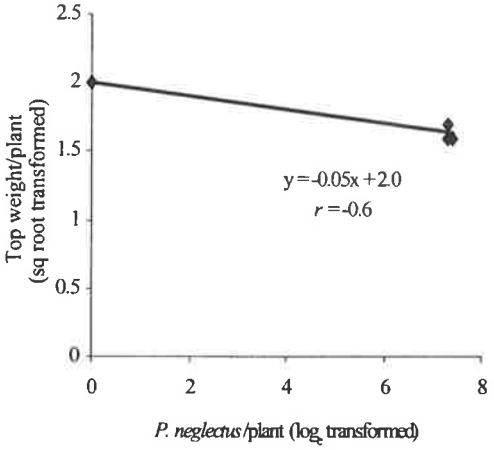
There were significant, negative relationships between root weight and nematode density at 8 weeks and 20 week harvest times although points were clumped ([4 weeks r = -0.25; 8 weeks r = -0.94, 12 weeks r = -0.86; 16 weeks r = -0.37, 20 weeks r = -0.91]. Graphs of these regressions are presented in Figure 6.7.

Figure 6.6 **Relationship between density of *P. neglectus* and top dry weight of wheat (cv. Machete) harvested every 4 weeks, July to December, 1997 n = 5.**

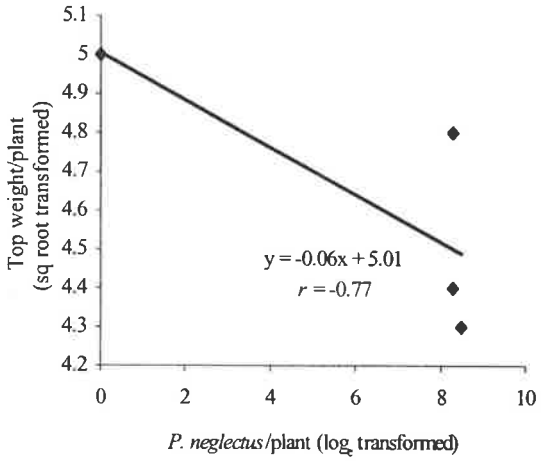
a) 4 weeks



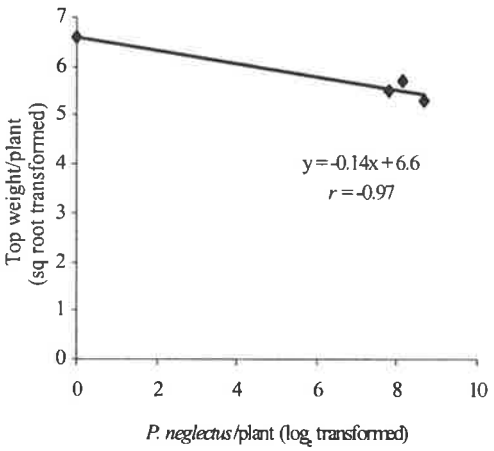
b) 8 weeks



c) 12 weeks



d) 16 weeks



e) 20 weeks

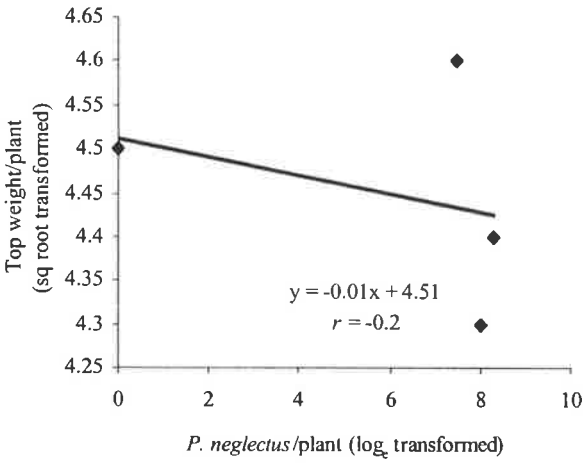
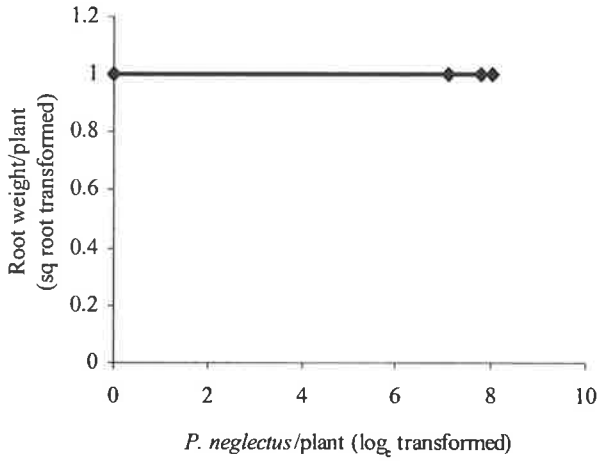
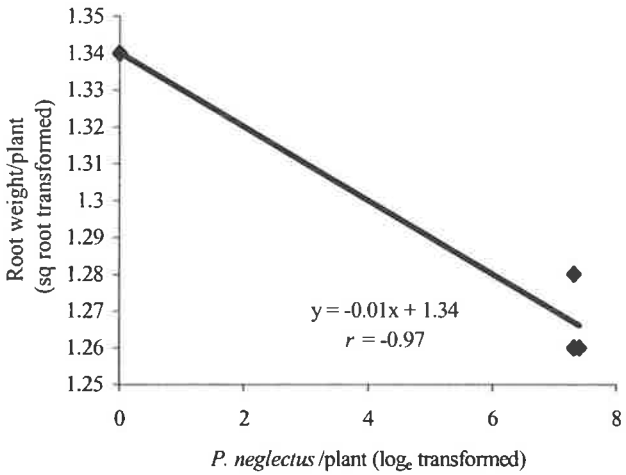


Figure 6.7 **Relationship between density of *P. neglectus* and root dry weight of wheat (cv. Machete) harvested every 4 weeks, July - December, 1997.**

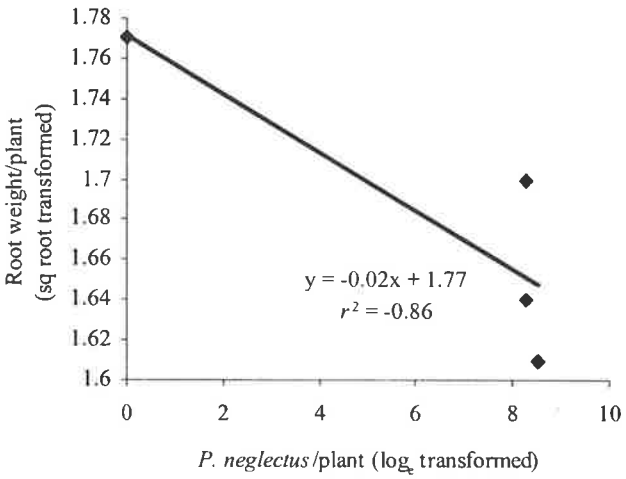
a) 4 weeks



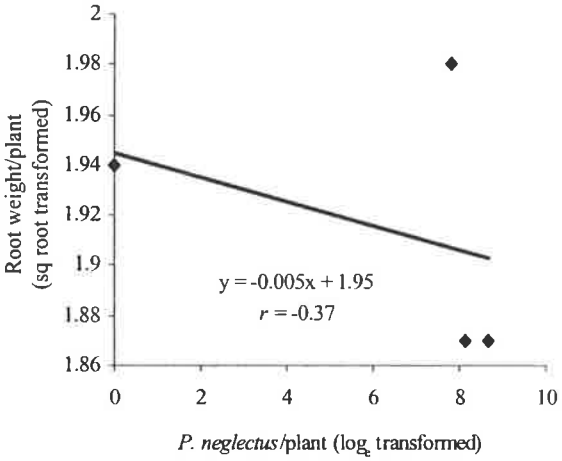
b) 8 weeks



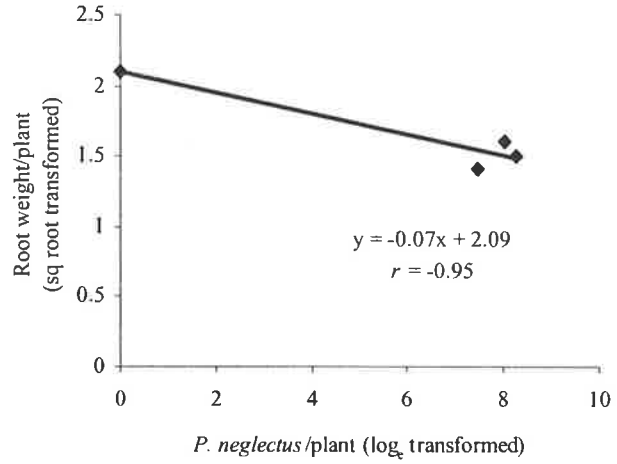
c) 12 weeks



d) 16 weeks



e) 20 weeks



6.2.5 Rainfall, soil temperature and air temperature

Rainfall was highest during the period prior to the 8 week harvest (117 mm) and decreased in the following harvest periods (Figure 6.8). The total rainfall/supplementary irrigation recorded over the 20 week period was 339 mm.

Soil and air temperatures (both day and night) increased over the 20 week assessment and are presented in Figure 6.9. Maximum soil (day) temperature peaked at 34.5°C in the 20 week assessment period (November) with an average of 25.3°C. Maximum night temperature in soil peaked at 9.7°C (average of 18.9°C) during the same period. Lowest soil temperature was recorded in the 8 week assessment period (August) with an average of 7.5°C and a minimum of 4.3°C. Air temperatures were high in September, October and November (averages of 25°C, 32°C and 36°C respectively) peaking at 54.6°C in November. The minimum average air temperature of 6.0°C was recorded in July, and the lowest air temperature was 2.1°C.

Figure 6.8 **Total rainfall/irrigation recorded at each harvest period in an outdoor experiment assessing population dynamics of *P. neglectus* within wheat roots (cv. Machete). Total rainfall = total rainfall plus irrigation.**

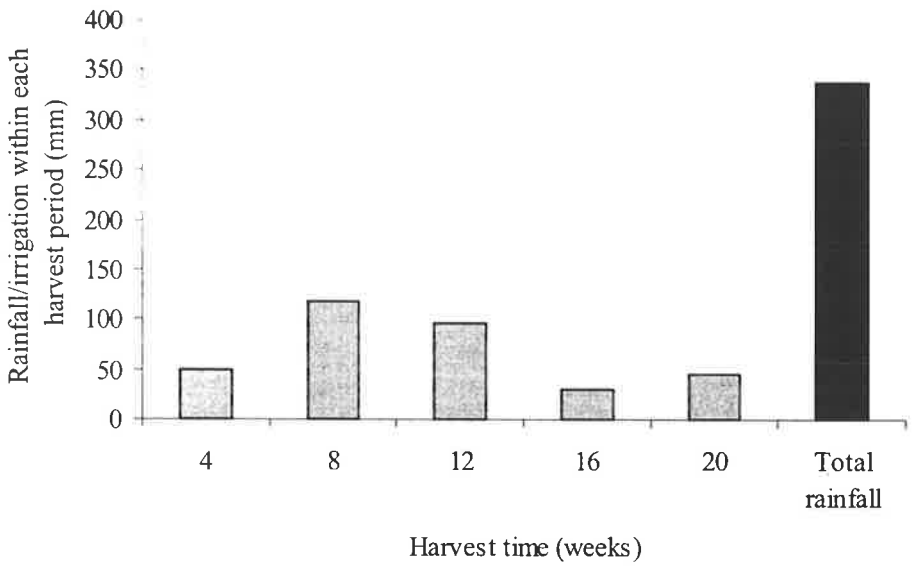
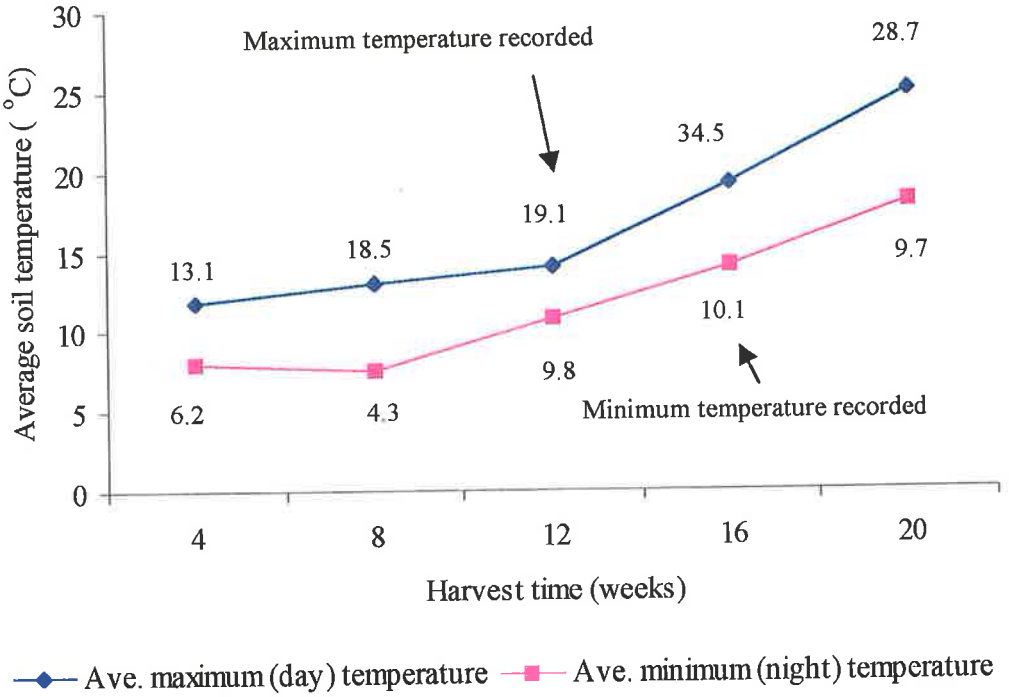
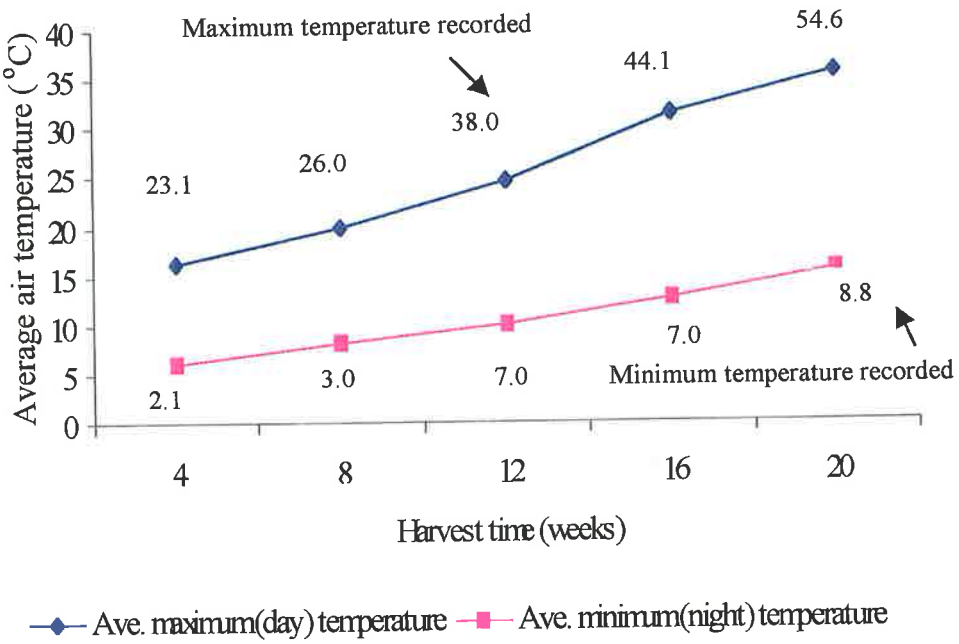


Figure 6.9 **Average temperature in a) soil (measured at a depth of 10 cm in 25 cm diameter pots) and b) air for each harvest period in outdoor experiment assessing population dynamics of *P. neglectus* within wheat roots (cv. Machete). Points represent average temperature and figures represent maximum and minimum temperatures recorded within each time period.**

a) Soil temperature



b) Air temperature



6.3 Discussion

The results presented show that *P. neglectus* can cause a reduction in wheat plant growth in the cultivar Machete, in the absence of other pathogens. From this pot study, no prediction could be made however, on the effect of differing initial nematode densities on nematode multiplication rates or yield loss. While significant differences were observed between initial density of *P. neglectus* in the soil inoculum at low, medium and high treatments, in general, this difference was not seen in number of *P. neglectus* observed within plant roots or in plant growth responses over the course of the experiment. It seems either that the number measured by the Whitehead tray method was an underestimation of the actual density of *P. neglectus*/g of dry soil in the initial soil inoculum, or the low inoculum treatment (1.2 *P. neglectus*/g of dry soil) was sufficient to cause damage to Machete wheat.

Both factors may have contributed to the lack of difference in plant response to the low compared to high nematode inoculum treatments. Problems may have occurred with the determination of number of nematodes in the soil inoculum, as the addition of carrot culture pieces infected with *P. neglectus* to soil may have resulted in the continued emergence of nematodes from the inoculum source after the original assessment, so the actual number of *P. neglectus*/g of soil in all inoculum treatments may have been underestimated. The lack of statistical significance between nematode inoculum treatments and damage seen at all inoculum densities may indicate that the low treatment (1.2 *P. neglectus*/g of soil) was above the tolerance limit for Machete wheat and was therefore sufficient to result in reduction of both root and shoot weight in this host. A yield loss of 15% was observed in Machete wheat in field experiments (estimated by use of nematicide) at initial levels as low as 1.0/g of soil (Chapters 7 and 8), suggesting that this host has a low tolerance limit to *P. neglectus*. In addition, there was no stimulatory effect of the low nematode rate on plant growth in this pot experiment. This response results from plant growth compensating for nematode attack (Olthof and Potter 1973) and was recorded with *P. thornei* on Machete, in a similar South Australian study, at levels of less than 3000 *P. thornei*/plant (Nicol 1996). In the results presented, initial densities in the low treatment were 3000 *P. neglectus*/plant and may have already been above a stimulatory level. In addition, competition has been observed with high initial nematode densities. Seinhorst (1967a) showed that for sedentary nematodes, the higher the initial density the smaller the proportion of juveniles that penetrated roots and became adults.

As a result of these factors, conclusions about the possible effects of differing initial nematode inoculum density on the population dynamics of *P. neglectus* are few. However, observations on changes in *P. neglectus* density over time and the overall effect of *P. neglectus* on plant growth can be made.

Significant, negative relationships between *P. neglectus* density and top weight at 8 and 16 weeks (Figure 6.6b and 6.6d) and root weight at 8 weeks (Figure 6.7) were observed using simple linear regression. In all cases, the distribution of data was uneven, due to the low numbers of data points and insufficient differences observed between low, medium and high treatments. Interpretation of the exact relationship between *P. neglectus* density and plant dry weight is therefore not possible, as further data points within a larger range of nematode densities would be required.

While the cluster of the data points for nematode treatments make defining a significant causal relationship between nematode density and yield difficult, the data do indicate an effect of *P. neglectus* on plant growth. This is evidenced by treatments inoculated with nematodes having lower top or root weights at all harvest times compared with the nil treatment, and suggests that at these nematode levels, the reduction in root and shoot weight caused by *P. neglectus* began very soon after invasion and continued to affect plant growth throughout the experiment. Although the inability to differentiate between nematode treatments prohibited the correlation of nematode density with plant growth, all *P. neglectus* levels were sufficient to cause a reduction in top and root weight compared with the nil treatment.

No difference between top weights in the various treatments was observed at the final assessment, probably as a result of the loss of grain (due to damage by birds). If grain had been harvested, it is expected the differences in top weight observed at 12 and 16 weeks would have been borne out in grain yield.

For nematode density, data were presented in both $\log_e$ transformed and untransformed forms, as relationships between final nematode density and initial density are obscured where transformed data were used. While the use of transformation stabilises variance within data sets, it has been observed that transformed data may be misinterpreted as the procedure can eliminate major effects (Wallace 1973; Vanderplank 1975). In untransformed data from this study, a large reduction was seen in *P. neglectus* from roots at 8 week and 20 assessments

compared with 12 and 16 weeks. This reduction was more difficult to observe using the $\log_e$ transformed data. Similar difficulties were seen with comparisons between final density of *P. neglectus* and initial density where relationships were obscured using transformed data.

The initial density of *P. neglectus* was designated as the number of nematodes per plant at the 4 week assessment. This was necessary because of the inconsistency observed between inoculum treatments in soil compared with the 4 week plant assessment. More importantly, there was difficulty in interpreting data for multiplication rates calculated using numbers per plant root system (Pf) divided by numbers per gram of soil (Pi), but initial density derived from the 4 week assessment allowed comparable assessment of levels per plant across the harvest periods. The 4 week assessment of numbers per plant should have given a close approximation of numbers at invasion, as limited multiplication would have occurred, given that the approximate life cycle of *P. neglectus* is 6 - 9 weeks (Section 2.2). Soil and air temperatures were also low in this 4 week period, limiting multiplication in this period. In addition, high levels of *P. neglectus* were recovered from the soil at 4 weeks, suggesting invasion could still have been occurring.

There was an overall trend (although non-significant) for the low nematode treatment to have the highest multiplication rate. While this experiment aimed to provide a range of initial *P. neglectus* densities, no significant differences were observed in multiplication rates of nematodes recovered from plants from low, medium and high inoculum rates. The trend for the highest multiplication rate to occur with the lowest initial density is in agreement with the bulk of research conducted on population dynamics of plant parasitic nematodes (e.g. Seinhorst 1970; Fisher and Hancock 1991; Wallace *et al.* 1995; Abdel-Momen and Starr 1997; Nicol *et al.* 1999).

Seinhorst (1970) suggested that final densities first increase then decrease with increasing initial density. This characteristic of nematode population dynamics was not observed in this experiment, as a sufficient range of initial densities was not achieved. At all harvest times however, the relationship between final and initial density levelled off (commencing at the low inoculum rate), fitting the curve proposed by Seinhorst (1967a; 1967b).

Lowest numbers of *P. neglectus* within plant roots were observed at the 8 and 20 week assessment times. Although at 8 weeks, there appeared to be little reason for the decline in nematode levels, in other studies where levels of nematodes were measured in soil rather than

roots, fluctuations in nematode density have been associated with seasonal effects or migration of nematodes from soil to roots when a host is present (Johnson *et al.* 1974; Plowright *et al.* 1990; Wallace *et al.* 1995). In the perennial crop, raspberry, Vrain *et al.* (1997) assessed *P. penetrans* levels in roots every two weeks over a 12 month period and established trends within seasons. They observed significant fluctuations in numbers, often between two week sampling periods, which did not appear to be associated with seasonal conditions or soil temperature.

In this study, the decline in *P. neglectus* at 8 weeks may have been due to a combination of both environmental and density dependent factors. Firstly, rainfall was highest during the period to 8 weeks. Drought stress has been implicated in increased nematode multiplication (Duncan and el-Morshedy 1996) and it is possible that high levels of moisture in this period suppressed nematode multiplication. The effect of seasonal conditions on the multiplication of *P. neglectus* under different field crops is further discussed in Chapter 7. Secondly, competition effects may have occurred after initial invasion at 4 weeks, i.e. the equilibrium density in this host was reached resulting in overpopulation (Seinhorst 1970).

Maximum numbers of *P. neglectus* in plant roots and maximum multiplication rates were seen at the 12 and 16 week assessments and corresponded with increases in average day and night soil and air temperatures coupled with significant growth of the wheat plant. In *Pratylenchus* spp., egg production has been shown to be highly correlated with temperature. For *P. penetrans*, greater numbers of eggs were produced at higher temperatures (up to 30°C) (Mizukubo and Adachi 1997) and for *P. thornei*, temperatures of 25°C compared with 20°C have been shown to increase multiplication rate of *P. thornei* either by directly favouring nematode reproduction or as a result of reduced vigour of the plant host (Nicol 1996; Castillo *et al.* 1995). Temperature would also have had a significant effect on the multiplication rate of *P. neglectus*. In addition, Seinhorst (1970) recorded a 10-fold increase in the number of *P. penetrans* in actively growing plants compared with dead or senescing plants, a response supported by these data.

At 20 weeks, lower numbers of *P. neglectus* were associated with higher temperatures and plant senescence. There was a small corresponding increase in soil levels of nematodes, indicating some migration from roots to soil. Assuming the 20 week harvest represented the final nematode density of *P. neglectus* in this experiment, the relationship between Pf and Pi (taken at

4 weeks) shows a reduction in nematode densities. As this occurred at all inoculum rates in this study, it further suggests the low inoculum density was sufficient to cause damage in Machete wheat and subsequent reduction in *P. neglectus* numbers. Levels of nematodes remaining in roots of the susceptible but intolerant host (cv. Machete) at the final assessment (plant senescence) were equivalent to initial levels. This effect has been observed in similar studies where overpopulation occurs (Seinhorst 1970).

In this study, although this host plant did not actually support an increase in *P. neglectus* levels at any inoculum density, sufficient densities would remain in the soil to infect a crop in the following year, and cause damage if the crop was intolerant. Initial densities of 1 - 3 *P. neglectus*/g of soil have been shown to result in yield loss (Chapter 8; Vanstone *et al.* 1998).

For most plant-parasitic nematodes, but particularly with multi-generational species such as *Pratylenchus*, multiplication rates and yield loss depend on factors affecting plant growth and therefore food source e.g. cultivar tolerance, moisture and nutrient availability, temperature and day length (Barker and Olthof 1976). Barker *et al.* (1981) suggest that for less damaging nematodes to affect the host, plants must be under environmental stress. Within the study presented in this chapter, simulated field conditions were established (i.e. uneven rainfall and fluctuating soil and air temperatures), to mimic environmental stresses on the plant host. Under these conditions, *P. neglectus* reduced both top and root weight of the wheat host even at the lowest nematode density (1.2 *P. neglectus*/g of soil). Further investigation of threshold densities and yield loss caused by *P. neglectus* in field experiments is described in Chapter 8.

Chapter 7 Resistance of cereal, pulse, oilseed and pasture varieties to *Pratylenchus neglectus* in field experiments

7.0 Introduction

Awareness of the field performance of varieties with regard to resistance to *P. neglectus* assists in both recommendations to growers and in understanding the dynamics of nematode populations. For growers, the definition of resistance categories for individual varieties is essential in developing an integrated approach to managing root disease in cropping rotations.

The multiplication rate of plant parasitic nematodes under field conditions in both susceptible and resistant plants will be affected by many factors. Edaphic factors such as soil moisture (Duncan and el-Morshedy 1996), texture (Delaville *et al.* 1996), nutrient status (Lopez *et al.* 1997), and temperature (Mani and Hinai 1997) affect both nematode and host. Synergistic (Taheri 1996; Walker 1997) and antagonistic (Wenefrida *et al.* 1998) soil biota may alter the extent or severity of damage and/or multiplication rate of nematodes within a host. Initial densities of plant-parasitic nematodes also affect multiplication rate, with lower initial densities resulting in higher multiplication (Fisher and Hancock 1991; Seinhorst 1966). Multiplication rate is therefore usually estimated in greenhouse experiments where temperature, moisture and soil are more easily controlled. Validation of greenhouse tests with field experiments is essential however, to better understand how nematode densities may change under different environmental conditions (Nombela and Romero 1999).

In addition to multiplication within a host, the number of nematodes remaining after a crop may also be affected by antagonistic or synergistic conditions within the plant and/or soil environment. For example, brassica crops such as canola have been associated with a reduction in number of *Meloidogyne* in soil (Johnson *et al.* 1992; Motjahedi *et al.* 1993). This reduction is believed to be the result of the nematicidal properties of isothiocyanates which are released as breakdown products from brassica tissue (Underhill 1980). Isothiocyanates have been shown to be effective in reducing densities of *P. penetrans* (McFadden *et al.* 1992) and *P. neglectus* (Potter *et al.* 1998).

Although resistance to root lesion nematodes is often compared between varieties of one crop species, few comparative studies within and between crops in field experiments have been conducted. In these experiments, two field trials were established to enable comparison of *P. neglectus* multiplication in 81 varieties of 12 crop and pasture species. The possible suppressive effects of canola on number of *P. neglectus* was also assessed. A field trial was also established to compare multiplication in 14 varieties from 4 different cereal species. These trials also contained nematicide (aldicarb) treatments and were used to assess yield loss in these crops and varieties, the results of which are presented in Chapter 8. For resistance studies within this chapter, the degree of resistance was defined by comparison with a susceptible wheat variety (cv. Machete) and either a resistant triticale (cv. Abacus) or wheat (cv. Krichauff), previously identified in field and pot experiments (Vanstone pers. comm.; Farsi 1995) and confirmed in these studies. Both multiplication rate and final nematode density were used to determine the susceptibility or resistance of crops and cultivars.

7.1 Materials and methods

7.1.1 Field trial establishment

Field trials were established in South Australia at Sandilands in 1996 and Paskeville in 1997 on the Yorke Peninsula, and at Condada on Eyre Peninsula in 1996 (see Figure 3.1 for location of these sites). Sowing, sampling, management and harvest details for Sandilands and Paskeville are outlined in Table 7.1.

At the Sandilands site, 17 wheat (*Triticum aestivum*), 2 durum (*T. turgidum* subsp. *durum*), 8 barley (*Hordeum vulgare*), 7 oat (*Avena sativa*), 3 triticale (*Triticosecale*), 6 chickpea (*Cicer arietinum*), 9 field pea (*Pisum sativum*), 3 faba bean (*Vicia faba*), 4 vetch (*V. sativa*), 7 canola (*Brassica napus*) and 8 medic (*Medicago* spp.) varieties were sown. At Paskeville, 16 wheat, 2 durum, 8 barley, 7 oat, 2 triticale, 2 cereal rye (*Secale cereale*), 6 chickpea, 6 field pea, 3 faba bean, 5 vetch, 7 canola and 8 medic varieties were sown.

Seeding rates for each crop at Paskeville and Sandilands (seeds/m^2) were: wheat, durum, triticale, rye and oat 180; barley 165; medic 200; vetch 50; desi chickpea 50; kabuli chickpea 35; field pea 35; faba bean 22; and canola 190. The cereal, faba bean, chickpea, vetch and field pea

varieties were sown at a depth of 5 - 6 cm. Medic and canola varieties were sown at a depth of 1 cm.

At the Condada site, 8 wheat, 2 barley, 3 oat and 1 triticale variety were sown at a rate of 180 seeds/m^2 and at a depth of 5 - 6 cm.

Herbicide and insecticide application details are presented in Table 7.1.

7.1.2 Experimental design

At both Sandilands and Paskeville, all crops were sown within the one trial, but separated into blocks for herbicide management. The separate blocks were as follows:

1) wheat, barley and triticale; 2) oat; 3) faba bean and vetch; 4) canola; 5) medic; 6) field pea; 7) chickpea.

All plots were 5 m long by 0.8 m wide with 6 rows per plot. Medic plots (cv. Caliph) were sown between blocks to allow separation for herbicide management. Each field trial was set up as a Complete Randomised Block Design with four replicates.

At Condada, the trial was set up as a Complete Randomised Block Design with six replicates. Plots were 5 m long by 1.7 m wide. The trial was sown on the 17th of June, 1996 and harvested on the 15th of December, 1996.

7.1.3 *Pratylenchus neglectus* and plant assessment

Details of seeding, sampling and harvest are presented in Table 7.1. Numbers of *P. neglectus* were determined by sampling moist soil at seeding and within 4 weeks either before or after harvest (see Section 3.7.4.2). Extraction and counting are described in Sections 3.2 and 3.3. The multiplication rate of *P. neglectus* was determined by dividing the final estimate of nematodes/g of soil by initial nematodes/g of dry soil.

To determine the possible effects of biofumigation of stubble from harvested canola varieties on *P. neglectus*, all canola plots and a susceptible wheat (cv. Machete) were sampled prior to the next season's sowing in the year following trials at Sandilands and Paskeville.

Grain yield was recorded for all plots, except the medic at Sandilands and Paskeville. At the Sandilands site only, top growth of medic varieties was assessed by taking all shoot material from three, randomly selected, 30 cm by 30 cm quadrats within each plot at flowering. Plant material was dried and weighed. Yields and relationships between yield and *P. neglectus* density for all trials are presented in Chapter 8.

Table 7.1 **Planting, assessment and management details of field trials conducted at Sandilands (1996) and Paskeville (1997), Yorke Peninsula, South Australia.**

Treatment	Sandilands		Paskeville	
Sown	20.6.96	All plots	4.6.97	All plots
Fertiliser	20.6.96	N:P:K 17:19:0, Zn 5% with seed 80 kg/ha (70g/plot)	4.6.97	N:P:K 17:19:0, Zn 5% with seed 80 kg/ha (70g/plot)
Nematicide	20.6.96	Aldicarb (Temik®) with seed 2.5 kg a.i./ha (13g/plot)	4.6.97	Aldicarb (Temik®) with seed 2.5 kg a.i./ha (13g/plot)
	2.7.96	All plots (average soil moisture 16%)	11.6.97	All plots (average soil moisture 10%)
Initial sampling	17.6.96	Sprayseed® 2.0 l/ha (pre-sowing)	1.6.97	Sprayseed® 2.0 l/ha (pre-sowing)
Herbicide	27.6.96	Diuron® 550g/ha: vetch & faba bean	7.6.97	Diuron® 650g/ha: pea, chickpea, vetch & faba bean
	26.7.96	Lexone® 250g/ha: pea & lentil; Glean® 20g/ha: oat; Targa® 300ml/ha: vetch, bean, pea & chickpea; Targa® 300ml/ha+Lontrel® 150ml/ha: canola; Achieve® 400g/ha + Lontrel® 120 ml/ha + LVE; MCPA® 400ml/ha: wheat, barley & triticale	17.7.97	Achieve® 22g/ha: wheat, barley & triticale; Verdict® 320ml/ha: pea, vetch, faba bean, chickpea, medic & canola
			23.10.97	Dominex 100EC® 300ml/ha: pea, faba bean, vetch, chickpea, medic & canola
	21.8.96	Broadstrike® 25 g/ha: chickpea		
Insect control	27.6.96	Endosulfan® 500 ml/ha	28.8.97	Endosulfan® 500ml/ha - all crops; 24D amine® 1.2l/ha: wheat, barley and triticale
Final sampling	19.11.96	All plots (average soil moisture 10%)	3.12.97	All plots (average soil moisture 6%)
Harvest	16–17.12.96	All plots	15.11.97	Pea, vetch, oat & early canola plots
			20.11.97	Wheat, barley, triticale, chickpea & late canola plots
Sampling prior to following season	21.5.97	Canola/Machete wheat (average soil moisture 12%)	1.5.98	Canola/Machete wheat (average soil moisture 12%)

7.1.4 Statistical analyses

7.1.4.1 Field trials at Sandilands and Paskeville

For the assessment of *P. neglectus* in each variety, the mean of four replicates were log transformed ($\log_e$ *P. neglectus*/g of soil) to normalise the data sets and an analysis of variance was conducted ($P < 0.05$). At Sandilands and Paskeville, differences between cultivars were compared with either the susceptible wheat (cv. Machete), or the resistant triticale (cv. Abacus), using Fisher's protected l.s.d. Cultivars were rated as susceptible (mean not significantly different from Machete and significantly > Abacus); moderate (mean not significantly different from Machete or Abacus); or resistant (mean not significantly different from Abacus and significantly < Machete).

In addition, to assess comparative differences between cultivars in both trials, data were analysed using the spatial techniques of Cullis and Gleeson (1991) and Gilmour *et al.* (1997). This analysis allows for edaphic differences between sites. The following models were compared:

$$Log_e \textit{ (multiplication rate)} = \textit{block} + \textit{mean} + \textit{cultivar}$$

$$Log_e \textit{ (final P. neglectus density/g of soil)} = \textit{block} + \textit{mean} + \textit{cultivar.}$$

The block factor was used to extract field variation due to trends within each trial and was a combination of row and/or column effects. The cultivar factor was fitted as a random effect to generate a genetic variance for each trial. Correlation between study sites determined the similarity of cultivars using the following model:

$$Log_e \textit{ (final P. neglectus/g soil or multiplication rate)} = \textit{block} + \textit{site} + \textit{site:cultivar.}$$

Analysis between crop species was determined using the model:

$$Log_e \textit{ (final P. neglectus/g soil or multiplication rate)} = \textit{block} + \textit{mean} + \textit{crop type.}$$

Cultivars were ranked against the site mean for each trial and an overall "common" ranking for each cultivar calculated from the between site correlations (Table 7.8).

7.1.4.2 Field trial at Condada

For the assessment of *P. neglectus* density for each variety, the mean of the six replicates was log transformed ($\log_e$ *P. neglectus*/g of soil) to normalise the data sets and an analysis of variance conducted ($P < 0.05$). Nematode numbers were expressed as initial number of nematodes/g of dry soil (Pi), final number/g of dry soil (Pf), or multiplication rate (Pf/Pi). Differences between varieties were compared with either the susceptible wheat (Machete), or resistant wheat (Krichauff), using a general ANOVA (Tukey test; h.s.d.). Cultivars were rated as susceptible (mean not significantly different from Machete and significantly > Krichauff); moderate (mean not significantly different from Machete or Krichauff); or resistant (mean not significantly different from Krichauff and significantly < Machete).

In many studies, where partial levels of resistance are present, the use of moderately resistant (MR) or moderately susceptible (MS) categories are common (e.g. Robbins *et al.* 1994; Thies *et al.* 1994; Alcañiz *et al.* 1996). In this study, use of the category moderate (M) was considered more appropriate than designation of either MR or MS, as final *P. neglectus* densities (and multiplication rates) differed between seasons. For example, in 1996 many cultivars were more resistant and would have been designated as moderately resistant while in 1997 these varieties would have been classified as moderately susceptible.

7.2 Results

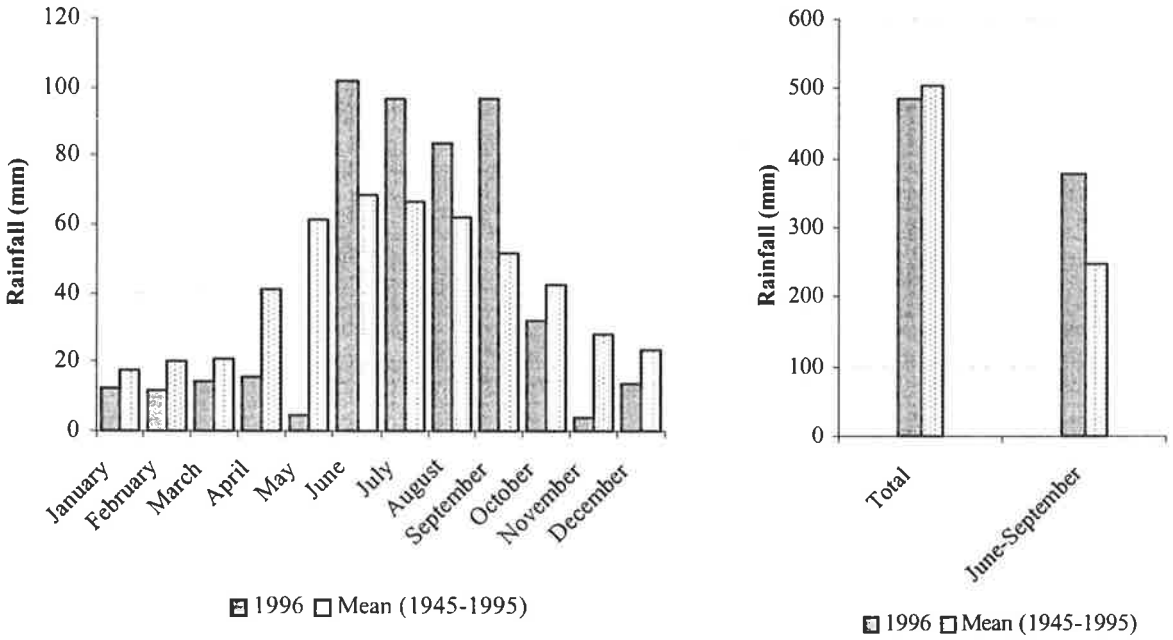
7.2.1 Sandilands, 1996

7.2.1.1 Rainfall

Rainfall for the Sandilands site is presented in Figure 7.1 and was similar to total annual rainfall for the 50 year average. However, most of the rainfall in 1996 was recorded between June and September, i.e. the active growing period of the crop, and, during this period, was 34% higher than the average.

Figure 7.1 **Seasonal and average (1945-1995) rainfall for a) Monthly rainfall b) June - September (growing period) and total annual rainfall at Sandilands, South Australia, 1996. Source: South Australian Bureau of Meteorology.**

a) Monthly rainfall **b) Growing period and total annual rainfall**



7.2.1.2 Initial density, final density and multiplication rate of *P. neglectus*

P. neglectus was identified at Sandilands. The initial and final nematode densities under cereal plots are shown in Table 7.2 and for pulse, oilseed and medic varieties in Table 7.3. Initial numbers in plots at Sandilands ranged from 0.3 *P. neglectus*/g dry soil (in Bluey field pea plots) to 2.4 *P. neglectus*/g dry soil (in Franklin barley plots) and the average initial density at this site was 1.0 *P. neglectus*/g dry soil. Final densities of *P. neglectus* ranged from 0.1 *P. neglectus*/g of soil in triticale, faba bean and field pea varieties to 2.8 *P. neglectus*/g of soil in Machete wheat and 3.2/g of soil in Dunkeld canola. The multiplication rate (Pf/Pi) of *P. neglectus* under cereal plots is shown in Figure 7.2 and under pulse, oilseed and medic plots in Figure 7.3. The multiplication rates for all crops/varieties at Sandilands were generally low and ranged from 0.1 on pea and triticale varieties to 1.7 in Desavic chickpea.

