The Distribution, Pathogenicity and Population Dynamics of *Pratylenchus thornei* on wheat in South Australia.

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Appendices

Summary

The root lesion nematode (*Pratylenchus thornei*) has been identified as a damaging pathogen on cereals worldwide and within Australia in Queensland and New South Wales. In South Australia, *P. thornei* and *P. neglectus* have been found, but their importance to the cereal industry has yet to be defined. Although the research reported here focused primarily on *P. thornei*, several experiments involved *P. neglectus*. The major objectives of the project were to determine the distribution of both *Pratylenchus* species in South Australia, to study the field and laboratory population dynamics of *P. thornei* in relation to wheat yields, to determine its host range on a variety of cereal and non-leguminous hosts and to identify possible sources of nematode resistant wheat cultivars/varieties. The involvement of root rotting fungi with the nematode in wheat disease was studied in preliminary experiments.

The statewide survey for *P. thornei* and *P. neglectus* in soil and plants from the cereal growing regions in South Australia showed that there was a 90% chance of finding one or both species of nematode in a given soil type. *P. neglectus* was more commonly found in sandy soils, while *P. thornei* tended to be associated with clay soils, although this distinction was not definitive. The survey confirmed that both nematodes had a wide host range.

An assay for screening cereal and non-leguminous hosts was developed. Plants could be effectively screened over two months instead of five, using plants grown in a sandy soil in small polyethylene tubes inoculated with a non damaging initial density of 400 *P. thornei*. From the plants examined, varying degrees of nematode multiplication were evident for both nematode species. The majority of commonly cultivated Australian wheats were highly susceptible to *P. thornei*. Triticale, rye, oats and durum were moderately susceptible to resistant, while the non-leguminous hosts showed suggested resistance to *P. thornei*. Similar results were obtained for *P. neglectus*. However, in some instances differences in nematode multiplication between some varieties/cultivars

within the *T. aestivum* species were evident. The variety AUS4930 was one of the least susceptible wheats tested for *P. thornei*, but the most susceptible for *P. neglectus*.

Laboratory studies on yield relations and population dynamics on wheat found that *P. thornei* significantly affected many growth variables. In general, low initial densities at early stages of growth (up to 5 weeks) were associated with a stimulus of many plant growth variables, possibly a host response to damage. However, higher initial densities significantly reduced many growth variables, verifying that *P. thornei* damages wheat in its own right.

The field population dynamics and yield relations of *P. thornei* were examined in a two year trial established in the Barossa Valley in South Australia. *P. thornei* caused significant yield losses up to 38% on commonly cultivated South Australian wheats, however the initial *P. thornei* density associated with yield reductions was seasonally variable. Two suspected resistant wheat varieties, AUS4930 and GS50A, were confirmed as resistant in the field, and a common South Australian wheat (Warigal) was found to be highly susceptible. The population dynamics of *P. thornei* followed the general pattern of nematode behaviour, with low initial densities associated with high multiplication and higher densities with reduced multiplication. However, the equilibrium density for *P. thornei* was approximately 10,000 *P. thornei*/200g OD soil, which was well above previously documented *P. thornei* thresholds on cereal crops.

Preliminary studies investigating the mechanism of *P. thornei* resistance showed that in both wheat varieties AUS4930 and GS50A the resistance acted post-penetration. Genetic inheritance studies with AUS4930 and a commonly grown South Australian wheat suggested further selection of both parents was necessary to define accurately the genetic basis of the resistance.

There were synergistic associations of wheat damage with *P. thornei* and *P. neglectus* and two commonly occurring South Australian root rotting fungi, *Fusarium acuminatum*

and *Microdochium bolleyi*. It will be necessary to further investigate such associations, particularly before adoption of resistant cultivars, because fungal infection might lower resistance.

From this study *P. thornei* is considered to be economically important in South Australia. The polyphagous host range and polycyclic nature of the nematode will make effective control of the nematode difficult, but not impossible. The two wheat varieties, field selections of GS50A from Queensland and AUS4930, originally from Iraq, offer potential sources of resistance to *P. thornei* in the field. The influence of root rotting fungi in combination with *Pratylenchus* on resistance needs to be carefully considered for successful nematode control, as well as the inherent differences in cultivar reaction to the two nematode species.

Declaration

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

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Abbreviations

ANOVA	analysis of variance
cm	centimetre
CRD	completely randomised design
d.f.	degrees of freedom
DDW	double distilled water
DNA	deoxyribose nucleic acid
f.wt.	fresh weight
g	gram
g	gravitational force
ha	hectare
kg	kilo gram
L	litre
M	molarity (g/L)
m	metre
m.s.	mean square
mg/ml	milligrams per millilitre
ml	millilitre
mM	milli molar
mm	millimetre
nm	nano metres
NSW	New South Wales
OD	oven dry
PDA	potato dextrose agar
pers. comm.	personal communication
Prob.	F probability
psi	pounds per square inch
RCBD	randomised complete block design
SARDI	South Australian Research and Development Institute
SDW	sterile distilled water
SED	standard error of difference
SPD	split plot design
t/ha	tonnes per hectare
USA	United States of America
V	volts
v.r.	variance ratio
vs'	versus

pto

w/w	wet weight
℃	degree Celsius
µ	micron
µg	micro-gram
µl	micro-litres
µm	micro-metre
µml	micro-millilitre

Chapter 1
Introduction

The world population is growing at an unsustainable rate with our resources being pushed to their limits. The population is expected to increase from 6.1 billion by the turn of the century to 8.2 billion by 2050 with more than 90% of this increase expected from developing countries. Worldwide, rice, wheat, corn and potatoes are the staple foods with wheat being the primary food in 43 countries (Reitz, 1967). An increase in wheat productivity would help to ameliorate the impact of this population increase.

In Australia, there are more than 45,000 farmers annually producing about 15 million tonnes of wheat of which over 85% is exported, making it Australia's third largest export earner. On a global scale, Australia is the fourth largest wheat exporter, contributing about 11% of the world market (National Farmers Federation, 1995). South Australia contributes about 14% of the total Australian production.

The Australian production per hectare of wheat is less than that of competitors, and despite an active research and extension program the production per hectare has not risen significantly in the last 20 years (National Farmers Federation, 1995). As a consequence current research is attempting to identify and rectify this problem. A contributor to the problem may be the root lesion nematode (*Pratylenchus* sp.) which reduces wheat yields in both southern and northern wheat belts of Australia. There are over 63 species in this genus (Loof, 1991), most of which are destructive pests on many plants. The nematodes move through the soil from root to root (Dropkin, 1989), producing characteristic narrow, elongated lesions on root surfaces or in the cortex.

In Australia, six species of *Pratylenchus* have been recorded on wheat but only two species, *P. thornei* and *P. neglectus*, are considered of economic importance principally

due to their widespread distribution (J. Thompson; A. Pattison; V. Vanstone and S. Taylor, pers. comm.). Both nematodes are morphologically similar, polyphagous and polycylic. *P. thornei* is known to markedly limit yield of wheat worldwide and within Australia is a known problem in the northern and eastern wheat belts and is also of importance in Southern Australia. *P. neglectus* is considered a potential problem only in South Australia. Terms such as "long fallow disorder" and "wheat sickness" have been used to describe the heavy yield losses caused by *P. thornei* on the Darling Downs in Queensland (Thompson *et al.*, 1981) and NSW (Doyle *et al.*, 1987). Preliminary evidence from Victoria and South Australia suggests *P. thornei* limits yield of wheat (Eastwood *et al.*, 1994; Nicol, 1991).

This study was undertaken to contribute to our understanding of the role of *P. thornei* in cereal production in South Australia. The main objectives of this research were to;

- determine the distribution of *P. thornei* and *P. neglectus* within the southern cereal belt.
- evaluate the host efficiency of the commonly cultivated cereal and non-leguminous crops to *P. thornei.*
- investigate in the field the relationship between nematode population density and yield in several cereal species.
- identify and investigate the possible mechanism and mode of inheritance of resistance in wheat to *P. thornei.*
- conduct preliminary investigations into the possible role of some root-rotting fungi in their association with *P. thornei* on the production of wheat.

In addition to the above, the first Australian investigation of the morphometrics of the two species *P. thornei* and *P. neglectus* was made and preliminary attempts were made to distinguish both species using molecular techniques.

Chapter 2
Literature Review

2.1 Systematics and Description

The genus *Pratylenchus* (Tylenchida: Pratylenchidae), established by Filipjev in 1936, which parasitizes a wide range of plant hosts has been difficult to classify taxonomically, with between 45-63 species depending on which key is used (Loof, 1991). On wheat, at least six species have been recorded: *P. thornei* (Fortuner, 1977), *P. neglectus* (Anderson & Townshend, 1976), *P. zeae* (Colbran & McCulloch, 1965), *P. crenatus* (Loof, 1978), *P. mediterraneus* (Corbett,1970)and *P. pinguicaudatus* (Corbett, 1970). In Australia, only three of these have been recorded on cereals: *P. thornei*, *P. neglectus* and *P. zeae* (Colbran & McCulloch, 1965).

Pratylenchus species are commonly known as root lesion nematodes. They are migratory obligate endoparasites with an average length of 0.5mm, (range 0.3 to 0.8mm) (Southey, 1978). *Pratylenchus* species have a long slender vemiform shaped body with a short, strong stylet (Southey, 1978).

In South Australia, two species, *P. thornei* and *P. neglectus,* are commonly associated with cereal growing areas (Ch. 4). The morphometric similarity of *Pratylenchus* species makes taxonomic identification a major impediment to sound ecological studies (Stirling & Stanton, 1993). Work described in Appendix D shows that the Australian forms of *P. thornei* and *P. neglectus* are indistinguishable from those in Europe, Africa, North America and the United Kingdom.

2.2 Host Range and Distribution

A host plant provides sustenance to a nematode parasite and allows reproduction (Caveness, 1974). *P. thornei* is polyphagous and attacks roots of plants from at least 18 botanical families (Table 2.1), including the basic food crops of cereals, legumes and

potatoes. Fortuner (1977) cites wheat (*Triticum aestivum* L.) as a primary host of *P. thornei.*

FAMILY	HOSTS WITHIN FAMILY
Chenopodiaceae	*Beta vulgaris, Spinacia oleracea*
Compositae	*Chrysanthemum sp., Helianthus spp., Lactuca sativa,*
Cucurbitaceae	*Citrullus lanatus*
Cupressaceae	*Chama ecyparis spp., Cupressus spp.*
Cruciferae	*Brassica oleracea* var.*botrytis, Iberis sp., Raphanus sativus*
Ericaceae	*Arctostaphylos pungens*
Fagaceae	*Quercus spp.*
Gramineae	*Echinochloa frumentacea, Hordeum vulgare, Panicum sp., Saccharum officinarum, Sorghum bicolor, Triticum aestivum, T.durum, Tritico secale, Zea mays*
Grossulariaceae	*Ribes rubrum*
Juglandaceae	*Juglans regia*
Leguminosae	*Cicer arietinum, Crotalaria juncea, Glycine max, Lens culinaris, Lupinus sp., Medicago sativa, Medicago rigidula, Phaseolus vulgaris, Pisum sativum, Trifolium repens, Vicia faba, Vicia sativa, Vigna unguiculata, Vigna sinesis,*
Liliaceae	*Allium cepa*
Linaceae	*Linum usitatissimum*
Pinaceae	*Pinus spp.*
Rosaceae	*Fragaria sp., Malus pumila, Prunus spp, Prunus domestica, Prunus persica var. nectarina, Pyrus communis, Rosa spp.*
Solanaceae	*Lycopersicon esculentum, Solanum tuberosum*
Theaceae	*Camellia sinensis*
Umbelliferae	*Daucus carota, Coriandrum sativum*
Vitaceae	*Vitis vinifera*

Table 2.1 : Host Range of *P. thornei* arranged by families
[Derived from Fortuner (1977), Greco *et.al.*(1984; 1988), Loof (1978) and O'Brien(1982), Evans & Webb (1989), Thompson (private comm. 1993)]

P. thornei has a world wide distribution but records are few. It has been reported in : **Europe** : England, Denmark, France, Spain, Greece (Thompson, private comm. 1993); Italy, Yugoslavia, Holland, Belgium and Germany (Fortuner, 1977). **Africa** : Canary Islands, South Africa, Egypt (Thompson, private comm. 1993); Morocco (Ammati, 1987); Algeria (Troccoli *et al.,* 1992). **North America** : Senora in Mexico, California in USA (Loof, 1978). **South America** : Venezuela (Loof, 1978). **Asia** : India (Fortuner, 1977); Korea (Thompson, private comm. 1993); Japan (Loof, 1978); and

Pakistan (Maqbool, 1987). **Middle East** : Syria (Greco *et al.,* 1988); Iran, Cyprus (Thompson, private comm. 1993); Israel (Loof, 1978) and **Australia :** (Baxter and Blake, 1967; Fortuner, 1977).

P. thornei has been recorded on wheat in the following countries : **Europe** : Yugoslavia (Fortuner, 1977); Italy and Mediterranean region (Lamberti, 1981). **Africa** : Morocco (Ammati, 1987), Algeria (Troccoli *et al.*, 1992). **North America** : West Senora in Mexico and Utah in USA (Fortuner, 1977) **Asia** : India and Pakistan (Maqbool, 1987) **Middle East** : Northern Negev in Israel (Orion *et al.,* 1982) and Syria (Greco *et al.,*1988) and **Australia** (Fortuner, 1977) .