Differences were observed between both final density and the multiplication rate of *P. neglectus* in varieties within most crops, with several wheat varieties rated as resistant (cvv. Krichauff, Bowie, Worrakatta, Tatiara and Excalibur). In general, barley varieties were rated as having moderate (or intermediate) resistance, with the exception of Sloop which was susceptible. All durum and triticale varieties tested were rated as resistant. Most chickpea varieties were intermediate. All medic and vetch cultivars were rated as moderately resistant and all field pea and faba bean varieties as resistant.

At Sandilands, a high proportion of the varieties tested were identified as resistant (20 cereal, and 20 pulse, oilseed and pasture varieties) compared with only 5 susceptible cereal and 7 susceptible pulse varieties.

Table 7.2 **Initial and final soil levels of *P. neglectus* in wheat, barley, oat and triticale varieties (n = 4) at Sandilands, Yorke Peninsula, 1996. S = susceptible; M = moderate; R = resistant.**

Crop	Variety	*P. neglectus*/g dry soil detransformed		*P. neglectus*/g dry soil $\log_e$ transformed + 1		Ranking
		Initial	Final	Initial	Final	
Wheat	Barunga	0.8	0.8	0.6	0.6	M
	Beulah	1.3	1.3	0.8	0.8	M
	Bowie	1.3	0.5	0.8	0.4	R
	Buckley	1.3	1.8	0.8	1.0	S
	Excalibur	1.3	0.5	0.8	0.4	R
	Frame	0.6	1.5	0.5	0.9	S
	Krichauff	1.0	0.1	0.7	0.1	R
	Janz	0.9	1.1	0.6	0.8	M
	Machete	1.0	2.8	0.7	1.3	S
	Meering	0.5	0.6	0.4	0.5	M
	Ouyen	1.0	1.0	0.7	0.7	M
	Silverstar	1.9	1.7	1.1	1.0	S
	Spear	1.4	0.9	0.9	0.7	M
	Tatiara	1.0	0.3	0.7	0.3	R
	Trident	1.2	0.8	0.8	0.6	M
	Worrakatta	1.0	0.3	0.7	0.2	R
	Yanac	1.1	1.1	0.8	0.7	M
	Average	1.1	0.9	0.7	0.6	
Durum	Yallaroi	0.6	0.1	0.5	0.1	R
	Tamaroi	0.7	0.3	0.5	0.2	R
	Average	0.7	0.2	0.5	0.2	
Barley	Arapiles	1.2	0.2	0.8	0.2	R
	Barque	1.4	0.6	0.9	0.4	R
	Chebec	1.0	0.6	0.7	0.5	R
	Franklin	2.4	0.7	1.2	0.5	R
	Galleon	0.8	0.2	0.6	0.2	R
	Schooner	0.8	1.2	0.6	0.8	M
	Sloop	0.7	1.6	0.5	0.9	S
	Average	1.0	0.7	0.7	0.5	
Triticale	Abacus	2.1	0.1	1.2	0.1	R
	Tahara	0.8	0.1	0.6	0.1	R
	Muir	1.2	0.1	0.8	0.1	R
	Average	1.4	0.1	0.9	0.1	
Oat	Bandicoot	0.8	0.5	0.6	0.4	R
	Bettong	0.8	0.6	0.6	0.4	R
	Carrolup	1.2	0.6	0.8	0.5	M
	Echidna	1.1	0.5	0.8	0.4	R
	Euro	1.3	0.4	0.8	0.3	R
	Potoroo	1.0	0.3	0.7	0.3	R
	Average	1.0	0.5	0.7	0.4	
	l.s.d.	-	-	0.5	0.4	

Table 7.3 **Initial and final soil levels of *P. neglectus* in chickpea, field pea, faba bean, vetch, canola and medic varieties (n = 4) at Sandilands, Yorke Peninsula, 1996. S = susceptible; M = moderate; R = resistant.**

Crop	Variety	*P. neglectus*/g dry soil detransformed		*P. neglectus*/g dry soil $\log_e$ transformed + 1		Ranking
		Initial	Final	Initial	Final	
Chickpea	Amethyst	0.8	0.9	0.6	0.6	M
	Desavic	1.3	1.3	0.8	0.8	M
	Dooen	1.3	0.8	0.8	0.6	M
	Kaniva	1.2	1.6	0.8	0.9	S
	Average	1.0	1.1	0.7	0.7	
Field Pea	Alma	0.8	0.1	0.6	0.1	R
	Bluey	0.3	0.2	0.3	0.2	R
	Dundale	0.4	0.2	0.3	0.1	R
	Early Dun	1.5	0.2	0.9	0.2	R
	Glenroy	0.8	0.2	0.6	0.2	R
	Laura	0.6	0.2	0.5	0.2	R
	Average	1.0	0.2	0.5	0.2	
Faba bean	Ascot	0.6	0.1	0.5	0.1	R
	Fiord	0.7	0.1	0.5	0.1	R
	Icarus	0.7	0.2	0.5	0.1	R
	Average	0.7	0.1	0.5	0.1	
Vetch	Blanchefleur	1.4	0.3	0.9	0.3	R
	Languedoc	1.6	0.5	1.0	0.4	R
	Popany	1.0	0.5	0.7	0.4	R
	Average	1.2	0.4	0.9	0.4	
Canola	Dunkeld	0.9	3.2	0.6	1.4	S
	Hyola 42	0.6	1.5	0.5	0.9	S
	Karoo	1.1	1.6	0.7	0.9	S
	Narendra	0.6	1.2	0.5	0.8	M
	Monty	1.3	1.6	0.8	0.9	S
	Oscar	0.6	1.6	0.5	0.9	S
	Rainbow	1.0	1.7	0.7	1.0	S
	Average	0.9	1.8	0.6	1.0	
Medic	Caliph	0.9	0.1	0.6	0.1	R
	Harbinger	1.6	0.5	1.0	0.4	R
	Herald	0.9	0.6	0.6	0.4	R
	Mogul	1.4	0.5	0.9	0.4	R
	Parabinga	1.4	0.4	0.9	0.3	R
	Paraggio	0.9	0.2	0.6	0.2	R
	Santiago	0.7	0.3	0.5	0.2	R
	Sava	0.7	0.2	0.5	0.2	R
	Average	1.1	0.4	0.7	0.3	
	l.s.d.	-	-	0.5	0.4	

Figure 7.2 **Multiplication of *P. neglectus* in wheat (w), barley (b), oat (o), durum (d) and triticale (t) varieties, Sandilands, South Australia, 1996. S = susceptible (*P. neglectus* multiplication not significantly different from Machete and > than Abacus); M = moderate (*P. neglectus* multiplication not significantly different from either Machete or Abacus); R = resistant (*P. neglectus* multiplication not significantly different from Abacus and < Machete). Varieties ranked in order of "Common" ranking shown in Table 7.8. = Machete wheat and Abacus triticale controls.**

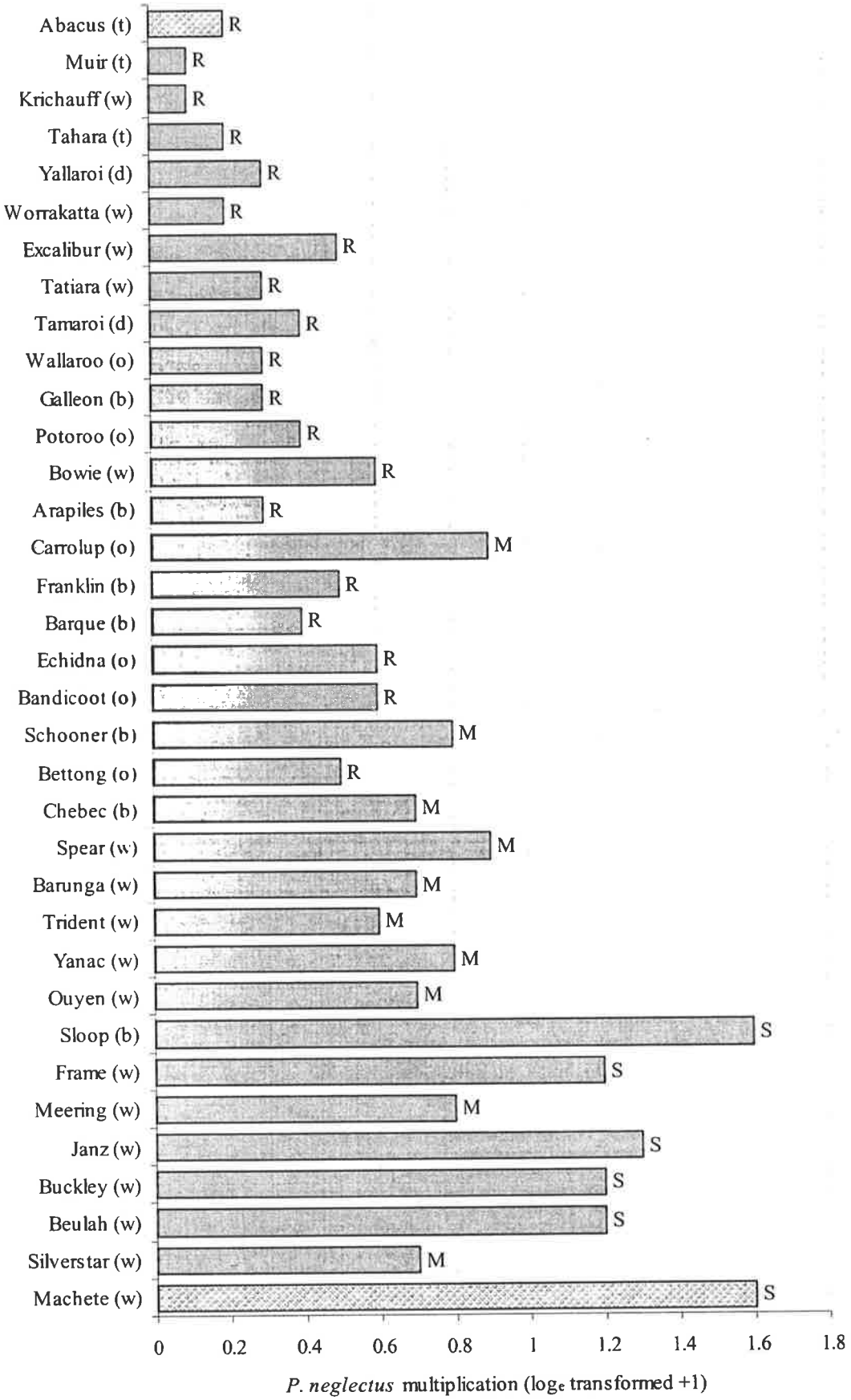

Abacus (t) R
Muir (t) R
Krichauff (w) R
Tahara (t) R
Yallaroi (d) R
Worrakatta (w) R
Excalibur (w) R
Tatiara (w) R
Tamaroi (d) R
Wallaroo (o) R
Galleon (b) R
Potoroo (o) R
Bowie (w) R
Arapiles (b) R
Carrolup (o) M
Franklin (b) R
Barque (b) R
Echidna (o) R
Bandicoot (o) R
Schooner (b) M
Bettong (o) R
Chebec (b) M
Spear (w) M
Barunga (w) M
Trident (w) M
Yanac (w) M
Ouyen (w) M
Sloop (b) S
Frame (w) S
Meering (w) M
Janz (w) S
Buckley (w) S
Beulah (w) S
Silverstar (w) M
Machete (w) S
0 0.2 0.4 0.6 0.8 1 1.2 1.4 1.6 1.8
P. neglectus multiplication (log_e transformed +1)

Figure 7.3 **Multiplication of *P. neglectus* in chickpea (ch), faba bean (f), vetch (v), field pea (p), canola (c) and medic (m) varieties at Sandilands, South Australia, 1996. S = susceptible (*P. neglectus* multiplication not significantly different from Machete and > than Abacus); M = moderate (*P. neglectus* multiplication not significantly different from either Machete or Abacus); R = resistant (*P. neglectus* multiplication not significantly different from Abacus and < Machete). Varieties ranked in order of "Common" ranking shown in Table 7.8. ☐ = Machete wheat and Abacus triticale controls.**


Abacus (t) R
Early Dun (p) R
Alma (p) R
Bonzer (p) R
Fiord (f) R
Icarus (f) R
Dundale (p) R
Ascot (f) R
Caliph (m) R
Harbinger (m) R
Paraggio (m) R
Santiago (m) R
Bluey (p) R
Laura (p) R
Glenroy (p) R
Blanchefleur (v) R
Sava (m) R
Parabinga (m) R
Mogul (m) R
Herald (m) R
Popany (v) R
Languedoc (v) R
Dooen (ch) R
Monty (c) M
Amethyst (ch) M
Oscar (c) S
Karoo (c) M
Kaniva (ch) M
Dunkeld (c) S
Rainbow (c) S
Hyola 42 (c) S
Narendra (c) S
Machete (w) S
Desavic (ch) S
0 0.2 0.4 0.6 0.8 1 1.2 1.4 1.6 1.8
P. neglectus multiplication ($\log_e$ transformed +1)

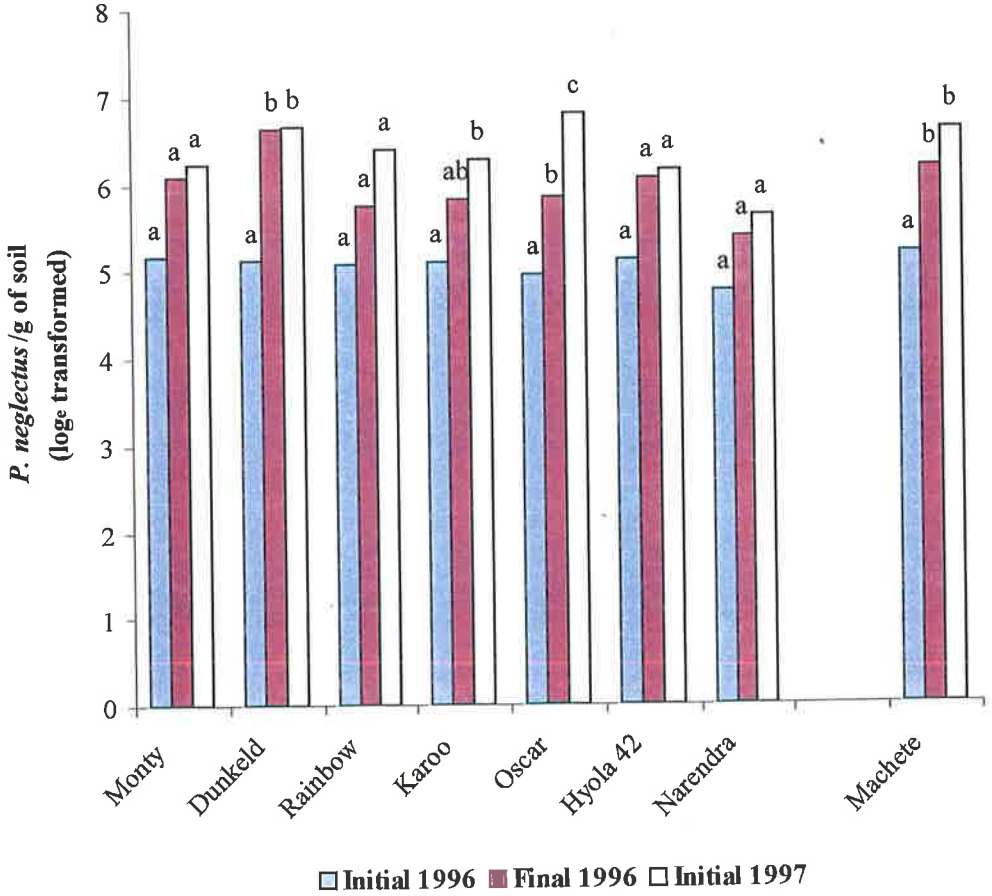
7.2.1.3 *Pratylenchus neglectus* density in canola plots

The ANOVA of results comparing numbers of *P. neglectus*/g of soil in canola varieties at sowing and harvest in 1996 and prior to sowing in 1997 are presented in Table 7.4. *P. neglectus* numbers/g of dry soil are shown in Figure 7.5. There was no significant difference in nematode numbers between any of the canola varieties and the susceptible control, Machete wheat, at any sampling time. The final number of *P. neglectus*/g of dry soil at harvest (compared with initial numbers/g of dry soil) increased for the varieties Monty, Dunkeld, Karoo, Hyola 42 and Oscar.

Table 7.4 The ANOVA of the results for difference between numbers of *P. neglectus* under canola and Machete wheat plots at initial sampling in June 1996, final sampling in December 1996 and initial sampling in May 1997, Sandilands variety trial, 1996/1997. * significant (P < 0.05).

Variety		d.f.	m.s.	P
Monty	Rep.	3	0.669	0.268
	Sampling time	2	1.362	0.053*
	Rep.*Sampling	3	0.230	
Dunkeld	Rep.	3	0.681	0.391
	Sampling time	2	3.059	0.047*
	Rep.*Sampling	5	0.574	
Rainbow	Rep.	3	0.522	0.356
	Sampling time	2	1.756	0.067
	Rep.*Sampling	6	0.400	
Karoo	Rep.	3	0.890	0.026
	Sampling time	2	1.361	0.013*
	Rep.*Sampling	6	0.148	
Oscar	Rep.	3	1.026	0.002
	Sampling time	2	3.385	0.0001*
	Rep.*Sampling	6	0.059	
Hyola 42	Rep.	3	0.139	0.733
	Sampling time	2	1.311	0.044*
	Rep.*Sampling	6	0.317	
Narendra	Rep.	3	0.623	0.230
	Sampling time	2	0.791	0.171
	Rep.*Sampling	4	0.308	
Machete	Rep.	3	0.277	0.484
	Sampling time	2	2.057	0.028*
	Rep.*Sampling	6	0.300	

Figure 7.4 **Numbers of *P. neglectus*/g of dry soil from plots of canola and Machete wheat control, Sandilands variety trial 1996/1997. Plots were sampled at sowing (Initial 1996), at harvest (Final 1996) and prior to the next season's crop (Initial 1997). Within each variety, columns with the same letters do not differ significantly (P < 0.05).**



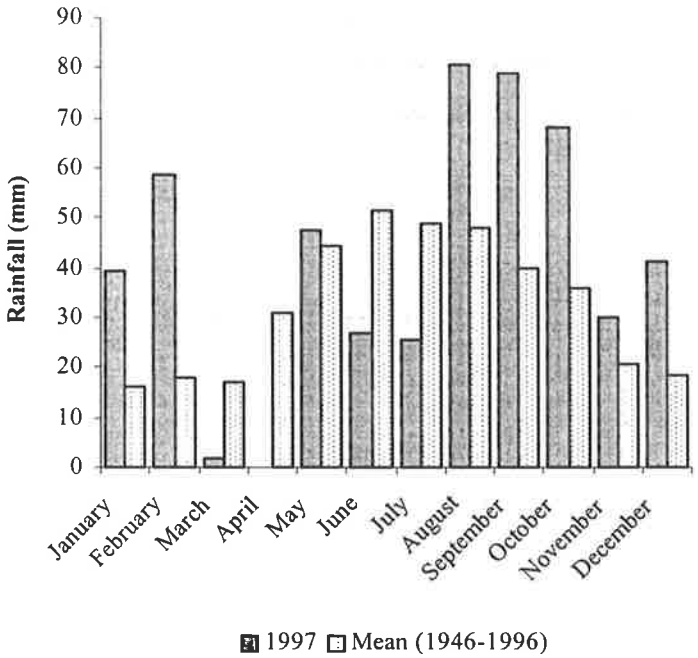
7.2.2 Paskeville, 1997

7.2.2.1 Rainfall

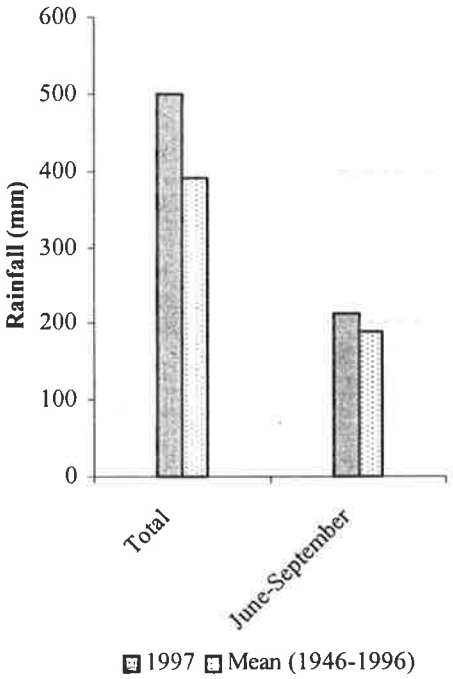
Rainfall for the Paskeville site is presented in Figure 7.5 and was above average (based on data from the previous 50 years for Paskeville) but lower than that recorded at Sandilands in 1996 (Figure 7.1). During the June - September growing period of the field trial, rainfall was 44% lower than during the same period at Sandilands in the previous season.

Figure 7.5 **Seasonal and average (1946-1996) rainfall for a) Monthly and b) June - September (growing period) and total annual rainfall at Paskeville, South Australia, 1997. Source: South Australian Bureau of Meteorology.**

a) Monthly rainfall



a) Growing period and total annual rainfall



7.2.2.2 Initial density, final density and multiplication rate of *P. neglectus*

P. neglectus was identified at this site. Initial and final nematode density at Paskeville for cereals are shown in Table 7.5 and for pulses in Table 7.6. Initial numbers were less variable than at Sandilands (Tables 7.2 and 7.3) and ranged from 0.3 *P. neglectus*/g in several varieties to 0.9 *P. neglectus*/g in Bowie wheat and Marloo oat. The average initial density at Paskeville was 0.5 *P. neglectus*/g of dry soil.

Final numbers of *P. neglectus* were higher than at Sandilands for most varieties. The highest final density was 3.0 *P. neglectus*/g of soil recorded for Silverstar wheat, and the lowest final density was 0.2 *P. neglectus*/g for triticale, rye and field pea varieties. The multiplication rate (Pf/Pi) of *P. neglectus* under cereal plots is shown in Figure 7.6 and under pulse, oilseed and medic plots in Figure 7.7. At Paskeville, the multiplication rates under all crops/varieties was

also generally higher than that at Sandilands, and ranged from 0.4 in field pea and triticale varieties to 2.5 in Silverstar wheat.

At Paskeville, more susceptible varieties were recorded than at Sandilands (19 cereal and 7 pulse and oilseed varieties). Eleven cereal and 18 pulse, oilseed and pasture varieties were identified as resistant.

Table 7.5 **Initial and final soil levels of *P. neglectus* in wheat, barley, oat and triticale varieties (n = 4), Paskeville variety trial, 1997. S = susceptible; M = moderate; R = resistant.**

Crop	Variety	*P. neglectus*/g dry soil detransformed		*P. neglectus*/g dry soil $\log_e$ transformed + 1		Ranking
		Initial	Final	Initial	Final	
Wheat	Barunga	0.5	1.4	0.4	0.9	S
	Beulah	0.5	2.5	0.4	1.3	S
	Bowie	0.9	1.1	0.6	0.1	R
	Carnamah	0.4	1.5	0.3	0.9	S
	Cascades	0.4	0.6	0.3	0.5	R
	Excalibur	0.5	1.0	0.4	0.7	M
	Frame	0.3	1.9	0.3	1.1	S
	Goldmark	0.3	1.4	0.3	0.9	S
	Krichauff	0.4	0.7	0.3	0.5	R
	Janz	0.7	1.9	0.5	1.1	S
	Machete	0.4	2.6	0.3	1.3	S
	Meering	0.4	2.2	0.3	1.2	S
	Ouyen	0.5	1.2	0.4	0.8	M
	Silverstar	0.4	3.0	0.3	1.4	S
	Spear	0.5	1.6	0.4	0.9	S
	Trident	0.4	1.4	0.3	0.9	S
	Worrakatta	0.5	0.7	0.4	0.5	R
	Yanac	0.6	2.3	0.5	1.2	S
	Average	0.5	1.6	0.4	0.9	
Durum	Yallaroi	0.4	0.7	0.3	0.5	R
	Tamaroi	0.5	0.5	0.4	0.4	R
	Average	0.5	0.6	0.4	0.5	
Barley	Arapiles	0.5	1.1	0.4	0.7	M
	Barque	0.4	1.5	0.3	0.9	S
	Chebec	0.3	1.5	0.3	0.9	S
	Franklin	0.4	1.5	0.3	0.9	S
	Galleon	0.6	1.2	0.5	0.8	M
	Mundah	0.7	1.6	0.5	0.9	S
	Schooner	0.5	0.7	0.4	0.5	R
	Sloop	0.6	1.4	0.5	0.9	S
	Average	0.5	1.3	0.4	0.8	
Triticale	Abacus	0.4	0.3	0.3	0.2	R
	Tahara	0.4	0.2	0.3	0.2	R
	Average	0.4	0.2	0.3	0.2	
Rye	Bevy	0.4	0.2	0.3	0.2	R
	SA Rye	0.4	0.2	0.3	0.2	R
	Average	0.4	0.2	0.3	0.2	
Oat	Bettong	0.4	1.3	0.3	0.8	M
	Carrolup	0.7	1.2	0.5	0.8	M
	Echidna	0.6	2.1	0.5	1.1	S
	Marloo	0.9	1.0	0.6	0.7	M
	Pallinup	0.5	1.9	0.4	1.1	S
	Potoroo	0.4	1.3	0.3	0.8	M
	Average	0.6	1.3	0.4	0.8	
	l.s.d.	-	-	0.5	0.5	

Table 7.6 **Initial and final soil levels of *P. neglectus* in chickpea, field pea, faba bean, vetch, canola and medic varieties (n = 4), Paskeville variety trial, 1997. S = susceptible; M = moderate; R = resistant.**

Crop	Variety	*P. neglectus*/g dry soil detransformed		*P. neglectus*/g dry soil $\log_e$ transformed + 1		Ranking
		Initial	Final	Initial	Final	
Chickpea	Amethyst	0.4	0.9	0.3	0.6	R
	Desavic	0.3	2.1	0.3	1.1	S
	Dooen	0.7	1.8	0.5	1.0	S
	Kaniva	0.8	1.6	0.6	1.0	S
	Lasseter	0.5	1.6	0.4	0.9	S
	Tyson	0.5	1.8	0.4	1.0	S
	Average	0.5	1.6	0.4	0.9	
Field Pea	Alma	0.4	0.2	0.3	0.2	R
	Bluey	0.7	0.2	0.5	0.2	R
	Dundale	0.4	0.2	0.3	0.2	R
	Average	0.5	0.2	0.4	0.2	
Faba bean	Ascot	0.6	0.5	0.5	0.4	R
	Fiord	0.5	0.4	0.4	0.4	R
	Icarus	0.5	0.5	0.4	0.4	R
	Average	0.6	0.5	0.4	0.4	
Vetch	Blanchefleur	0.6	1.2	0.5	0.8	M
	Languedoc	0.6	1.9	0.5	1.1	S
	Average	0.6	1.6	0.5	1.0	
Canola	Dunkeld	0.7	1.3	0.5	1.8	S
	Hyola 42	0.4	0.9	0.3	0.6	R
	Karoo	0.7	0.9	0.5	0.7	M
	Monty	0.6	1.4	0.5	0.8	M
	Oscar	0.5	0.8	0.4	0.6	R
	Rainbow	0.6	0.6	0.5	0.4	R
	Average	0.6	1.0	0.5	0.9	
Medic	Caliph	0.5	0.4	0.4	0.4	R
	Harbinger	0.4	0.3	0.3	0.3	R
	Herald	0.5	0.6	0.4	0.5	R
	Mogul	0.4	1.9	0.3	0.6	R
	Parabinga	0.5	0.9	0.4	0.6	R
	Paraggio	0.6	0.7	0.5	0.5	R
	Santiago	0.6	0.6	0.5	0.5	R
	Sava	0.4	0.5	0.4	0.4	R
	Average	0.5	0.7	0.4	0.5	
	l.s.d.	-	-	0.5	0.6	

Figure 7.6 **Multiplication of *P. neglectus* in wheat (w), barley (b), oat (o), durum (d) and triticale (t) varieties, Paskeville variety trial, 1997. S = susceptible (*P. neglectus* multiplication not significantly different from Machete and > than Abacus); M = moderate (*P. neglectus* multiplication not significantly different from either Machete or Abacus); R = resistant (*P. neglectus* multiplication not significantly different from Abacus and < Machete). Varieties are ranked in order of the "Common" ranking shown in Table 7.8. ☐ = Machete wheat and Abacus triticale controls.**

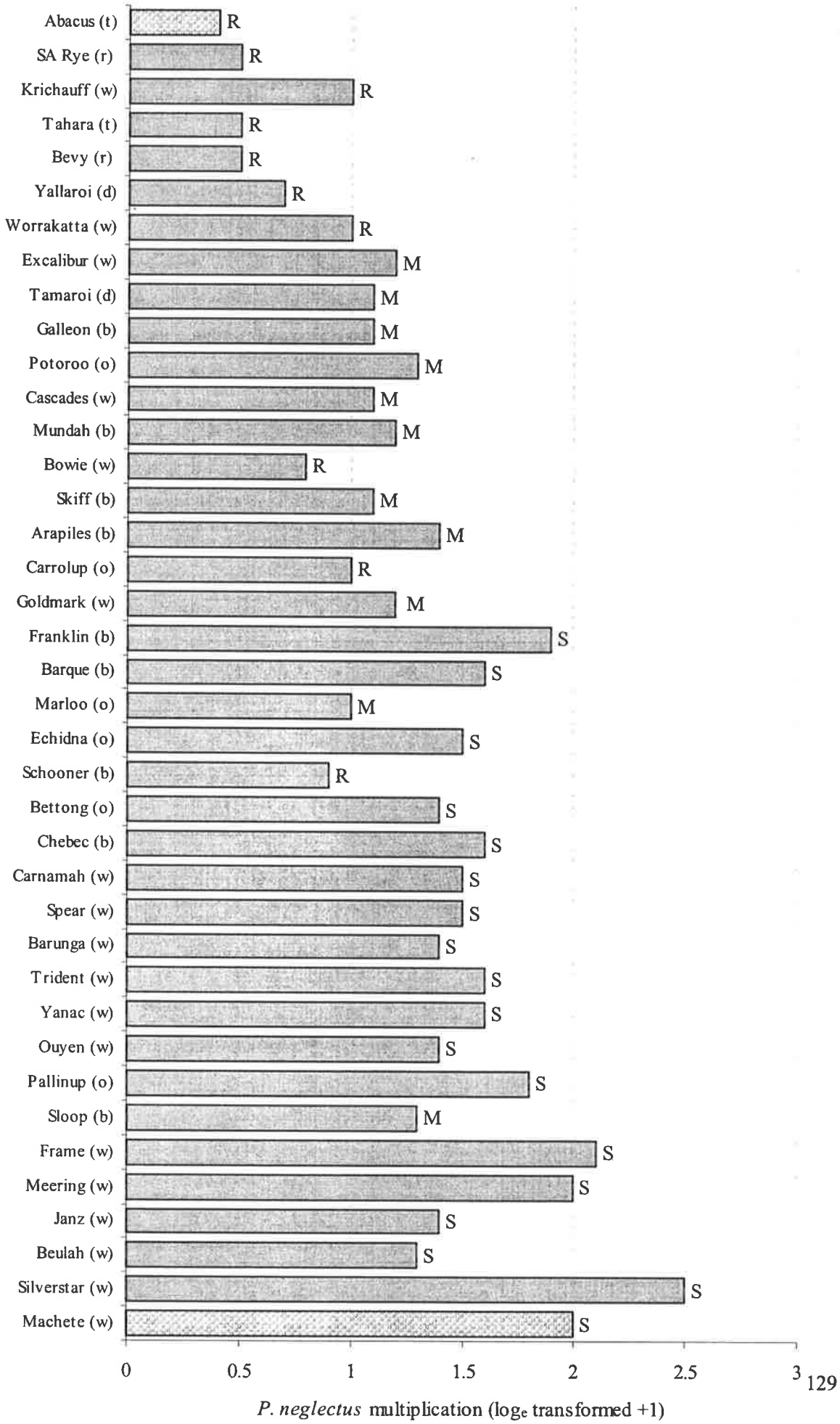
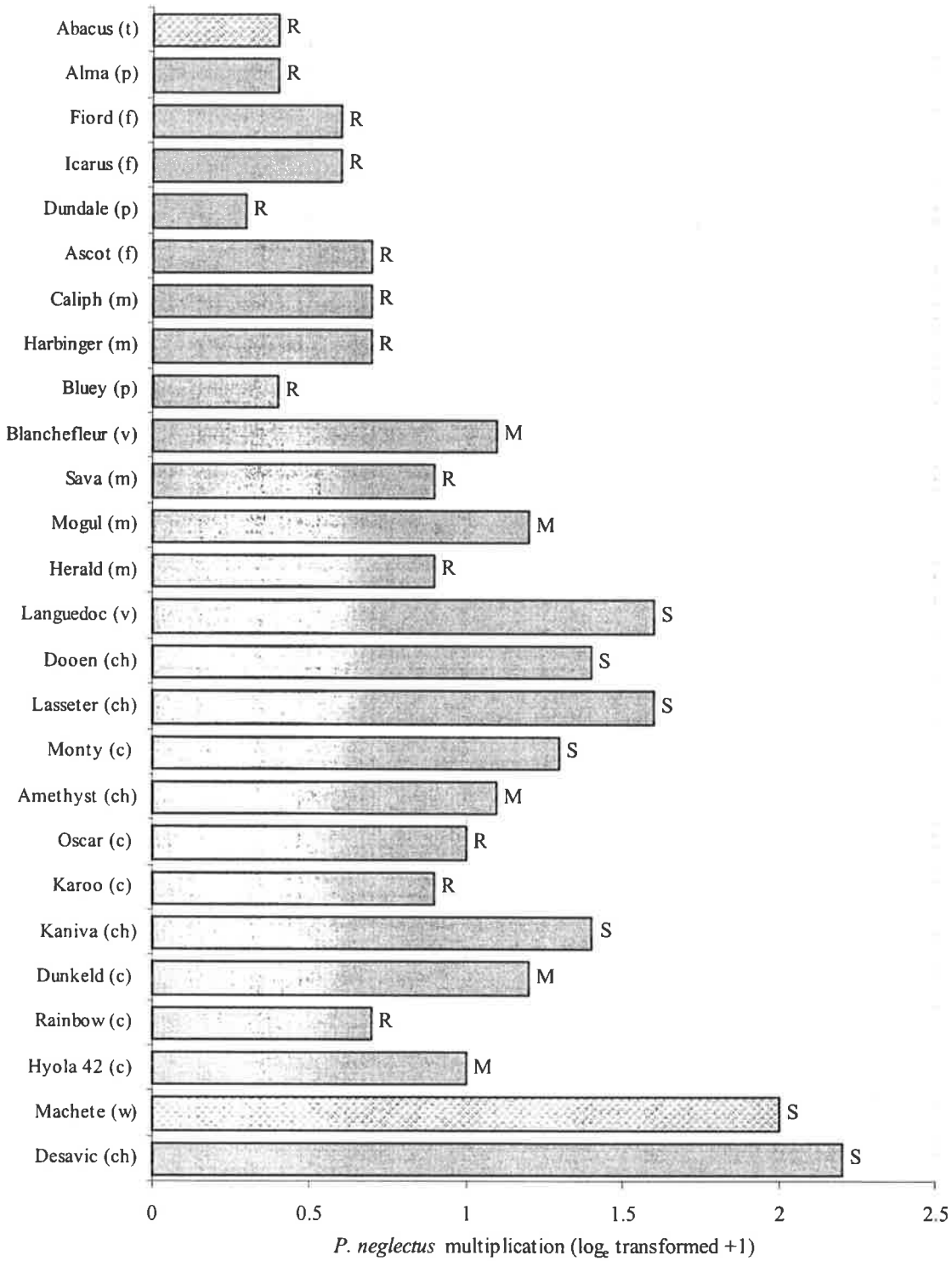

Abacus (t) R
SA Rye (r) R
Krichauff (w) R
Tahara (t) R
Bevy (r) R
Yallaroi (d) R
Worrakatta (w) R
Excalibur (w) M
Tamaroi (d) M
Galleon (b) M
Potoroo (o) M
Cascades (w) M
Mundah (b) M
Bowie (w) R
Skiff (b) M
Arapiles (b) M
Carrolup (o) R
Goldmark (w) M
Franklin (b) S
Barque (b) S
Marloo (o) M
Echidna (o) S
Schooner (b) R
Bettong (o) S
Chebec (b) S
Carnamah (w) S
Spear (w) S
Barunga (w) S
Trident (w) S
Yanac (w) S
Ouyen (w) S
Pallinup (o) S
Sloop (b) M
Frame (w) S
Meering (w) S
Janz (w) S
Beulah (w) S
Silverstar (w) S
Machete (w) S
0
0.5
1
1.5
2
2.5
3
P. neglectus multiplication ($\log_e$ transformed +1)

Figure 7.7 **Multiplication of *P. neglectus* in chickpea (ch), faba bean (f), vetch (v), field pea (p), canola (c) and medic (m) varieties, Paskeville variety trial 1997. S = susceptible (*P. neglectus* multiplication not significantly different from Machete and > than Abacus); M = moderate (*P. neglectus* multiplication not significantly different from either Machete or Abacus); R = resistant (*P. neglectus* multiplication not significantly different from Abacus and < Machete). Varieties are ranked in order of the "Common" rankng shown in Table 7.8. ☐ = Machete wheat and Abacus triticale controls.**


Abacus (t) R
Alma (p) R
Fiord (f) R
Icarus (f) R
Dundale (p) R
Ascot (f) R
Caliph (m) R
Harbinger (m) R
Bluey (p) R
Blanchefleur (v) M
Sava (m) R
Mogul (m) M
Herald (m) R
Languedoc (v) S
Dooen (ch) S
Lasseter (ch) S
Monty (c) S
Amethyst (ch) M
Oscar (c) R
Karoo (c) R
Kaniva (ch) S
Dunkeld (c) M
Rainbow (c) R
Hyola 42 (c) M
Machete (w) S
Desavic (ch) S
0
0.5
1
1.5
2
2.5
P. neglectus multiplication (log transformed +1)

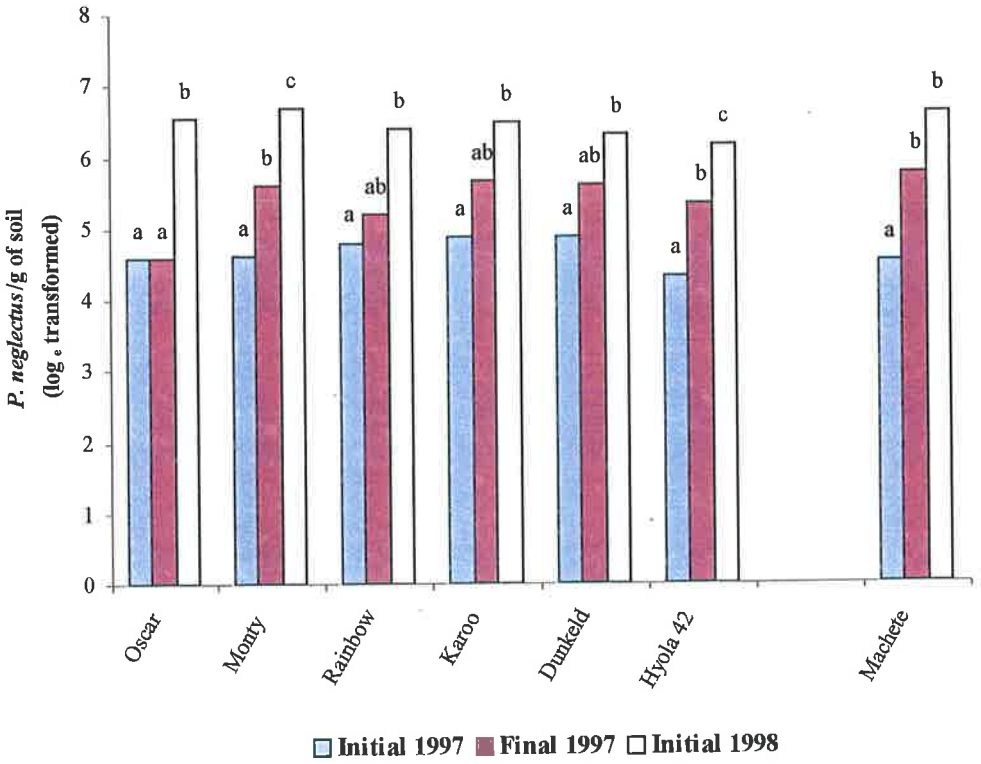
7.2.2.3 *Pratylenchus neglectus* density in canola plots

The ANOVA of the results comparing *P. neglectus* numbers/g of soil under canola varieties at sowing and harvest in 1997 and prior to sowing in 1998 are presented in Table 7.7. *P. neglectus* numbers/g of soil are shown in Figure 7.8. At Paskeville, there were significantly fewer *P. neglectus* at harvest under Rainbow and Oscar compared with the susceptible control, Machete wheat. There were significantly higher numbers of *P. neglectus*/g of soil prior to sowing in 1998 compared with final numbers in 1997 under Oscar, Monty, Karoo and Hyola 42. In May 1998, there was no significant difference in numbers in any of the canola varieties and the susceptible wheat variety Machete.