Within the Australian wheat belt, *P. thornei* is known to occur in four States and is predominantly found in the heavy-textured soils. At present, the distribution encompasses Dubbo in the south of NSW, north to the Darling Downs and to Chinchilla in the north of the Queensland cereal belt (Thompson, private comm. 1993). In Victoria, *P. thornei* occurs in clay and loam soils at Nhill in the Wimmera and at Elmore and Rochester in the North-Central area (Thompson, private comm. 1993) and Charlton and Horsham (J. Fisher, pers. comm.). In South Australia, *P. thornei* was identified by Fisher in 1956 (J. Fisher, pers. comm.). It has been recorded in the Adelaide metropolitan area (Grandison, 1972; Singh, 1984) and is widespread throughout the cropping regions of South Australia (S. Taylor, pers.comm.; V. Vanstone, pers. comm.).

The closely related species *P. neglectus* occurs in temperate regions in Europe, Canada, United States, Japan, South Africa and North-Western India (Townshend & Anderson, 1976). *P. neglectus* has a widespread distribution in the cereal areas of Victoria (Meagher, 1970), Western Australia (J. Stanton, pers. comm.), the North-West wheat belt of Queensland (McCulloch & Thompson, unpub. data), NSW (R. McLeod, pers. comm.) and South Australia (Stynes & Veitch, 1983, S. Taylor, pers. comm.). Reports

of *P. neglectus* in Queensland are sporadic compared with *P. thornei* (Thompson, private comm. 1993). In the cropping regions of South Australia, *P. neglectus* is widespread on the Eyre Peninsula and is also commonly found in combination with *P. thornei* on Yorke Peninsula, in the South-East and to a lesser extent the Lower to Upper North (Ch. 4).

2.3 Biology, Histopathology and Life Cycle

2.3.1 Biology

P. thornei is a primary root parasite of wheat (Fortuner, 1977). There is some difference in opinion relating to the form of parasitism; Dropkin (1989) considered *Pratylenchus* spp. to be endoparasitic while Fulton *et al.* (1960) thought they were both ectoparasitic and endoparasitic. Reproduction in the genus *Pratylenchus* is bisexual (Dropkin, 1989), but males of *P. thornei* are rare (Fortuner, 1977). Females of *P. thornei* do not have functional spermathecae (Suatmadji; in Thompson, private comm. 1993) and reproduction is by mitotic parthenogenesis. A closely related species *P. mediteraneus* (formerly known as *P. thornei*) has similar morphology, however the presence of males has led to its recognition as a new species (Orion *et al.*, 1984)

2.3.2 Histopathology

Pratylenchus spp. invade the root cortex and kill cells during feeding, resulting in brownish elongated lesions (Dropkin, 1989). Hence they are known as root-lesion nematodes. *P. thornei* invades roots in a non-random fashion, being attracted to parts of the root already invaded and their subsequent reproduction results in groups of nematodes at intervals along both seminal and nodal roots (Baxter and Blake,1967). As a consequence, nematodes are concentrated in localised areas and cause damage to restricted parts of the root. The percentage of *P. thornei* invading wheat roots at a particular site decreases as the nematode numbers increase due to a shortage of locations

for penetration and/or interference between individual nematodes (Baxter & Blake, 1967).

Studies by Baxter and Blake (1968) on invasion of wheat roots by *P. thornei* under field and aseptic conditions established that *P. thornei* alone is pathogenic. In both aseptic and field conditions, *P. thornei* causes lysis of the parenchyma cells resulting in cell wall disintegration and formation of cavities in the cortex after three weeks. Following destruction of the cortex, the epidermis is sloughed off exposing the uninvaded stele which is often necrotic. Lesions (0.5-2mm) in the proximal parts of the seminal roots were noted after six weeks, but did not affect the structure of the stele. In stained root segments, *P. thornei* was usually located in the cortex lying parallel to the long axis of the root. Orion and Lapid (1993) found similar histopathology with *P. mediteraneus*, which is closely related to *P. thornei*. Males, females and larvae of *P. mediteraneus* invaded the cortical cells resulting in root collapse. Scanning electron microscope studies of *P. mediteranues* on *Vicia sativa* revealed that the nematodes invaded the roots at the root hair region by forming a clear "drilled" hole in the root epidermis and the cortical parenchyma, suggesting enzymatic lysis combined with mechanical force (Kurppa and Vrain, 1985). A dense layer of bacteria surrounded the invaded region. Inside the cortex, the nematode moved within the parenchymal tissue destroying the cell cytoplasm. Movement of the nematode through cell walls again suggested use of enzymatic activity. The surface of the invaded region appeared as a lesion on which the root hairs were shed and the epidermis was densely punctured. At the edges of the lesion, abnormally long root hairs were observed. In cross and longitudinal sections, aggregation of nematodes was observed in the root cortex where the plant tissue was completely destroyed. Eggs were deposited in cavities apparently formed by the nematode. Orion and Lapid (1993) described *P. mediteraneus* as a migratory endoparasite although there is evidence of ectoparasitic activity causing damage to root apices. It is possible that *P. thornei* may have similar behaviour.

Probably no two species of *Pratylenchus* produce an identical plant response to parasitism. Even plant reactions which appear outwardly similar may differ biochemically. A wide range of histopathological reactions may arise from the enormous number of possible combinations of plant species or cultivars and nematodes and the varied physiology of both (Southey, 1986). Cortical invasion and necrosis is also associated with other species of *Pratylenchus.* Ogiga and Estey (1975) found that *P. penetrans* invaded the cortex and caused necrosis on turnip and corn and refer to other authors with similar findings on alfalfa, pea, peach, apple, carrot, celery and strawberry. *P. coffeae* on *Musa* sp. also has similar histopathology (Pinochet, 1978). However, the histology of *P. thornei* differs from that of the closely related species *P. neglectus,* which is also a pathogen of wheat. Studies by Anderson and Townshend (1976) found that *P. neglectus* invades in a non-random manner, being attracted to the root tips in particular. This is probably a chemokinetic response. *P. neglectus* does not feed predominantly on the cortical cells, but rather externally on the meristematic tissue behind the root cap, causing root elongation to cease, after which the nematode invades and migrates to more mature areas of the root (Anderson and Townshend, 1976). Studies on wheat by Kimpinski *et al.* (1976) found that *P. neglectus* invades the seminal roots first, then the crown roots and observed that there were much lower numbers in the latter.

P. fallax, also pathogenic to wheat, is another species closely related to *P . thornei*, and it has a non-random invasion similar to that of *P. neglectus* (Corbett, 1972). *P. fallax* invades the main roots of wheat, barley and sugarbeet at the root tips, the region of root hair development, and at points where the laterals emerge. Unlike *P. thornei,* browning of the endodermis produced visible lesions before the cortex became necrotic. However, like *P. thornei*, *P. fallax* did not penetrate the stele.

Studies by Townshend *et al.* (1989) on the histopathology of *P. penetrans* on alfalfa roots, indicate the nematode moves through the cortex intracellularly, with nematodes once again lying parallel to the stele. The cortical parenchyma cells are penetrated and fed

upon, containing only cytoplasmic debris after 48 hours of feeding. The proximal cells had increased tannin deposits, degenerate mitochondria, increased numbers of ribosomes and no internal membrane structure. Often the endodermis had collapsed and contained massive tannin deposits on the inner cell wall and cell lumen.

Similar studies by Vovlas and Troccoli (1990) of *P. penetrans* in broad-bean roots found the nematode located entirely inside the cortex and generally longitudinal to the vascular cylinder with stelar tissues unaffected. Nematodes fed intracellularly causing extensive rupturing of cell walls, cavities and thickening of cell walls, or necrosis of the cells around feeding sites. There was no associated hyperplasia or hypertropy of the cortical cells but thickening of two to three layers of cells adjacent to the nematode were observed.

Some species of *Pratylenchus* appear to cause different histopathology in different hosts. *P. penetrans* invaded the endodermis and stele in corn, but the endodermis was a barrier to invasion in apple, peach, celery, strawberry and pea (Ogiga and Estey, 1975). Root exudates have been considered the most likely stimulus attracting *Pratylenchus* to a host plant (Baxter and Blake, 1967; Wallace, 1974; Wallace, 1989). Production of toxins, enzymes and unidentified compounds by both the host and nematode may play a role in root invasion and destruction by respective *Pratylenchus* spp. *P. penetrans* was found to induce lateral root formation in turnip and corn (Chen *et al.*,1963). It has been suggested that this response is associated with a phenolic defence mechanism of the host, which inactivates the hydrolytic enzymes of the nematodes and signals growth response by regulating indole acetic acid (Ogiga and Estey, 1975). In peach, strawberry and celery, root necrosis was found to extend beyond the infection court of *P. penetrans* (Baxter and Blake, 1968) suggesting toxins produced directly or indirectly by the nematode induced necrosis in advance of the feeding site. However, there are few histochemical investigations of plants infected with *P. thornei.* Such studies are essential for the understanding of the disease complex between pathogen and host.

2.3.3 Life Cycle

Invasion, movement through and exit from roots, may be accomplished by both adult and juvenile forms (Fulton *et al.,* 1960). Root-lesion nematodes possess four similar developmental stages, each separated by a moult, with juveniles resembling the adult forms except for maturation of the reproductive system (Fulton *et al.,* 1960). The first juvenile stage and first moult occur in the egg, and the emerging second-stage juveniles move about in the soil or enter roots (Dropkin, 1989). Subsequent juvenile stages develop until the fourth moult from which the juveniles emerge as adults. Females require a host plant to oviposit (Larson,1953), but juveniles of *P. thornei* can moult into females independent of a host (Thompson, private comm. 1993).

Figure 2.1 illustrates the general disease cycle for *Pratylenchus* spp., which can be split into 3 components (Fulton *et al.*, 1960):

1. Penetration in which the larvae and adults of various stages enter and leave the roots of susceptible hosts.

2. Dissemination of eggs, larvae and adults. The female, with or without fertilisation, may lay eggs singly or in small groups inside infected roots (Fortuner, 1977); the eggs hatch or are released into the soil following root breakdown (Agrios, 1988).

3. Oversummering in which the egg stage is probably most persistent, but most other growth stages are quiescent in the soil or when hosts are unavailable (Fulton *et al.*, 1960).

P. thornei attacks crops in the winter growing season and subsequently survives over drought conditions in a desiccated state (see section 2.7) until reactivated by rainfall the following winter (Grandison and Wallace, 1974; Glazer and Orion, 1983). *Pratylenchus* spp. are thought to leave the root for the surrounding soil environment when the roots become hard and inhospitable (Fortuner, 1977).

The life cycle of *Pratylenchus* spp. is completed 45-65 days (Agrios, 1988), depending on the species, temperature, as well as other factors contributing to this variability (Fulton *et al.*, 1960). Optimum conditions for development are species-specific. Few studies on the life cycle and optimum conditions of *Pratylenchus* spp. have been made. Larson (1959) found the complete life cycle of *P. thornei* took 40-45 days at 27°C. This included two days for the female to penetrate the root, one day to lay eggs, seven days for the egg to hatch into a second stage juvenile, seven days for the next moult to a third stage juvenile, four days to the third moult, fourteen days until the fourth moult was complete and about eight days until the female commenced laying. However, Siyanand *et al.* (1982) found the life cycle (egg to egg) of *P. thornei* required 25-29 days under laboratory conditions at 30± 2°C. Baxter and Blake (1968) observed appearance of second stage juveniles 24 days after wheat had been inoculated with adults, and the work of Orion *et al.* (1979) indicates the optimal soil temperature for reproduction of *P. mediteraneus* is between 18°C and 22°C.

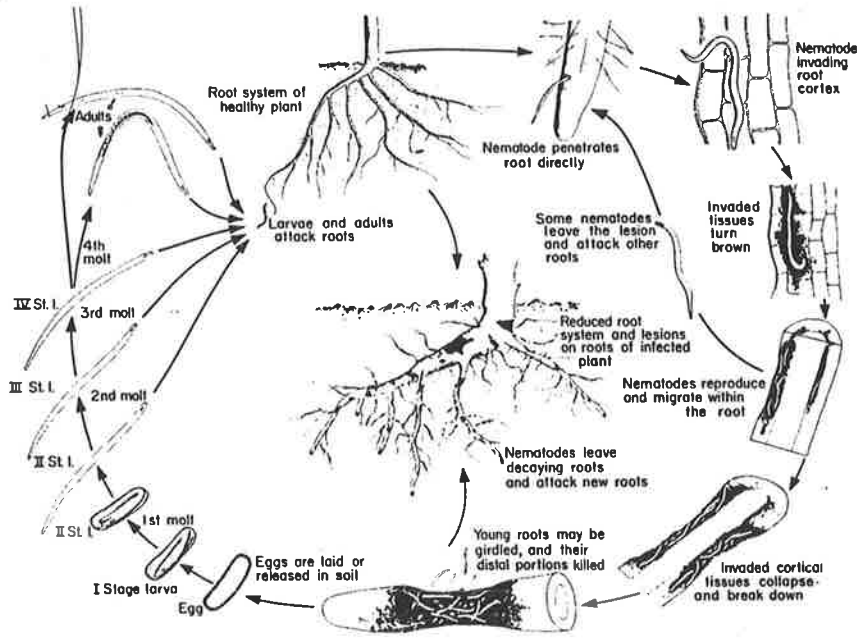


Fig. 2.1 : Life cycle of the root lesion nematode, *Pratylenchus* spp.(source: Agrios, 1988)

Larson (1953) demonstrated the reproduction and completion of the life cycle of *P. thornei* in wheat roots. Thompson (private comm. 1993) suggests that *P. thornei*

completes several generations within the life span of a single wheat crop and infers that nematode multiplication follows the compound interest epidemiological model described by Van der Plank (1975). Such exponential multiplication in the early stages of growth has been recorded by Baxter and Blake (1968). Thompson (private comm. 1993) further suggests that in a monoculture system *P. thornei* survives about six months fallow between successive wheat crops. This would mean that the population increases in a series of interrupted compound interest curves until population equilibrium is reached.