Table 7.7 **The ANOVA of the results for difference between numbers of *P. neglectus* under canola and Machete wheat plots from initial sampling in June 1997, final sampling in December 1997 and initial sampling in May 1998, Paskeville variety trial 1997/1998 (n = 4). * significant (P < 0.05).**

Variety		d.f.	m.s.	P
Oscar	Rep.	3	0.600	0.1260
	Sampling time	2	5.143	0.0010*
	Rep.*Sampling	6	0.029	
Monty	Rep.	3	0.972	0.0030
	Sampling time	2	4.307	0.0001*
	Rep.*Sampling	6	0.058	
Rainbow	Rep.	3	0.495	0.3530
	Sampling time	2	2.774	0.0380*
	Rep.*Sampling	4	0.341	
Karoo	Rep.	3	0.059	0.6830
	Sampling time	2	2.563	0.0060*
	Rep.*Sampling	4	0.111	
Dunkeld	Rep.	3	0.390	0.3440
	Sampling time	2	2.060	0.0260*
	Rep.*Sampling	6	0.289	
Hyola 42	Rep.	3	0.102	0.4280
	Sampling time	2	3.480	0.0020*
	Rep.*Sampling	4	0.088	
Machete	Rep.	3	0.235	0.740
	Sampling time	2	9.357	0.003*
	Rep.*Sampling	5	0.550	

Figure 7.8 **Numbers of *P. neglectus*/g of dry soil from plots of canola and Machete wheat control, Paskeville variety trial 1997/1998. Plots were sampled at sowing (Initial 1997), at harvest (Final 1997) and prior to the next season's crop (Initial 1998) (n = 4). Within each variety, columns with the same letters do not differ significantly (P < 0.05).**



7.2.3 Comparison between varieties using final density or multiplication rate

In 1996 at Sandilands, varieties were generally less susceptible, with 13 of the 69 varieties assessed rated as susceptible, compared with 44 of the 71 varieties assessed at Paskeville in 1997.

In 1996, there were small differences between the ranking obtained using final density of *P. neglectus*/g of soil or using multiplication rate. Using final density, 21 cereals were resistant, 10 were moderate and 4 were susceptible; while using multiplication rate, 20 were resistant, 10 were moderate and 5 were susceptible. For the pulse, oilseed and pasture varieties, use of final density identified 21 resistant, 4 moderate and 7 susceptible varieties. Using multiplication rate, 22 were rated as resistant, 4 as moderate and 6 as susceptible.

In 1997, minor differences in the estimation of resistance were observed by using final density of *P. neglectus*/g of dry soil or multiplication rate. For the cereals, using final density, 19 were classified as susceptible, 8 as moderate and 11 as resistant; while using multiplication rate, 17 were susceptible, 12 moderate and 10 were resistant. For the pulse, oilseed and pasture varieties using final density 7 varieties were susceptible, 3 were moderate and 18 were resistant. Use of multiplication rate identified 7 susceptible, 6 moderate and 13 resistant varieties.

7.2.4 Combined field trial results for Sandilands and Paskeville

The "common" ranking, i.e. analysis of combined data for 1996 and 1997, is presented in Table 7.8. The ranking for each variety (resistant, moderate or susceptible) were identified using combined data from both sites.

Of the wheat varieties, 13 were rated as susceptible, 3 moderate and 4 as resistant. For barley, 8 were moderate and 1 was resistant. One oat variety was susceptible, 8 were moderate and 1 was resistant. All triticale and rye varieties were rated as resistant. Of the pulses, 5 chickpea varieties were susceptible and one was moderate, 2 vetch varieties were moderate and 1 resistant, and all faba bean and field pea varieties were resistant. Eight canola varieties were rated as susceptible and 1 as moderate. Four medic varieties were moderate and 4 were resistant.

Table 7.8 **Combined data for final density (Pf) and multiplication (Pf/Pi) of *P. neglectus* under cereal, pulse, oilseed and pasture varieties for 1996 (Sandilands) and 1997 (Paskeville) (n = 4). S = susceptible (*P. neglectus* multiplication not significantly different from Machete and > than Abacus); M = moderate (*P. neglectus* multiplication not significantly different from either Machete or Abacus); R = resistant (*P. neglectus* multiplication not significantly different from Abacus and < Machete).**

Variety	Crop	Combined data from 1996 and 1997 ("Common" ranking)		Overall variety ranking
		Pf	Pf/Pi	
Desavic	Chickpea	0.81	2.00	S
Machete	Wheat	1.38	1.71	S
Narendra	Canola	0.74	1.66	S
Silverstar	Wheat	1.03	1.55	S
Hyola 42	Canola	0.66	1.46	S
Beulah	Wheat	0.96	1.43	S
Rainbow	Canola	0.75	1.43	S
Buckley	Wheat	1.15	1.38	S
Janz	Wheat	0.71	1.37	S
Meering	Wheat	0.59	1.35	S
Frame	Wheat	0.40	1.35	S
Sloop	Barley	0.73	1.34	S
Tyson	Chickpea	0.70	1.33	S
Pallinup	Oat	0.55	1.27	S
Dunkeld	Canola	0.80	1.25	S
Ouyen	Wheat	0.60	1.22	S
Yanac	Wheat	0.86	1.19	S
Trident	Wheat	0.53	1.16	S
Barunga	Wheat	0.55	1.14	S
Spear	Wheat	0.63	1.09	S
Grouse	Canola	0.56	1.09	S
Kaniva	Chickpea	0.69	1.09	S
Karoo	Canola	0.59	1.07	S
Carnamah	Wheat	0.40	1.05	S
Oscar	Canola	0.33	1.03	S
Amethyst	Chickpea	0.45	1.03	S
Monty	Canola	0.55	1.01	S
Lasseter	Canola	0.30	1.00	S
Bettong	Oat	0.38	0.98	M
Schooner	Barley	0.35	0.97	M
Dooen	Chickpea	0.60	0.97	M
Bandicoot	Oat	0.21	0.97	M
Languedoc	Vetch	0.65	0.96	M
Echidna	Oat	0.35	0.90	M
Marloo	Oat	0.46	0.88	M
Barque	Barley	0.28	0.88	M
Franklin	Barley	0.30	0.87	M
Goldmark	Wheat	0.42	0.84	M

Variety	Crop	Combined data from 1996 and 1997 ("Common" ranking)		Overall variety ranking
		Pf	Pf/Pi	
Carrolup	Oat	0.31	0.79	M
Pinnacle	Canola	0.24	0.76	M
Arapiles	Barley	-0.31	0.76	M
Skiff	Barley	0.07	0.68	M
Popany	Vetch	-0.17	0.63	M
Herald	Medic	-0.04	0.60	M
Bowie	Wheat	-0.03	0.60	M
Mundah	Barley	0.26	0.60	M
Mogul	Medic	-0.03	0.59	M
Cascades	Wheat	-0.26	0.58	M
Potoroo	Oat	-0.17	0.58	M
Parabinga	Medic	-0.04	0.47	M
Galleon	Barley	-0.08	0.46	M
Sava	medic	-0.48	0.45	M
Wallaroo	oat	0.08	0.44	M
Chebec	barley	0.05	0.39	R
Tamaroi	durum	-0.37	0.38	R
Blanchefleur	vetch	0.07	0.36	R
Tatiara	wheat	-0.17	0.31	R
Glenroy	field pea	-0.59	0.30	R
Excalibur	wheat	-0.19	0.29	R
Worrakatta	wheat	-0.38	0.29	R
Laura	field pea	-0.71	0.29	R
Bluey	field pea	-1.01	0.28	R
Santiago	medic	-0.49	0.24	R
Paraggio	medic	-0.42	0.15	R
Yallaroi	durum	-0.75	0.14	R
Harbinger	medic	-0.57	0.06	R
Caliph	medic	-0.81	0.03	R
Ascot	faba bean	-0.81	0.02	R
Bevy	rye	-0.98	-0.01	R
Dundale	field pea	-1.28	-0.06	R
Icarus	faba bean	-0.92	-0.06	R
Tahara	triticale	-1.00	-0.06	R
Fiord	faba bean	-0.84	-0.16	R
Euro	oat	-0.78	-0.20	R
Krichauff	wheat	-0.93	-0.24	R
Muir	triticale	-0.73	-0.30	R
SA Rye	rye	-1.27	-0.33	R
Bonzer	field pea	-0.87	-0.35	R
Alma	field pea	-1.28	-0.50	R
Early Dun	field pea	-0.93	-0.53	R
Abacus	triticale	-1.3	-0.76	R

7.2.5 Condada cereal variety trial, 1996

P. neglectus was identified on soil at Condada. The ANOVA of the results for differences in initial number, final number and multiplication rate of *P. neglectus* is shown in Table 7.9. Initial number, final number and multiplication rate of *P. neglectus* are shown in Table 7.10. The distribution of initial numbers of nematodes across the site is shown in Figure 7.9. There was no significant difference in initial numbers between varieties and large variation was observed across the site, ranging between 8.7 and 0.7 *P. neglectus*/g of soil (average = 3.1/g). Significant differences were seen between both final numbers and multiplication following different varieties. Highest final numbers were observed in the wheat varieties Machete, Frame, Janz and Barunga, while lowest numbers were recorded for Chebec barley, BT-Schomburgk wheat, Tahara triticale and Krichauff wheat. Multiplication rates of *P. neglectus* were low, with the highest rate recorded in Machete wheat (1.9) and the lowest in Krichauff (0.2).

The resistance rating for each variety was similar to that derived in the overall ranking from Sandilands and Paskeville (Table 7.8), with the exception of the barley varieties Chebec and Schooner, which were rated as resistant at Condada and moderate in combined data from Sandilands and Paskeville.

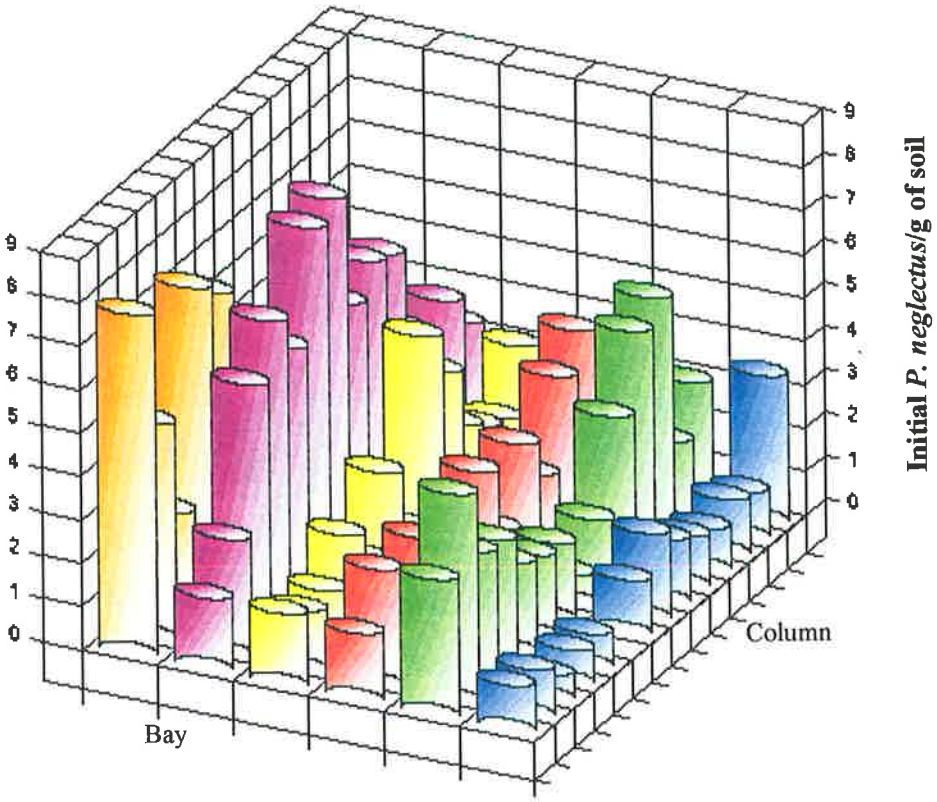
Table 7.9 The ANOVA of the results for multiplication rate and initial and final numbers of *P. neglectus* on wheat, barley, oat and triticale varieties, Condada variety trial 1996 (n = 6).

		d.f.	m.s.	P
Initial *P. neglectus*/g of dry soil (Pi)	Rep.	5	2.661	0.000
	Variety	13	0.221	0.769
	Rep.*Variety	64	0.322	
Final *P. neglectus*/g of dry soil (Pf)	Rep.	5	2.930	0.000
	Variety	13	3.142	0.000
	Rep.*Variety	65	0.347	
Multiplication rate of *P. neglectus* (Pf/Pi)	Rep.	5	0.209	0.920
	Variety	13	3.364	0.000
	Rep.*Variety	64	0.733	

Table 7.10 **Multiplication rate, initial and final number of *P. neglectus* at Condada variety trial, 1996. Figures in brackets are detransformed means (n = 6). S = susceptible (*P. neglectus* multiplication not significantly different from Machete and > than Krichauff); M = moderate (*P. neglectus* multiplication not significantly different from either Machete or Krichauff); R = resistant (*P. neglectus* multiplication not significantly different from Krichauff and < Machete).**

Variety	Initial/g soil ($\log_e + 1$)	Final/g soil ($\log_e + 1$)	Multiplication ($\log_e + 1$)	Rating
Machete (wheat)	1.5 (3.6)	2.0 (6.6)	1.1 (1.9)	S
Janz (wheat)	1.1 (2.1)	1.4 (3.0)	0.9 (1.6)	S
Barunga (wheat)	1.3 (2.6)	1.3 (2.7)	0.9 (1.4)	M
Frame (wheat)	1.3 (2.7)	1.4 (3.2)	0.8 (1.3)	S
Potoroo (oat)	1.3 (2.8)	1.3 (2.9)	0.7 (1.1)	M
Euro (oat)	1.0 (1.8)	1.0 (1.7)	0.7 (1.1)	M
Stiletto (wheat)	1.3 (2.6)	1.2 (2.5)	0.7 (1.0)	M
Echidna (oat)	1.2 (2.2)	0.9 (1.5)	0.7 (1.0)	M
Schooner (barley)	1.3 (2.7)	0.9 (1.6)	0.5 (0.6)	R
Excalibur (wheat)	1.4 (2.9)	0.7 (1.1)	0.3 (0.4)	R
Chebec (barley)	1.3 (2.8)	0.6 (0.8)	0.3 (0.3)	R
BT-Schomburgk (wheat)	1.5 (3.3)	0.6 (0.9)	0.3 (0.3)	R
Tahara (triticale)	1.3 (2.6)	0.6 (0.8)	0.3 (0.3)	R
Krichauff (wheat)	1.4 (3.0)	0.5 (0.6)	0.2 (0.2)	R
h.s.d	ns	0.7	0.6	

Figure 7.9 **Initial number of *P. neglectus* sampled in individual plots from the Condada variety trial, 1996. Each bar represents one sample from an individual plot within the trial (each sample consisted of 12 - 15 sub-samples). Different coloured bars represent different bays within the field trial.**



7.3 Discussion

Results from these field trials show the broad host range of *P. neglectus*. There were major effects of crop species on *P. neglectus* multiplication, with the overall ranking of crops (most to least susceptible) being chickpea > wheat > canola > barley > oat > vetch > medic > durum > faba bean > triticale > rye > field pea. It is important to note that there was a wide range of resistance within some crops. For wheat, superior resistance was identified in three varieties (Excalibur, Krichauff and Worrakatta). In contrast, the majority of barley varieties were more resistant, with the exception of Sloop which appeared to be susceptible.

In the northern hemisphere, while *P. neglectus* has been associated with minor damage on spring barley in pot experiments (Wolny 1989; Umesh and Ferris 1992), barley varieties appear to be poorer hosts. Wheat varieties have been recorded as good hosts for *P. neglectus* (Lasserre *et al.* 1994; Mojtahedi and Santo 1992) and oat varieties also hosted this species (Lasserre *et al.* 1994; Rivoal *et al.* 1995). *P. neglectus* was observed to feed ectoparasitically on oilseed rape but endoparasitically on its preferred host maize (Webb 1995). In Australia, wheat has been shown to be a good host for *P. neglectus* (Vanstone *et al.* 1998). In this study, wheat was confirmed as a good host, with 13 susceptible, 2 moderate and 3 resistant varieties identified (based on the "common" ranking shown in Table 7.8). In contrast to results from the northern hemisphere, oat varieties were generally poorer hosts to *P. neglectus,* with only one susceptible and 7 moderate varieties.

Canola was the only crop where the susceptibility ranking changed significantly between seasons. It was rated as the most susceptible crop in 1996, but only the fifth most susceptible in 1997. To determine whether a biofumigation effect occurred as canola stubble degraded, plots were sampled immediately prior to seeding in the year following each trial. In 1996, there were high *P. neglectus* numbers at harvest and no reduction in *P. neglectus* densities 5 months after harvest. In 1997 however, there were lower numbers of *P. neglectus* at harvest compared with the Machete wheat control. Confusingly, numbers in May 1998 were significantly higher under Oscar, Monty, Karoo and Hyola 42 and no canola variety was significantly different to Machete wheat. While this implies that *P. neglectus* multiplied during the 5 month period between harvest in 1997 and seeding in 1998, this hypothesis is unlikely as little moisture was recorded and no growth occurred on the plots during this period. It is therefore suggested that the increase in *P. neglectus* numbers was probably due to higher soil moisture in May 1998.

Soil moisture has been shown to affect recovery of *P. neglectus* (Chapter 5) and in May 1998, soil moisture was 12% compared with only 6% in December 1997.

While this offers an explanation for the overall increase in *P. neglectus* numbers in May 1998, the reason for significant differences observed between canola varieties and Machete wheat in December 1997 but not in May 1998 is unclear. In May 1998, there was a trend for reduction in *P. neglectus* numbers under canola, and the lack of statistical significance may have been a result of insufficient replication.

While not conclusive, the trend at the Paskeville site may be an example of a potential role of biofumigation by breakdown of canola stubble in reducing *P. neglectus* numbers. Potter *et al.* (1998) suggested this effect is due to 2-phenylethyl glucosinolate, and that levels within root tissue may be increased through breeding. As canola appears to be a good host for *P. neglectus*, varieties with increased levels of this compound may be beneficial in reducing *P. neglectus* levels.

In general, the rating of varieties within each trial was the same using either final density of *P. neglectus*/g of soil or multiplication rate. This may not always be the case, as interactions between initial density and plant tolerance will influence both final nematode density and multiplication rate, i.e. damage caused by *P. neglectus* may limit plant growth in intolerant plants and therefore nematode density. At both Sandilands and Paskeville, low initial *P. neglectus* densities were present (average < 1 *P. neglectus*/g) and it was assumed that there was little effect of plant damage influencing nematode multiplication. This is supported by the absence of a significant response to nematicide or relationship between initial density of *P. neglectus* and yield (Chapter 8). At Condada however, while moderate initial numbers of *P. neglectus* were present (average 3.1/g), multiplication rates were similar to those observed at Sandilands and Paskeville. A tolerance response (calculated by regression analyses of initial number of nematodes and yield) was observed for Machete wheat, Euro and Echidna oat and Schooner barley at this site (Chapter 8). The low multiplication rates observed at Condada may therefore be explained by higher initial numbers of *P. neglectus* and potential interactions between nematode multiplication and damage to the host. In contrast, given the low initial densities, surprisingly low rates of multiplication were observed at both Sandilands and Paskeville.

Extremely high rates of multiplication have been observed in similar studies by others. For example, Nicol *et al.* (1999) reported *P. thornei* multiplication rates of 20-30 at initial densities of approximately 12 *P. thornei*/g of soil, and Noling and Ferris (1986) found a maximum rate of reproduction of up to 360 eggs/female for *M. hapla* at initial levels of 4 nematodes/kg of soil. For *P. neglectus*, a multiplication rate of up to 30 has been observed for Machete in cereal field trials at initial densities of only 0.6 *P. neglectus*/g of dry soil (V. Vanstone pers. comm.), indicating that significantly higher rates than those observed within this study can occur and that seasonal and site characteristics greatly influence multiplication.

At Paskeville, while the multiplication rate of *P. neglectus* was low, there were higher final *P. neglectus* densities and a greater number of varieties were designated as susceptible compared with results at the Sandilands site. It is suggested that differences in multiplication rate between Sandilands and Paskeville may have reflected seasonal conditions. In 1996 at Sandilands, rainfall was average to above average, especially for the period of crop establishment and growth (June - September), compared with conditions at Paskeville in 1997 where rainfall was below average for this period.

Dry conditions have been observed to increase multiplication of *P. neglectus* (Vanstone pers. comm.). Drought also increases densities of the citrus nematode *Tylenchulus semipenetrans*, possibly because hydraulic lift in the root xylem resulting from lower soil moisture prolongs the activity of this nematode (Duncan and el-Morshedy 1996). This may also be the case with *P. neglectus*. Higher soil moisture in turfgrass was associated with lower levels of both *Tylenchorhynchus* sp. and *Hoplolaimus galeatus,* thought to result from increased bacterial activity (Browning *et al.* 1999). It is also possible that low soil moisture may decrease ectoparasitic feeding by *P. neglectus* as movement in soil pores is restricted under dry conditions, possibly encouraging feeding and multiplication within the root systems.

In addition to seasonal conditions, the lower initial nematode densities observed at Paskeville may have resulted in higher multiplication rates compared with either Sandilands or Condada. However, regardless of the overall multiplication rate or final nematode densities recorded, comparison between crop species and/or varieties at Sandilands, Paskeville and Condada was possible as multiplication rates across all varieties were relative to the susceptible and resistant controls.

At Sandilands and Paskeville, overall ranking of varieties, with seasonal and site effects removed, allowed comparative assessment of varieties in relation to each other. In this analysis, it was more difficult to assign varieties into resistant, moderate or susceptible categories, as varieties could only be compared with the site mean and not against a susceptible or resistant control. Judgement therefore had to be made based on overall results from both sites, and with inclusion of results from the Condada site.

Similarities in the resistance rating of many varieties were observed at the three sites. For example, Machete and Frame wheat, Desavic chickpea and Dunkeld canola were consistently rated as susceptible while superior levels of resistance were recorded in the wheat varieties Worrakatta and Krichauff (sister lines) and Excalibur. For *P. thornei*, no wheat variety assessed has proved to be completely resistant (i.e. no multiplication occurs) although superior levels of resistance have been found in wheat selections (Nicol 1996; Thompson *et al.* 1999) and from *Aegilops tauschii* (the D-genome donor of wheat) (Thompson and Haak 1997). The results from these field trials assessing resistance to *P. neglectus* also suggest that while no variety tested was completely resistant (i.e. immune), useful levels of resistance were identified in wheat. Of the other crops tested, superior resistance was identified in field pea, faba bean and triticale varieties.

However, differences in the rating of selected varieties occurred between sites. In Chapter 9, differential multiplication on four cereal varieties was observed between geographically isolated populations of *P. neglectus* and *P. thornei.* At field sites in this study, the barley variety Schooner was susceptible only at Sandilands while the wheat varieties Frame and Machete were susceptible and Excalibur wheat was resistant at all field sites. Results from growth room experiments in Chapter 9 strongly support this finding, as multiplication of the Sandilands population (collected from the same site as the Sandilands field site reported in this chapter) was high in Schooner, Frame and Machete and low in Excalibur.

Alcañiz *et al.* (1996) attributed inconsistent responses in assessment of resistance to *P. vulnus* in *Prunus* rootstocks to variability between isolates of this nematode. Differences in the pathogenicity of *P. neglectus* populations on wheatgrass have also been observed (Griffin 1991). In addition, variation has been identified in populations of *Pratylenchus* either by morphological or molecular assays (Duncan *et al.* 1999). The results presented in this chapter suggest differences occur between South Australian populations of *P. neglectus* and appears to

be the first evidence of variation being supported by results from field assessment. Differences between the pathogenicity of populations of *P. neglectus* and *P. thornei* are further assessed in Chapter 9 in growth room experiments.

In Chapter 4, differences were observed between the rate of extraction of *P. neglectus* from the root systems of different crops when assessed using the misting technique. This was partially attributed to continued multiplication and egg hatch within the misting chamber and highlighted difficulties with the use of the misting technique for extraction when comparing different crop species. These trials have shown that determination of the resistance or susceptibility of crops/varieties to *P. neglectus* can be achieved by assessing soil plus roots in field plots (using the Whitehead tray for extraction), providing comparisons can be made with previously characterised check varieties.

Chapter 8 Population dynamics of, and yield loss caused by, *Pratylenchus neglectus* and *P. thornei* in field experiments

8.0 Introduction

Damage caused by *P. neglectus* and *P. thornei* is often difficult to identify and quantify, as symptoms may be indistinct or masked by plant responses to biotic and abiotic factors. Nematicides have therefore been used extensively to measure yield loss caused by root lesion nematodes (Doyle *et al.* 1987; McDonald *et al.* 1987; Badra and Adesiyan 1990; Thompson 1990).

In addition to nematicidal effects, nematicides can have non-target effects on plant growth as a result of environmental and site factors such as rainfall, soil type and cultivar, which may alter the effects of these chemicals. For example, the nematicide aldicarb is believed to leach faster in soils with higher sand and lower organic matter content resulting in differential responses between soil types (Barker *et al.* 1988). Varietal response to aldicarb in tobacco and soybean in the absence of nematodes has also been observed (Barker and Powell 1988; Barker *et al.* 1988).

As a result, Barker and Noe (1987) suggest that the use of resistant varieties to manipulate nematode densities is a more appropriate method by which to measure damage thresholds and yield loss in plants. Establishing a range of nematode densities in field trials also assists in determining relationships between nematode density and yield using correlation analyses (Stynes and Veitch 1983).

Correlation analyses have been used in southern Australia to determine yield loss caused by both *P. neglectus* and *P. thornei*. Yield loss of up to 27% caused by *P. thornei* was determined by correlation analyses of *P. thornei* numbers and yield following the establishment of a range of initial nematode densities using resistant and susceptible cereals (Nicol *et al.* 1999). Damage caused by *P. neglectus* was determined using natural variation in densities of *P. neglectus*, and a significant negative correlation was obtained between nematode density and yield for this species, with 56 - 74% of the varietal yield differences attributed to nematode damage (Vanstone *et al.* 1998).

In Chapter 6, yield loss caused by *P. neglectus* in the absence of other pathogens was shown in a pot trial using Machete wheat. However, the lack of a range of initial densities prevented any meaningful assessment of the population dynamics of this nematode. In this chapter, yield loss in cereals, pulses, oilseeds and pasture legumes caused by *P. neglectus* is further investigated under field conditions. In addition, the population dynamics of *P. neglectus* and the related species, *P. thornei,* are assessed.

8.1 Materials and methods

8.1.1 Field sites

8.1.1.1 Aldicarb rate trial

A field trial was established in 1996 at Condada within the same field as the rotation trial. Two wheat varieties (Machete and Janz) were included at this site and the trial was set up as a Complete Randomised Block Design with six replicates. Plots were 10 m long by 1.7 m wide. Six rates of aldicarb were applied either only at seeding (early) as described in Section 3.7.2, or at seeding with a second application at the same rate 12 weeks after seeding (late). The late application was applied using a fertiliser spreader, custom built to a plot width of 1.7 m and raised to a height of 1 m to minimise damage to plants during application. The following rates of aldicarb were used: 0, 1.0, 1.5, 2.5, 4.0 and 6.0 kg a.i/ha. Sowing and harvest details are presented in Table 8.1.

8.1.1.2 Variety trials

Field trials were established at Sandilands (Yorke Peninsula) and Condada (Eyre Peninsula) in 1996 and Paskeville in 1997 (see Figure 3.10 for site locations). Sowing, sampling, management and harvest details and the experimental design for the Sandilands and Paskeville trial sites are described in Chapter 7 (Table 7.1 and Section 7.2.2). Experimental design for the Condada site is described in Section 7.2.2 and sowing and harvest details in Table 8.1.

At all sites, the nematicide aldicarb was applied to selected plots at sowing at a rate of 2.5 kg a.i./ha, as described in General Techniques (3.7.2).

8.1.1.3 Rotation trials

Field trials were established over two growing periods (1996 and 1997) at Minnipa and Condada, Eyre Peninsula (See Figure 3.10 for the location of these sites). These experiments were established to assess the effect of producing a range of nematode densities by using resistant and susceptible cereal varieties in the first year on the yield of an intolerant cereal in the second year. In 1996 at each site, two susceptible wheat varieties (Machete and Spear), a resistant wheat (Excalibur), a resistant barley (Chebec) and a resistant triticale (Tahara) were sown. Levels of resistance of these varieties had been determined in previous field studies (Vanstone pers. comm.) and confirmed in Chapter 7 (Table 7.8). In 1997 at each site, the intolerant oat variety Echidna (as determined in Section 8.2.1.3) was sown over these plots. Echidna is also resistant to the other main cereal pathogens in this region, take-all (*G. graminis* var. *tritici*) and cereal cyst nematode (*H. avenae*). This variety was therefore selected as a precaution against interaction with these pathogens, even though no symptoms were observed in the trials in 1996.

Trials were set up as a Complete Randomised Block Design with four replicates. Plots were 10 m long by 1.7 m wide. In 1996 only, aldicarb was applied at sowing at a rate of 2.5 kg a.i./ha as described in General Techniques (3.7.2). Sowing and harvest details are shown in Table 8.1.

Table 8.1 Sowing and harvest details for variety rotation and aldicarb rate trials at Condada and Minnipa, Eyre Peninsula, South Australia.

Site	Trial	Sowing	Harvest
Condada 1996	Rotation (1st year)	21 June	16 December
	Aldicarb rate	21 June	16 December
	Variety trial	17 June	15 December
Condada 1997	Rotation (2nd year)	4 June	1 December
Minnipa 1996	Rotation (1st year)	25 June	17 December
Minnipa 1997	Rotation (2nd year)	5 June	18 November
Fertiliser applied to all sites at seeding (N:P:K; 10:22:0) Zn 5% at 55 kg/ha			

8.1.2 *Pratylenchus* and plant assessment

Numbers of either *P. neglectus* or *P. thornei* were determined in soil at seeding in untreated plots only. Final numbers of *P. neglectus* or *P. thornei* were determined in moist soil within 3 weeks either before or after harvest. Nematode sampling, extraction and counting are described in Sections 3.7.4, 3.2 and 3.3. The multiplication rate of *P. neglectus* was determined by dividing final nematodes/g of soil by initial nematodes/g of dry soil.

In the variety trials at Sandilands and Paskeville, grain yield was recorded for all plots except medic. At Sandilands only, the top growth of medic varieties was assessed by taking all shoot material from three, randomly selected, 30 cm by 30 cm areas of each plot at flowering. Plant material was dried and weighed. In the variety trial (Condada) and the rotation (Condada and Minnipa) and aldicarb rate (Condada) trials, grain yield was recorded for all plots.

8.1.3 Statistical analyses

For assessment of the numbers of *Pratylenchus* in each variety, the means of four replicates were log transformed ($\log_e$ *Pratylenchus*/g of soil) to normalise the data sets and an analysis of variance was conducted ($P < 0.05$). Number of nematodes was expressed either as initial number of nematodes/g of dry soil (Pi) or final number/g of dry soil (Pf). The multiplication rates of nematodes were calculated by dividing final numbers of nematodes by initial numbers (Pf/Pi). In all trials, a general ANOVA was conducted and a Tukey test (h.s.d.) was used to compare nematode densities and multiplication rates between varieties.

Yield loss was calculated either from comparison of aldicarb treated and untreated plots or from the regression analyses produced using a simple linear correlation analysis to determine the relationship between mean grain yield (kg/plot) and either final or initial nematode number or multiplication rate. For the Sandilands and Paskeville sites, differences between yields of aldicarb treated and untreated plots were separated using a general linear model (least squares means). At these sites, yield data for cereals were analysed separately to yield of pulse, oilseed and pasture varieties ($\log_e$ transformed for Sandilands pulse, oilseed and medic only). In the Condada variety trial, rotation trials at Condada and Minnipa, and aldicarb rate trial at Condada, data for yield were not transformed and differences between yield in aldicarb treated and untreated plots were determined using Fisher's protected l.s.d.

The relationship between multiplication rate and initial density of *P. thornei* (Minnipa rotation trial) and *P. neglectus* (Condada rotation trial) in Echidna oat in 1997 was presented as both untransformed and $\log_e$ transformed data. For the relationship between multiplication rate and initial density was determined by the following negative exponential decay model, which was fitted using untransformed and $\log_e$ transformed data from both sites:

$$Pf/Pi = a + ce^{-bPi} \text{ (Zar 1998)}$$

The relationship between the $\log_e$ transformed final and initial densities of *P. thornei* (Minnipa rotation trial) in Echidna oat in 1997 was assessed using a non-linear exponential model at Minnipa:

$$Pf = ce^{bPi}/(e^{bPi} - 1) + d \text{ (Zar 1998)}$$

P. neglectus data were fitted using a linear exponential model for the Condada rotation trial.

8.2 Results

8.2.1 Aldicarb rate trial

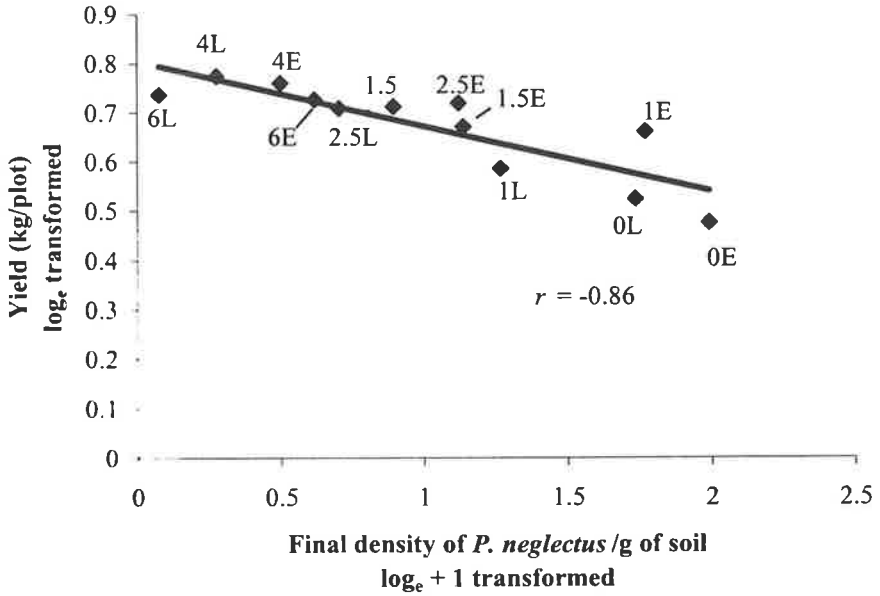
The regression analyses of final *P. neglectus* density and yield for Machete and Janz wheat treated with 6 rates of aldicarb at Condada are presented in Figure 8.1. The ANOVA of the results for yield differences in Machete and Janz treated with differing rates of aldicarb are shown in Table 8.2. Highly significant negative relationships were observed between the final numbers of *P. neglectus*/g of soil and yield for both Machete (r = -0.855) and Janz (r = -0.860) (tabulated r value = -0.708; $P<0.01$; n = 6; d.f. = 4).

Table 8.2 The ANOVA of the results for yield of Machete and Janz wheat treated with different rates and times of aldicarb application, Condada aldicarb rate trial, 1996.

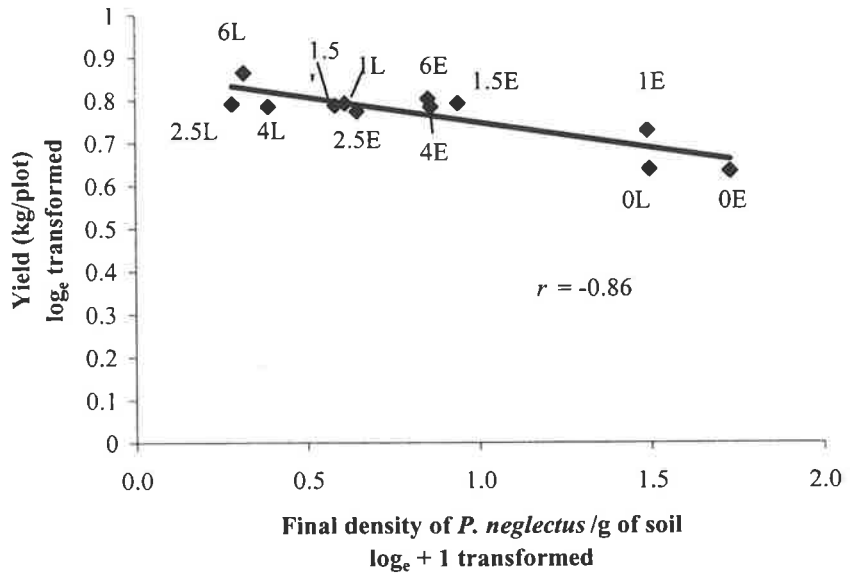
	d.f.	m.s.	P
Rep.	3	0.065	0.000
Aldicarb rate	11	0.048	0.000
Variety	1	0.213	0.000
Rate*Variety	11	0.006	0.010
Rep.*Rate*Variety	61	0.002	

Figure 8.1 **Relationship between final numbers of *P. neglectus* and yield in plots treated with differing rates of aldicarb, Condada aldicarb rate trial, 1996. Each point represents the mean of 6 replicates. 0E and 0L = Untreated; 1E, 1.5E, 2.5E, 4E and 6E = 1.0, 1.5, 2.5, 4.0 and 6.0 kg a.i. aldicarb respectively at sowing (Early); 1L, 1.5L, 2.5L, 4L, 6L = 1.0, 1.5, 2.5, 4.0 and 6.0 kg a.i. aldicarb respectively at sowing and a second application at these rates 12 weeks following sowing (Late).**

a) Machete wheat

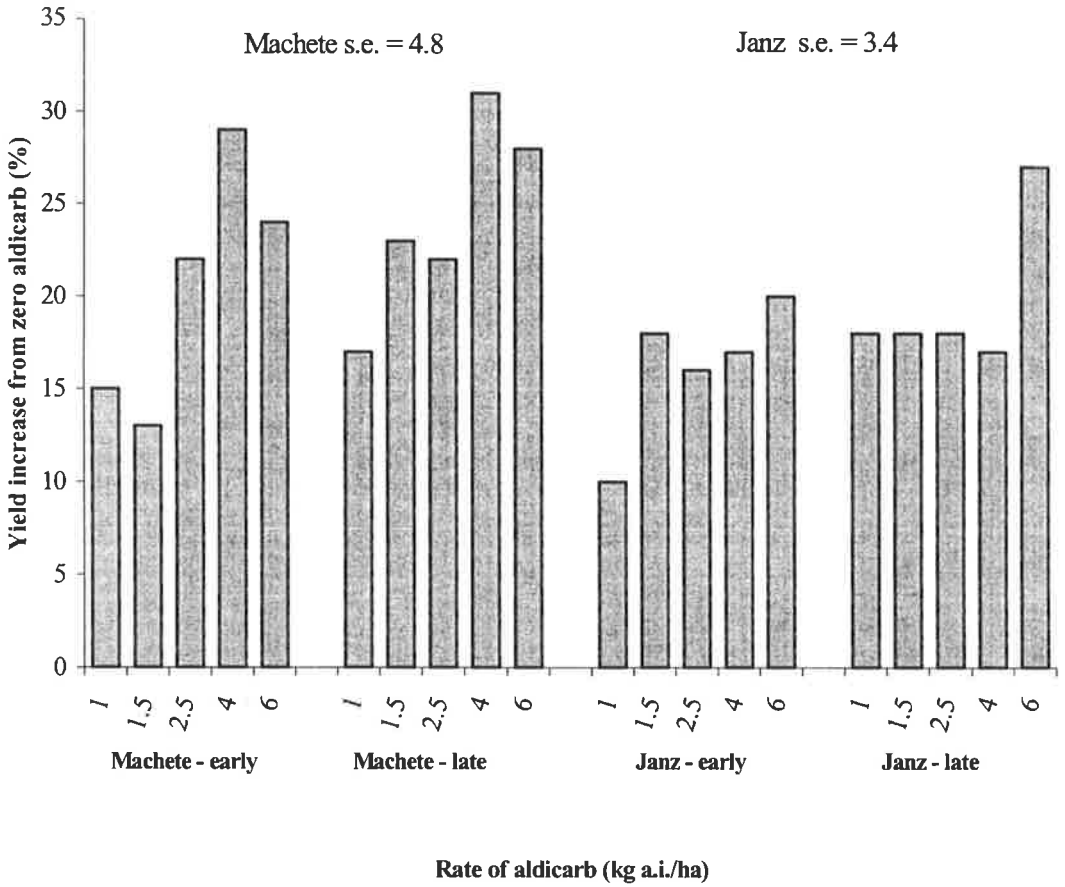


b) Janz wheat



Responses in aldicarb treated compared with untreated plots for Machete and Janz wheat are shown in Figure 8.2. For both varieties, significant yield increases were observed in aldicarb treated plots compared with untreated plots in all except the 1.5 kg a.i./ha early treatment for Machete and the 1 kg a.i./ha early treatment for Janz. For both varieties, largest responses were seen at the higher rates of aldicarb. For Machete wheat, yield responses of 31%, 29% and 28% were observed in the 4 kg a.i./ha late, 4 kg a.i./ha early and the 6 kg a.i./ha late, respectively, compared with untreated plots. However, differences were only significant between aldicarb treatments at 4.0 kg a.i./ha late (i.e. 8.0 kg total applied) and aldicarb at 1.5 kg a.i./ha early (i.e. 1.5 kg total applied at sowing only). For Janz wheat, responses of 27% and 20% were seen at rates of 6 kg a.i./ha late and 6 kg a.i./ha early. Yield response in plots with aldicarb applied at 1 kg a.i./ha early was significantly lower than for all other treatments (10%).