2.4 Symptoms

As mentioned in Section 2.3, root lesions are the main symptom for hosts infected with *Pratylenchus* spp. Thompson (private comm. 1993) found wheat plants severely damaged by *P. thornei* with dark brown lesions within the cortex of the roots and sometimes with discoloration extending to the stele. At lower population levels or with tolerant wheat cultivars lesions were not seen readily, but roots had general light-brown discoloration compared with white uninvaded roots (Thompson, private comm. 1993).

Portions of plants affected by *Pratylenchus* spp. above ground do not express definitive symptoms. In general, damaged plants appear stunted and unthrifty, unspecific symptoms which could be caused by restricted or non-functional root systems, drought or mineral imbalance in the soil (Fulton *et al.,* 1960). Effects of *P. thornei* on growth, yield and vigour have not been demonstrated consistently. Van Gundy *et al.*(1974) noted infected wheat plants were rarely killed but usually stunted and chlorotic, sometimes with necrosis at leaf tips accompanied by reduced tillering, size and number of ears, and only one head being produced instead of two to four per plant. Ear sterility was a symptom in Yugoslavia, while in India plants appeared sickly with poor growth (Fortuner, 1977); however, infestation occurred without browning or lesions. Severe stunting and shrunken grain are reported for wheat in the USA infested with *P. thornei* (Thorne, 1961), but Larson (1953) was not able to demonstrate any adverse effects of *P. thornei* in inoculated soil or in infested fields in California.

In Australia, Thompson's studies (private comm. 1993) in Queensland seem in part agreement with the work of Van Gundy *et al.* (1974). Wheat attacked by *P. thornei* appears stunted with few tillers and yellowed lower leaves with green and upright upper leaves. Affected wheat wilts prematurely compared with barley or nematode-free wheat under slight moisture stress. Doyle *et al.* (1987) record similar findings of stunting, restricted tillering and yellow lower leaves in NSW, particularly in old farming country where wheat has been grown regularly for many years while adjacent fields were often unaffected.

2.5 Associations with Other Pathogens

Pratylenchus spp. are successful in penetrating the root beyond its protective barriers and as a result infected roots are almost always invaded by secondary microorganisms including pathogens (Mountain,1954). Subsequently, rotting and further deterioration of root tissues occurs such that a disease complex between the nematode and secondary pathogen is established which may play a significant role in reducing crop yields (Powell, 1971).

The most widespread disease assoication with *Pratylenchus* spp. is with fungi, particularly rootrot complexes (Powell, 1971). Fungi found associated with the roots of plants containing *Pratylenchus* spp. include *Verticillium*, *Phytophthora*, *Pythium*, *Perenospora*, *Aphanomyces*, *Cylindrocarpon*, *Fusarium* and *Trichoderma* (Dropkin, 1989). Thorne (1961) found that oats and maize, infested with *P. thornei* were more susceptible to severe attack by smut (Ustilaginales). Synergistic interactions occurred between *Pratylenchus* sp. and *Verticllium dahliae* and/or *Erwinia carotovora* (Faulkner and Skotland, 1965; Krikun and Orion, 1977; Riedel and Rowe, 1985; Santo and Huan, 1992). Benedict and Mountain (1956) found *Pratylenchus* spp. associated with the fungus *Sclerospora*, causing root rot of sugar cane.

With respect to wheat, the work of Van Gundy *et al.* (1974) in Mexico suggests other soil organisms may contribute to the damage caused by *P. thornei.* They found *Rhizoctonia solani*, *Pythium* sp., *Penicillium* spp., *Alternaria* sp. and *Bipolaris* sp. in the roots of declining plants infected with *P. thornei.* They proposed that the field disorder was caused by a disease complex of *P. thornei* and *R. solani.* Such a complex involving *R. solani* and *P. neglectus* has been shown to cause almost twice the yield reductions on wheat than with the nematode alone (Benedict and Mountain, 1956). In South Australia, *P. neglectus* has been associated with *R. solani* causing bare patch on wheat and barley (de Beer, 1965). Siddiqi (1986) also found a negative association of *P. brachyurus* and *R. solani* on peanuts, together causing greater damage than either organism alone. The work of Taheri *et al.* (1994) found *P. neglectus* increased severity of lesioning on wheat roots with the following fungi: *Pyrenocheta terrestris*, *Pythium irregulare*, *Fusarium oxysporum*,and *Gaeumannomyces graminis* in combination with *Fusarium equiseti.* These are all found commonly in the cropping regions of South Australia.

In addition to interactions with fungi, some bacterial infections are enhanced by *Pratylenchus* spp. Dropkin (1989) found *Agrobacterium* caused more damage to roses in the presence of *Pratylenchus* than on its own. The disease complex of *P. penetrans* and *Pseudomonas* spp. caused significantly more growth reduction on alfalfa than the sum of either pathogen alone, suggesting a synergistic relationship (Bookbinder *et al.,* 1982). The plant height, root weight and root score of apple was significantly reduced when *P. penetrans* was combined with *Bacillus subtilis* (Utkhede *et al.,* 1992). Similarly bacterial wilt in tomato caused by *Pseudomonas* was enhanced by *Pratylenchus* (Dropkin, 1989).

Complexes between nematodes occur, although few are cited in the literature. *P. zeae*, which is a known pathogen of cereals, has a synergistic association with the nematode *Tylenchorhynchus vulgaris* on maize (Upadhyay and Swarup, 1981). Recently, much work has investigated the association of migratory and sedentary nematodes and suggests that the effects are varied and dependent upon species of nematode. For example, Umesh and Ferris (1994) found *P. neglectus* suppressed *M. chitwoodi* by reducing egg production, final population levels, reproductive index and in addition reduced the damage caused to potato and barley. Field observations of Lasserre *et al.* (1994) indicated that a reduction in population densities of *P. neglectus* on wheat coincided with the development of the cereal cyst nematode (*Heterodera avenae*) in wheat roots. In support, *Pratylenchus* spp. and *H. avenae* were also negatively correlated on barley (Esmenjaud *et al.,* 1990). Other associations include *M. incognita*, which is known to suppress *P. brachyurus* on soybean (Herman *et al.,* 1988), and *P. penetrans* on tomato (Estores *et al.,* 1972).

2.6 Environmental Influences

The impact of a nematode community on a population of host plants is the result of all the interrelationships both within and outside the community, with the total environment, including interactions with the host plants. For example, the physico-chemical aspects of the environment constitute one set of parameters governing nematode populations (Norton, 1979).

Despite Fortuner's (1977) view that *P. thornei* has a "cosmopolitan" distribution, certain ecological conditions favour its activity, reproduction and survival. The rate of population increase and the final equilibrium population density of *P. thornei* will depend on the environment as well as such factors as the initial nematode density and the host species. In discussing some of the ecological factors influencing *P. thornei* , the

following subsections on Climate, Soil and Nutrition will concentrate on the nematode with wheat as its host.

2.6.1 Climate

As previously noted in Section 2.3, *P. thornei* is active in the winter (growing season) in South Australia and subsequently survives drought conditions (over summer) in a desiccated state until reactivated by rainfall in the following autumn winter (Grandison and Wallace, 1974). *P. thornei* appears to be associated with the semi-arid zones of Utah (Thorne, 1961), Mexico (Van Gundy *et al.,* 1974) and southern Australia (Baxter and Blake, 1968; Grandison, 1972), which all experience a Mediterranean climate characterised by winter rainfall and summer drought, with hot summers and cool and mild winters (Sale, 1982). However, Grandison's (1972) studies implied that temperature, rainfall and precipitation-evaporation ratio were unimportant in influencing the population and density of *P. thornei.* Similarly, Thompson *et al.* (1989) found that high populations of *P. thornei* caused yield loss independent of seasonal conditions in Australia.

In contrast, work with the closely related species, *P. mediteraneus* found low moisture levels was a major ecological factor affecting the nematode multiplication in Northern Negev, where Orion *et al.* (1984) recorded the highest populations during a drought year and the lowest numbers in exceptionally wet years. Further, irrigated soil was a sub-optimal environment for the nematode which had no effect on yield under these conditions (Orion *et al.,* 1984). However, opposing results have been found with *P. neglectus* and *P. penetrans* . Low soil moisture was associated with fewer *P. neglectus* (Kimpinski, 1972), and under soil moisture conditions of 115 bars, reproduction, root invasion and survival of *P. penetrans* were greatly suppressed (Kable and Mai, 1968).

The inference of this suggests further work on the effect of climatic components need to be examined for *P. thornei.*

The work by Glazer and Orion (1983) showed that *P. mediteraneus* was able to withstand desiccation for up to 7-8 months, remaining infective. Moistening the soil activates dormant stages of *P. mediteraneus* or causes hatching of eggs in soil or plant remnants (Orion *et al.*, 1979). Under experimental conditions, wheat plants kept at 14°C during the early part of growth were not significantly affected by *P. thornei* , but plants kept at 25°C showed maximum reduction in yield (Van Gundy *et al.*, 1974). Similar studies with *P. neglectus* on wheat found maximum penetration and movement at 20°C (Umesh and Ferris, 1992), while the life cycle was completed most rapidly at 25-30°C. However, Townshend and Anderson (1976) found that greatest invasion and penetration of *P. neglectus* in maize roots was at 30°C. Orion *et al.*(1979) recorded the optimal temperature for *P. thornei* reproduction was 18 - 22°C. This suggests that differing physiological activities of the nematode have different optimal temperatures which are determined according to the host and surrounding environment.

2.6.2 Soil

Both soil type and depth influence *P. thornei.* Fortuner (1977) stated that *P. thornei* preferred heavier textured soils. Most global records of *P. thornei* are associated with clay soil: in Utah on heavy clay loams (Thorne, 1961), Holland on heavy soils (Loof, 1960), Mexico on fine textured clay soils (Van Gundy *et al.,* 1974), Netherlands, England and Wales on clay soils (Loof, 1960; Corbett, 1970).

In Australia, *P. thornei* is also associated with heavy soils. Thompson (private comm. 1993) found *P. thornei* in heavy cracking clay soils or vertisols from Dubbo in NSW to Chinchilla in Queensland. In the northern cereal belt on the Darling Downs in

Queensland *P. thornei* occurred on a full range of heavy textured soils including self-mulching, massively-structured black earths, grey clays and red loam soils (Thompson, 1989). Doyle *et al.* (1987) noted *P. thornei* on dark heavy-medium textured clays in northern NSW. In South Australia the distribution of *P. thornei* is not clearly defined.

Grandison and Wallace (1974) concluded that soil type was one of the main factors governing the distribution and abundance of *P. thornei* in Adelaide. Their studies found the hot dry summers markedly inhibited reproduction of *P. thornei* in sandy soil, but in clay soils higher moisture retention reduced such inhibition. The cool wet winters increased nematode reproduction in both sand and clay soils, but clay soils may be subject to waterlogging. Grandison (1972) concluded higher numbers of *P. thornei* were associated with clay than with sand soils in the Adelaide Metropolitan area.

P. thornei has been found from the topsoil to depths of 1.2 metres (Doyle *et al.,* 1987), with maximum numbers generally located between 30-60cm. In Queensland wheat fields, Thompson (private comm. 1993) and Doyle *et al.* (1987) noted *P. thornei* was found at depths of 20-60cm particularly after a period of fallow. This is in contrast to the soil distribution of *H. avenae* which occurs largely in the top 30cm of soil, and as a result control of *P. thornei* is more difficult. Depth distribution of the nematode may be influenced by the host. In Egypt, *P. thornei* occurred at 40cm depth under fallow, but moved upwards in the soil profile when sugarcane was planted (Fortuner, 1977).

2.6.3 Nutrition

Nitrogen, commonly applied as ammonia or urea, has important effects on plant growth and *P. thornei,* however there is confusion in the literature on what the possible effects are. Van Gundy *et al.* (1974) claimed that nitrogen application offered some measure of

control, but only when the *P. thornei* population was near the threshold for economic damage (42 *P. thornei* /100cc soil). If the population was 5-7 times higher, nitrogen had no effect. Kimpinski (1972) found that the concentration of ammonium nitrate was correlated with fewer numbers and lower densities of *P. neglectus* in wheat roots. In addition, the effects on the disease complex of *R. solani* and *P. neglectus* was lessened by the addition of nitrogen fertilizer (deBeer, 1965). However, Thompson (private comm. 1993) found the rate of response in wheat yield to increased nitrogen fertilizer was less in soil infested with *P. thornei* than in soil treated with aldicarb (nematicide). Thompson (1987) demonstrated that wheat roots infested with *P. thornei* take up soil nitrogen less efficiently than uninfested roots. However, growth of such infested roots was stimulated by fertilizer application, leaving higher populations of the nematode to attack subsequent crops. Doyle *et al.* (1987) found wheat yields in northern NSW were not significantly increased by the addition of nitrogen, and Orion *et al.* (1984) found no change in numbers of *P. mediteraneus* with the use of 150kg/ha nitrogen.