Figure 8.2 **Percentage increase in yield of wheat (cvv. Machete and Janz) with differing rates and times of aldicarb application relative to zero aldicarb treatment, Condada aldicarb rate trial, 1996. Early = aldicarb applied at sowing; late = aldicarb applied at sowing with a second application at the same rate applied 12 weeks after sowing (n = 6).**



8.2.2 Variety trials

8.2.2.1 Sandilands variety trial, 1996

P. neglectus was identified at Sandilands. Initial and final nematode numbers are shown in Chapter 7 (Tables 7.2 and 7.3). Low initial levels were observed at this site, with numbers ranging from 0.3 - 2.4 *P. neglectus*/g of soil (average density of 1 *P. neglectus*/g of soil).

The ANOVA of the results comparing yield in aldicarb treated and untreated plots is shown in Table 8.3. The percent yield differences between aldicarb treated and untreated plots and the significance between treatments are shown in Table 8.4. Significant yield increases were observed in aldicarb treated plots compared with untreated plots for only 8 of the 70 varieties assessed at this site. In the cereals, significant yield increases of 15%, 13%, 12% and 12% were recorded for the wheat varieties Machete, Silverstar, Beulah, Buckley and Worrakatta respectively. These varieties, with the exception of Worrakatta, also had higher final densities of *P. neglectus* (2.8, 1.7, 1.3, 1.8, 0.3 *P. neglectus*/g of soil, respectively). For the pulse, pasture and oilseed varieties, large yield increases in aldicarb treated plots were observed in Amethyst and Tyson chickpea and the vetch line 3323 (46%, 24% and 15%, respectively). Although yield increases were observed in aldicarb treated plots of medic varieties, differences were not significant.

Table 8.3 The ANOVA of the results for effect of aldicarb treatment on yield of cereals, pulses and oilseeds, Sandilands variety trial, 1996.

	d.f.	m.s.	P
	Wheat, barley, oat and triticale varieties		
Rep.	3	1.353	0.0001
Variety	37	0.737	0.0001
Rep.*Variety	125	0.126	0.0001
Aldicarb	1	1.105	0.0001
Variety*Aldicarb	37	0.062	0.2314
Res.	124	0.052	
	Chickpea, faba bean, field pea, vetch, medic and canola varieties		
Rep.	3	0.160	0.0001
Variety	33	0.406	0.0001
Rep.*Variety	85	0.031	0.0001
Aldicarb	1	0.041	0.020
Variety*Aldicarb	33	0.007	0.495
Res.	120	0.049	

Table 8.4 **Percent response in yield from aldicarb treated compared with untreated plots for wheat (w), barley (b), oat (o), triticale (t), rye (r), durum (d), chickpea (ch), canola (c), faba bean (fb), field pea (p) and vetch (v) varieties, Sandilands variety trial, 1996. P value derived from general linear model (least squares means) are significant at P < 0.05. Yields from aldicarb treated and untreated plots are presented in Appendix 5. na = analysis not available due to missing values.**

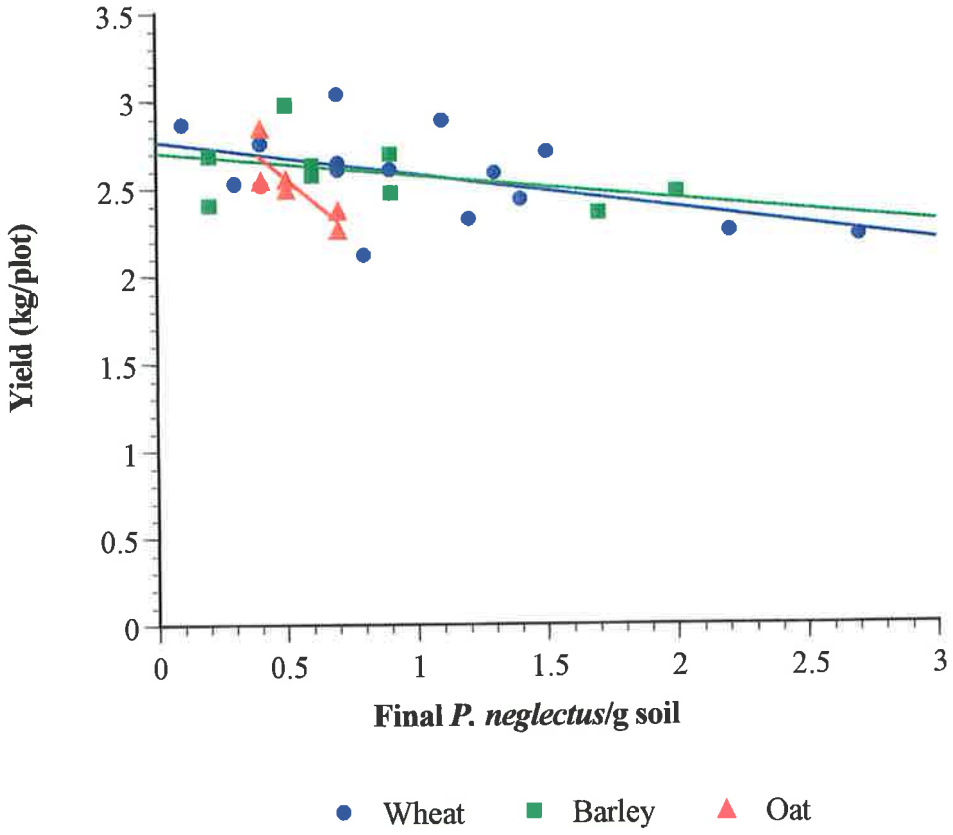
Cereal varieties			Pulse, pasture and oilseed varieties		
Variety	Response in aldicarb plots (%)	P value	Variety	Response in aldicarb plots (%)	P value
Abacus (t)	4	0.507	Alma (p)	0	0.726
Arapiles (b)	-3	0.643	Amethyst (ch)	46	0.006
Bandicoot (o)	7	0.423	Ascot (fb)	-1	0.379
Barque (b)	10	0.097	Blanchefleur (v)	10	0.145
Barunga (w)	7	na	Bluey (p)	4	na
Bettong (o)	-7	0.279	Bonzer (p)	-2	na
Beulah (w)	12	0.046	Caliph (m)	10	0.541
Bowie (w)	6	0.302	Desavic (ch)	13	na
Buckley (w)	10	0.045	Dooen (ch)	-3	na
Carrolup (o)	10	0.110	Dundale (p)	-6	0.367
Chebec (b)	0	0.914	Dunkeld (c)	3	na
Echidna (o)	7	0.274	Early Dun	0	0.929
Euro (o)	5	0.414	Fiord (fb)	-1	0.823
Excalibur (w)	3	0.623	Harbinger (m)	17	0.291
Frame (w)	-5	na	Herald (m)	13	0.193
Franklin (b)	11	0.078	Hyola 42 (c)	3	0.666
Galleon (b)	1	0.865	Icarus (fb)	4	0.428
Janz (w)	3	0.558	Kaniva (ch)	4	na
Krichauff (w)	-9	0.132	Karoo (c)	6	0.500
Machete (w)	15	0.020	Languedoc (v)	-10	na
Meering (w)	-12	0.723	Laura (p)	6	na
Mundah (b)	-12	na	Mogul (m)	-19	0.501
Ouyen (w)	9	0.162	Monty (c)	5	0.399
Potoroo (o)	4	0.537	Narendra (c)	0	0.995
Schooner (b)	8	0.300	Oscar (c)	10	na
Silverstar (w)	13	0.043	Parabinga (m)	7	0.431
Sloop (b)	6	0.332	Paraggio (m)	0	0.983
Spear (w)	2	0.734	Popany (v)	-7	0.881
Tahara (t)	6	0.339	Rainbow (c)	4	0.591
Tamaroi (d)	8	0.242	Sava (m)	16	0.115
Trident (w)	0	0.902	Tyson (ch)	24	0.062
Wallaroo (o)	9	0.248	3323 (v)	15	0.009
Worrakatta (w)	12	0.035			
Yallaroi (d)	8	0.300			
Yanac (w)	0	na			

The *r* values for significance of relationships between either initial or final nematode number and yield at Sandilands, using simple linear regression, are shown in Table 8.5. No relationship was observed between yield and initial number of *P. neglectus* at this site (graphs not shown). The relationship between final density and yield for cereals is shown in Figure 8.3. Significant negative relationships were observed for wheat and oat when crop species were assessed separately (wheat $r = -0.525$; oat $r = -0.870$), but not for cereals when all data were combined ($r = -0.026$). For pulse, oilseed and pasture varieties, no significant relationships were observed either with combined data, or within each crop species, and graphs of these relationships are not presented.

Table 8.5 ***r* values for correlation between initial or final number of *P. neglectus* and yield in untreated plots Sandilands variety trial, 1996, (n = 4), * = significant at P < 0.05.**

Crop	d.f.	tabulated *r* (P < 0.05)	calculated *r* value	
			Initial density	Final density
		Cereal varieties		
All varieties	40	0.304	0.09	-0.03
Wheat	18	0.444	0.14	-0.53*
Barley	7	0.666	0.11	-0.44
Oat	4	0.811	0.06	-0.87*
		Pulse and oilseed varieties		
All varieties	24	0.388	0.17	-0.27
Field pea	6	0.707	0.25	-0.22
Chickpea	3	0.878	0.70	-0.85
Canola	5	0.754	0.61	0.03
Medic	6	0.707	0.33	0.25

Figure 8.3 **Relationship between final density of *P. neglectus* and yield for wheat varieties: (r = -0.53: y = -0.193x + 2.77); barley varieties (r = -0.44: y = -0.133x + 2.70); oat varieties (r = -0.87: y = -1.412x + 3.28), Sandilands variety trial, 1996 (Each point represents the average for each variety [n = 4]).**



8.2.2.2 Paskeville variety trial, 1997

P. neglectus was identified at this site. Initial and final numbers are shown in Chapter 7 (Tables 7.5 and 7.6). Initial numbers of *P. neglectus* at this site were very low, with densities ranging from 0.3 - 0.9 *P. neglectus*/g of soil (average density of 0.5 *P. neglectus*/g of soil).

The ANOVA of the results comparing yield in aldicarb treated and untreated plots is shown in Table 8.6. The percent yield difference between aldicarb treated and untreated plots and significance between treatments are shown in Table 8.7. For cereal, pulse and oilseed varieties, there was no overall significant effect of aldicarb on yield at this site. For individual cereal varieties, a significant yield increase in aldicarb treated compared to untreated plots was observed for Echidna oat (11%), and the wheat varieties Worrakatta (14%) and Excalibur (20%). A significant decrease in yield from aldicarb compared to untreated plots was measured for the barley varieties Skiff (14%) and Sloop (20%).

Table 8.6 The ANOVA of the results for effect of aldicarb treatment on yield for cereal, pulse and oilseed varieties, Paskeville variety trial, 1997.

	d.f.	m.s.	P
	Wheat, barley, oat, durum, rye and triticale varieties		
Rep.	3	0.198	0.0001
Variety	40	0.540	0.0001
Rep.*Variety	118	0.045	0.0001
Aldicarb	1	0.031	0.1894
Rep.*Variety*Aldicarb	40	0.025	0.0894
	Chickpea, faba bean, field pea, vetch and canola varieties		
Rep.	3	0.331	0.0001
Variety	20	0.445	0.0001
Rep.*Variety	59	0.056	0.0001
Aldicarb	1	0.013	0.3011
Rep.*Variety*Aldicarb	20	0.018	0.0895

Table 8.7 **Percent response in yield from aldicarb treated compared with untreated plots for wheat (w), barley (b), oat (o), triticale (t), rye (r), durum (d), chickpea (ch), canola (c), faba bean (fb), field pea (p) and vetch (v) varieties, Paskeville variety trial, 1997. P value derived from general linear model (least squares means) are significant at $P < 0.05$. Yields from aldicarb treated and untreated plots are presented in Appendix 6. na = analysis not available due to missing values.**

Cereal varieties			Pulse, pasture and oilseed varieties		
Variety	Response in aldicarb plots (%)	P value	Variety	Response in aldicarb plots (%)	P value
Abacus (t)	-5	0.545	Alma (p)	10	0.220
Arapiles (b)	-8	0.371	Ascot (fb)	0	0.974
Barque (b)	-2	0.792	Blanchefleur (v)	23	0.009
Barunga (w)	-1	0.854	Bluey (p)	11	0.102
Bettong (o)	-6	0.344	Desavic (ch)	-2	0.697
Beulah (w)	7	0.293	Dooen (ch)	10	0.156
Bevy (r)	0	1.000	Dundale (p)	12	0.166
Bowie (w)	0	1.000	Dunkeld (c)	-8	0.456
BT Schomburgk (w)	-13	na	Fiord (fb)	-10	0.095
Carnamah (w)	0	0.979	Grouse (c)	-13	0.263
Carrolup (o)	9	0.110	Hyola 42 (c)	-1	0.871
Cascades (w)	-4	0.494	Icarus (fb)	-8	0.316
Chebec (b)	-10	0.149	Kaniva (ch)	-14	0.166
Echidna (o)	11	0.053	Karoo (c)	8	0.581
Euro (o)	0	0.937	Languedoc (v)	13	0.115
Excalibur (w)	20	0.010	Lasseter (ch)	6	0.286
Frame (w)	-9	0.270	Monty (c)	-9	0.418
Franklin (b)	-2	0.792	Oscar (c)	-10	0.348
Galleon (b)	-4	0.598	Pinnacle (c)	-4	0.745
Janz (w)	-9	0.217	Rainbow (c)	14	na
Krichauff (w)	-11	0.135	Tyson (ch)	-2	0.697
Machete (w)	13	0.248			
Marloo (o)	-7	0.293			
Meering (w)	2	0.792			
Mundah (b)	-10	0.173			
Ouyen (w)	-7	0.371			
Pallinup (o)	-1	0.813			
Potoroo (o)	0	0.732			
Schooner (b)	3	0.732			
Silverstar (w)	-3	0.772			
Skiff	-14	0.006			
Sloop (b)	-20	0.004			
Spear (w)	0	0.958			
Tahara (t)	-4	na			
Tamaroi (d)	4	0.673			
Trident (w)	-5	0.511			
Worrakatta (w)	-14	0.147			
Yallaroi (d)	-5	0.583			
Yanac (w)	-2	0.813			

The *r* values for significance of relationships between density of *P. neglectus* and yield are presented in Table 8.8. Relationships between final number of *P. neglectus* and yield of cereals are shown in Figure 8.4, and between final numbers and yield of pulses and oilseeds in Figure 8.5. As at Sandilands, no significant relationships were observed between yield and initial density of *P. neglectus* for cereals, either with combined data or when analysed as separate crop species. A significant, negative relationship was observed only between the final number of *P. neglectus* and yield of wheat varieties (when analysed as a separate crop).

In contrast to the Sandilands site, significant relationships were observed between the initial number of *P. neglectus* and yield of chickpeas (assessed as a separate group),and also for the combined pulse and oilseed data. No significant relationships were observed for pulse and oilseed varieties with the final density of *P. neglectus* at this site.

Table 8.8 ***r* values for correlation between initial or final number of *P. neglectus* and yield in untreated plots, Paskeville variety trial, 1997, (n = 4), * = significant at P < 0.05.**

Crop	d.f.	Tabulated *r* (P < 0.05)	calculated *r* value	
			Initial density	Final density
Cereal varieties				
All varieties	38	0.312	0.095	0.278
Wheat	18	0.444	0.030	-0.445*
Barley	7	0.666	0.155	0.221
Oat	5	0.745	0.203	0.741
Pulse and oilseed varieties				
All varieties	22	0.404	0.624*	0.024
Chickpea	3	0.878	0.943*	-0.808
Canola	6	0.707	0.548	-0.114
Faba bean and field pea	4	0.811	0.789	0.502

Figure 8.4 **Relationship between final density of *P. neglectus* and yield for wheat varieties (r = -0.45: y = -0.064x + 1.41); barley varieties (r = 0.22: y = 0.092x + 1.22); and oat varieties (r = 0.74: y = 0.088x + 1.49), Paskeville variety trial, 1997. Each point represents the average for each variety (n = 4).**

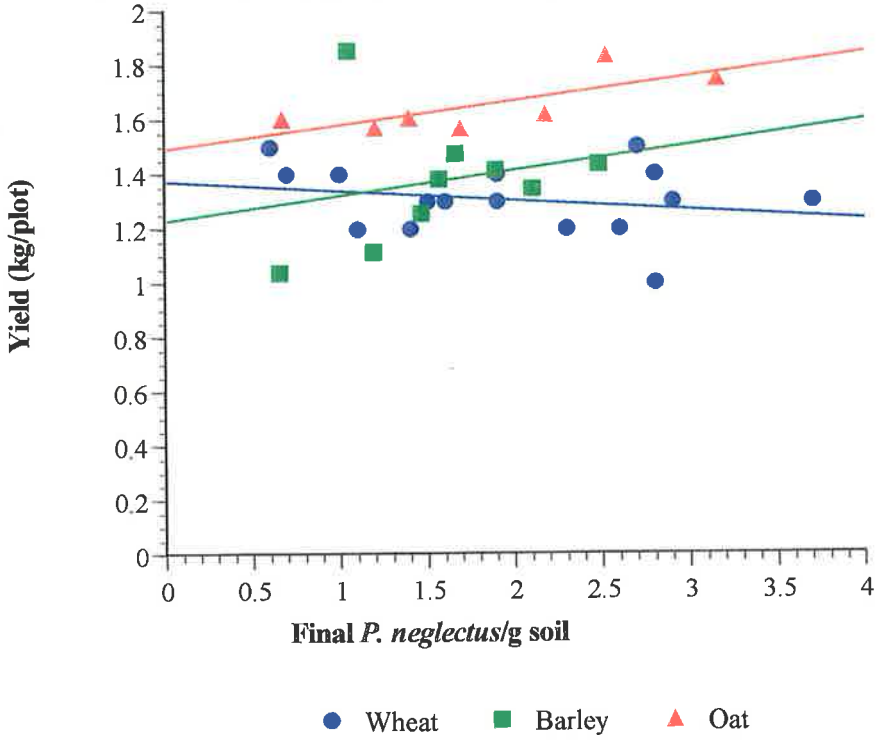
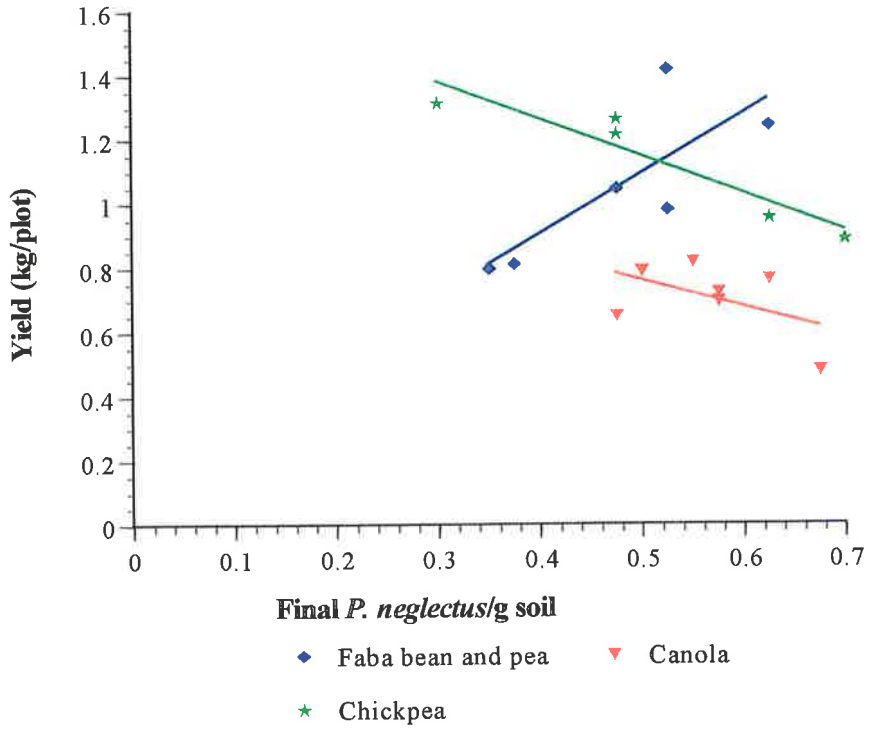


Figure 8.5 **Relationship between initial density of *P. neglectus* and yield for chickpea varieties r = -0.94: y = -1.17x + 1.73); canola varieties (r= -0.55: y = -0.77x + 1.17); faba bean and field pea varieties (r = 0.79: y = 0.189x + 0.16) Paskeville variety trial, 1997. Each point represents the average for each variety (n = 4).**



8.2.2.3 Condada variety trial, 1996

P. neglectus was identified at Condada. Initial number, final number and multiplication rate of *P. neglectus* are shown in Table 7.10. There was no significant difference in initial numbers between varieties and large variation was observed across the site (Figure 7.9). Significant differences were seen between both final numbers and multiplication rates following different plant varieties. Highest final numbers were observed in the wheat varieties Machete, Frame, Janz and Barunga, while lowest numbers were recorded in Chebec barley, BT-Schomburgk wheat, Tahara triticale and Krichauff wheat.

The regression equations and *r* values for initial and final numbers and yield and *r* values for multiplication rate and yield in untreated plots are presented in Table 8.9. The relationships between the mean of initial numbers and yield for individual varieties are shown in Figure 8.6. Significant, negative relationships between initial numbers of *P. neglectus* and yield in untreated plots were observed for Machete wheat, Echidna oat, Schooner barley and Euro oat, and these varieties were therefore designated as intolerant. All other cereals in this trial were designated tolerant, even though significant relationships were observed between final numbers and yield in Frame wheat and Schooner barley. No relationship was observed between multiplication rate and yield.

Figure 8.7 shows the relationship between initial numbers of *P. neglectus* and yield for the intolerant varieties (Machete wheat, Schooner barley, and Euro and Echidna oat) and tolerant varieties (BT-Schomburgk, Krichauff, Janz, Frame, Stiletto, Barunga and Excalibur wheat, Potoroo oat and Chebec barley) expressed for all replicates. As with means from individual varieties, a significant negative relationship was observed for intolerant varieties but not for tolerant varieties.

The relationship between final numbers of *P. neglectus* and yield for intolerant and tolerant varieties is shown in Figure 8.8. A significant negative relationship was observed for intolerant varieties only.

Table 8.9 **Regression equations and *r* values (P < 0.05) for correlation between initial number/g of soil (Pi), final number (Pf) or multiplication rate of *P. neglectus* and yield in untreated plots for Condada variety trial, 1996 (n = 6). [A] Intolerant varieties (Machete, Echidna, Euro and Schooner) tabulated *r* value (22 d.f.) = 0.404; [B] Tolerant varieties (Excalibur, Frame, Barunga, Janz, Stiletto, BT Schomburgk, Krichauff, Potoroo, Chebec and Tahara), tabulated *r* value (58 d.f.) = 0.250; [C] tabulated *r* value (4 d.f.) = 0.811. * = significant at P < 0.05.**

	Pi/g of soil		Pf/g of soil		Multiplication rate
	r value	Equation	*r* value	Equation	*r* value
Intolerant varieties [A]	-0.576*	y = -0.07x + 1.31	-0.488*	y = -0.07x + 1.28	-0.012
Tolerant varieties [B]	-0.083	y = -0.01x +1.14	-0.245	y = -0.04x + 1.25	-0.033
Individual varieties[C]					
Machete wheat	-0.821*	y = -0.07x + 1.22	-0.544	y = -0.01x + 1.02	-0.209
Excalibur wheat	-0.735	y = -0.05x + 1.42	-0.757	y = -0.11x + 1.39	-0.054
Frame wheat	-0.450	y = -0.08x + 1.54	-0.918*	y = -0.18x + 1.93	-0.539
Barunga wheat	-0.096	y = -0.01x + 1.11	-0.384	y = -0.03x + 1.19	-0.044
Janz wheat	0.258	y = 0.02x + 0.97	-0.619	y = -0.01x + 1.07	-0.801
Stiletto wheat	0.082	y = 0.02x + 1.22	-0.201	y = -0.04x + 1.34	-0.082
BT-Schomburgk wheat	0.330	y = 0.01x + 1.04	-0.096	y = -0.03x + 1.11	-0.571
Krichauff wheat	0.073	y = 0.05x + 1.31	-0.655	y = -0.35x + 1.53	-0.449
Potoroo oat	-0.178	y = -0.01x + 1.29	-0.674	y = -0.04x + 1.34	-0.577
Euro oat	-0.897*	y = -0.16x + 1.52	-0.659	y = -0.18x + 1.93	-0.540
Echidna oat	-0.894*	y = -0.04x + 1.11	-0.569	y = -0.07x + 1.04	-0.324
Schooner barley	-0.958*	y = -0.09x + 1.63	-0.830*	y = -0.18x + 1.66	-0.215
Chebec barley	-0.556	y = -0.04x + 1.22	-0.697	y = -0.13x + 1.22	-0.104
Tahara triticale	-0.358	y = -0.09x + 1.31	-0.361	y = -0.20x + 1.25	-0.255

Figure 8.6 **Relationship between initial numbers of *P. neglectus* and yield in untreated plots for individual intolerant varieties (Machete wheat, Echidna oat, Schooner barley and Euro oat), Condada variety trial 1996. Each point represents one replicate.**

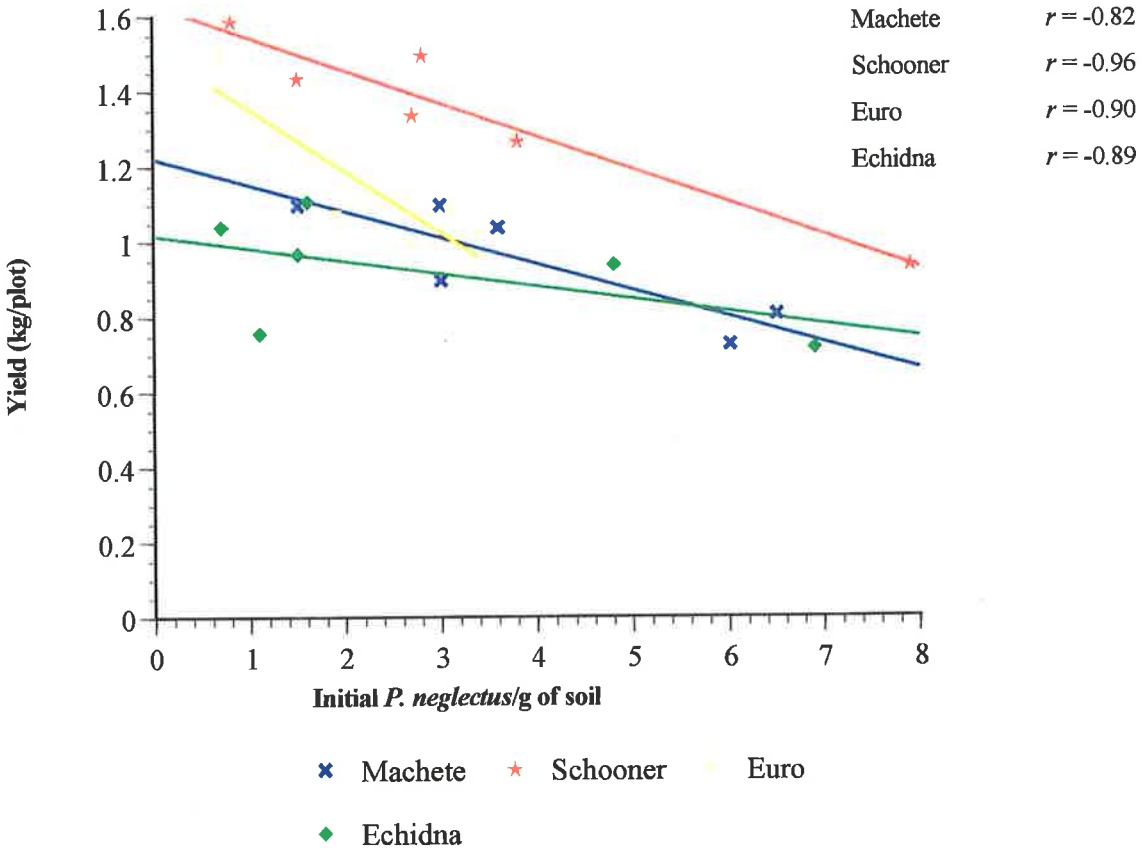


Figure 8.7 **Relationship between initial numbers of *P. neglectus*/g of soil and yield in untreated plots, for intolerant varieties (Machete wheat, Schooner barley, Euro and Echidna oat; tabulated *r* value [22 d.f.] = 0.404) and tolerant varieties (Tahara triticale, BT Schomburgk, Krichauff, Janz, Frame, Stiletto, Barunga, Excalibur wheat, Potoroo oat and Chebec barley; tabulated *r* value [58 d.f] = 0.262), Condada variety trial, 1996. Each point represents one replicate.**

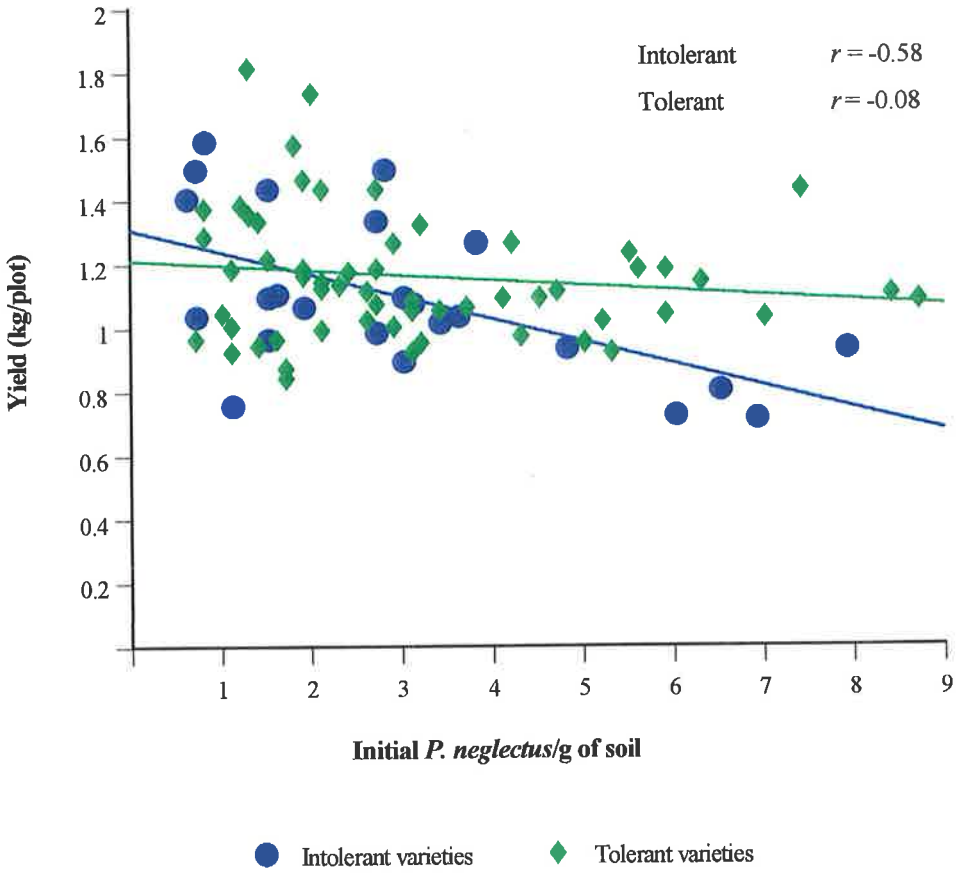
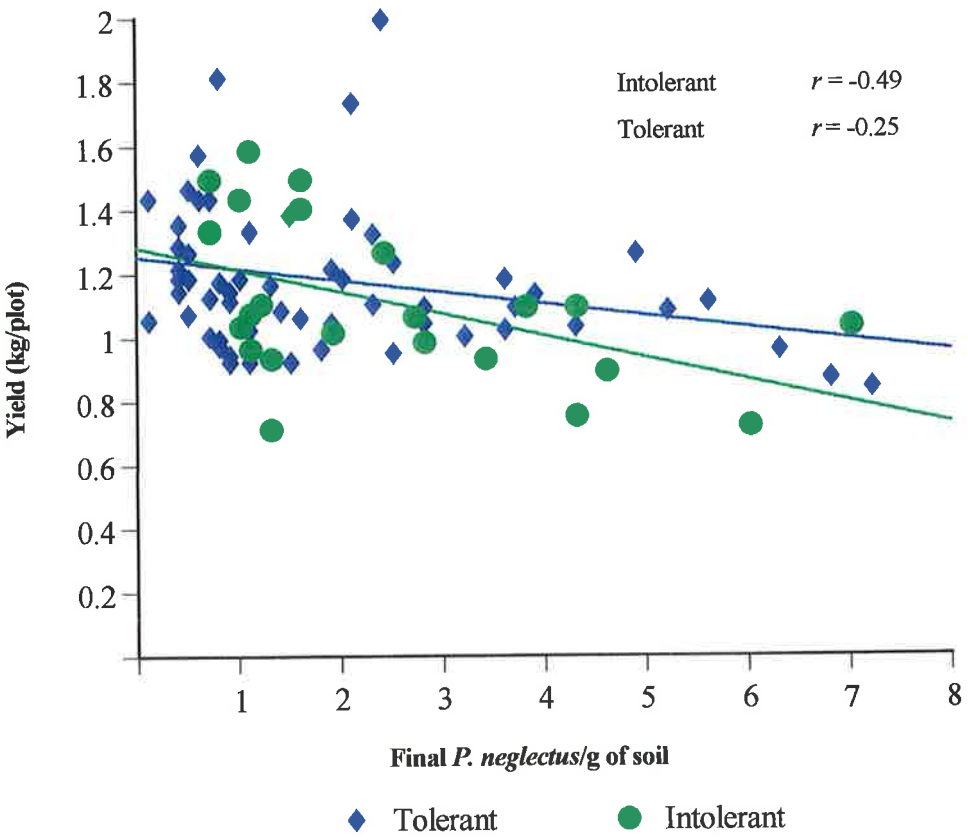


Figure 8.8 **Relationship between final numbers of *P. neglectus*/g of soil and yield in untreated plots, Condada variety trial, 1996. For intolerant varieties (Machete wheat, Schooner barley, Euro and Echidna oat; tabulated *r* value [22 d.f.] = 0.404). For tolerant varieties (Tahara triticale, BT-Schomburgk, Krichauff, Janz, Frame, Stiletto, Barunga, Excalibur wheat, Potoroo oat and Chebec barley; tabulated *r* value [58 d.f] = 0.262). Each point represents one replicate.**



The ANOVA of the results of the effect of variety and aldicarb treatment on yield is presented in Table 8.10, and significance between aldicarb treated and untreated plots in the Condada variety trial is shown in Table 8.11. Percentage yield increase in aldicarb treated compared with untreated plots is presented in Figure 8.9. Significant yield responses in aldicarb treated compared with untreated plots were observed in Tahara triticale, Stiletto wheat, Chebec barley, Janz wheat, Machete wheat and Echidna oat (10%, 10%, 10%, 11%, 17% and 21%, respectively).

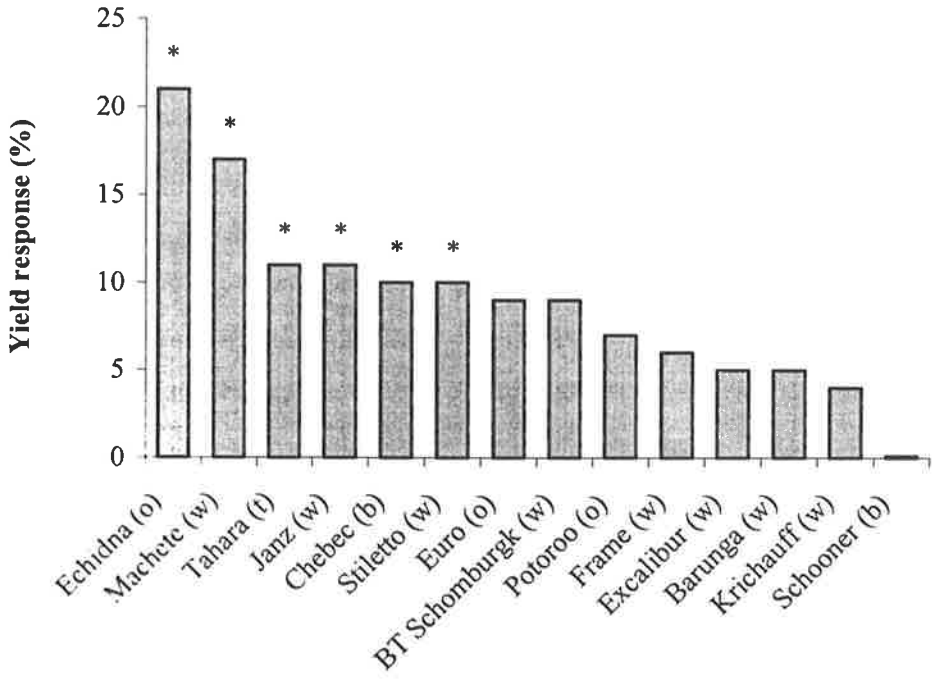
Table 8.10 **The ANOVA of the results of variety and aldicarb treatment on yield, Condada cereal variety trial, 1996.**

	d.f.	m.s.	P
Rep.	5	0.449	0.0000
Variety	13	0.170	0.0012
Rep.*Variety	65	0.055	
Aldicarb	1	0.505	0.0000
Variety*Aldicarb	13	0.010	0.0176
Rep.*Variety*Aldicarb	70	0.007	

Table 8.11 **Comparison between yield in aldicarb treated and untreated plots, Condada variety trial, 1996 (n = 6). n.s. = non-significant; * = significant at P < 0.05; ** = significant at P < 0.01. Percentage yield loss is shown in Figure 8.9.**

Variety	Yield (kg/plot)		Difference (A – U)
	Untreated (U)	Aldicarb treated (A)	
	Wheat		
Barunga	1.09	1.15	0.06 n.s.
BT Schomburgk	1.08	1.19	0.11 n.s.
Excalibur	1.26	1.33	0.07 n.s.
Frame	1.29	1.37	0.08 n.s.
Janz	1.02	1.15	0.13 *
Krichauff	1.33	1.38	0.05 n.s.
Machete	0.94	1.13	0.19 **
Stiletto	1.27	1.41	0.14 *
	Barley		
Chebec	1.09	1.21	0.12 *
Schooner	1.35	1.36	0.01 n.s.
	Oat		
Echidna	0.92	1.16	0.24 **
Euro	1.18	1.29	0.11 n.s.
Potoroo	1.26	1.36	0.1 n.s.
	Triticale		
Tahara	1.09	1.22	0.13 *
l.s.d. (5%)			0.12
l.s.d. (1%)			0.16

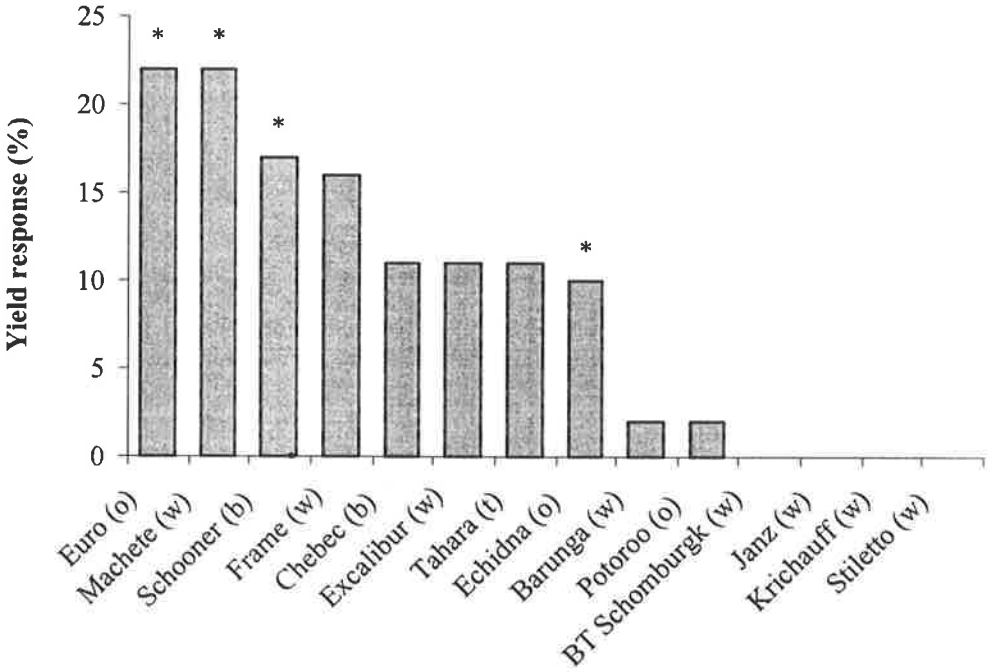
Figure 8.9 **Percentage increase in yield from aldicarb compared with untreated plots for wheat (w), barley (b), oat (o) and triticale (t) varieties, Condada variety trial, 1996. * Significant response from aldicarb treated plots (P < 0.05).**



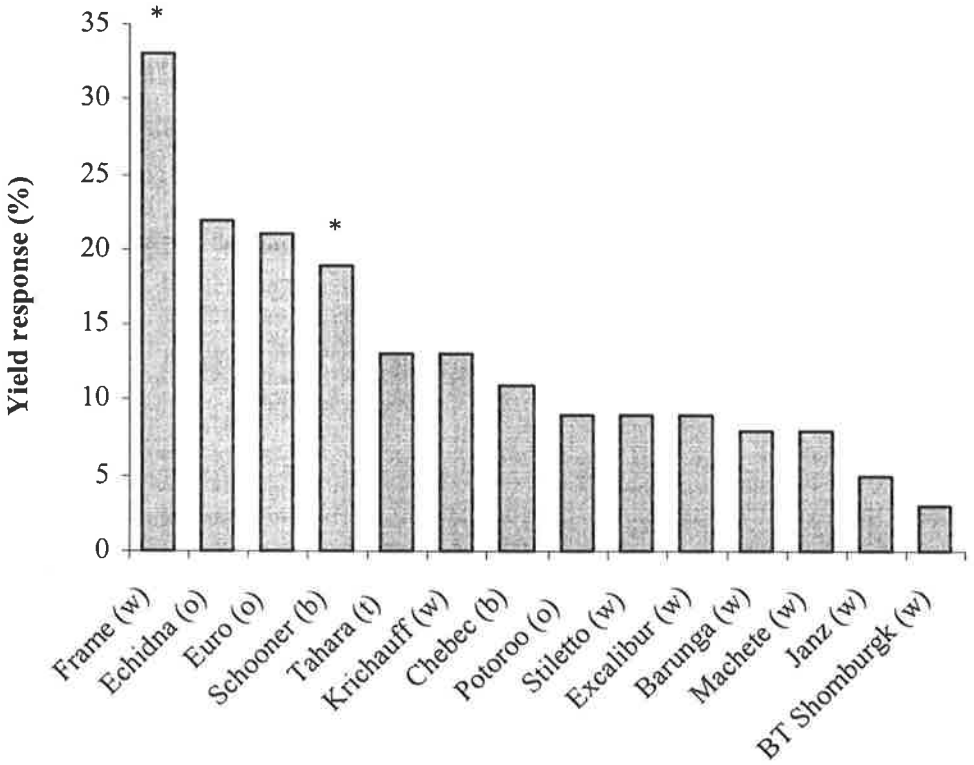
Yield losses calculated from the regression equations (Table 8.9) for each variety using both initial and final numbers of *P. neglectus* are shown in Figure 8.10. Greater similarities were seen between yield loss measured by aldicarb or determined from the regression with initial density and yield than with final density and yield. Using either aldicarb or correlation of yield with initial numbers, yield losses of approximately 21% were recorded for Machete wheat, and significant yield responses were observed in Echidna oat. Lower yield loss (< 10%) was recorded using either aldicarb or regression analysis for Potoroo oat, and the wheat varieties Excalibur, Krichauff, Barunga and BT-Schomburgk. Less consistent results were produced for Schooner barley and Euro oat, with low yield response to aldicarb, but significant negative relationships were observed between both initial and final nematode numbers and yield.