Potassium and phosphorus fertilizers did not significantly increase wheat yields in the *P. thornei* -infested sites of northern NSW (Doyle *et al.*, 1987). Similarly, number and density of *P. neglectus* were not altered by the addition of phosphorus and potassium to wheat (Kimpinski, 1972), and populations of *P. penetrans* on red clover and alfalfa were not affected by potassium fertilization (Willis, 1976). Again wheat yields were not significantly increased by application of copper, magnesium, boron, manganese, molybdenum or sulphur in a *P. thornei* infested site in NSW (Doyle *et al.*, 1987), but zinc did increase yield.

2.7 Survival

The closely related species, *P. mediteraneaus* is only active in winter in non-irrigated wheat fields in Israel (Orion *et al.*, 1979), emphasising the need for an efficient survival

mechanism for the nematode, especially during the summer periods before subsequent reactivation by rainfall in the following winter. All stages of *P. thornei* survive desiccation (Thompson *et al.,* 1981), during which the nematode is thought to enter a state of "anhydrobiosis". *P. mediteraneaus* have been observed to coil about themselves, shrink the body wall, and distort somatic muscles (Glazer and Orion, 1983). Adaptations of all organisms to anhydrobiotic survival are manifested by a reduction in metabolic rate (Womersley, 1987).

Thorne (1961) suggested that *P. thornei* migrated into the soil when roots became inhospitable and remained there until the next crop was planted. In an experimental area kept fallow for 5 years, 5% of the initial *P. thornei* population survived (Thompson and Clewett, 1990). Survival of *P. thornei* in 200g soil samples was reduced by 80% by drying from 19.5% to 5% moisture and/or high temperatures (Baxter and Blake, 1968). At temperatures of 40°C, mortality was induced within a two week period. Similarly, only 5% of the original population of *P. thornei* survived in an aerated steam treated soil for 30 minutes at 50°C but was completely eliminated at 70°C (Thompson, private comm. 1993).

Heavy clay based soils, commonly found associated with *P. thornei,* may give more protection against heat than lighter textured soils, presumably because of moisture retention, hence may be associated with nematode survival (Thompson, private comm. 1993). In Queensland vertisols where stubble is burnt and retained, the greatest number of *P. thornei* occurred at a depth of 30cm (Haak *et al.*, 1993). Studies on *P. mediteraneus* in northern Negev of Israel, with a similar climate to Australia, showed little annual variation in population density (Orion *et al.*, 1979). As suggested for *P. thornei, P. mediteraneus* is known to survive the unfavourable hot dry summer by entering an anhydrobiotic state (Glazer and Orion, 1983). Anhydrobiosis is induced

when the relative humidity is reduced to 97.7%. Only 3% of nematodes survived three cycles of desiccation and rehydration. This has implications for fallowing in winter, where the nematode population could be reduced by 80% because of intermittent wetting of soil (Orion *et al.,* 1984). S. Taylor (pers. comm.) found that several false breaks appeared to reduce *P. neglectus* populations in the field in South Australia. Neutral lipids in *P. mediteraneus* are accumulated by juvenile stages in unfavourable conditions (Storey *et al.*, 1982) and may have a role in survival.

When Baxter and Blake (1968) extracted *P. thornei* from soil, nematodes were inactive and irregularly shaped at first but slowly became active. Thompson (1989) reported *P. thornei* collected from dry fields can survive in undiminished numbers for at least two years when stored at ambient temperatures. Viability of *P. thornei* decreased rapidly during the first five weeks of storage and more slowly during the next fifty weeks, with the loss being increased at higher temperature and moisture (Baxter and Blake, 1968). *P. neglectus* also appears to survive desiccation (Townshend and Anderson, 1976). Survival was best at 2°C with mortality increasing with increasing temperature and soil moisture. Survival of adults and 4th stage juveniles was greater than 3rd and 2nd stage juveniles (Townshend and Anderson, 1976). *P. neglectus* can be considered "freezing susceptible" and cannot survive sub-zero temperatures. It declined slowly over 15 months at low moisture to less than half the initial population (Meagher, 1970).

2.8 Economic Importance

As previously discussed in section 2.3, *P. thornei* invades both seminal and nodal roots and causes cortical degradation. Cortical loss in wheat is thought to reduce the absorptive capacity of roots of certain grasses (Jacques and Schwass, 1956 ; in Baxter and Blake, 1968). Simmonds and Sallans (1933) showed loss of seminal roots in wheat can reduce grain yield, but loss of nodal roots is more significant. However, Krassousky (1926) indicated that on a weight-for-weight basis, seminal roots absorb

twice as much as nodal roots. Thompson (private comm. 1993) found that *P. thornei* severely limited the uptake of available nitrogen from the soil. Baxter and Blake (1968) suggested that *P. thornei* would reduce yields in crops grown during the dry season, if a high population was present in the soil at seeding. *P. mediteraneus* reduced capability of root systems to absorb water and nutrients from the soil, subsequently causing water stress and thereby affecting yield (Orion *et al.*, 1984).

Severe wheat decline in northern NSW and the Darling Downs in Queensland is associated with *P. thornei* (Doyle *et al.*, 1987; Colbran and McCulloch, 1965; Thompson, private comm. 1993). Thompson and Clewett (1986) reported that *P. thornei* caused yield losses up to 50% in wheat cultivars on the Darling Downs. Thompson (private comm. 1993) noted that with intolerant wheat cultivars such as Gatcher and Banks the yield loss was higher with up to 85% reduction. Although well fertilized crops are less affected by *P. thornei* (section 2.6.3), high yielding varieties can still cost an average loss of 0.5 tonnes/hectare (Thompson *et al.*, 1981). Populations of *P. thornei* in Queensland exceeding 500/200g soil in any of the upper layers of the profile constitute the economic threshold for intolerant wheats (Thompson, 1993). In northern NSW, Doyle *et al.*(1987) observed reduction in wheat yields of 50% in paddocks cropped with wheat alone for more than ten years. In South Australia, aseptic *P. thornei* on wheat reduced dry weight of plants by 50% after 9 weeks in the laboratory (Nicol, 1991), strongly suggesting *P. thornei* limited yield. Preliminary field work by Taylor and McKay (1993) indicate densities of 7.5 *P. thornei*/g soil before sowing caused yield losses up to 70% in wheat. The work of Eastwood *et al.* (1994) in Victoria showed *P. thornei* has the potential to reduce wheat yields by at least 44% and smaller amounts in other crops. It was estimated that per nematode per g soil *P. thornei* caused approximately 2% yield loss in the wheat cultivar Meering. Care should be taken with the *P. thornei* damage estimates recorded on wheat because most of these documented

yield losses were obtained using nematicides which may have influenced the growth of the plant and the microflora of the soil environment.