Figure 8.10 **Yield loss calculated from the regression equations (presented in Table 8.9) for a) initial number of *P. neglectus* and yield, and b) final number of *P. neglectus* and yield for varieties of wheat (w), barley (b), oat (o) and triticale (t), Condada variety trial, 1996. * regression significant ($P < 0.05$).**

a)



b)



8.2.3 Rotation trials

8.2.3.1 Minnipa rotation trial, 1996 and 1997

P. thornei was identified at this site. The ANOVA of the results for the effect of variety on initial or final density of *P. thornei* in 1996 and 1997 is shown in Table 8.12, and initial and final densities and multiplication rates of *P. thornei* at Minnipa are shown in Table 8.13. In 1996, there were no significant differences between initial numbers of *P. thornei* under cereal plots. Significant effects of cereal variety on numbers of *P. thornei* were observed in the final sampling in 1996, and both initial and final sampling in 1997.

Machete was the most susceptible variety, followed by Spear, Excalibur, Chebec and Tahara with final nematode densities in 1996 of 28, 23, 7, 2 and 1 *P. thornei*/g of dry soil respectively. In 1996, significant differences were observed between multiplication rates under different varieties with highest multiplication in Machete (8.0) and lowest in Tahara (0.2). The effect of initial number on multiplication rate of *P. thornei* in 1997 (following manipulation with resistant and susceptible varieties in 1996) using both $\log_e$ transformed and untransformed data is presented in Figure 8.11. For both untransformed and $\log_e$ transformed data, a negative exponential decay model was fitted to the data (Section 8.1.3). Data were also fitted using a linear exponential model (figure not presented). For both untransformed and $\log_e$ transformed data, little difference in significance was observed between either the negative exponential decay or the linear model ($\log_e$ transformed: data $R = 0.76$ and 0.75, respectively; untransformed $R = 0.60$ and 0.57, respectively). Higher rates of multiplication were observed with lower initial densities of *P. thornei*.

The relationship between final and initial density of *P. thornei* is shown in Figure 8.12. Final density increased with increasing initial density, until a ceiling level was reached. In this trial, initial densities of approximately 7.4 *P. thornei*/g of soil produced a ceiling level of approximately 17.4 *P. thornei*/g.

Table 8.12 **The ANOVA of results for the effect of cereal varieties on initial and final density of *P. thornei* in 1996 (Machete, Spear and Excalibur wheat, Chebec barley and Tahara triticale) and 1997 (Echidna oat following these varieties), Minnipa rotation trial (n = 4).**

	d.f.	m.s.	P
	1996 Initial density of *P. thornei*/g of soil		
Rep.	3	0.798	
Variety	4	0.157	0.052
Rep.*Variety	11	0.225	0.608
	1996 Final density of *P. thornei*/g of soil		
Rep.	3	0.983	
Variety	4	5.837	0.007
Rep.*Variety	11	0.136	0.0000
	1997 Initial density of *P. thornei*/g of soil		
Rep.	3	0.772	
Variety	4	3.602	0.019
Rep.*Variety	12	0.157	0.0000
	1997 Final density of *P. thornei*/g of soil		
Rep.	3	0.461	
Variety	4	2.450	0.107
Rep.*Variety	12	0.183	0.0002

Table 8.13 **Initial density, final density and multiplication rate of *P. thornei*, Minnipa rotation trial 1996 and 1997. 1996: Machete wheat, Spear wheat, Excalibur wheat, Chebec barley and Tahara triticale; 1997: Echidna oat (n = 4). Figures in brackets are detransformed means.**

	Initial *P. thornei*/g of soil ($\log_e$ +1)	Final *P. thornei*/g of soil ($\log_e$ + 1)	Multiplication rate (Pf/Pi; $\log_e$ + 1)
		1996	
Machete	1.88 (5.6)	3.38 (28.4)	2.2 (8.0)
Spear	2.09 (7.0)	3.18 (23.0)	1.4 (3.1)
Excalibur	1.76 (4.8)	2.06 (6.8)	0.9 (1.5)
Chebec	1.92 (5.8)	1.01 (1.7)	0.4 (0.5)
Tahara	1.56 (3.8)	0.75 (1.1)	0.2 (0.2)
h.s.d.	1.09	0.85	0.9
	1997 (Echidna oat following these varieties)		
Machete	2.71 (14.0)	2.94 (17.9)	0.9 (1.5)
Spear	2.36 (9.5)	2.57 (12.1)	0.9 (1.5)
Excalibur	1.28 (2.6)	1.86 (5.4)	1.1 (2.1)
Chebec	0.51 (0.7)	1.02 (1.8)	1.3 (2.6)
Tahara	0.88 (1.4)	1.49 (3.4)	1.3 (2.6)
h.s.d.	0.89	0.97	0.9

Figure 8.11 **The effect of manipulation of initial density of *P. thornei* with resistant and susceptible varieties in 1996 (Machete, Spear and Excalibur wheat, Chebec barley and Tahara triticale) on multiplication rate of *P. thornei* in Echidna oat, Minnipa rotation trial, 1997. a) untransformed data b) $\log_e$ transformed data (fitted using $Pf/Pi = a + ce^{bPi}$). Each point represents one replicate.**

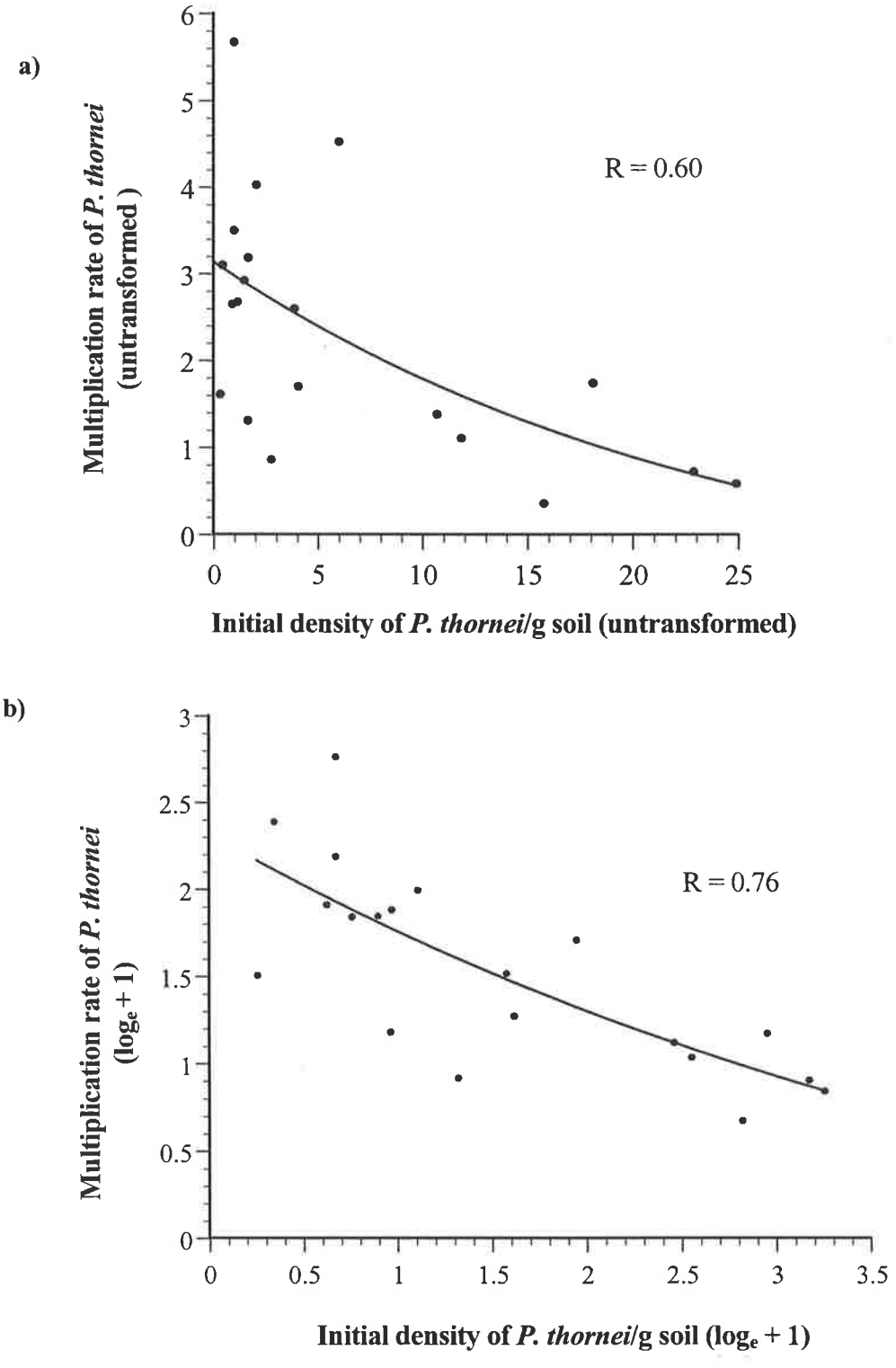
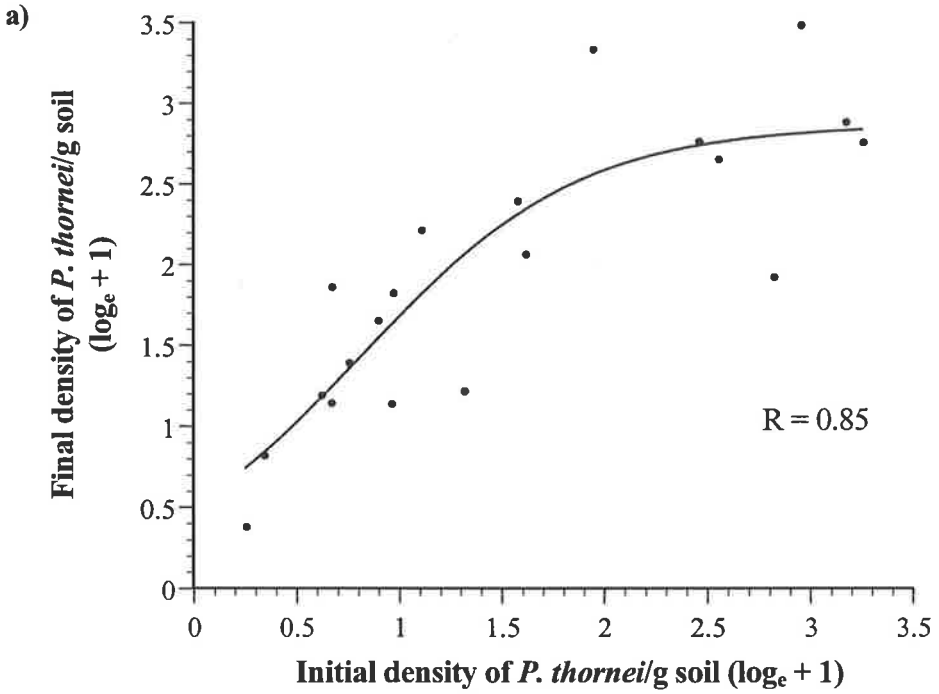


Figure 8.12 **The effect of manipulation of initial density of *P. thornei* by resistant and susceptible varieties in 1996 (Machete, Spear and Excalibur wheat, Chebec barley and Tahara triticale) on the relationship between initial density and final density of *P. thornei* in Echidna oat, Minnipa rotation trial, 1997. Each point represents one replicate. Data fitted using $Pf = ce^{bPi}/(e^{bPi} - 1) + d$.**



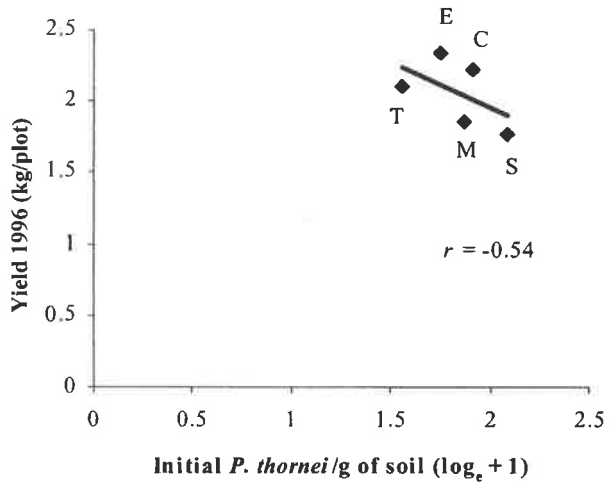
Using simple linear regression, the relationships between both initial and final *P. thornei* density and yield for the average of each variety (n = 4) in 1996 and 1997 are shown in Figure 8.13. The relationship between number of *P. thornei* and yield of Echidna oat for individual plots in 1997 (following resistant or susceptible varieties in 1996) is shown in Figure 8.14. The regression equations and *r* values are presented in Table 8.14. Significant, negative relationships were observed between the initial and final density of *P. thornei* and yield in 1997 for both the average of each variety (tabulated *r* value [3 d.f.] = 0.878) and individual plots (tabulated *r* value [18 d.f.] = 0.444). No relationship was observed between the initial density or final density of *P. thornei* and yield in 1996.

Table 8.14 **Regression equations and *r* values for correlation between initial or final number of *P. thornei* and mean yield in untreated plots, Minnipa rotation trial, 1996 (Machete, spear and Excalibur wheat, Chebec barley and Tahara triticale) and 1997 (Echidna oat following these varieties [n = 4]). Tabulated *r* value (P < 0.05; 3 d.f. = 0.878 for average of variety; 18 d.f. = 0.444 for individual plots). * significant.**

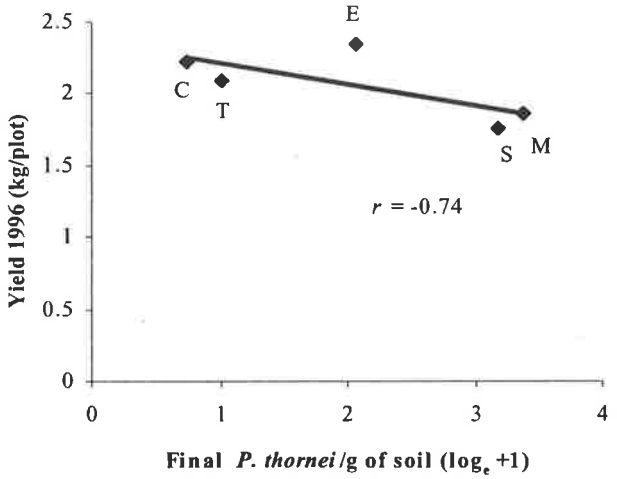
	r value	Regression equation
	Yield 1996	
Initial density 1996	-0.538 n.s.	y = -0.655x + 3.26
Final density 1996	-0.736 n.s.	y = -0.146x + 2.36
	Yield 1997 (average for each variety) 3 d.f.	
Initial density 1997	-0.917*	y = -0.096x + 1.84
Final density 1997	-0.881*	y = -0.118x + 1.92
	Yield 1997 (individual plots following each variety) 18 d.f.	
Initial density 1997	-0.495*	y = -0.069x + 1.80
Final density 1997	-0.445*	y = -0.089x + 1.87

Figure 8.13 **Relationship between a) Initial number of *P. thornei* and yield, 1996 b) Final number of *P. thornei* and yield, 1996 c) Initial number of *P. thornei* and yield, 1997 d) Final number of *P. thornei* and yield 1997, Minnipa rotation trial. In 1996, Machete (M), Spear (S), and Excalibur (E) wheat, Chebec barley (C) and Tahara triticale (T) were sown. In 1997, Echidna oat was sown over these varieties. Each point represents the average for each variety (n = 4).**

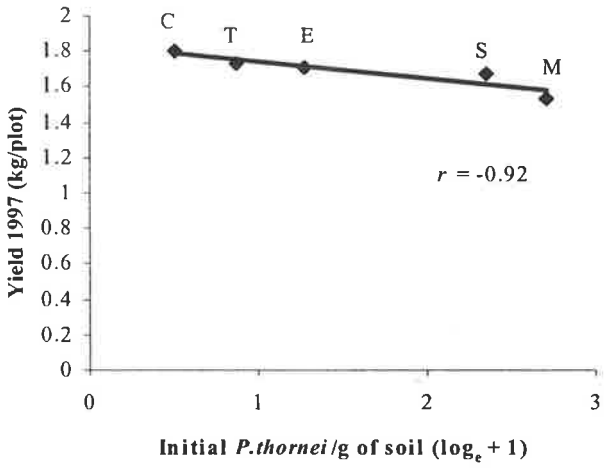
a) Initial number of *P. thornei* and yield, 1996.



b) Final number of *P. thornei* and yield, 1996.



c) Initial number of *P. thornei* and yield, 1997.



d) Final number of *P. thornei* and yield, 1997.

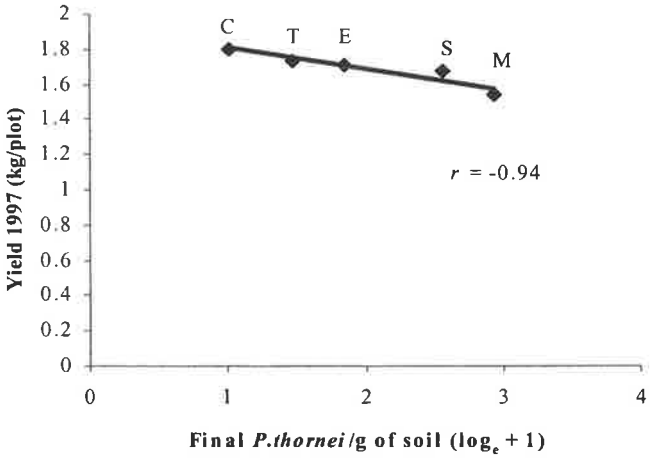
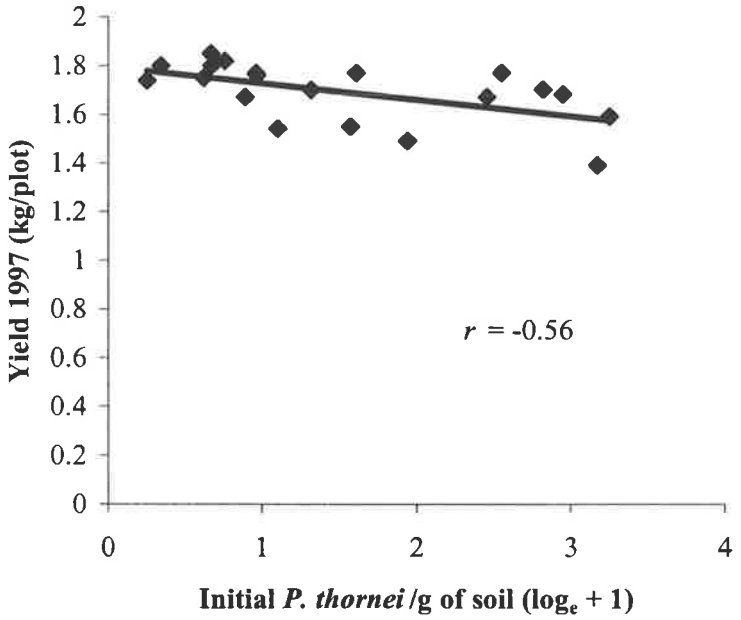
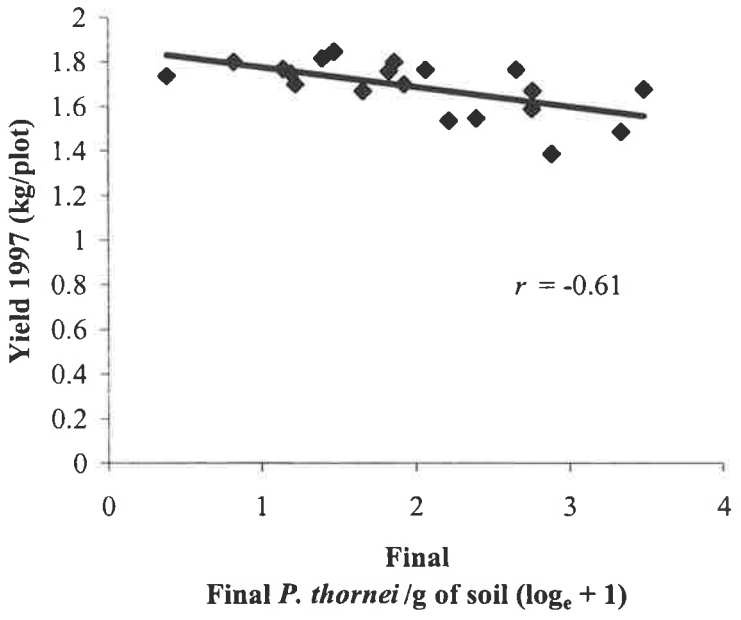


Figure 8.14 **Relationship between a) initial number of *P. thornei* and b) final number of *P. thornei* and the yield of Echidna oat, Minnipa rotation trial, 1997. Each point represents an individual replicate.**

a) Initial number of *P. thornei*



b) Final number of *P. thornei*



The ANOVA of the results of the effect of variety and aldicarb treatment on yield of Machete, Spear and Excalibur wheat, Chebec barley and Tahara triticale in 1996 and the effect of varieties grown in 1996 on the yield of Echidna oat in 1997 are shown in Table 8.15. Yield responses in Machete, Spear and Excalibur wheat, Chebec barley and Tahara triticale were calculated using either aldicarb or from the regression equation of final number of *P. thornei* and yield in 1996, and are shown in Figure 8.15. Yield losses measured using aldicarb were observed only in Spear and Machete (17% and 10%, respectively). A negative response to aldicarb was observed in Chebec barley. Higher yield responses were calculated using regression analyses, with losses of 21% and 25% respectively in Machete and Spear.

Yield losses calculated from the regression equation of both initial and final number of *P. thornei* and yield in Echidna oat in 1997 are presented in Figure 8.16. Slightly higher yield losses were calculated using final compared with initial numbers of *P. thornei*, although results were very similar.

Table 8.15 **The ANOVA of results for differences in yield of cereals in 1996 (Machete, Spear and Excalibur wheat, Chebec barley and Tahara triticale) and 1997 (Echidna oat), Minnipa rotation trial (n = 4).**

	d.f.	m.s.	P
		1996	
Rep.	3	0.036	
Variety	4	0.237	0.0890
Rep.*Variety	12	0.013	0.0001
Aldicarb	1	0.127	
Variety*Aldicarb	4	0.096	0.0039
Rep.*Variety*Aldicarb	15	0.011	0.0007
		1997	
Rep.	3	0.031	0.0490
Variety	4	0.231	0.0000
Rep.*Variety	12	0.009	

Table 8.16 **Yield from aldicarb treated and untreated plots for Machete, Spear, Excalibur wheat, Chebec barley and Tahara triticale in 1996 and for Echidna oat following these plots in 1997 (n = 4), Minnipa rotation trial. * significant (P < 0.05), # significantly different from yield following Machete (P < 0.05).**

	1996			1997 (Echidna oat following these varieties)
	Untreated (U)	Aldicarb (A)	Difference (A-U)	Untreated plots from 1996
Machete wheat	1.87	2.09	0.22*	1.54
Spear wheat	1.76	2.14	0.38*	1.67
Excalibur wheat	2.34	2.44	0.10	1.71
Chebec barley	2.23	2.01	-0.22*	1.73
Tahara triticale	2.10	2.19	0.09	1.80#
			l.s.d. 0.16	h.s.d. 0.20

Figure 8.15 **Difference between yield reduction caused by *P. thornei* measured by aldicarb (compared with untreated plots) or calculated from the regression equation comparing final number of *P. thornei* and yield in 1996 (y = -0.655x + 3.26) in Machete, Spear and Excalibur wheat, Chebec barley and Tahara triticale, Minnipa rotation trial, 1996.**

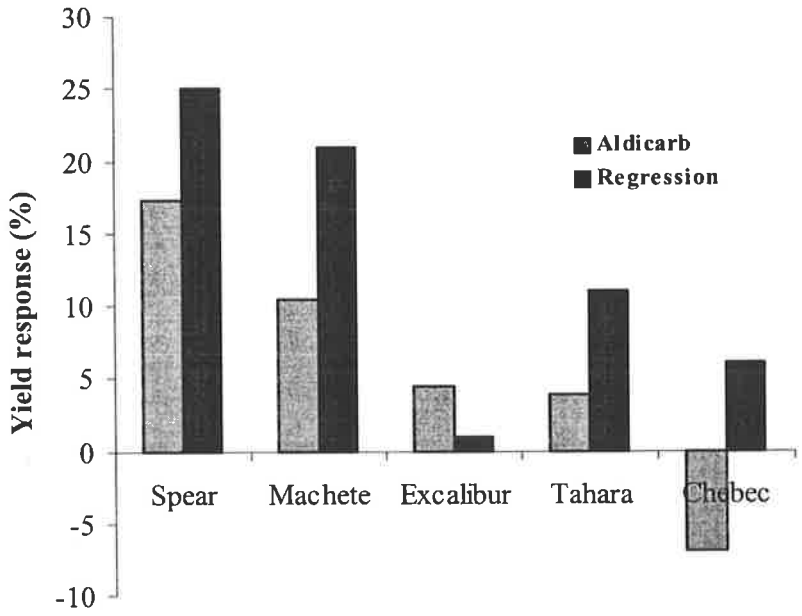
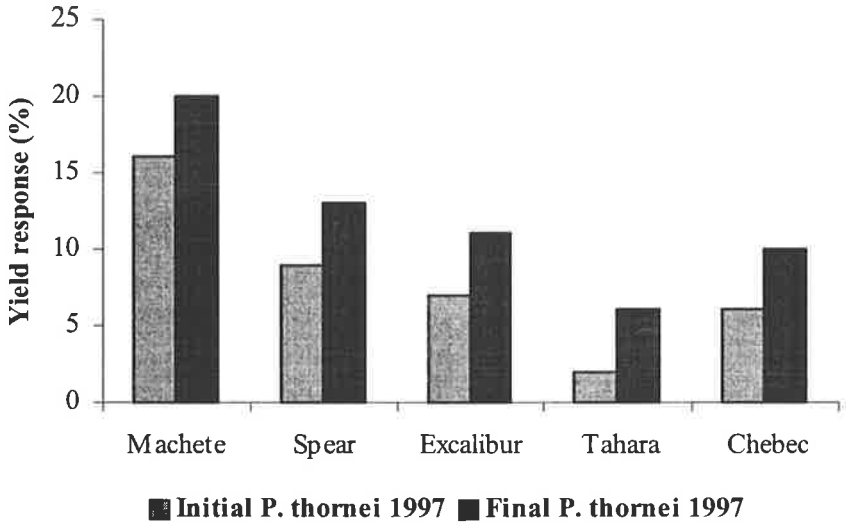


Figure 8.16 **Yield reduction caused by *P. thornei* calculated from the regression equation of either initial number and yield (y = -0.096x + 1.84) or final number and yield (y = - 0.118x + 1.92) in Echidna oat following Machete, Spear and Excalibur wheat, Chebec barley and Tahara triticale, Minnipa rotation trial 1997 (n=4).**



8.2.3.2 Condada rotation trial, 1996 and 1997

P. neglectus was identified at this site. The ANOVA of the results for the effect of variety on initial and final density and multiplication rates of *P. neglectus* in 1996 and 1997 is presented in Table 8.17, and initial and final densities of *P. neglectus* are shown in Table 8.18. As observed with *P. thornei* at Minnipa, there was no significant difference between initial numbers of *P. neglectus* under different cereal plots in 1996. Significant differences were observed between final numbers under each variety in 1996, with highest numbers under Machete wheat, Spear wheat, Excalibur wheat, Tahara triticale and Chebec barley (12, 4, 1, 0.5 and 0.4 *P. neglectus*/g of dry soil respectively). These differences were also observed in the initial sampling in 1997 although, in contrast to results for *P. thornei* at Minnipa, there was no significant difference in final numbers of *P. neglectus* recovered under Echidna oat in 1997 following the varieties sown in 1996.

Table 8.17 **The ANOVA of results for the effect of cereal varieties on initial and final density of *P. neglectus* in 1996 (Machete, Spear and Excalibur wheat, Chebec barley and Tahara triticale) and 1997 (Echidna oat following these varieties), Condada rotation trial (n = 4).**

	d.f.	m.s.	P
		1996 Initial density of *P. neglectus*/g of soil	
Rep.	3	0.043	0.751
Variety	4	0.355	0.055
Rep.*Variety	12	0.105	
		1996 Final density of *P. neglectus*/g of soil	
Rep.	3	0.166	0.230
Variety	4	3.469	0.0000
Rep.*Variety	11	0.099	
		1997 Initial density of *P. neglectus*/g of soil	
Rep.	3	0.284	0.314
Variety	4	2.178	0.0008
Rep.*Variety	12	0.216	
		1997 Final density of *P. neglectus*/g of soil	
Rep.	3	0.271	0.448
Variety	4	0.515	0.192
Rep.*Variety	12	0.285	

Table 8.18 **Initial density, final density and multiplication of *P. neglectus*, Condada rotation trial, 1996 and 1997. Figures in brackets are detransformed means (n = 4).**

	Initial *P. neglectus*/g of soil ($\log_e$ +1)	Final *P. neglectus*/g of soil ($\log_e$ + 1)	Multiplication rate (Pf/Pi; detransformed)
		1996	
Machete	2.06 (6.9)	2.54 (11.6)	1.7
Spear	2.48 (11.0)	1.50 (3.5)	0.3
Excalibur	1.95 (6.0)	0.68 (1.0)	0.2
Chebec	2.53 (11.6)	0.40 (0.5)	0.04
Tahara	1.91 (5.8)	0.34 (0.4)	0.07
		1997 (Echidna oat following these varieties)	
Machete	2.95 (18.1)	2.36 (9.6)	0.5
Spear	2.24 (8.3)	1.85 (5.4)	0.7
Excalibur	1.57 (3.8)	1.76 (4.8)	1.3
Chebec	1.23 (2.4)	1.64 (4.2)	1.8
Tahara	1.26 (2.5)	1.64 (4.2)	1.7

The multiplication rate of *P. neglectus* for individual varieties of cereals grown in 1996 is presented in Table 8.18. As observed with *P. thornei* at Minnipa in 1996, multiplication rates were highest in Machete (1.7) and lowest in Chebec (0.04). In 1997, multiplication rates in Echidna oat were lowest following Machete (0.3) and highest following Chebec (1.8).

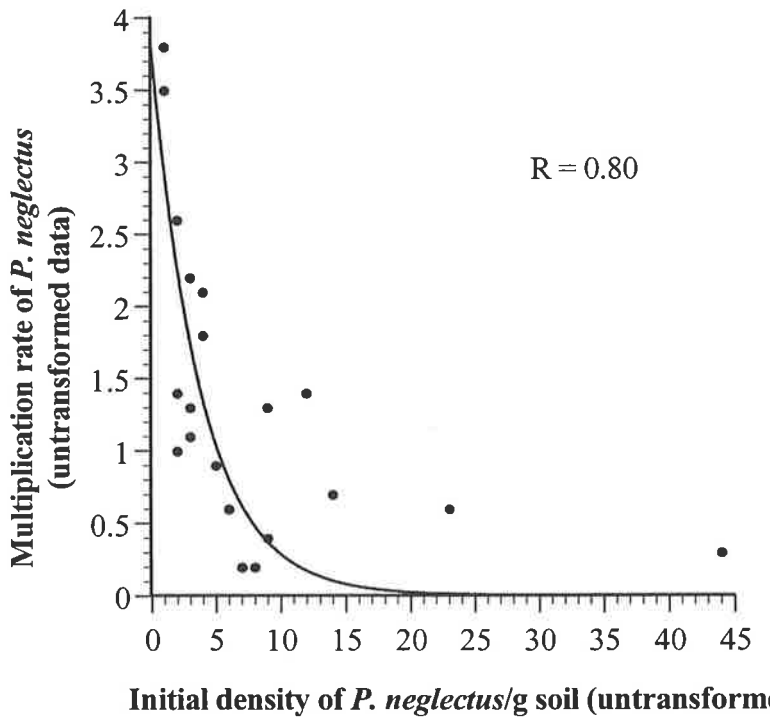
In 1997, where a range of densities of *P. neglectus* was produced by varieties sown in 1996, the relationship between multiplication rate and initial density of *P. neglectus* using both untransformed and $\log_e$ transformed data is shown in Figure 8.17. Multiplication rates for *P. neglectus* in this trial were similar to those observed for *P. thornei.* An exponential linear model was more significant than for *P. thornei*, with R = 0.85.

The relationship between final and initial density of *P. neglectus* in 1997 is presented in Figure 8.18. In contrast to data presented for *P. thornei* in the previous section, data for *P. neglectus* had a poor fit with the non-linear exponential decay model R = 0.75 (Figure 8.18a) and no asymptotic ceiling level for final nematode density at high initial densities was produced.

Figure 8.18b shows same the data set with 4 points removed (from low final densities) fitted using the non-linear exponential model. Data showed an improved fit (R = 0.81). No ceiling level could be defined however, and the relationship could also be represented using a linear regression model (R = 0.81; figure not presented).

Figure 8.17 **The effect of manipulation of initial density of *P. neglectus* by resistant and susceptible varieties (Machete, Spear and Excalibur wheat, Chebec barley and Tahara triticale) in 1996 on the multiplication rate of *P. neglectus* under Echidna oat in the Condada rotation trial, 1997. a) untransformed data, b) $\log_e$ transformed data (fitted using the non-linear equation $Pf/Pi = a + ce^{-bPi}$). Each point represents one replicate.**

a)



b)

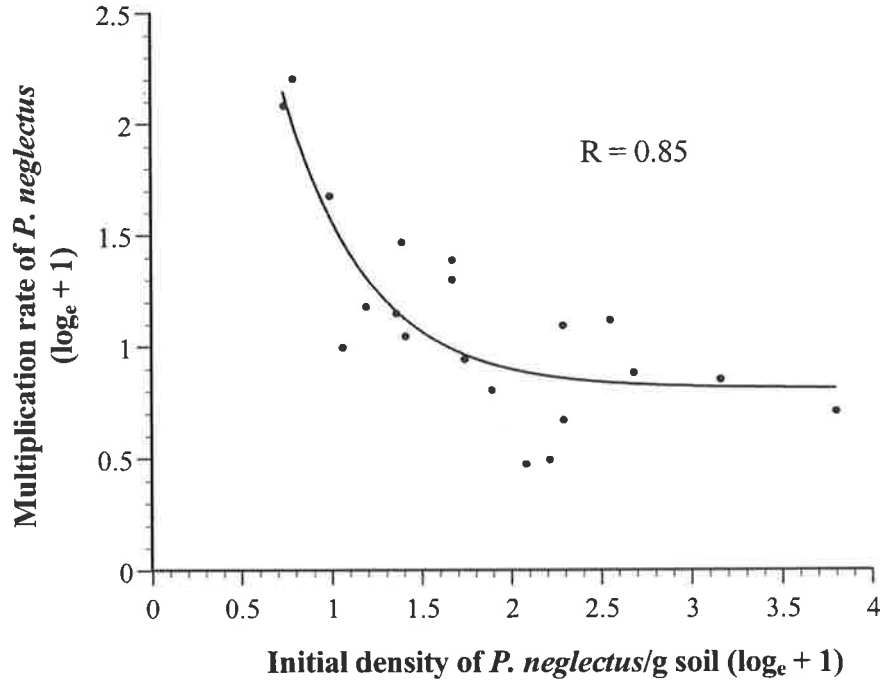
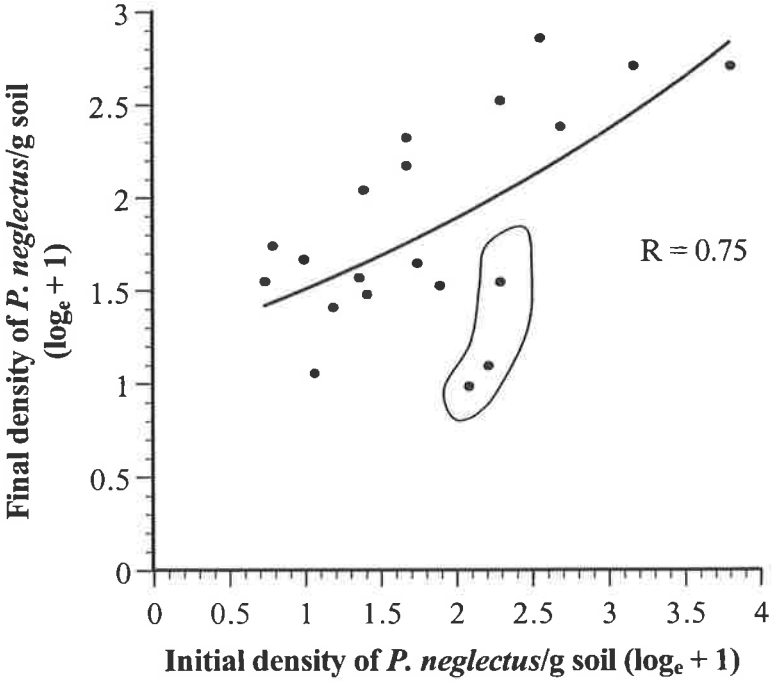
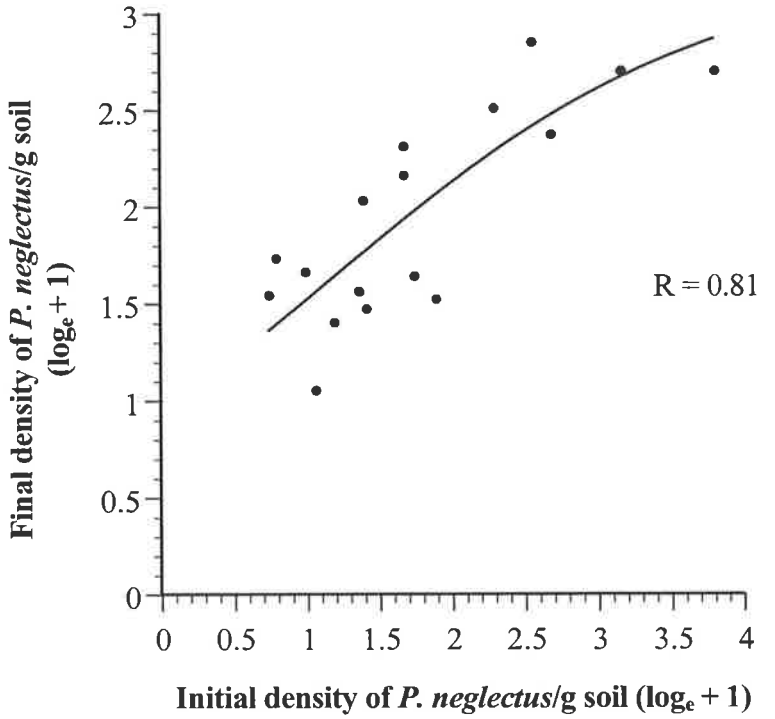


Figure 8.18 **The effect of manipulation of initial density of *P. neglectus* by resistant and susceptible varieties (Machete, Spear and Excalibur wheat, Chebec barley and Tahara triticale) in 1996 on the final density of *P. neglectus* under Echidna oat in the Condada rotation trial, 1997, represented by a) non-linear exponential decay Pf = $ce^{bPi}/(e^{bPi} - 1) + d$ all data points, b) non linear exponential decay with 3 data points shown in a) removed. Each point represents one replicate.**

a)



b)



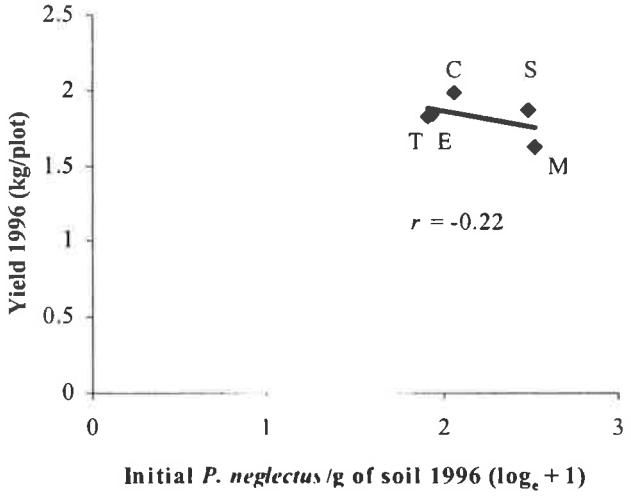
Using simple linear regression, the relationships between both initial and final *P. neglectus* density and yield for the average of each variety (n = 4) in 1996 and 1997 are shown in Figure 8.19. The relationship between number of *P. neglectus* and yield of Echidna oat for individual plots in 1997 (following resistant and susceptible varieties in 1996) is shown in Figure 8.20. The regression equations and *r* values are presented in Table 8.19. As with *P. thornei*, significant negative relationships were observed between both the initial and final density of *P. neglectus* and yield in 1997 for the average for each variety (tabulated *r* value [3 d.f.] = 0.878) and no relationship was observed between the initial density or final density of *P. neglectus* and yield in 1996. In contrast to *P. thornei*, for individual plots a significant relationship was observed only between initial density in 1997 and yield (tabulated r value [18 d.f.] = 0.444).

Table 8.19 **Regression equations and *r* values for correlation between initial or final number of *P. neglectus* and mean yield in untreated plots at Condada rotation trial, 1996 (Machete, Spear and Excalibur wheat, Chebec barley and Tahara triticale) and 1997 (Echidna oat following these varieties) n = 4. Tabulated *r* value (P < 0.05; 3 d.f. = 0.878 for average of variety; 18 d.f. = 0.444 for individual plots) * significant.**

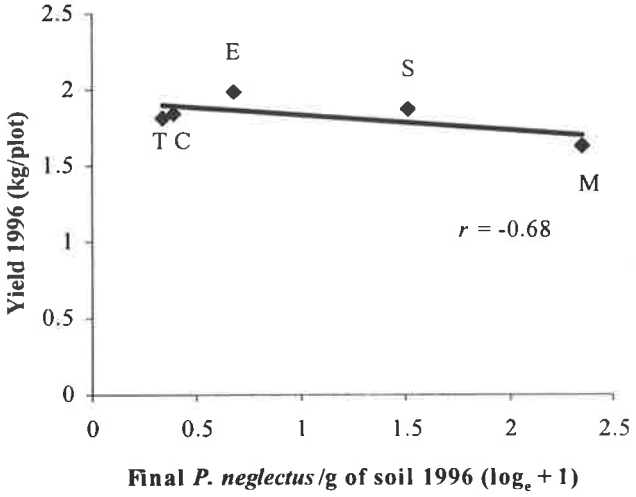
	r value	Regression equation
	Yield 1996	
Initial density 1996	-0.223 n.s.	y = -0.212x + 2.29
Final density 1996	-0.683 n.s.	y = -0.1001x + 1.93
	Yield 1997 (average for each variety) 3 d.f.	
Initial density 1997	-0.900*	y = -0.181x + 1.94
Final density 1997	-0.856	y = -0.439x + 2.41
	Yield 1997 (individual plots following each variety) 18 d.f.	
Initial density 1997	-0.474*	y = -0.154x + 1.94
Final density 1997	-0.420	y = -0.217x + 2.04

Figure 8.19 **Relationship between a) Initial number of *P. neglectus* and yield, 1996 b) Final number of *P. neglectus* and yield, 1996 c) Initial number of *P. neglectus* and yield, 1997 d) Final number of *P. neglectus* and yield 1997, Condada rotation trial. In 1996, Machete (M), Spear (S), and Excalibur (E) wheat, Chebec barley (C) and Tahara triticale (T) were sown. In 1997, Echidna oat was sown over these varieties. Each point represents the average for each variety (n = 4).**

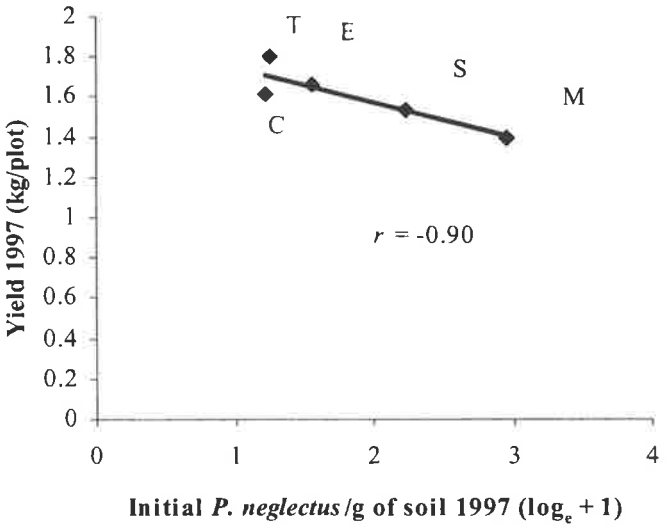
a) Initial number of *P. neglectus* and yield, 1996.



b) Final number of *P. neglectus* and yield, 1996.



c) Initial number of *P. neglectus* and yield, 1997.



d) Final number of *P. neglectus* and yield, 1997.

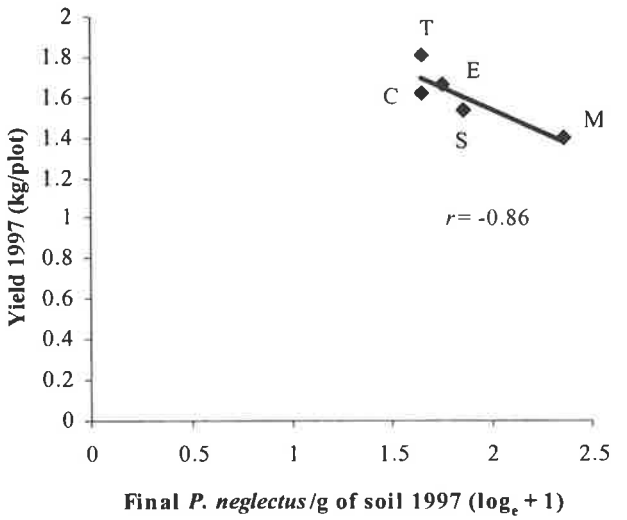
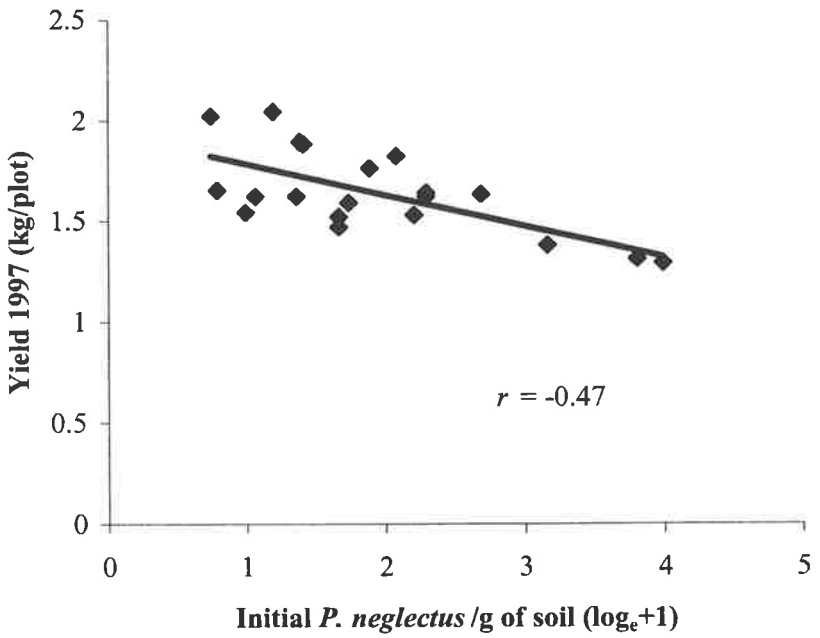
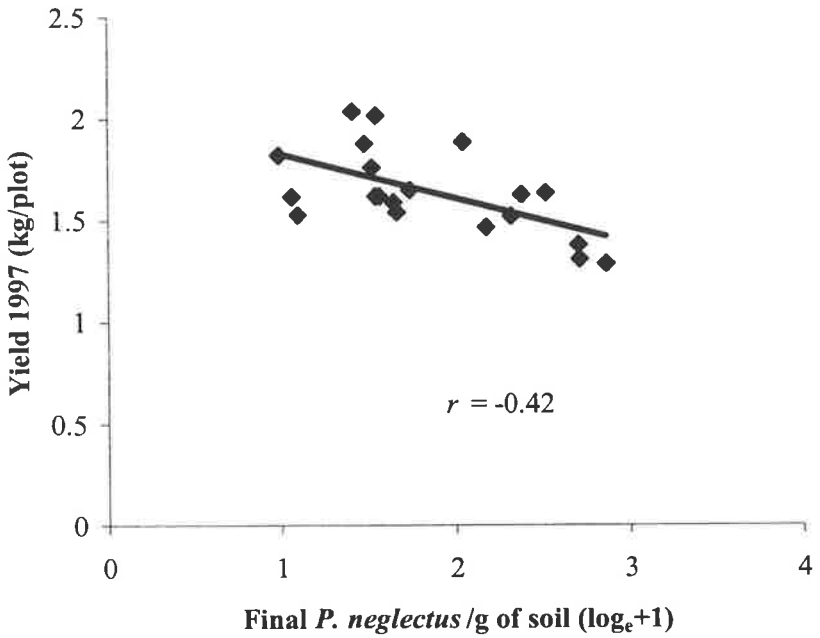


Figure 8.20 **Relationship between a) initial number of *P. neglectus* and b) final number of *P. neglectus,* and the yield of Echidna oat, Condada rotation trial, 1997. Each point represents an individual replicate.**

a) **Initial density of *P. neglectus***



b) **Final density of *P. neglectus***



The ANOVA of the results of the effect of variety and aldicarb treatment on yield of Machete, Spear and Excalibur wheat, Chebec barley and Tahara triticale in 1996 and the effect of varieties grown in 1996 on the yield of Echidna oat in 1997 are shown in Table 8.20. The yield in aldicarb treated and untreated plots in 1996 for Machete, Spear and Excalibur wheat, Chebec barley and Tahara triticale and in Echidna oat following these plots in 1997 are presented in Table 8.21. Yield responses calculated using either aldicarb or from the regression equation of final number of *P. neglectus* and yield in 1996 are shown in Figure 8.21. No significant responses were measured in 1996 using aldicarb.

Yield losses calculated from the regression equation of both initial and final number of *P. neglectus* and yield in Echidna oat in 1997 are presented in Figure 8.22. Higher yield losses were calculated using final compared with initial numbers of *P. neglectus*.

Table 8.20 The ANOVA of results for differences in yield of cereals in 1996 (Machete, Spear and Excalibur wheat, Chebec barley and Tahara triticale) and 1997 (Echidna oat following these varieties), Condada rotation trial (n = 4).

	d.f.	m.s.	P
		1996	
Rep.	3	0.105	0.110
Variety	4	0.066	0.246
Rep.*Variety	12	0.042	
Aldicarb	1	0.112	0.714
Variety*Aldicarb	4	0.030	0.833
Rep.*Variety*Aldicarb	15	0.083	
		1997	
Rep.	3	0.148	0.005
Variety	4	0.091	0.018
Rep.*Variety	12	0.200	

Table 8.21 **Yield in aldicarb treated and untreated plots for Machete, Spear, Excalibur wheat, Chebec barley and Tahara triticale in 1996 and for Echidna oat in 1997 (n = 4), Condada rotation trial. # significantly different from yield following Machete (P < 0.05).**

	1996			1997 (Echidna oat following these varieties)
	Untreated (U)	Aldicarb (A)	Difference (A-U)	Untreated plots from 1996
Machete wheat	1.63	1.69	0.06	1.40
Spear wheat	1.87	1.71	-0.16	1.53
Excalibur wheat	2.0	1.80	-0.20	1.66
Chebec barley	1.84	1.90	0.06	1.62
Tahara triticale	1.82	1.86	0.04	1.81#
			l.s.d. 0.44	h.s.d. 0.32

Figure 8.21 **Difference between yield reduction caused by *P. neglectus* measured by aldicarb (treated compared with untreated plots) or calculated from the regression equation comparing final *P. neglectus* number and yield in the first year of the rotation trial (1996) in Machete, Spear and Excalibur wheat, Chebec barley and Tahara triticale, Condada rotation trial, 1996 (n = 4). Regression analysis y = -0.1001x + 1.93.**

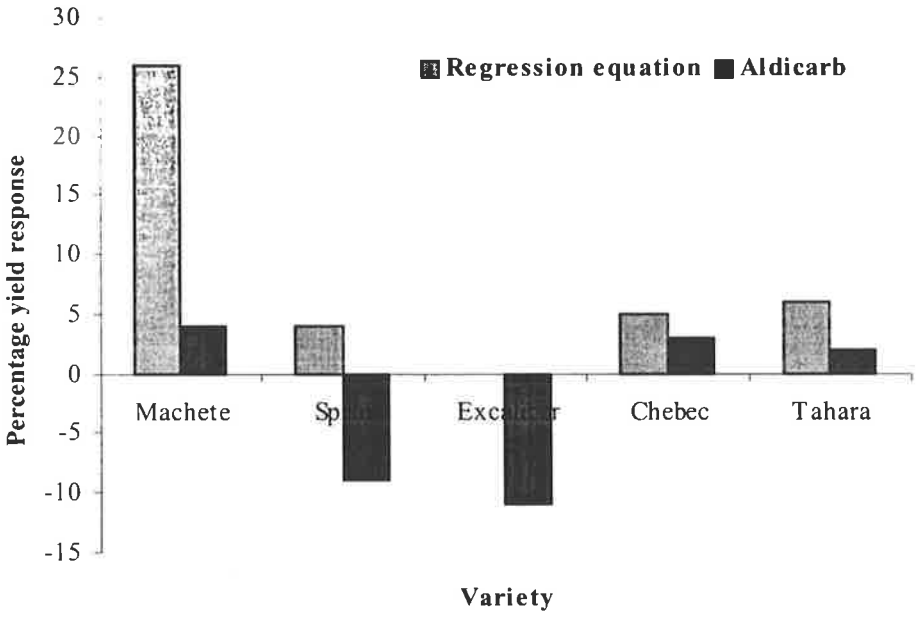
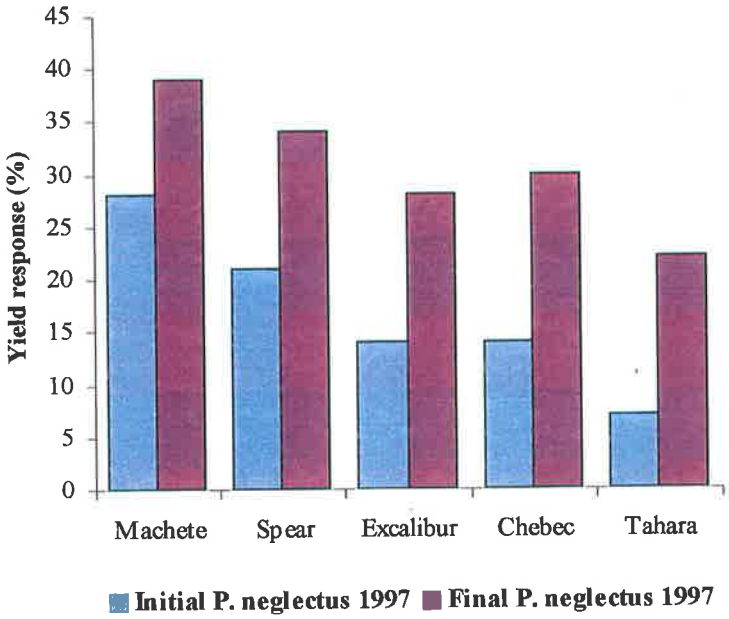


Figure 8.22 **Yield reduction caused by *P. neglectus* calculated from the regression equation of initial number and yield (y = -0.181x + 1.94) and final number and yield (y = -0.380x + 2.31) in Echidna oat following Machete, Spear or Excalibur wheat, Chebec barley and Tahara triticale in the second year of the Condada rotation trial, 1997 (n = 4).**



8.3 Discussion

8.3.1 Use of aldicarb to measure yield loss

Plant response due to treatment with aldicarb has been shown to be influenced by interactions with variety or soil type (Barker *et al.* 1988) and also by rainfall (Barker and Powell 1988). Lower rates of aldicarb (1.5 - 4.5 kg a.i./ha) have been shown to stimulate growth of tobacco plants while rates > 4.5 kg a.i./ha have been shown to be phytotoxic (Barker and Powell 1988).

In the aldicarb rate trial within this study, no phytotoxic response was seen in wheat at aldicarb rates of up to 6.0 kg a.i./ha. A growth promotion response may have occurred however, as significant responses to aldicarb were seen at comparatively low rates (e.g. a 15% increase at a rate of 1.0 kg a.i./ha in the wheat variety Machete and a 17% response at a rate of 1.5 kg a.i/ha in the wheat variety Janz).

8.3.2 Comparison between aldicarb and regression analyses to calculate yield loss

While yield loss in wheat and chickpea caused by *P. thornei* has been established in numerous studies (Orion *et al.* 1984; Doyle *et al.* 1987; Greco *et al.* 1988; DiVito *et al.* 1992; Nicol *et al.* 1999), fewer records of yield loss caused by *P. neglectus* are available. *P. neglectus* has been shown to be a minor pathogen on barley in pot experiments (Umesh and Ferris 1992; 1994) and on corn in field trials (Todd and Oakley 1996). Lasserre *et al.* (1994) reported damage to wheat at densities of 3000 *P. neglectus*/g root, but not at densities of 300 *P. neglectus*/g root. Damage has also been recorded on alfalfa (Griffin and Gray 1990; Griffin and Jensen 1997).

In studies reported in this chapter, where a range of *P. neglectus* or *P. thornei* densities was established, significant relationships were observed between both initial or final nematode density and yield. These relationships occurred where nematode numbers had been manipulated either by the use of nematicide (aldicarb rate trial) or with susceptible and resistant varieties (rotation trials). Significant relationships for selected varieties were also recorded in the variety trial at Condada, where differences between initial numbers were a result of natural site variation. This was possible as a reasonably high replication was used (n = 6) and a large range of initial numbers of *P. neglectus* was present, thus satisfying a criterion indicated by Seinhorst

(1966) that a range of densities is required before damage by plant-parasitic nematodes can be predicted.

In the Condada cereal variety trial, four varieties were determined to be intolerant on the basis of significant, negative relationships between initial numbers of *P. neglectus* and yield. In contrast, using aldicarb to measure yield (compared with untreated plots), six varieties were determined to be intolerant. Only Machete wheat and Echidna oat recorded significant responses using both analyses. Based on data from one trial, it was difficult to conclude which analysis gave the most accurate results.

Low rates of aldicarb in the aldicarb rate trial may have resulted in a possible growth promotion response. In plots treated with aldicarb at 2.5 kg a.i./ha in the Paskeville variety trial, where very low initial numbers of *P. neglectus* were present, no yield increase was observed in aldicarb compared with untreated plots for any variety, suggesting that this rate did not stimulate plant growth or yield.

Trudgill and Cotes (1983) also found no growth promoting effects of the nematicide, oxamyl, in several trials with potato. Variation in response to both aldicarb and oxamyl was observed however, and the application of nematicide did not always produce maximum yield increases. For these reasons, determining yield loss using regression analyses is generally considered more reliable (Trudgill and Cotes 1983; Barker and Noe 1987), and results from the studies reported here tend to support this finding.

It was concluded that, for the majority of varieties, a tolerance rating could only be designated by using data from several trials. Machete wheat and Echidna oat were intolerant to *P. neglectus,* as measured by both aldicarb and correlation between nematode density and yield. However, results for other varieties were variable and it was difficult to designate a tolerance rating. Using Tahara triticale as an example, for the majority of sites, this variety appeared tolerant. There were non-significant yield increases of 3% (aldicarb) and 11% (regression) measured at an initial density of 5.8 *P. neglectus*/g of soil in the first year of the rotation trial at Condada, and non-significant responses of 6% and –4% using aldicarb at low initial densities at both Paskeville and Sandilands. However, in the variety trial at Condada in 1996, in a different field to the rotation trial, significant yield responses of 10% were recorded using aldicarb

(although not regression analyses) at an initial level of 2.6 *P. neglectus*/g of soil. It is assumed that the results produced using aldicarb in this instance were aberrant and that Tahara triticale is tolerant.

In addition, using aldicarb, significant increases in yield were observed for the wheat variety Excalibur at low initial numbers of *P. neglectus* in both the Paskeville and Sandilands trials and for the variety Worrakatta at Paskeville. These results are also assumed to be spurious as Excalibur showed no significant yield response at higher initial nematode numbers in either the Condada cereal variety or rotation trials using aldicarb or regression analyses. Similarly, both Excalibur and Worrakatta wheat were shown to be tolerant using regression analyses (Vanstone *et al.* 1998; Taylor *et al.* 1999). It is possible that the presence of different populations of *P. neglectus* between sites (see Chapter 9) may also have been a factor influencing differences in yield loss between sites.

These results therefore question the reliability of using aldicarb in all instances to produce varietal ratings for tolerance to *P. neglectus*. Several trials are required using aldicarb and/or correlation of nematode density with yield to confirm a varietal rating. The most consistent determination of tolerance was produced by manipulation of nematode densities. For screening large quantities of germplasm in field trials, this may prove uneconomic, as accurate nematode quantification is required on a large number of plots: a time consuming and labour intensive task.

8.3.3 Comparisons between varietal tolerance and resistance to *P. neglectus* and *P. thornei*

Despite the discrepancies between results obtained using either aldicarb or regression analyses discussed above, it is apparent that *P. neglectus* causes significant yield loss to cereals in South Australia, and that some cereal varieties are tolerant of *P. neglectus* and/or *P. thornei*. Interestingly, considering the marked molecular difference between the two nematode species (Chapter 9; Curran pers. comm.), there were many similarities between varieties for both resistance and tolerance. For example, in the first year of the rotation trials, while multiplication was higher for *P. thornei* than for *P. neglectus*, Chebec barley, Tahara triticale and Excalibur

wheat had both superior tolerance and resistance to each nematode. The wheat varieties Machete and Spear were both more susceptible and more intolerant to each nematode.

In soybean, varieties have been shown to have tolerance to both *H. glycines* and *Hoplolaimus columbus,* and it was concluded that plant characteristics responsible for tolerance to one nematode may confer tolerance to other species (Boerma and Hussey 1984). This is supported by evidence that tolerance of soybean varieties to *H. glycines* has been linked to an increased efficiency in uptake of phosphorus (Price *et al.* 1995) as well as a tolerance to chloride toxicity (Parker *et al.* 1985). For varieties used in the studies reported here, a greater efficiency in the uptake of zinc has been shown for Excalibur wheat compared with Machete wheat (Howes pers. comm.). As many South Australian soils are zinc deficient, this may offer explanation for the increased levels of tolerance to *P. neglectus* seen in these trials.

For *P. neglectus*, the mechanism of resistance in wheat and triticale has been shown to be both a result of lower nematode penetration and also factors after invasion such as delay in moulting and egg laying (Farsi 1995). It appears that selected varieties may share this mechanism in relation to both *P. neglectus* and *P. thornei,* e.g. in these trials, Chebec barley, Excalibur wheat and Tahara triticale. Conversely, Echidna oat was more susceptible to *P. thornei* than to *P. neglectus,* so resistance to one species will not always confer resistance to the other.

8.3.4 Modelling yield loss

The results reported here support the linear relationship between the number of *P. neglectus* or *P. thornei* and yield, proposed by Brown (1969), rather than the proportional relationship (Seinhorst 1965; Phillips *et al.* 1998). With a proportional relationship, a tolerance limit (defined as the nematode population below which a plant can compensate for nematode damage) and a minimum yield (defined as yield limit below which no further decrease will result irrespective of the nematode density) occur. Elston *et al.* (1991) found that for *G. pallida* in potato, the relationship between yield and either untransformed or log transformed initial densities could not be represented by a straight line, as the relationships had asymptotic properties. Elston *et al.* (1991) suggested an inverse linear model best represented the relationship with yield and initial density and that with *G. pallida* there was unlikely to be zero yield (complete crop failure) even at very high initial nematode densities.

For *P. neglectus* in the Condada variety trial in 1996 and the rotation trial in 1997, and for *P. thornei* in the rotation trial in 1997, a simple linear regression was the most suitable relationship between yield and initial nematode density. These results therefore support the work of Oostenbrink (1966) who proposed that most nematode damage functions can be described as linear, as it is rare that very low nematode numbers can be measured or that very high nematode densities are achieved in trials. In these trials, the linear relationship suggests both a lack of resolution at lower densities and also that a tolerance limit was not achieved in the varieties assessed. In the Condada variety trial, varieties were separated into tolerant and intolerant categories based on information provided from all trials. For tolerant varieties, no significant relationship was seen between yield and nematode density. For the intolerant varieties, 33% of the variation at the site was attributed to *P. neglectus* using a simple linear relationship. Although over 24 data points were available, insufficient resolution at both low and high nematode densities was available to determine a tolerance or minimum yield limit. Even where a range of densities was established for *P. neglectus* and *P. thornei* in the Condada and Minnipa rotation trials, a linear relationship was also the most appropriate analysis. In these trials, for the intolerant oat variety Echidna, approximately 25% of the variation at these sites was attributed to *Pratylenchus*. Although initial densities were manipulated in 20 plots, the linear relationship observed showed that densities were not achieved that were high enough to suggest a tolerance limit. Insufficient resolution was available at the lower nematode densities to determine a minimum yield limit.

8.3.5 Determining relationships between either initial or final nematode density and yield

In the variety trials established at Sandilands, no relationships were observed between initial numbers of *P. neglectus* and yield. This was expected as a result of low replication (n = 4) of individual varieties, lower average initial nematode densities and a limited range of initial densities for comparison. A significant, negative correlation was seen between initial numbers of *P. neglectus* and the yield of chickpea varieties (when analysed as a separate group) and between all pulse and oilseed varieties (when combined data were analysed) at Paskeville in 1997. This result is surprising given that initial numbers ranged between only 0.1 and 0.7 *P. neglectus*/g of soil and would be expected only if pulses (and particularly chickpea) were

very intolerant. At Sandilands, where the same comparisons were made, no such correlation was found. Environmental conditions at Paskeville may have contributed strongly to yield loss in these crops. It is known that environment has a large impact on damage caused by plant-parasitic nematodes, e.g. yield loss caused by potato cyst nematode differed between sites with similar initial nematode densities (Trudgill 1986). In dry conditions (which occurred at Paskeville during the main period of crop growth) damage to root systems by *P. neglectus* may have impaired the uptake of water, thus increasing yield loss. For *P. thornei*, dry conditions during chickpea growth have been suggested to exacerbate yield loss, with losses recorded at only 0.031 *P. thornei* per gram of soil (Orion *et al.* 1984), well below the minimum for *P. neglectus* recorded here.

Significant negative relationships were also observed between final numbers of *P. neglectus* or *P. thornei* and yield. While relationships between initial density and yield are common where a range of numbers are present (Seinhorst 1972; Evans and Haydock 1990; Trudgill and Phillips 1994; Seinhorst 1995; Phillips *et al.* 1998), relationships between final density and yield are not as well documented. From this study, the significant relationship between final density and yield suggests that resistance and tolerance may occur together i.e. a more resistant variety will also be more tolerant. This may be more likely with species such as *P. neglectus* or *P. thornei* that have more than one generation per season where the more multiplication is limited, the greater the increase in yield.

No significant relationships were observed between initial density of *P. neglectus* and yield of cereals at either Sandilands or Paskeville. In contrast, significant relationships were observed between the final density of *P. neglectus* and yield for wheat varieties at these sites. Vanstone *et al.* (1998) showed that in South Australia, relationships between final density and yield have been observed consistently with wheat varieties. Significant relationships between final density and yield do not occur as often in oat, barley or triticale varieties however (Vanstone pers. comm.). This suggests that, in the majority of wheat varieties tested, resistance and tolerance occur together, i.e. those varieties that are more susceptible (such as Machete) are also more intolerant. In barley and oat this is not the case as the varieties tested within this study were generally more resistant and a relationship between final density and yield was less likely. This relationship is highlighted by results at Condada where, after the establishment of a range in *P. neglectus* densities, a strong relationship between initial (but not final) density and yield was

seen for Echidna oat. Conversely, for *P. thornei* at Minnipa a significant negative relationship was observed for Echidna oat between both initial and final numbers of *P. thornei* and yield. Multiplication of *P. thornei* was higher than for *P. neglectus* in Echidna and suggests a stronger relationship between tolerance and resistance for *P. thornei* in Echidna oat.

8.3.6 Population dynamics of *P. neglectus* and *P. thornei* in field trials

Where initial densities of *P. thornei* were manipulated using resistant or susceptible cereals in the first season of the field trial, the effect on final density in Echidna oat agreed well with the non-linear exponential model proposed by Seinhorst (1966). As the initial density of *P. thornei* increased, the final density also increased until a ceiling level of approximately 28 nematodes/g of soil (at initial levels of approximately 6 *P. thornei*/g) was reached.

In contrast, for *P. neglectus* at Condada (following establishment of a range of initial nematode densities), the relationship between final and initial numbers of nematodes was best described using a linear exponential model. The apparent lack of fit with a non-linear exponential model may be explained by the spread of data points at the low-medium range of final and initial nematode densities. While the lack of resolution at these lower nematode densities prohibits the fit with this model, it is suggested the relationship between final and initial numbers of *P. neglectus* also follows the relationship observed for *P. thornei* at Minnipa.

A similar relationship between initial and final density for *P. thornei* was observed in the wheat host Warigal, although ceiling levels were much higher (approximately 74 *P. thornei*/g of soil after initial numbers of 33 *P. thornei*/g) (Nicol *et al.* 1999) and is probably a reflection of the higher reproduction in this host compared with Echidna oat.

The relationship between final and initial nematode density may also be represented by a quadratic equation, where maximum numbers of nematodes are followed by a reduction at highest initial numbers of nematodes. This relationship has been observed for *H. avenae* in wheat (Fisher and Hancock 1991), *P. thornei* in wheat (Nicol *et al.* 1999) and was also extrapolated for *G. pallida* in potato (Phillips *et al.* 1998). The reduction in final density at high initial numbers of nematodes may occur as a result of overpopulation, competition for feeding sites or the effect of damage to the plant by nematodes (Seinhorst 1970).

In the first year of the rotation trials, for either *P. neglectus* or *P. thornei*, varietal selection had the largest effect on multiplication rate. Multiplication rates were higher for *P. thornei* with a level of 8.0 on Machete wheat compared with only 1.7 for *P. neglectus*. As with final density, Nicol *et al.* (1999) observed much higher multiplication rates for *P. thornei* in Warigal wheat (20 – 30 times at low initial nematode densities).

In 1997, where multiplication rates on one variety were compared at differing initial densities, there was a trend for the lowest initial densities to produce higher multiplication rates. For *P. neglectus* at Condada, the relationship between initial number and multiplication rate was best described using a negative exponential decay model. For *P. thornei* at Minnipa, a similar trend was observed, but for either $\log_e$ transformed or untransformed data, a linear model could also be used to describe the data. The poorer fit with the negative exponential decay model compared with data for *P. neglectus* appeared to be a result of both the large amount of variation within the data and an insufficient number of data points to allow resolution of the curve, particularly at very high or very low initial densities.

The negative exponential decay model is similar to that proposed by Ferris (1985) and, in these models, the value b is designated as a rate determining variable. Higher values of b suggest a greater dependence of multiplication on nematode density. In the study reported here, the b values for *P. neglectus* and *P. thornei* were similar (b = 2.16 and 2.0 respectively), but without comparison with other nematode species using this specific model, it is not possible to infer whether this indicates a high dependence between multiplication and initial density. A negative exponential relationship may also have been observed for *P. thornei* on wheat, although no analysis was attempted (Nicol *et al.* 1999).

For *P. neglectus* in the Condada rotation trial, using the negative exponential decay model, a value for a of 0.8112 (detransformed a = 1.25) was determined. The value a represents the asymptotic level where the minimum rate of multiplication occurs and suggests that at high initial densities, a maximum rate of multiplication of 1.3 would be achieved in Echidna oat. From Table 8.18, an average minimum rate of multiplication of 0.5 was observed in Echidna oat (at initial densities of 18 *P. neglectus*/g of dry soil) and highlights that the model accounted for only 73% of the variation seen in the data. For *P. thornei* in the Minnipa rotation trial, using the

negative exponential decay model, a similar result occurred (detransformed a = 1.09), and suggests that in the host Echidna, a maximum multiplication rate of 1.1 was predicted at high initial nematode densities. From Table 8.13, the average rate of multiplication in Echidna was 1.5 at the highest initial nematode density of 14 *P. thornei*/g of dry soil, similar to that suggested by the model (although the model only accounted for 53% of the variation in the data at this site).

For *P. thornei* in the rotation trial, a linear model best described the relationship between initial density and multiplication rate. This has also been observed between the initial density and multiplication rate for *M. hapla* in alfalfa (Noling and Ferris, 1986). A linear relationship suggests that an insufficient range of *P. thornei* densities has been achieved. In this study, it is suggested that higher initial *P. thornei* densities were required and for this reason, no maximum rate of nematode multiplication occurred as reported for *P. neglectus*. The lack of resolution at high initial densities of *P. thornei* was also seen between final and initial densities (as previously discussed). The absence of a reduction in final numbers after the ceiling level was reached supports the observation that a linear relationship between multiplication rate and initial density resulted from insufficient data points at high initial densities.

In this study, results from rotation trials clearly show the importance of initial density on determining both the multiplication rate and final density of both *P. neglectus* and *P. thornei*. While an attempt can be made to rate the resistance of a variety using the maintenance level concept (where birth rate equals death rate) proposed by Jones (1956) and the definition by Trudgill *et al.* (1998) of a resistant plant as one that restricts or prevents nematode multiplication, the population dynamics of *P. neglectus* in field trials can make such a rating difficult.

In the Condada trial in 1997, with high initial numbers of *P. neglectus* (18 *P. neglectus*/g), final numbers were reduced (9.6 *P. neglectus*/g) and Echidna behaved as resistant based on the maintenance level concept. In contrast, where initial densities were lower (2.4 *P. neglectus*/g), final numbers increased to 4.2 *P. neglectus*/g and Echidna would have been designated as susceptible. For *P. neglectus* at this site, a maintenance level of between 4 - 5 *P. neglectus*/g therefore occurred in Echidna oat. For *P. thornei* in the Minnipa rotation trial, Echidna appeared to be a better host, as multiplication occurred at all initial densities. At the highest

initial density of 14 *P. thornei*/g of soil, final densities increased to 18 *P. thornei*/g. A maintenance level greater than 14 *P. thornei* therefore occurred in Echidna oat at this site.

Based on overall results for all trials (see also Chapter 7), Echidna oat was rated as a moderate host for *P. neglectus*, because multiplication occurred where low initial *P. neglectus* numbers were present and conditions were favourable for nematode multiplication. Care must be taken in recommending varietal selection for control of *P. neglectus* or *P. thornei.* Confusion can occur amongst growers as in one season at a lower initial density, a variety may behave as susceptible, while at a higher initial density it may behave as resistant. As proposed in Chapter 7, resistance may be better rated using final density and only where it can be compared with resistant and susceptible check varieties.

These results have confirmed that, in South Australia, tolerance is present in cereal varieties to both *P. neglectus* and *P. thornei* and the levels of tolerance present can result in economic benefit to South Australian growers. Further research is required for pulse and oilseed varieties, and field trials must be established over a greater range of nematode densities than reported in this study.

Chapter 9 Variation within and between Australian populations of *Pratylenchus neglectus* and *P. thornei*

9.0 Introduction

While the dispersal of nematodes can occur by water or wind there are, in general, limited opportunities for the introduction of new genetic material into nematode populations, particularly where reproduction is parthenogenic. This, coupled with the intensive monoculture practised in many agricultural regions, produces selection pressures between populations which may lead to the formation of races.

The presence of a race or population within a species may be suggested if differences are observed between the ability of a characterised host to support nematodes, i.e. a resistant cultivar allows multiplication. Differential reproduction in potential host plants is therefore an extremely useful tool in determining differences between nematode populations, as it is both practical and measurable. For example, differences in virulence have been observed between isolates of *P. penetrans* in potato (France and Brodie 1995), *P. vulnus* in *Prunus* (Pinochet *et al.* 1994), and *P. neglectus* in alfalfa (Griffin 1991).

Morphological variation can also be used to assess differences between geographically isolated populations, but its usefulness may be limited as some characters will vary depending on the food source (Section 2.11) and, usually, nematodes have relatively few useful morphological characters for taxonomy. As a result, techniques such as isozyme, RFLP or RAPD analyses are now commonly used to identify differences between species and populations.

Within the *Heterodera avenae* species complex, clear protein differences were observed between races collected from seven countries (Bossis and Rivoal 1996). Variation was also reported between ten populations of *H. avenae* from the former USSR, and these were supported with morphological differences (Subbotin *et al.* 1996). For *Pratylenchus*, isozyme profiles have successfully differentiated between species (Payan and Dickson 1990), as well as between populations of *P. brachyurus* (Ibrahim *et al.* 1995).

In this study, possible differences within and between Australian populations of *P. neglectus* and *P. thornei* were evaluated using allozyme electrophoresis and assessment of reproduction in cereals.

9.1 Collection and maintenance of *Pratylenchus* populations

9.1.1 Materials and Methods

9.1.1.1 Populations

Pratylenchus were extracted from plants or soil, hand-picked from the resulting nematode suspension, surface sterilised and transferred to tubs containing sterile carrot culture (Section 3.4.1). Ten - fifteen nematodes per carrot culture tub (Plate 3.5) were used to establish the original populations and several cultures were set up per population. Details of the date of establishment and collection site are presented in Table 9.1 and site locations in Figure 3.2. The "Original" population was initially established in 1991 from *P. neglectus* obtained from Palmer, South Australia by V. Vanstone, and maintained continuously in culture since this date.

9.1.1.2 Clonal lines

Following establishment of the populations, approximately 30 clonal lines were set up per population by transferring a single surface sterilised nematode to sterile carrot callus. Clonal lines assessed in either allozyme or virulence studies were chosen randomly. To maintain populations in culture, pieces of culture containing nematodes were transferred asceptically to sterile, uninoculated carrot culture, as soon as nematodes could be seen on the culture or tub wall (Plate 3.5). This depended on the rate of multiplication of each population, and ranged between 3 and 9 months after inoculation.

Table 9.1 **Collection site and date for *P. neglectus* and *P. thornei* populations used in pathogen variability studies. Location of each site is shown in Figure 3.2.**

Collection site	Collection date
P. neglectus	
Port Clinton, Yorke Peninsula, South Australia	December 1995
Swan Hill, Mallee, Victoria	December 1995
Pinnaroo, Mallee, South Australia	December 1995
Sandilands, Yorke Peninsula, South Australia	December 1995
Buckleboo, Upper Eyre Peninsula, South Australia	February 1996
Palmer, Mallee, South Australia	December 1995
Yacka, Mid-north, South Australia	November 1996
Lake Grace, Western Australia	March 1997
Original culture (set up using *P. neglectus* from Palmer, South Australia).	February 1991
P. thornei	
Nunjikompita, Upper Eyre Peninsula, South Australia	March 1996
Waite Campus, Adelaide, South Australia	July 1996
Toowoomba, Darling Downs, Queensland	July 1996
Horsham, Wimmera, Victoria	August 1996

9.2 Identification of differences within and between *Pratylenchus* populations using allozyme electrophoresis

9.2.1 Materials and methods

Preparation of nematode pellets for allozyme electrophoresis and methods used for running nematode proteins on cellulose acetate gels are described in Section 3.9. Buffers used are given in Appendices 3 and 4.

9.2.1.1 Experiment 1

Six *P. neglectus* (Port Clinton, Palmer, Pinnaroo, Sandilands Buckleboo and Original) and three *P. thornei* (Nunjikompita, Toowoomba and Waite) populations were compared. In addition, a population of the oat race of *Ditylenchus dipsaci* (also obtained from carrot culture) and a control of uninoculated carrot culture were included. Loci assessed are presented in Table 9.2.

9.2.1.2 Experiment 2

Twenty-two clonal lines from seven *P. neglectus* populations (Port Clinton, Swan Hill, Pinnaroo, Sandilands, Buckleboo, Lake Grace and Original) and 19 clonal lines from four *P. thornei* populations (Nunjikompita, Waite, Toowoomba and Horsham) were compared. Loci assessed are presented in Tables 9.3 and 9.4.

9.2.2 Results

9.2.2.1 Experiment 1

Of the 27 loci assessed, eight gave bands too poor to resolve and three gave identical patterns for all nematode cultures. Phenotype scores for the remaining 16 loci are presented in Table 9.2. For *P. neglectus* populations, the banding patterns at the loci Got2, Gpi, 6Gpd, Pgk and Tpi suggested mixed genotypes in the Port Clinton, Palmer, Sandilands and Original populations, although it was not possible to determine how many genotypes or phenotypes were present. *P. thornei* populations were more uniform with only minor differences observed in banding patterns across all loci.

For *P. neglectus* and *P. thornei*, no alleles were shared in ten of the 19 loci assessed, i.e. these species differed from each other at 53% fixed differences. *P. thornei* and *P. neglectus* also differed from *D. dipsaci* at 53%.

9.2.2.2 Experiment 2

Nineteen loci were scored from a total of 27, of which three were the same for all clonal lines and populations. For *P. neglectus* and *P. thornei,* there were differences in 11 of these 19 loci, representing 57% fixed differences between these two species. The phenotypes of the 16 loci scored are presented in Tables 9.3 (*P. neglectus*) and 9.4 (*P. thornei*). For *P. neglectus*, differences in banding patterns were observed both within and between populations. As with Experiment 1, *P. thornei* populations were more uniform.

Inferred genotypes were determined by grouping clonal lines and populations that did not share any alleles and are presented in Table 9.5. Based on groupings of *P. neglectus* that did not share alleles, four populations were identified:

1) Swan Hill
2) Buckleboo
3) Port Clinton and Sandilands
4) Pinnaroo, Lake Grace and Original.

As all *P. thornei* populations were more uniform (very similar banding patterns were observed for each loci) all were placed into one group on the basis of allozyme results.

Table 9.2 **Allozyme phenotype profiles for *P. neglectus* and *P. thornei* populations (originally derived from up to 15 females). Invariant loci: Ap1, Ap2, Enol. Inactive loci: Acp, Acyc, Lap, PepB, PepC. Variable loci but not reliably scored: Ak, Fum, Ga3pd, Hk, Ldh, Mdh, PepA, PepD. Each letter corresponds to a band on the cellulose acetate gel.**

Population	Acon	Ald	Est	Fdpase1	Got1	Got2	Gpi	Idh	Me	Mpi	Pgam	6Pgdd	Pgk	Pgm1	Pk	Tpi
							P. neglectus									
Port Clinton	b	a	f?	b	a	c	abcd	b	b	bd	c	ace	e	b	b	abd
Palmer	b	a	f?	b	a	cde	abcd	b	b	bd	bc	bd	bd	ab	b	abd
Pinnaroo	b	a	f?	b	a	cde	abcd	b	b	bd	bc	bcd	de	b	b	abd
Sandilands	b	a	f?	b	a	abcd	abcd	b	b	bd	bc	-	de	b	b	abd
Buckleboo	b	a	f?	b	a	c	c	b	b	-	c	ace	e	-	-	ad
Original	b	a	e	b	a	ab	ab	b	b	bd	bc	cd	cde	bc	b	d
							P. thornei									
Nunjikompita	c	b	cd	a	b	d	d	a	a	ce	a	c	ac	bc	a	c
Waite	c	b	cd	a	b	d	d	a	a	ce	a	c	ac	bc	a	c
Toowoomba	c	b	cd	a	b	d	d	a	a	ce	a	c	ac	bc	a	c
							Controls									
D. dipsaci	a	c	b	c	-	def	def	-	c	a	e	f	d?	c	b?	f
Uninoculated carrot	-	-	a	-	b?	d	f	-	d	-	-	-	-	-	-	e

Table 9.3 **Allozyme phenotype profile between clonal lines (established from one *P. neglectus* female) from seven geographically isolated *P. neglectus* populations. Invariant loci: Ap1, Ap2, Enol. Inactive loci: Acp, Acyc, Lap, PepB, PepC. Variable loci but not reliably scored: Ak, Fum, Ga3pd, Hk, Ldh, Mdh, PepA, PepD. Each letter corresponds to a band on the cellulose acetate gel.**

Pop^{n}/clonal line	Acon	Ald	Fdpase1	Got1	Got2	Gpi	Idh	Me	Mpi	Pgam	6Pgd	Pgk	Pgm1	Pgm3	Pk	Tpi
Port Clinton 8	b	ab	-	a	c	bc	bc	b	bd	c	ace	e	ab?	ab	bc	abd
Swan Hill 4	b	b	b	a	cde	a	-	b	bd	bc	bd	e	a	b	bc	abd
Pinnaroo 4	b	ab	b	a	cde	a	bc	b	bd	c	bd	e	a	b	bc	abd
Pinnaroo 9	b	ab	b	a	cde	a	bc	b	bd	bc	bd	e	b	ab	bc	abd
Pinnaroo 11	b	ab	b	a	cde	a	bc	b	bd	bc	bd	de	b	ab	bc	abd
Sandilands 4	b	ab	b	a	c	abc	bc	b	bd	c	ace	de	b	ab	bc	abd
Sandilands 10	b	a	b	a	c	abc	bc	b	bd	c	ace	de	b	ab	bc	abd
Sandilands 13	b	a	b	a	c	abc	bc	b	bd	c	ace	de	b	ab	bc	abd
Sandilands 20	b	ab	b	a	c	abc	bc	b	bd	c	ace	de	b	ab	bc	abd
Buckleboo 10	b	a	b	a	c	c	b	b	d	bc	bd	e	ab	b	bc	a
Buckleboo 16	b	a	b	a	c	c	b	b	d	bc	bd	e	ab	b	bc	a
Buckleboo 17	b	a	b	a	c	c	b	b	d	bc	bd	e	ab	b	bc	a
Buckleboo 28	b	a	b	a	c	c	b	b	d	bc	bd	e	ab	b	bc	a
Lake Grace 9	b	a	b	a	c	a	bc	b	-	c	-	cde	-	-	bc	d
Lake Grace 11	b	-	b	a	cde	a	bc	b	d	bc	-	cde	-	-	bc	d
Lake Grace 13	b	-	b	a	cde	a	bc	b	d	c	-	cde	b	a	bc	d
Lake Grace 18	b	a	b	a	cde	a	bc	b	d	-	-	cde	b	a	bc	d
Lake Grace 20	b	-	b	a	cde	a	bc	b	d	-	-	cde	b	a	bc	d
Original 15	b	a	b	a	cde	a	bc	b	d	-	-	cde	b	a	bc	d
Original 18		a	b	a	-	-	bc	b	-	c	-	-	b	-	bc	-
Original 24		a	b	a	cde	a	bc	b	bd	bc	-	cde	b	a	bc	d

Table 9.4 **Allozyme phenotype profile between clonal lines (established from one *P. thornei* female) from four geographically isolated *P. thornei* populations. Invariant loci: Ap1, Ap2, Enol. Inactive loci: Acp, Acyc, Lap, PepB, PepC. Variable loci but not reliably scored: Ak, Fum, Ga3pd, Hk, Ldh, Mdh, PepA, PepD. Each letter corresponds to a band on the cellulose acetate gel.**

Pop^{n}/clonal line	Acon	Ald	Fdpase1	Got1	Got2	Gpi	Idh	Me	Mpi	Pgam	6Pgd	Pgk	Pgm1	Pgm3	Pk	Tpi
Nunjikompita 3	c	b	a	b	abc	d	a	a	ce	a	c	ac	bc	c	a	c
Nunjikompita 5	c	b	a	bc	abc	d	a	a	ce	a	c	ac	bc	c	a	c
Nunjikompita 17	c	b	a	bc	abc	d	a	a	ce	a	-	ac	bc	c	a	c
Nunjikompita 8	c	b	a	bc	abc	d	a	a	ce	a	-	ac	bc	c	a	c
Nunjikompita 19	c	b	a	bc	abc	d	a	a	ce	a	-	ac	-	c	a	c
Nunjikompita 20	c	b	a	bc	abc	d	a	a	ce	a	c	ac	-	c	a	c
Waite 10	c	b	a	bc	abcde	d	a	a	ce	a	c	ac	bc	c	a	c
Waite 23	c	b	a	bc	abcde	d	a	a	ce	a	-	ac	bc	c	a	c
Waite 25	c	bc	a	bc	abcde	d	a	a	ce	a	-	ac	bc	c	a	c
Waite 28	c	b	a	bc	abcde	d	a	a	ce	a	c	ac	bc	c	a	c
Waite 38	c	b	a	bc	abcde	d	a	a	ce	a	c	ac	bc	c	a	c
Toowoomba 15	c	b	a	bc	abc	d	a	a	ce	a	c	ac	bc	c	a	c
Toowoomba 19	c	b	a	bc	abc	d	a	a	ce	a	c	ac	bc	c	a	c
Horsham 2	c	b	a	bc	abc	d	a	a	ce	a	c	ac	bc	c	a	c
Horsham 6	c	b	a	bc	abc	d	a	a	ce	a	c	ac	bc	c	a	c
Horsham 11	c	b	a	bc	abc	d	a	a	ce	a	c	ac	bc	c	a	c
Horsham 12	c	b	a	bc	abc	d	a	a	ce	a	c	ac	bc	c	a	c
Horsham 27	c	b	a	bc	abc	d	a	a	ce	a	c	ac	bc	c	a	c
Horsham 34	c	b	a	bc	abc	d	a	a	ce	a	c	ac	bc	c	a	c

Table 9.5 **Inferred genotypes for seven *P. neglectus* populations (based on no shared alleles). Invariant loci: Ap1, Ap2, Enol. Inactive loci: Acp, Acyc, Lap, PepB, PepC. Variable loci but not reliably scored: Ak, Fum, Ga3pd, Hk, Ldh, Mdh, PepA, PepD. Each letter corresponds to an allele.**

Pop^{n}/clonal line	Ald	Got2	Gpi	Idh	Me	Mpi	Pgam	6Pgd	Pgk	Pgm1	Pgm3	Tpi
Port Clinton	ab	aa	bc	ab	bb	ab	bb	ace	cc	ab	ab	ad
Swan Hill 4	bb	ab	aa	-	bb	ab	ab	bd	cc	bb	aa	ad
Pinnaroo 4	ab	ab	aa	ab	bb	ab	bb	bd	cc	bb	aa	ad
Pinnaroo 9	ab	ab	aa	ab	bb	ab	ab	bd	cc	bb	ab	ad
Pinnaroo 11	ab	ab	aa	ab	bb	ab	ab	bd	bc	bb	ab	ad
Sandilands 4	ab	aa	ac	ab	bb	ab	bb	ae	bc	bb	ab	ad
Sandilands 10	aa	aa	ac	ab	bb	ab	bb	ae	bc	bb	ab	ad
Sandilands 13	aa	aa	ac	ab	bb	ab	bb	ac	bc	bb	ab	ad
Sandilands 20	ab	aa	ac	ab	bb	ab	bb	-	bc	bb	ab	ad
Buckleboo 10	aa	aa	cc	aa	bb	bb	ab	bd	cc	ab	bb	aa
Buckleboo 16	aa	aa	cc	aa	bb	bb	ab	bd	cc	ab	bb	aa
Buckleboo 17	aa	aa	cc	aa	bb	bb	ab	bd	cc	ab	bb	aa
Buckleboo 28	aa	aa	cc	aa	bb	bb	ab	bd	cc	ab	bb	aa
Lake Grace 9	-	aa	aa	ab	bb	bb	-	-	-	-	-	dd
Lake Grace 11	-	ab	aa	ab	bb	bb	bb	-	ac	-	-	dd
Lake Grace 13	-	ab	aa	ab	bb	bb	ab	-	ac	bb	aa	dd
Lake Grace 18	aa	ab	aa	ab	bb	bb	bb	-	ac	bb	aa	dd
Lake Grace 20	-	ab	aa	ab	bb	bb	-	-	ac	bb	aa	dd
Original 15	aa	-	-	ab	bb	-	b	-	-	bb	-	dd
Original 18	aa	ab	aa	ab	ab	ab	ab	-	ac	bb	aa	dd
Original 24	aa	ab	aa	ab	ab	ab	ab	-	ac	bb	aa	dd

9.3 Differential reproduction of *Pratylenchus* populations in cereals

9.3.1 Materials and methods

Differences in multiplication between either clonal lines (originally established from one *P. neglectus* or *P. thornei* female) or populations of *P. neglectus* or *P. thornei* (originally established from up to 15 individuals) were assessed in growth room experiments.

After germination (Section 3.5.1), a single cereal seed was sown into a tube and transferred to controlled temperature growth room (Section 3.5.2). Ten replicates were included per clonal line or population. After emergence, each plant was inoculated with 500 *Pratylenchus* by making two holes in the soil with a thin skewer on either side of the seedling and pipetting 0.5 ml of nematode suspension into each hole. Nematodes from each population or clonal line were obtained from one carrot culture tub only.

Plants were harvested 6 weeks after inoculation and nematodes were extracted from roots in a misting chamber (Section 3.1.3). Numbers of *P. neglectus* were counted and expressed as nematodes/plant. For Experiments 1 and 2, numbers of nematodes were $\log_e (x + 1)$ transformed. For Experiment 3, numbers were square root transformed to normalise data sets before analysis of variance was conducted and means separated ($P < 0.05$). Differences between extraction treatments for Experiments 1 and 2 were separated using a Tukey test (h.s.d.) and for Experiment 3 using least mean squares.

9.3.1.1 Experiment 1

Multiplication of 21 clonal lines from 7 *P. neglectus* populations (Buckleboo, Sandilands, Lake Grace, Palmer, Pinnaroo, Yacka and the Original population) and 9 lines from 2 *P. thornei* populations (Nunjikompita and Waite) was assessed in Frame wheat.

9.3.1.2 Experiment 2

The multiplication of 8 clonal lines from 3 *P. neglectus* populations and 3 clonal lines from 1 *P. thornei* showing the greatest variability from Experiment 1 (Section 9.3.1.1) were evaluated in Frame wheat. *P. neglectus,* freshly extracted from wheat roots (cv. Machete) grown in pots in the glasshouse, were also included. When selecting populations for assessment in this experiment, care was taken to ensure that all cultures used for nematode

inoculum were at a similar stage of development, i.e. nematodes were present in small clusters on tub walls and the infected carrot culture was a uniform brown colour.

9.3.1.3 Experiment 3

The multiplication of 7 *P. neglectus* (Original, Pinnaroo, Swan Hill, Port Clinton, Buckleboo, Sandilands and Walpeup) and 3 *P. thornei* (Nunjikompita, Toowoomba and Horsham) populations (established using between 10-15 nematodes) were compared in three wheat (Frame, Machete and Excalibur) and one barley (Schooner) cultivar in a growth room experiment. Three separate carrot cultures from the Original population were also compared.

9.3.2 Results

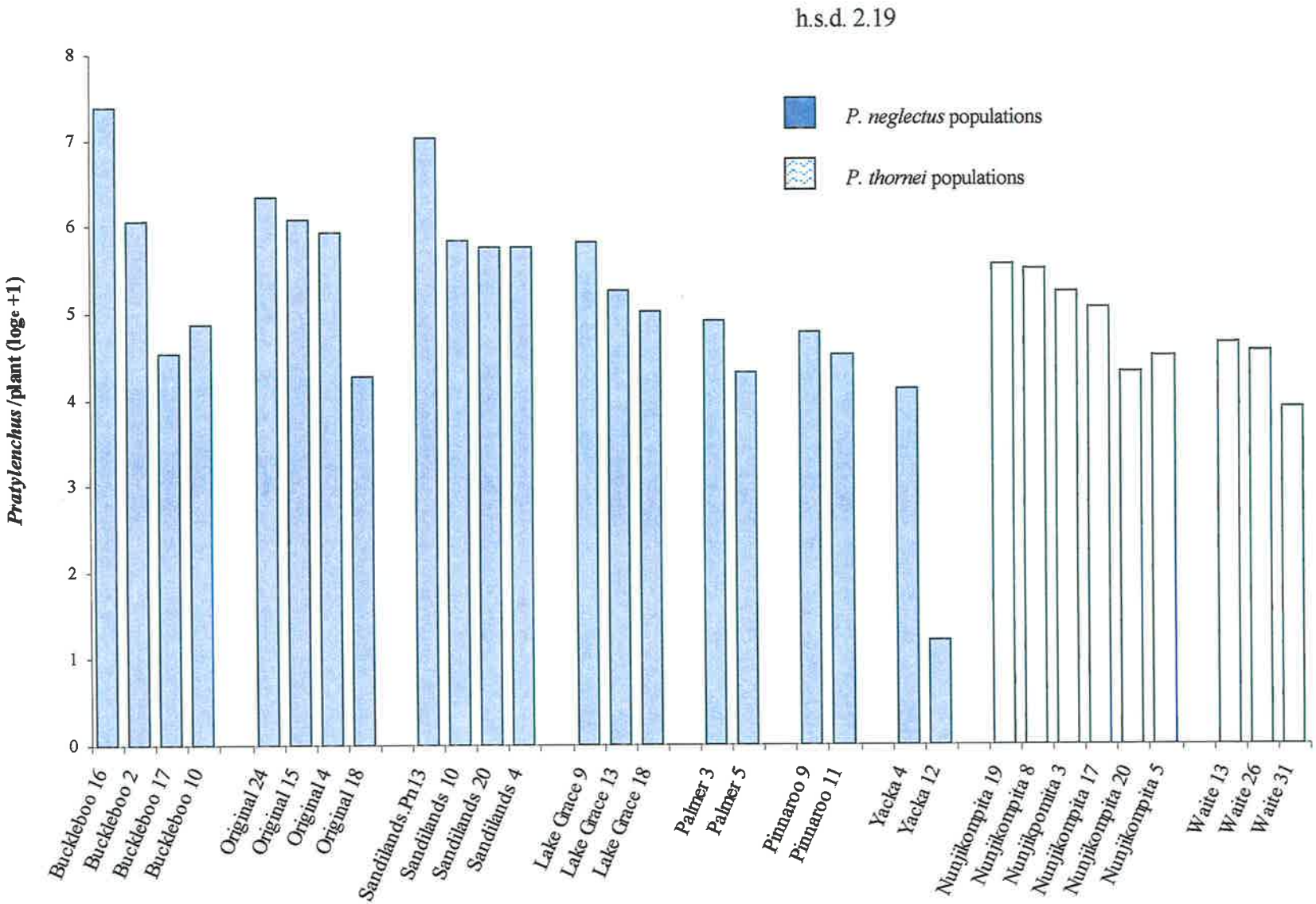
9.3.2.1 Experiment 1

Differences in the multiplication of clonal lines from *P. neglectus* and *P. thornei* populations are shown in Figure 9.1 and results from the ANOVA comparing reproduction of populations in Frame wheat in Table 9.6. There were significant differences both between clonal lines within each population and between *P. neglectus* and *P. thornei* populations. There was no correlation between numbers of *Pratylenchus*/plant and either shoot or root weight ($r = 0.17$ and $r = 0.1$ respectively).

Table 9.6 ANOVA of results for differences between clonal lines on Frame wheat (Experiment 1; n = 10).

	d.f.	m.s.	P
Rep.	9	1.984	0.320
Clonal line	29	12.628	0.0000
Rep.*Clonal line	259	1.707	

Figure 9.1 **Comparison of multiplication of 21 clonal lines (established from single nematodes) from eight *P. neglectus* and two *P. thornei* populations in Frame wheat (Experiment 1; n = 10).**


h.s.d. 2.19
P. neglectus populations
P. thornei populations
Pratylenchus /plant (loge +1)
0
1
2
3
4
5
6
7
8
Buckleboo 16
Buckleboo 2
Buckleboo 17
Buckleboo 10
Original 24
Original 15
Original 4
Original 18
Sandilands Pn13
Sandilands 10
Sandilands 20
Sandilands 4
Lake Grace 9
Lake Grace 13
Lake Grace 18
Palmer 3
Palmer 5
Pinnaroo 9
Pinnaroo 11
Yacka 4
Yacka 12
Nunjikompita 19
Nunjikompita 8
Nunjikpomita 3
Nunjikompita 17
Nunjikompita 20
Nunjikompita 5
Waite 13
Waite 26
Waite 31

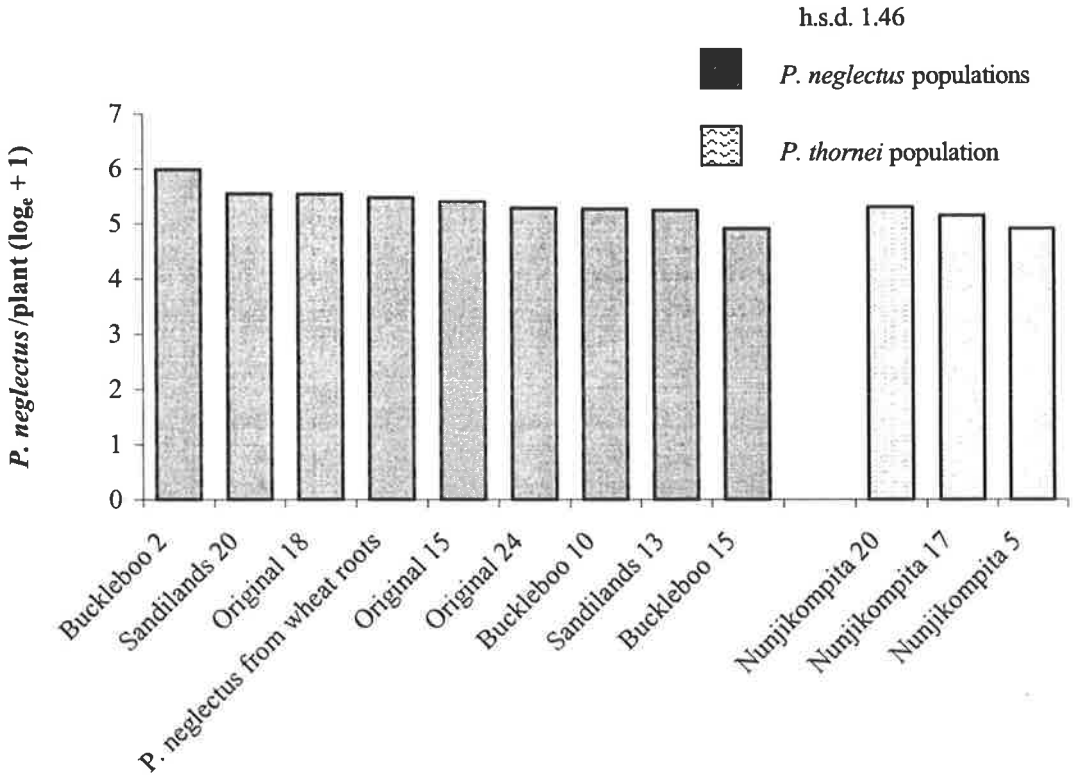
9.3.2.2 Experiment 2

Differences in multiplication of clonal lines from *P. neglectus* and *P. thornei* populations are shown in Figure 9.2 and results from the ANOVA comparing reproduction of populations in Frame wheat given in Table 9.7. There was no significant difference between either the *P. neglectus* or *P. thornei* populations or between clonal lines within each population. There was also no significant difference between multiplication in Frame wheat of clonal lines or *P. neglectus* freshly extracted from wheat roots. As with Experiment 1, there was no correlation between top (r = 0.36) or root weight (r = 0.22) and number of nematodes recovered.

Table 9.7 **The ANOVA of the results comparing differences between clonal lines on Frame wheat (Experiment 2; n = 10).**

	d.f.	m.s.	P
Rep.	9	0.984	0.4162
Clonal line	11	0.832	0.5655
Rep.*Clonal line	94	0.948	

Figure 9.2 **Comparison of multiplication on Frame wheat of eight clonal lines (established from single nematodes) from three *P. neglectus* populations, one *P. thornei* population and *P. neglectus* extracted from wheat roots (Experiment 2; n = 10).**



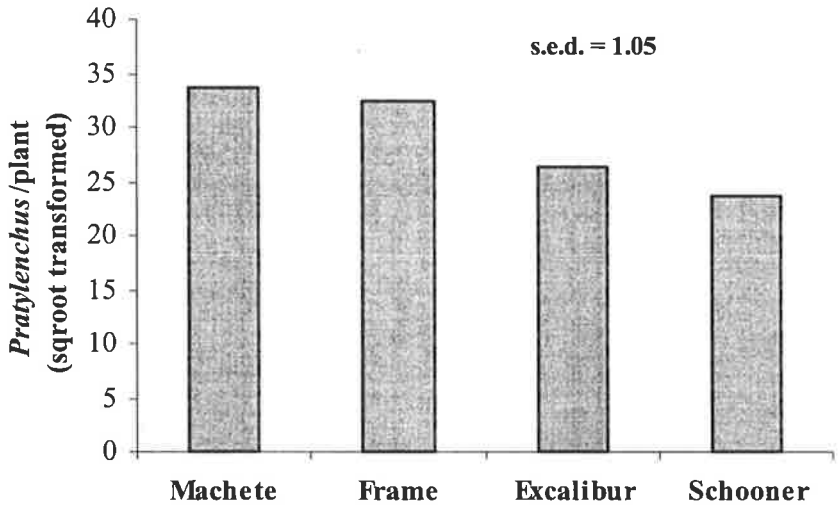
9.3.2.3 Experiment 3

Results from the ANOVA comparing differences in numbers of nematodes between populations and varieties after 6 weeks is shown in Table 9.8 and the overall difference in multiplication on the four cultivars in Figure 9.3. Significantly higher numbers of *Pratylenchus* were recorded on Machete and Frame wheat compared with Excalibur wheat, and numbers recorded on Excalibur were significantly higher than on Schooner barley. No correlation was observed between numbers of nematodes recovered and either top weight (r = 0.1) or root weight (r = 0.14).

Table 9.8 **The ANOVA of the results comparing the effect of population and variety on numbers of *Pratylenchus*/plant (Experiment 3; n =10).**

	d.f.	m.s.	P
Rep	9	0.955	0.034
Population	12	5.44	0.000
Variety	3	14.027	0.000
Population*Variety	36	0.963	0.005
Rep.*Population*Variety	451	0.47	

Figure 9.3 **Overall multiplication of 7 *P. neglectus* and 3 *P. thornei* geographically isolated populations (established from multiple nematodes from each site) on 3 wheat (Frame, Machete and Excalibur) and 1 barley (Schooner) cultivar (Experiment 3; n = 10).**



Multiplication of individual populations on Frame, Machete and Excalibur wheat and Schooner barley is shown in Figure 9.4. Three groups of *P. neglectus* and 2 groups of *P. thornei* were delineated and are presented below. Although there was a significant difference between overall numbers of *P. neglectus* from the three individual Original populations tested (obtained from separate carrot culture tubs of the same population) there was no difference in the relative ranking of the cultivars for these three tubs and all were included within *P. neglectus* Group 1.

P. neglectus ***Group 1:*** Comprised Original and Pinnaroo populations. Within this group numbers from Machete and Frame were significantly higher than from Excalibur and Schooner.

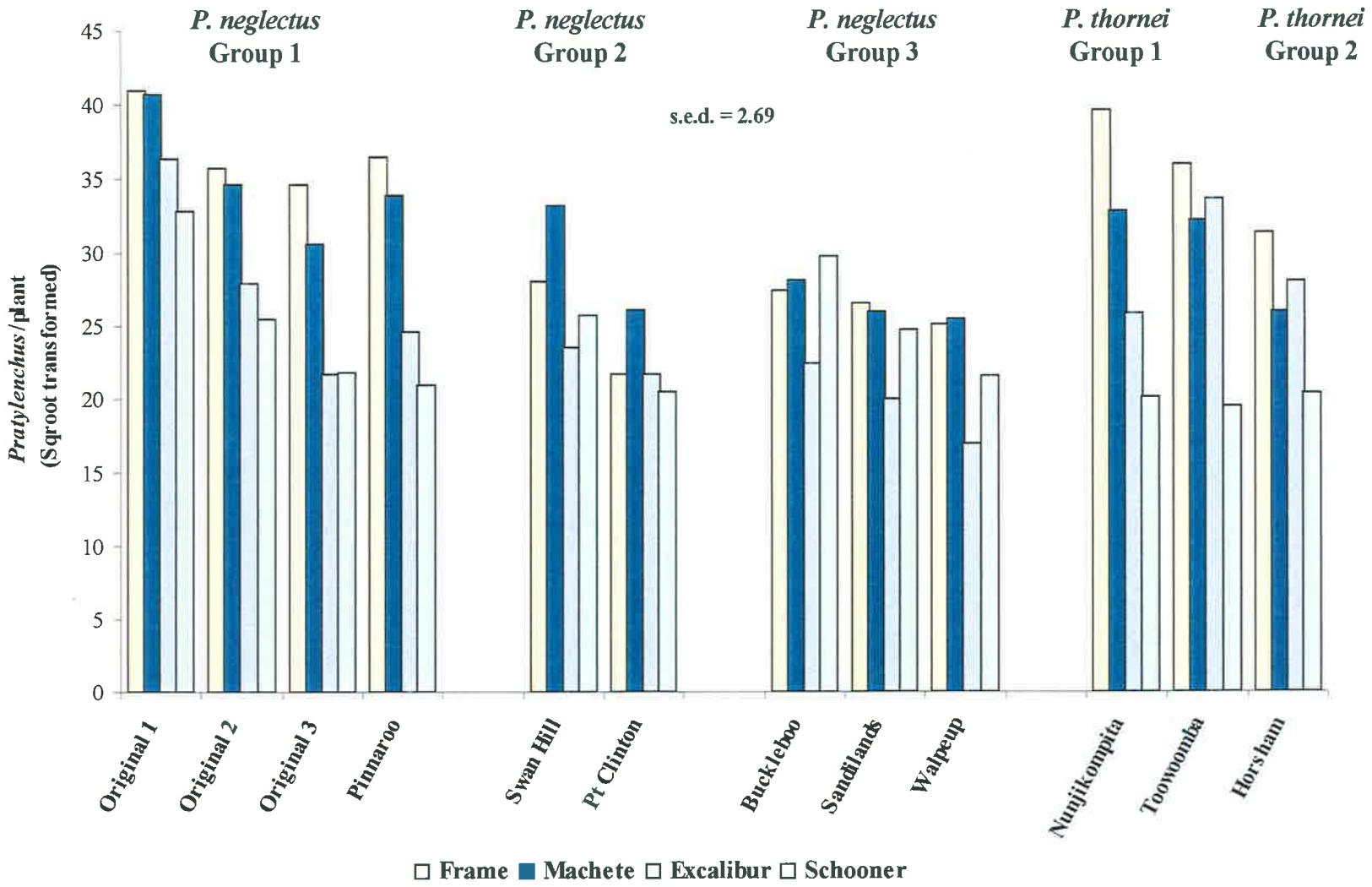
P. neglectus ***Group 2:*** Comprised Swan Hill and Port Clinton populations. This group was differentiated on the basis of a significantly higher rate of multiplication in Machete compared with Frame, Excalibur and Schooner.

P. neglectus ***Group 3:*** Comprised Buckleboo, Sandilands and Walpeup populations, which showed significantly lower multiplication in Excalibur compared to Machete, Frame and Schooner.

P. thornei ***Group 1:*** Comprised the only South Australian *P. thornei* population in this experiment. For this population, significantly higher numbers of nematodes were recovered from Frame, Machete, Excalibur and Schooner.

P. thornei ***Group 2:*** Comprised Toowoomba (Queensland) and Horsham (Victoria) populations and multiplication was significantly greater in Frame. Multiplication in Machete and Excalibur was not significantly different and recovery from all wheat cultivars was greater than from Schooner barley.

Figure 9.4 **Differences in multiplication between *P. neglectus* and *P. thornei* geographically isolated populations (established from multiple nematodes from each site) on four wheat (Machete, Frame and Excalibur) and one barley (Schooner) cultivar (Experiment 3; n = 10).**


P. neglectus Group 1
P. neglectus Group 2
P. neglectus Group 3
P. thornei Group 1
P. thornei Group 2
s.e.d. = 2.69
Pratylenchus /plant (Sqroot transformed)
45
40
35
30
25
20
15
10
5
0
Original 1
Original 2
Original 3
Pinnaroo
Swan Hill
Pt Clinton
Buckleboo
Sandilands
Walpeup
Nunjikompita
Toowoomba
Horsham
Frame
Machete
Excalibur
Schooner

9.4 Discussion

9.4.1 Allozyme electrophoresis

In Experiment 1 (Section 9.2.1.1), the *P. neglectus* populations tested were derived from a mixed source (i.e. from up to 15 individuals) and this made interpretation of allozyme results difficult as the multiple bands seen on gels may have been the result of mixed genotypes within populations.

In Experiment 2 (Section 9.2.1.2), although clonal lines (derived from individual females) were used, banding patterns still suggested mixed genotypes. It was concluded that either the populations exhibited extreme variability, or *P. neglectus* is a polyploid species. It seems likely that the latter applies as high ploidy level has been observed for other *Pratylenchus* species (Roman and Triantaphyllou 1969). If *P. neglectus* is polyploid, two or more homologous loci may be expressed on gels and this would explain the multiple banding seen within populations.

Interestingly, although the inferred genotypes proposed from the allelic differences differ from those seen in pathogenic differences (Section 9.3), the allozyme results are more reflective of the geographic location of the populations tested. Swan Hill (Victoria) and Buckleboo (Eyre Peninsula, South Australia) were discrete populations and are separated by approximately 730 km, while the two Yorke Peninsula (South Australia) populations (Port Clinton and Sandilands) are separated by only 40 km are genetically similar and fell within the same group. The Western Australian population was similar to two South Australian populations, and may indicate introduction of nematodes from one Australian state to the other.

Ibrahim *et al.* (1995) showed that in the isozyme profiles of six species of *Pratylenchus* tested, there were 28% fixed differences between *P. neglectus* and *P. thornei* with the largest difference being 41% (between *P. thornei* and *P. pinguicaudatus*). For this study, larger differences (up to 57%) were recorded for *P. neglectus* and *P. thornei*, suggesting greater diversification between the Australian populations of these species. No morphological differences have been observed, however, between Australian populations of *P. neglectus* and *P. thornei* and those from Europe, Africa and North America (Nicol 1996).

Within *P. brachyurus,* isozyme analysis suggested either two or three population groups (depending on the enzymes used) (Payan and Dickson 1990). As with *P. neglectus*, *P. brachyurus* reproduces by mitotic parthenogenesis (Roman and Triantaphyllou 1969) and is probably a polyploid species. The results supported the findings in this study that interpretation of isozyme profiles is extremely difficult for species using this reproductive strategy, as differentiation of allozymes is usually made with controlled single pair crosses.

9.4.2 Comparison between reproductive fitness of nematode populations

In addition to direct observation of genetic differences between populations of plant-parasitic nematodes using techniques such as allozyme electrophoresis, the measurement of phenotypic differences between populations can be made by evaluating reproduction within plant hosts.

In the virulence tests reported here, multiplication of the original *P. neglectus* culture was not significantly different from more recently established populations or from *P. neglectus* extracted freshly from wheat roots. This indicates no loss in infectivity after continuous culture on carrots for 8 years and has important implications for use of these cultures for routine screening of cultivars for resistance to *P. neglectus* in glasshouse studies.

While variation between clonal lines was observed in Experiment 1, results from Experiment 2 suggest that this was probably due to differences between carrot culture tubs rather than genetic differences. It was therefore difficult to assess true variation between populations based on multiplication on a single host, as the number of nematodes recovered from each tub may differ depending on the relative fitness of the culture. In virulence Experiment 3, this was highlighted by the differences observed between total numbers obtained from each cultivar for the three culture tubs tested from the Original population. The lack of variation between clonal lines in Experiment 2 also tended to support a minimal effect of mixed genotypes within populations. When care was taken to select carrot culture tubs of the same developmental stage in virulence Experiment 2, no differences were seen between clonal lines on the wheat variety Frame. Variation in fitness has also been observed in *P. brachyurus* from soybean callus. Although large numbers of nematodes were recovered from cultures after 18 months, many individuals were inactive. It was concluded that greatest reproductive fitness was observed in cultures harvested after 4-6 months (Koenning and Schmitt 1986).

To establish genetically based differences between populations, it was therefore concluded that differences must be assessed in the relative ranking of multiplication in different varieties and, in this study, hosts that displayed differences between multiplication (i.e. virulence) of populations were termed "differential" varieties. In virulence Experiment 3, significant differences were observed between the number of *Pratylenchus* recovered from each variety for each nematode population/carrot tub (i.e. the fitness differed between individual nematode carrot callus), but populations were separated into three *P. neglectus* and two *P. thornei* groups based on the relative ranking of multiplication in the four cultivars tested (Figure 9.4).

Multiplication within the wheat variety Machete was high for all groups. Machete has been chosen as a susceptible (and intolerant) check variety in field and pot trials and has shown consistent reactions across sites and seasons (Vanstone *et al.* 1998, Chapters 6, 7 and 8). Based on these preliminary laboratory experiments, the consistent susceptible ranking observed in this variety between sites in field studies may be due, in part, to consistent reaction to different populations of *P. neglectus*.

In contrast, variation in multiplication of *P. neglectus* was observed in Frame wheat in growth room tests. *P. neglectus* Group 2 showed low multiplication in Frame and numbers of nematodes recovered were comparable to the more resistant Excalibur wheat and Schooner barley.

Low multiplication for all *P. neglectus* populations was recorded in Excalibur wheat. Significant differences occurred between *P. thornei* populations, however, with low multiplication of the South Australian compared with the Queensland and Victorian populations. This correlates with field trial data, where Excalibur has been assessed as being susceptible in Queensland field trials (Thompson pers. comm.) and as having intermediate resistance in Victoria (Hollaway *et al.* in press). In South Australia, this variety has also been variable in field trials, with ranking ranging from resistant (Chapter 8) to moderately susceptible (Vanstone pers. comm.). Although a high degree of segregation was recognised within the variety after release (Hollamby pers.comm.), some differences may also be due to variation in the nematode populations attacking it.

While numbers of nematodes recovered from all varieties in *P. neglectus* Group 3 were generally low, this group was differentiated based on increased multiplication in Schooner barley. As previously observed in comparison with field data (Chapter 7), the relative ranking of Machete, Frame, Schooner and Excalibur of the Sandilands population within this group was supported by field data observations, i.e. multiplication in Schooner was not significantly different to the susceptible varieties Frame and Machete at Sandilands, and was more resistant to *P. neglectus* at the Condada and Paskeville field sites.

In most cases, similar geographic locations showed disparate results in terms of nematode multiplication (i.e. Sandilands and Port Clinton populations [Figure 3.2]). Large geographic distances separated both Group 2 sites (Swan Hill and Port Clinton) and Group 3 sites (Buckleboo, Sandilands and Walpeup) with sites within these groups separated by distances of 500 km and 600 km respectively. This suggests that "local" populations may be quite variable.

Further assessments are required to determine if these responses are true differences in the virulence among these populations. If confirmed, these results have clear implications for breeding and screening for tolerance and resistance. The number of populations within *P. neglectus* would need to be determined, and indicator varieties evaluated that differentiate between these populations before meaningful assessment of resistance and tolerance could be undertaken.

These preliminary results indicate that for *P. neglectus*, Frame wheat and Schooner barley may be useful differential varieties to determine differences between populations. For the limited number of *P. thornei* populations tested, Excalibur wheat appears to distinguish between South Australian compared with Victorian and Queensland populations. While Schooner and Excalibur were originally chosen for comparison within this study as a result of variation observed in data from field sites, the differences observed between multiplication of populations in Frame is surprising as field data had suggested more consistency (Vanstone *et al.* 1998).

In this study, a small selection of populations has been compared, with a limited range of varieties, and suggests that there is potential variation between populations of *P. neglectus* and *P. thornei* within Australia, requiring greater efforts in screening for resistance.

There was no indication that the differences observed in multiplication of *P. neglectus* or *P. thornei* in cereals were also reflected in a plant growth response as there was no significant relationship between final nematode densities and top or root growth. This is not surprising, as a low inoculation density was selected to reduce the possibility of a high initial nematode density inhibiting multiplication (Seinhorst 1970). Further studies are therefore required to determine if the populations identified also affect plant growth (i.e. tolerance) and this relationship would be best assessed in growth room experiments, where environmental conditions are controlled, using a range in nematode densities as shown in Chapter 8.

9.5 Conclusions

In studies where clear differences between host range or morphological characters have been observed, it has been possible to correlate differences between populations using several techniques. For example, differences between the normal and giant faba bean races of *Ditylenchus dipsaci* were supported by morhological characters, host range studies and RAPD analysis (Esquibet *et al.* 1998). The giant and normal races of *D. dipsaci* are very distinct however, and have been termed sibling species based on their failure to produce fertile progeny when crossed (Sturhan 1983).

In general, despite the availability of many methods for differentiation between nematode populations, clear distinctions within nematode species often remain confusing. For example, using RAPD analysis, variation was observed between isolates of *Radopholus similis* but could not be linked with differences in either morphological characters or in reproduction on banana hosts (Fallas *et al.* 1996; Marin *et al.* 1999). Using both morphological and RAPD analyses in an extensive study to differentiate within *P. coffeae* and between related species, it was not possible to identify relationships because of variation, and virulence tests were recommended (Duncan *et al.* 1999). In addition, although differences in reproduction of different *P. vulnus* isolates in *Prunus* were considered sufficient to separate the isolates into two races, there was no correlation with differences observed in the RAPD analysis (Pinochet *et al.* 1994).

The preliminary experiments presented in this study indicate that differences may occur between populations of *P. neglectus* or *P. thornei*. Further work is required to define these differences including the collection of more populations, and additional experiments on reproductive fitness.

These studies also showed that although differences between populations were identified using either allozyme electrophoresis or virulence tests, the groups obtained did not concur. This may occur for several reasons. Firstly, virulence tests usually only use a limited number of varieties and this may be insufficient to identify all differences between populations. Secondly, the enzymes chosen within allozyme studies may not necessarily reflect differences in reproductive fitness. Thirdly, for parthenogenic species, allozyme results are often difficult to interpret as it is not possible to study progeny from crosses between individuals.

Both allozyme studies and virulence tests indicated that parthenogenic species such as *P. neglectus* can generate and maintain a high degree of variability. The apparently greater variability of *P. neglectus* may also suggest that it has been present in southern Australia for longer than *P. thornei*. Previously, determining whether *P. neglectus* and *P. thornei* are endemic or introduced has relied upon records of these species under native vegetation where agriculture has not been practised, but this may not be useful as it is likely that both nematodes are carried to new regions by wind dispersal.

As allozyme profiles represent protein differences between populations or species, they allow assessment of the degree of relatedness between groups. The high variability between *P. neglectus* groups indicates temporal separation and therefore a large period of time since introduction into Australia. In comparison, minor variation was observed between *P. thornei* populations suggesting a more recent introduction into Australia.

While it was determined that the allozyme data presented in this study were too preliminary to allow the assessment of related of populations of *P. neglectus* and *P. thornei*, a more detailed study of allozymes differences is required and should allow a more meaningful interpretation of population differences.

Chapter 10 General conclusions and discussion

Assessing the impact of plant-parasitic nematodes within cropping systems is dependent on knowledge of a number of factors concerning the biology of individual nematode species. For field studies, an understanding of how sampling techniques may be affected by survival strategies and the horizontal and vertical distribution of each nematode species is important. In addition, factors affecting the population dynamics of plant-parasitic nematodes must be assessed.

10.1 Sampling and extraction

Results obtained from this study (Chapters 4 and 5) confirmed that both separation and catch efficiency are critical factors for research on plant-parasitic nematodes, and that care must be taken when choosing methods to extract plant-parasitic nematodes from plants and soil. As no single extraction method will be suitable for all purposes, the limitations imposed by each system must be evaluated with reference to the purpose of the study. In this study, estimates of the density of *P. neglectus* from both field and laboratory trials were used for assessing population dynamics, defining resistance, correlating density with yield loss and comparing virulence of different populations of both *P. neglectus* and *P. thornei*. Often, large numbers of samples were evaluated, and both the efficiency and reliability of estimates of density from both plants and soil were therefore of importance.

In the cropping systems of Southern Australia, very little rainfall occurs over summer and the majority of crops are autumn/winter-sown with no irrigation. An extended period of fallow occurs over the summer. *P. thornei* can survive these conditions in a state of anhydrobiosis (Glazer and Orion 1983; Tobar *et al.* 1996) and, although not demonstrated experimentally, it appears *P. neglectus* must also over-summer in this state as high densities remain in the soil during the summer period (Chapter 5). This survival strategy has implications for sampling methods particularly when a method of extraction is being used that relies on nematode motility. This was illustrated by the greater recovery of *P. neglectus* following addition of moisture to the soil either as rain or as water immediately prior to sampling. These studies emphasise that caution must be taken when sampling for *Pratylenchus* in dry soil as estimates of numbers may be up to 35% fewer than those obtained by sampling in wet soil.

South Australian agricultural soils are generally shallow and nutrient-poor, and this affects the distribution of host root systems which in turn, affects both the vertical and horizontal distribution of *Pratylenchus*. In results presented in Chapter 5, the majority of *Pratylenchus* were found between 5 and 20 cm in the soil profile. In shallower soils, between 47 - 84% of *Pratylenchus* were found in the top 10 cm of the soil layer and this depth was considered adequate for obtaining the major proportion of this species. In deeper soils, only 39 - 43% were found in the top 10 cm and a sampling depth of 20 cm was considered more appropriate. Sampling strategies may therefore change with different site characteristics.

Once soil has been sampled, the efficiency of extraction of *Pratylenchus* will also influence results. In studies reported in Chapter 4, higher rates of extraction from soil using the Whitehead tray method were achieved after only 72 hours compared with extraction from plants using the misting chamber, possibly because it did not provide an environment as suitable for nematode multiplication. The continued multiplication observed within plant roots in the misting chamber supported the use of the modified Whitehead tray method (Section 4.1) as the most efficient and simplest method for extraction of *P. neglectus* or *P. thornei* from soil. When assessing resistance in field trials, the evaluation of nematodes from soil samples (plus root material) compared with extraction from root samples was useful, as the collection of entire plant root systems (impossible to achieve in the field) was not required.

Storage of soil samples prior to extraction may be a source of variation between numbers of nematodes obtained using the Whitehead tray method. While storage at 4°C for up to a week resulted in increased recovery of *P. neglectus* for the sandy loam soil tested in this study, no significant difference was observed between either storage temperature or period (up to 12 weeks) for the clay loam soil. This result highlights differences that may occur between sites with different soil characteristics or soil moisture. The effect of sample storage should be an important consideration when comparing densities of nematodes from different sites or within the same trial, if samples must be processed over a prolonged time period.

Where extraction of *P. neglectus* from plant roots is required (e.g. in pot experiments), misting resulted in estimations comparable to those from the staining technique at all plant ages except 4 week old plants (Chapter 4). For older plants, misting was suitable for

estimation of *P. neglectus,* and has the advantage that living nematodes are obtained if using this technique for diagnostic purposes.

Although the misting technique (Section 4.2.4) was considered effective for the extraction of *P. neglectus* from older plant roots, there was variation between rates of extraction from different crops. This variation, coupled with the difficulty of recovering whole root systems from field plots, led to routine estimation of *Pratylenchus* density by sampling soil (including roots) under each plot and expressing results as nematodes/g of soil. An additional benefit of using soil cores was the ability to take larger numbers of small cores compared with sampling whole plants. Accuracy in estimating nematode densities was therefore increased as larger numbers of sub-samples could be taken and bulked for each field plot.

10.2 Determining resistance ratings in field experiments

Estimating numbers of *Pratylenchus* by extraction from soil does not allow determination of numbers actively feeding and multiplying within roots. However, for resistance ranking of cultivars in field trials, a reduction in densities of *P. neglectus* in soil at the end of the season was assumed to indicate resistance for three reasons. Firstly, final densities in soil under all crops were compared with a resistant check variety (Abacus or Tahara triticale) and a susceptible check variety (Machete wheat). These controls were previously identified from field and laboratory trials (Vanstone pers. comm.; Farsi 1995; Farsi *et al.* 1995) and supported by findings from Chapters 6, 7 and 8. Secondly, final densities (Pf) were assessed relative to initial densities (Pi). Thirdly, soil samples contained root material, so *P. neglectus* both within and surrounding plant roots were included. Estimates from soil samples were thus assumed to give an indication of the total numbers of nematodes present. The estimation of nematodes both within and surrounding roots may be particularly important for migratory endoparasites such as *Pratylenchus*, which move freely between roots and soil.

A disadvantage of using soil samples when evaluating resistance to *P. neglectus* was the inability to differentiate between nematodes feeding ectoparasitically and endoparasitically. *P. penetrans* can feed ectoparasitically before invasion, and extended feeding on root hairs causes hypertrophy (Zunke 1990). In contrast, although *P. neglectus* appears to reduce numbers of root hairs (Plate 2.2) it appears that this also suggests ectoparasitic feeding behaviour by this species.

It could be argued that the assessment of *P. neglectus* from soil (containing root material) fails to give a true indication of plant resistance as it does not directly measure invasion and multiplication within plant roots or distinguish between transitory ectoparasitic feeding behaviour. However, the measurement of *P. neglectus* from within plant roots alone (using a technique such as misting) would present the same difficulties, i.e. this would not differentiate between nematodes that are transitory within roots and those that are actively multiplying.

A further problem associated with assessment of numbers of *P. neglectus* within plant roots could be the timing of sampling and extraction. Migratory nematodes may leave plant roots when the food source becomes depleted at the end of the growing season (Loof 1991). If sampling occurs at the end the growth period for the plant (harvest), the measurement of *P. neglectus* from soil plus roots under each plot is assumed to overcome these difficulties. In addition, this measurement gives a practical assessment of nematode densities for growers for management purposes.

In Chapter 7, when determining resistance status of plants, the "moderate" category was used rather than moderately susceptible and moderately resistant ratings. The moderate rating implies an intermediate category between resistant and susceptible and appears to more accurately reflect resistance ratings. When comparing results from different sites and seasons, varieties such as Chebec barley and Echidna oat were rated as resistant in the Sandilands variety trial but susceptible in the trial at Paskeville. It is proposed varieties within the moderate category are more likely to be affected by site and seasonal conditions, and may show either increased multiplication in seasons more conducive to nematode reproduction or more limited multiplication in non-conducive seasons.

The estimation of final density was preferred to the use of multiplication rate (Pf/Pi) to estimate resistance, given that the magnitude of the Pf:Pi ratio varies inversely with initial density (Ferris and Noling 1987). The evaluation and comparison of final density against initial density in field trials allowed an estimation of whether *P. neglectus* levels increased or decreased under different hosts during the season. However, a range of initial densities is required if multiplication rate is to be used to define host plant resistance (McSorley and Gallaher 1992). In the trials reported in Chapter 7, at Sandilands and Paskeville, only a limited range of initial densities was available, and to minimise the influence of initial density on final density, sites with reasonably uniform, low initial densities were chosen.

The dependence of multiplication rate on initial density was seen clearly in the field trials reported in Chapter 8, where five differing initial densities of *P. neglectus* or *P. thornei* were established prior to over-sowing with an intolerant crop (Echidna oat) in the second season. For both *P. neglectus* and *P. thornei*, the multiplication rate decreased as initial density increased. If multiplication rate was used to define resistance, Echidna oat appeared resistant (Pf/Pi < 1) to *P. neglectus* at the highest initial density, but susceptible (Pf/Pi = 1.8) to *P. thornei* at all initial densities.

In these trials, plotting the final density of *P. neglectus* against initial density gave an equilibrium density (i.e. where the food source was sufficient to maintain the population) of 17 nematodes/g with an initial density of 11 *P. neglectus*/g soil. For *P. thornei*, a higher equilibrium density of 28 *P. thornei*/g was estimated with a lower initial density of 6 nematodes/g soil, also suggesting Echidna oat is more susceptible to *P. thornei* than to *P. neglectus*. Use of either final density or multiplication rate indicated that Echidna oat is a moderate host to *P. neglectus* and a susceptible host to *P. thornei,* and these ratings were supported by the differences in equilibrium density.

Thus, if multiplication rate alone is used to determine resistance in independent field trials, the resistance rating obtained may depend on the initial density of nematodes. For the assessment of the resistance rating of commercial varieties, if several field trials (with a range of initial densities) are established, differences in resistance ratings may appear to be obtained. In South Australia, inconsistencies have been observed in scoring resistance ratings for varieties in field trials, probably as a result of the interaction of multiplication rate and initial density. For management purposes, interpretation of trials bearing in mind the population dynamics of the nematode species is critical, particularly when assessing results for growers. Without comparison from several trials and use of known check varieties confusing results may be obtained. In these studies, a number of trials and techniques were used to confirm the resistance rating of individual varieties.

10.3 Resistance within field crops to *P. neglectus*

Screening for resistance to *P. neglectus* in wheat appears particularly difficult, as complete resistance is rare and results from Chapter 9 suggest development of resistant varieties may be further complicated by the possibility of several co-existing strains of *Pratylenchus*. In South Australia, considerable research has been undertaken to identify and evaluate resistant or susceptible check varieties for *P. neglectus* (Vanstone pers. comm.; Chapters 7 and 8; Farsi 1995). For *P. thornei*, Arapiles barley (resistant) and Meering wheat (susceptible) have been identified as check varieties for *P. thornei,* and have assisted in characterising the resistance rating of 83 varieties of field crops commonly grown in Victoria (Hollaway *et al.* 2000).

Results from Hollaway *et al.* (2000) for *P. thornei* and for *P. neglectus* (Chapter 7) highlight the broad host range of both nematodes, with multiplication observed in varieties of wheat, barley, oat and chickpea. Resistance to both species was identified in faba bean, field pea and triticale varieties. Within crops, particular varieties were found to have reduced susceptibility to both *P. neglectus* and *P. thornei* (e.g. Yallaroi and Tamaroi durum; Franklin, Galleon, Schooner, Arapiles and Barque barley; and Excalibur wheat). However, resistance to one species did not necessarily confer resistance to the other. As previously discussed, trials in Chapter 8 showed that Echidna oat was more susceptible to *P. thornei* than to *P. neglectus*. Of the nine canola varieties tested within trials at Sandilands and Paskeville (Chapter 7), eight were observed to be susceptible to *P. neglectus*, with one rated as moderate. Hollaway *et al.* (2000) found all canola varieties tested were rated as having moderate resistance to *P. thornei.* In addition, the wheat varieties Worrakatta and Krichauff were rated as susceptible and moderate respectively to *P. thornei* by Hollaway *et al.* (2000), while these varieties were rated as moderate and resistant respectively to *P. neglectus* (Chapter 7).

In wheat and triticale, resistance to *P. neglectus* has been shown to be a result of both lower nematode penetration and factors after invasion such as a delay in moulting and egg laying (Farsi 1995). Resistance to *P. neglectus* has been identified in the wheat variety Virest, and was hypothesised to be under the control of a single gene for resistance (Farsi 1995). A single dominant gene for resistance has also been tentatively identified from Excalibur wheat for which a molecular marker has been produced, and this gene appears to be present in the largely unrelated variety Krichauff (Williams *et al.* in press). Resistance associated with this single gene may be affected by modifying genes, the presence or absence of which alter the effectiveness of resistance. This hypothesis is supported by results from field trials at

Sandilands and Paskeville (Chapter 7), where Worrakatta wheat was identified as having moderate resistance to *P. neglectus* while Krichauff was resistant. As Worrakatta and Krichauff are closely related sister lines, modifying genes may be present in one and not the other, resulting in a higher level of resistance in Krichauff. Further research is currently being conducted to define the level of resistance conferred by the Virest, Excalibur and Krichauff genes using doubled haploid populations (Williams, Howes and Taylor, unpublished data).

The implementation of a marker for resistance to *P. neglectus* in wheat will prove useful, given that the phenotypic identification of resistance using glasshouse, growth-room or field trial screening systems is time consuming and labour intensive. In addition, results from Chapters 4, 5, 7 and 9 have highlighted the difficulty of assessing resistance using either field or growth room screening systems. In either field or growth room studies, inherent variation may be produced by the inoculation source (including differences in the infectivity or virulence of inoculum), site and seasonal conditions for plant growth, and the extraction and assessment of nematodes from plants or soil. Although the use of a molecular marker for the identification of resistance is expensive and limited to material containing the marker as well as by the precision of the marker, molecular tools should greatly assist in the development of resistant varieties for *Pratylenchus* where phenotypic assessment is difficult.

For situations where a single gene for resistance is affected by the presence of modifying genes that may increase the level of resistance, a molecular marker could be used to eliminate susceptible material. Lines with potential resistance identified by the marker could then be tested using traditional growth-room or glasshouse screening systems to determine the effectiveness of resistance (i.e. whether modifying genes were present).

10.4 Determining tolerance and yield loss

Although results are often subject to variation, estimation of differences in final nematode density (or multiplication) in either field or laboratory experiments is relatively straightforward compared with the estimation of crop tolerance. The assessment of tolerance requires evaluation of yield and is best conducted in field experiments. In addition, the expression of yield loss may only be measurable when the plant host is compromised by factors such as water or nutrient deficiency (Barker *et al.* 1981). Yield loss will therefore be directly affected by site and seasonal conditions. For *Pratylenchus* under South Australian

conditions, relatively small losses (< 15%) are common, and difficult to measure accurately within field trials.

Despite these difficulties, yield loss caused by *P. neglectus* under field conditions (Chapter 8) was demonstrated using both a) regression analyses between a range of nematode densities and yield and b) the application of the nematicide, aldicarb. Although the magnitude of damage caused by *P. neglectus* was similar using both methods, discrepancies were observed between results from trials where nematicide and regression analyses were compared. In trials to assess the effect of different rates of aldicarb on the yield of cereal varieties, potential stimulatory responses were observed at low rates of nematicide. Natural site variation or the presence of different pathotypes of *P. neglectus* between sites may also have contributed to aberrant results between different sites. It was concluded that although time consuming, establishing a range of nematode densities in field sites prior to these trials gave the most consistent yield loss and/or tolerance rating results.

In South Australia, the establishment of long-term field trials which establish varying densities of *P. neglectus* is difficult, as the majority of paddocks have *Pratylenchus*, take-all, cereal cyst nematode and *Rhizoctonia solani* at varying levels (McKay pers. comm.). Rotations that allow the multiplication of *P. neglectus* in a long-term rotation trial, without the concurrent build up of other damaging pathogens, are therefore limited.

Natural variation in *P. neglectus* densities across field sites (selected because levels of take-all and cereal cyst nematode were below detection) was used successfully to measure yield loss in the variety trial at Condada (Chapter 8) when there was sufficient replication. An obvious limitation of this system is that variation in nematode density is produced by chance and there may be insufficient range in initial densities for individual varieties.

The preparation of short-term, two year trials that produce a range of *P. neglectus* densities in the first season for assessment of varieties in the second season, therefore appears to be the most efficient and effective method for the assessment of tolerance/yield loss. As with defining resistance, however, the presence of different populations/strains of *Pratylenchus* may present difficulties when using field trials to identify tolerance and yield loss, leading to inconsistent results or differences in the magnitude of yield loss between sites.

10.5 Population dynamics and modelling yield loss – *P. neglectus* and *P. thornei*

In Chapter 6, an attempt to define the relationship between initial densities and either final densities or multiplication rate of *P. neglectus* was dubious due to the insufficient range of initial densities produced in the pot experiment. This experiment did, however, demonstrate that *P. neglectus* can cause a reduction in both top and root growth of wheat in the absence of other pathogens. The population dynamics of both *P. neglectus* and *P. thornei* in Echidna oat were seen more clearly in field trials (Chapter 8), where a range of initial nematode densities was established using resistant and susceptible varieties in the first year of the experiment. In these field experiments, the lowest multiplication rates of *P. neglectus* and *P. thornei* occurred at the highest initial densities. The relationship between multiplication rate and initial density could be described using either a non-linear exponential decay or a linear regression model. The relationship between final density and initial density of *P. neglectus* or *P. thornei* was either non-linear exponential or linear. Linear models were largely applicable for representing data following $\log_e$ transformation as this reduced spread between data points. However, the use of non-linear models may make greater sense with respect to the biology of plant-parasitic nematodes. With both multiplication rate and final density, non-linear models suggest an asymptotic limit and therefore, at very high initial nematode densities, both a maximum final density and a minimum rate of multiplication would be reached.

Similarly, although a linear model best described the relationship between the density of *Pratylenchus* and yield in Chapter 8, Elston *et al.* (1991) demonstrated a non-linear model was most applicable. It is assumed that, in the studies reported in Chapter 8, a greater number of data points (especially at higher initial densities), would also have shown a non-linear relationship as this indicates an asymptotic level of maximum yield loss. It is expected that, at least for *P. neglectus*, maximum yield loss would not be zero, as complete crop loss has not been attributed to this species even at very high initial densities (120 *P. neglectus*/g dry soil) (V. Vanstone pers. comm.).

10.6 Variability of *P. neglectus* and *P. thornei*

Results from Chapter 9 suggest the presence of three to four different strains of *P. neglectus* and two of *P. thornei*. These were identified from a randomly selected group of only nine *P. neglectus* and four *P. thornei* populations. This indicates that either the level of variation between geographically isolated populations of both *P. neglectus* and *P. thornei* is large or the test used to detect differences in virulence was too sensitive. The results from field trials at Sandilands appear to support the differences observed in the growth-room tests, with the differences observed in multiplication in Frame wheat and Schooner barley corresponding to results from the field sites. No further comparisons between field sites and laboratory studies were made, further work is required to confirm differences seen in growth room trials and to determine the possible number and difference between populations of *P. neglectus* in South Australia. If confirmed, the presence of populations of *Pratylenchus* with different reproductive fitness in varieties makes defining resistance or tolerance and screening for these characteristics difficult.

Populations of *Pratylenchus* within Australia require characterisation of their pathogenicity to, or reproductive fitness within, differential varieties. To achieve this, nematode populations will need to be assessed on the basis of differences in damage to, or multiplication within, different hosts. Differential varieties, i.e. those that display a known tolerance and/or resistance response, should be identified. In Chapter 9, Frame wheat and Schooner barley were identified as potential differential varieties for *P. neglectus* and Excalibur wheat as a potential differential for *P. thornei*. Further work is required to confirm these results, and a larger, more conclusive survey of populations of *P. neglectus* and *P. thornei* in Australia should be carried out.

If differential varieties are identified, *Pratylenchus* populations from field sites may be characterised on the basis of multiplication in, or damage to, these varieties. Once populations have been identified using differences in reproductive fitness or virulence, it may be possible to produce molecular probes for identification of individual populations based on techniques such as sequence characterised amplified region (SCAR) analyses (Zijlstra *et al.* 2000), assessment of differences between the ITS region (Subbotin *et al.*2000) or differences between enzyme phenotypes (Carneiro *et al.* 2000).

10.7 Conclusions – the importance of *P. neglectus* in South Australia

In South Australia, *P. neglectus*, take-all (*Gaeumannomyces graminis*) and cereal cyst nematode (*Heterodera avenae)*, are common pathogens in field crops. Both take-all and *H. avenae* are considered major pathogens, with take-all having the potential to cause 80% yield loss in wheat (Roget pers. comm) and *H. avenae* the potential to cause 90% yield loss if initial inoculum levels are high and an intolerant cereal variety is grown (J. Lewis pers. comm).

In the northern hemisphere, *P. neglectus* is regarded as a minor pathogen in wheat grass (Griffin 1992) and barley (Umesh and Ferris 1992). In Chapter 8, *P. neglectus* was demonstrated to cause yield loss of up to 27% in Echidna oat at initial levels of 18 *P. neglectus*/g soil. This suggests that, while probably not as damaging as either take-all or *H. avenae*, *P. neglectus* is a significant pathogen under South Australian conditions. In addition, the broad host range of *P. neglectus* demonstrated in Chapter 7 indicates this species should be an important consideration in cropping rotations in this region.

The manipulation of densities of *P. neglectus* or *P. thornei* using resistant and susceptible varieties in 1996 and 1997 allowed assessment of the impact of these nematodes in broad acre cropping systems in South Australia. Partial calculation of the cost of these nematodes to a grower could be made assuming rotations using either Machete wheat or Tahara triticale in the first year followed by Echidna oat. At Condada, the yield increase observed in Echidna following Tahara triticale, compared with Machete wheat, was 23%. A rotation which included the resistant triticale/intolerant oat rotation (combined figures for 1996 and 1997) would have resulted in returns of $AUS587/ha compared with the susceptible, intolerant wheat/intolerant oat rotation with returns of $AUS503/ha (based on yield in untreated Tahara and Machete plots in 1996 and yield in Echidna following either Machete wheat or Tahara triticale and prices of $AUS147/tonne for Machete and Tahara and $AUS118/tonne for Echidna [Figures presented in Appendix 7]). At Minnipa, the yield increase in Echidna following Tahara, compared with Machete wheat, was 14% which would have resulted in returns of $AUS647/ha in the resistant triticale/intolerant oat rotation compared with $AUS559/ha in the susceptible, intolerant wheat/intolerant oat rotation.

While these figures do not account for costs involved in producing the crop, costs are assumed to be comparable as triticale and wheat have similar nutrition, machinery and

herbicide requirements. Additional benefits may also result from a reduction in nematode levels up to two seasons following triticale, particularly for *P. thornei*. Although the figures do not allow for the difficulty in selling triticale (for which few markets are available in South Australia), the increases observed could make a substantial difference in the profitability of cereal growing in the low-input, low yield environments of South Australia.

Understanding the importance of pathogens within cropping systems is critical if information on rotations and sampling are to be used successfully to reduce the impact of each pathogen. The research presented within this thesis has shown that *P. neglectus* can be a significant factor in lost production within southern Australia if population levels within paddocks are left unchecked. This study has also demonstrated that the inclusion of resistant and or tolerant varieties within cropping systems is an economical and effective management option for reducing the impact of both *P. neglectus* and *P. thornei*.

Appendix 1 Specifications for Tailem Bend sand (all results calculated on an oven dry basis).

Sand content	
- Coarse (%)	88
- Fine (%)	11
Clay content	1.2
Silt content	0.4
pH ($CaCl_2$)	7.1
Total carbon (%)	0.03
Total nitrogen (%)	< 0.005
Exchangeable cations (Cmol/kg)	
- Ca	0.75
- Mg	0.42
- K	0.07
- Na	0.19
Cu (mg/kg)	0.1
Fe (mg/kg)	1.8
Mn (mg/kg)	0.7
Zn (mg/kg)	0.5

Appendix 2 Specifications for University of California (U. C.) soil mix (Waite Precinct version)

400 l of coarse washed sand sterilised at 100°C for 30 minutes
300 l of Euroturf peatmoss added and mixed (at 80°C).
Mixture is cooled and the following fertilisers are added:

Calcium hydroxide 700 g
Calcium carbonate 480 g
Nitrophoska (15:4:12) 600 g
- Total nitrogen 15% (5% NH_4, 4% NO_3, 1% NH_2, 5% IBDU)
- Total phosphorus 3.9%
- Potassium sulphate 12.4%
- Magnesium carbonate 1.25%
- Dicalcium phosphate 3.4%
- Sulphate 5.3%
- Iron oxide 0.3%
- Copper oxide 0.0002%
- Zinc oxide 0.007%
- Calcium borate 0.01%
- Molybdenum oxide 0.0003%

Appendix 3 **Loci, buffers and substrates required for allozyme electrophoretic studies described in Chapter 9 (taken from Richardson *et al.* 1986). [A] Buffers are presented in Appendix 4.**

Loci	Electrophoretic buffers[A]	Staining buffer	Substrate	Stock solutions
Aconitate hydratase (Acon)	B, A, C, L	0.1M Tris-HCl pH8.0 (2 ml)	Aconitate (0.2 ml)	NADP, $MgCl_2$, MTT, PMS (0.1 ml each)
Acid Phosphatase (Acp)	D, A	0.05M Citric acid pH 4.0 (2.5 ml)	Methlyumbelliferyl phosphate (0.5 mg)	
Adenylate kinase (Ak)	B, A	0.1M Tris-HCl pH8.0 (2 ml)	ADP (6 mg), glucose (2 mg)	NADP, MgC_{l2}, MTT, PMS (0.1 ml each)
Aldolase (Ald)	A	0.1M Tris-HCl pH7.4 (2 ml)	Fructose-1, 6 diposphate (10 mg), NADH (3 mg)	NAD, arsenate, MTT, PMS (0.1 ml each)
Alkaline phosphatase (Ap)	D	0.1M Tris-HCl pH8.6 (2 ml)	β-Napthyl acid phosphate (10 mg), Fast blue BB salt (8 mg)	$MgCl_2$ (0.1 ml)
Enolase (Enol)	A, B	0.1M Tris-HCl pH8.0 (2 ml)	2PGA (4 mg), ADP (4 mg), NADPH (3 mg)	$MgCl_{2,}$, KCl (0.1 ml each)
Esterases (Est)	G, C, D, A	0.1 Tris-maleate pH 6.5 (2 ml)	Methlyumbelliferyl phosphate (0.5 mg)	
Fructose-1, 6-Diphosphatase (Fdpa)	D	0.1M Tris-HCl pH8.0 (2 ml)	Fructose-1, 6 diphosphate (10 mg)	NADP, 1M $MgCl_2$, MTT, PMS (0.1 ml each)
Fumarate hydratase (Fum)	A	0.1M Tris-HCl pH8.0 (2 ml)	Fumarate (0.2 ml)	NADP, MTT, PMS (0.1 ml each)
Glyceraldehyde-3-phosphate dehydrogenase (Ga3pd)	D, L	0.1M Tris-HCl pH8.0 (1.5 ml)	Glyceraldehyde-3-phosphate (0.5 ml)	NAD, arsenate, MTT, PMS (0.1 ml each)
Aspartate Aminotransferase (Got)	B, J	0.1M Tris-HCl pH8.0 (2 ml)	Fast Garnet GBC salt (6 mg)	
6-phosphogluconate dehydrogenase (6pgd)	B, D	0.1M Tris-HCl pH8.0 (2 ml)	6PGA (5 mg)	NADP, 1M $MgCl_2$, MTT, PMS (0.1 ml each)
Glucose-phosphate isomerase (Gpi)	A, K	0.1M Tris-HCl pH8.0 (2 ml)	Fructose-6-phosphate (5 mg)	NADP, 1M $MgCl_2$, MTT, PMS (0.1 ml each)
Hexokinase (Hk)	C, D, L	0.1M Tris-HCl pH8.6 (2 ml)	Glucose (2 mg), ATP (6 mg)	NADP, 1M $MgCl_2$, MTT, PMS (0.1 ml each)
Isocitrate dehydrogenase (Idh)	A, C	0.1M Tris-HCl pH8.0 (2 ml)	DL-Isocitric acid (10 mg)	NADP, $MgCl_2$, MTT, PMS (0.1 ml each)
Lactate dehydrogenase (Ldh)	A, D, C, L	0.1M Tris-HCl pH8.0 (2 ml)	Lactate (0.1 ml)	NADP, MTT, PMS (0.1 ml each)
Malate dehydrogenase (Mdh)	A, L	0.1M Tris-HCl pH8.0 (2 ml)	Malate (0.2 ml)	NADP, MTT, PMS (0.1 ml each)
Malic Enzyme (Me)	B, G, C	0.1M Tris-HCl pH7.4 (2 ml)	Malate (0.2 ml)	NADP, $MgCl_2$, MTT, PMS (0.1 ml each)
Mannose-phosphate isomerase (Mpi)	B, A	0.1M Tris-HCl pH8.0 (2 ml)	Mannose-6-phosphate (8 mg)	NADP, $MgCl_2$, MTT, PMS (0.1 ml each)
Peptidases (Pep B or PepC)	A	0.1M Tris-HCl pH8.0 (2 ml)	valine-leucine (PepA), leucine-glycine-glycine (PepB), lysine-leucine (PepC), phenylalanine-proline (PepD) (10 mg)	$MgCl_2$ (0.1 ml each)
Phosphoglycerate mutase (Pga)	A	0.1M Tris-HCl pH8.0 (2 ml)	3PGA (6 mg), 2,3 diPGA (2 mg), ADP (5 mg), NADPH (3 mg)	$MgCl_{2,}$, KCl (0.1 ml each)
Phosphoglycerate kinase (Pgk)	A	0.1M Tris-HCl pH8.0 (2 ml)	3PGA (6 mg), ATP (6 mg), NADH (3 mg)	$MgCl_{2,}$, EDTA (0.1 ml each)
Phosphoglucomutase (Pgm)	C, B	0.1M Tris-HCl pH8.0 (2 ml)	Glucose-1-phosphate (10 mg)	NADP, $MgCl_2$, MTT, PMS (0.1 ml each)
Pyruvate kinase (Pk)	A	0.1M Tris-HCl pH8.0 (2 ml)	PEP (2 mg), ADP (4 mg), NADH (3 mg)	$MgCl_{2,}$, KCl (0.1 ml each)
Triose-phosphate isomerase (Tpi)	B, A, L	0.1M Tris-HCl pH8.0 (2 ml)	DHAP solution (1 ml)	NAD, arsenate, MTT, PMS (0.1 ml each)

Appendix 4 Recipes for running buffers used in allozyme electrophoresis studies in Chapter 9 (taken from Richardson *et al.* 1986).

Code	Buffer	Final molarity of each buffer constituent
A	0.01M Citrate-phosphate pH 6.4	10mM Na_2HPO_4; 2.5mM Citric acid
B	0.02M Phosphate pH 7.0	11.6 mM Na_2HPO_4; 8.4mM $Na_2H_2PO_4$
C	0.05M Tris-maleate pH 7.8	50mM Tris; 20mM Maleic acid
D	0.015M Tris-EDTA-borate-$MgCl_2$ pH 7.8	15mM Tris; 5mM Na_2EDTA; 10mM $MgCl_2$
G	0.1M Tris-maleate pH 7.8	100mM Tris; 40mM Maleic acid
J	0.1M Tris-citrate pH 8.2	100mM Tris; 10mM Citric acid
K	0.015M Tris-maleate pH 7.2	15mM Tris; 7mM Maleic acid
L	0.05M Tris-maleate-EDTA-$MgCl_2$ pH 7.8	50mM Tris; 1 mM Na_2EDTA; 1mM $MgCl_2$; 20mM Maleic acid

Appendix 5 Yield in aldicarb treated and untreated plots, Sandilands variety trial, 1996. Figures in brackets are detransformed means (n=4)

Crop/Variety	Yield of cereals (kg/plot) Untransformed		Crop/Variety	Yield of pulse, oilseed and medic (kg/plot) Log_e transformed	
	Untreated	Aldicarb treated		Untreated	Aldicarb treated
Wheat			***Field Pea***		
Barunga	2.13	2.28	Alma	1.02 (2.77)	1.04 (2.83)
Beulah	2.44	2.76	Bluey	0.68 (1.97)	0.71 (2.03)
Bowie	2.61	2.78	Bonzer	0.86 (2.36)	0.84 (2.32)
Buckley	2.89	3.21	Dundale	0.97 (2.63)	0.92 (2.51)
Excalibur	2.65	2.72	Early Dun	1.01 (2.75)	1.03 (2.80)
Frame	2.76	2.64	Laura	0.91 (2.48)	0.96 (2.61)
Janz	2.71	2.81			
Krichauff	2.87	2.63	***Chickpea***		
Machete	2.23	2.61	Amethyst	0.20 (1.22)	0.37 (1.45)
Meering	2.63	2.57	Desavic	0.42 (1.52)	0.49 (1.63)
Ouyen	2.59	2.82	Dooen	0.32 (1.38)	0.31 (1.36)
Silverstar	2.26	2.59	Kaniva	0.50 (1.65)	0.53 (1.70)
Spear	3.04	3.10	Tyson	0.36 (1.43)	0.48 (1.62)
Tatiara	2.52	2.68			
Trident	2.61	2.59	***Faba bean***		
Yanac	2.33	2.31	Ascot	0.76 (2.14)	0.71 (2.03)
			Fiord	0.97 (2.64)	0.95 (2.59)
Barley			Icarus	1.09 (2.97)	1.14 (3.13)
Arapiles	2.41	2.34			
Barque	2.58	2.85	***Vetch***		
Chebec	2.64	2.62	3323	0.93 (2.53)	1.09 (2.97)
Franklin	2.70	2.99	Blanchefleur	0.79 (2.20)	0.88 (2.41)
Galleon	2.69	2.71	Langeudoc	0.56 (1.75)	0.51 (1.67)
Mundah	2.98	2.66	Popany	0.61 (1.84)	0.60 (1.82)
Schooner	2.36	2.55			
Sloop	2.48	2.64	***Canola***		
			Dunkeld	0.73 (2.08)	0.75 (2.12)
Durum			Hyola 42	0.77 (2.16)	0.80 (2.23)
Tamaroi	2.13	2.30	Karoo	0.70 (2.01)	0.74 (2.10)
Yallaroi	2.30	2.50	Monty	0.79 (2.20)	0.82 (2.27)
			Narendra	0.80 (2.23)	0.80 (2.23)
Triticale			Oscar	0.56 (1.75)	0.66 (1.93)
Abacus	2.44	2.54	Rainbow	0.72 (2.05)	0.75 (2.12)
Muir	2.31	2.38			
Tahara	2.39	2.54	***Medic***		
			Caliph	3.50 (32.97)	3.60 (36.63)
Oat			Harbinger	3.86 (47.44)	4.05 (57.48)
Bandicoot	1.78	1.91	Herald	3.90 (49.80)	4.03 (56.44)
Bettong	2.38	2.21	Mogul	3.96 (52.85)	3.79 (44.45)
Carrolup	2.27	2.53	Parabinga	3.72 (41.13)	3.82 (45.44)
Echidna	2.55	2.73	Paraggio	3.79 (44.09)	3.79 (44.24)
Euro	2.56	2.70	Santiago	3.83 (46.00)	3.83 (46.14)
Potoroo	2.85	2.96	Sava	3.59 (36.29)	3.76 (43.09)
Wallaroo	2.86	2.05			

Appendix 6 Yield in aldicarb treated and untreated plots, Paskeville variety trial, 1997 (n = 4).

Crop/Variety	Yield of cereal, pulse and oilseed varieties (kg/plot, untransformed means)				
	Untreated	Aldicarb treated	Crop/Variety	Untreated	Aldicarb treated
Wheat			***Oat***		
Barunga	1.20	1.18	Bettong	1.57	1.48
Beulah	1.41	1.51	Carrolup	1.61	1.76
BT Schomburgk			Echidna	1.75	1.94
Bowie	1.41	1.41	Euro	1.61	1.60
Carnamah	1.29	1.29	Marloo	1.57	1.47
Cascades	1.53	1.47	Pallinup	1.62	1.60
Excalibur	1.21	1.46	Potoroo	1.83	1.87
Frame	1.20	1.30			
Janz	1.35	1.23			
Krichauff	1.39	1.25	***Field Pea***		
Kukri	1.28	1.17	Alma	0.82	0.91
Machete	1.25	1.41	Bluey	1.05	1.18
Meering	1.48	1.51	Dundale	0.80	0.91
Ouyen	1.20	1.12			
Silverstar	1.10	1.07			
Spear	1.30	1.29	***Chickpea***		
Trident	1.35	1.28	Desavic	1.32	1.29
Worrakatta	1.38	1.57	Dooen	0.96	1.07
Yanac	1.01	1.03	Kaniva	0.89	0.78
Barley			Lasseter	1.22	1.30
Arapiles	1.12	1.03	Tyson	1.27	1.24
Barque	1.38	1.36			
Chebec	1.44	1.30			
Franklin	1.48	1.45	***Faba bean***		
Galleon	1.26	1.21	Ascot	1.25	1.25
Mundah	1.35	1.22	Fiord	1.42	1.29
Schooner	1.04	1.07	Icarus	0.98	0.91
Skiff	.89	1.59			
Sloop	1.42	1.14			
Durum			***Canola***		
Tamaroi	1.08	1.12	Dunkeld		0.75
Yallaroi	1.03	1.15	Hyola 42		0.89
			Karoo		0.52
Rye			Monty		0.66
Bevy	0.89	0.89	Oscar		0.72
			Pinnacle		0.63
Triticale			Rainbow		
Abacus	1.33	1.27			
Tahara					

Appendix 7 Yield (t/ha) and price (Aus$/t) for cereals in two year rotation field trials at Condada (*P. neglectus*) and Minnipa (*P. thornei*).

	Condada (*P. neglectus*)		Minnipa (*P. thornei*)	
	Price $/t 1999/00	Yield in untreated plots 1996 (t/ha)[A]	Price $/t 1999/00	Yield in untreated plots 1996 (t/ha)[A]
Machete wheat (ASW)	147[B]	2.3	147[B]	2.6
Spear wheat (ASW)	147[B]	2.6	147[B]	2.5
Excalibur wheat (ASW)	147[B]	2.8	147[B]	3.3
Chebec barley (Feed)	137[C]	2.6	137[C]	3.1
Tahara triticale (Feed)	150[D]	2.5	150[D]	2.9
	Price $/t 1999/00[E]	Yield in Echidna oat following these varieties, 1997	Price $/t 1999/00[E]	Yield in Echidna oat following these varieties, 1997
Machete	118	1.4	118	1.5
Spear	118	1.5	118	1.7
Excalibur	118	1.7	118	1.7
Chebec	118	1.6	118	1.7
Tahara	118	1.8	118	1.8

A Yield in untreated plots expressed as tonnes per hectare, 1996

B Price for Australian Standard Wheat (ASW) (obtained from the Australian Wheat Board, May 2000).

C Price for feed barley (obtained from the Australian Wheat Board, May 2000).

D Price for triticale (obtained from the Australian Wheat Board, May 2000).

E Price for feed oat, 1999/00 (obtained from the Australian Wheat Board, May 2000).

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