In countries other than Australia, *P. thornei* has been associated with major yield reductions of wheat in Utah, USA (Thorne, 1961) and Senora, Mexico with an economic threshold of 42 *P. thornei*/100cc soil (Van Gundy *et al.,* 1974). *P. mediteraneus* is also associated with severe yield reductions of wheat in the arid areas of Negev in Israel (Orion *et al.,* 1984). *P. neglectus* damages winter wheat in France when densities approach 3000/g root (Lasserre *et al.,* 1994). Laboratory tests have indicated that *P. neglectus* is damaging on cereals (Griffin, 1992; Mojtahedi *et al.,* 1992; Umesh and Ferris, 1994). In South Australia, Taylor and McKay (1993) found nematode densities up to 4 *P. neglectus*/g soil but these did not result in yield loss. However, A. Taheri (pers. comm.), found at high initial densities *P. neglectus* caused up to 20% yield reduction of the wheat cultivar Machete.

Although *P. thornei* is associated with major yield loss, there are also other economic aspects to be considered. As previously documented in section 2.2, the host range of *P. thornei* is wide and includes an extensive range of important plant species associated with wheat rotations. Several legumes, especially chickpea (*Cicer arietinum*), soybean (*Glycine max*) and various other grams and cowpea (genus *Vigna*) favour nematode multiplication. Among the cereals, maize (*Zea mays*) and Triticale (*Tritico secale*) in particular favour nematode multiplication, whereas barley (*Hordeum vulgare*) and sorghum (*Sorghum* sp.) are associated with few nematodes (Thompson, private comm. 1993). The significance of this is a loss in flexibility of crop choice to the producer, such that some common practices of legume/wheat rotations in certain areas of the grain belt may no longer be a viable cropping sequence. However, crops such as sorghum may be substituted for legumes in wetter areas. In the more marginal areas of South Australia,

opportunities for crops other than wheat are few thereby reducing the opportunities for control through crop rotation.

Some farmers have attempted to control *P. thornei* by applying high rates of fertilizer, but results in increased growth of weeds (Thompson and Clewett, 1988). Thompson (private comm. 1993) noted that the need to control weeds by greater herbicide usage increased the cost of production, or if not controlled led to a greater weed seed bank for subsequent crops. This has resulted in a change from winter to summer crops on some properties in Queensland.

The northern cereal belt supplies 25% of total wheat production in Australia, and the presence of *P. thornei* has been a major factor in farmers seeking to use alternative crops (Thompson, private comm. 1993). So significant has the impact of *P. thornei* been in Eastern Australia that control measures are actively being researched. Similar research has been conducted in Mexico by Van Gundy *et al.* (1974) who described a pest management approach involving the use of variety selection, nitrogen fertilizer, planting in cool soil (15°C) and crop rotation (avoiding wheat after wheat) as the most practical solution to control *P. thornei* on a commercial scale.

The closely related species *P. mediteraneus* was controlled successfully on wheat in Israel; with yield increases of 40-90% attained by biannual fallowing and 50-70% by the use of soil fumigation (Orion *et al.*, 1984). Other *Pratylenchus* species associated with cereals, *P. pinguicaudatus*, *P. fallax* and *P. crenatus* in England (Corbett, 1970) and *P. zeae* in Queensland (Colbran and McCulloch, 1965), have not been investigated with respect to yield-reducing potential.

As discussed previously, *Pratylenchus* species are known to be associated with other soil organisms, particularly fungi. The fungus *R. solani* has often been associated with high numbers of *P. neglectus* in crops which yielded poorly in South Australia (Stynes and Veitch, 1983). The preliminary work of A. Taheri (pers. comm.) suggests that fungi could be a major determinant of the degree of damage caused by *P. neglectus* in South Australian cropping regions. A similar situation may also exist for *P. thornei.*

The following section provides some insight into possible control measures of *P. thornei.* However, the type of control used is ultimately determined by the farmers' financial and technical resources (Dropkin, 1989). At present there is no current adequate control of *P. thornei* and research is only preliminary.

2.9 Population Dynamics and Control Measures

The impact of plant parasitic nematodes on plant health and crop yield varies with biogeographic location, cropping sequence and intensity, cultivar selection, soil characteristics and nematode community structure (McKenny and Ferris, 1983). Although *Pratylenchus* is capable of multiplying for several generations during a single season, they spread only from plant to plant due to their relative immobility. It is unrealistic to attempt to design methods to eliminate plant parasitic nematodes. Practically, control is adequate if the population remaining after harvest is smaller than would cause harm if the crop were planted again (Jones and Kempton, 1978). Therefore, control of a particular nematode requires sufficient understanding of the nematode population dynamics, biology, ecology and interactions with other organisms.

Models of nematode population and plant yield are mathematical statements and computer programs that represent portions of presumed reality (Dropkin, 1989). The aim is to

predict nematode population and crop yields based on limited information about a particular situation. If the initial density of a nematode is known when a crop is planted then prediction of the final density at the end of the growing season can be made, and again at the start of the following year (Dropkin, 1989).

In order to study population dynamics nematologists need to obtain adequate estimates of numbers of nematode present. Sampling plans must be custom made for many different situations since the relationship between the number of sample cores and relative error changes in response to many factors, including nematode species, field size, crop and soil type (McSorley and Parrado, 1982). Along with the sampling accuracy, the specific proportion of nematodes extracted using a particular technique and soil type is different and needs to be established.

Fig. 2.2 is a standard population curve for nematodes. The curves are uncomplicated by emigration, immigration or the persistence of individuals not participating in reproduction. The scales of P_f and P_i are the same so a line drawn at 45° through the origin is where neither population increase or decrease occurs, namely the multiplication rate is x1. As P_i increases the multiplication rate increases to its maximum and then decreases to x1 or less, largely because of increasing competition between individuals and decreasing food supply (Jones and Kempton, 1978).

Many authors (Barker and Olthof, 1976; Ferris, 1981; Jones and Kempton, 1978; Oostenbrink, 1966; Seinhorst, 1965) have investigated damage functions in annual crops by relating a parameter of plant performance to a single estimate of nematode population density and age structure. A generalised model for damage is a linear regression of plant growth against log-transformed initial nematode population density. Seinhorst (1965,

1973) produced models based on theoretical consideration then tested them in a series of careful experiments. The model, based on the general biology of nematodes involved data collection from a wide range of samples including low to high nematode densities. His model is based on the competition curve of Nicholson and assumed that "the average nematode" does not vary as population density changes and the ability of nematode to cause damage at high densities is unaltered (Dropkin, 1989).

The model of Seinhorst is illustrated in Fig. 2.3. The yield of a crop under attack by a nematode is the sum of the low yield when nematodes are at the maximum level known for that crop plus an increment that depends on the particular nematode population observed. This increment (1-m) where m is the minimum yield, is affected by the proportion of roots that escape infection. This proportion Z is raised to an exponent. The exponent consists of the differences between the observed density of nematodes and that at which no damage results (P_i-T), where P_i is the initial population density and T is the tolerance limit. From such models the economic injury level and economic threshold can be determined. The Economic Injury Level is the density of a particular nematode species that will cause a yield loss equal to the cost of nematode control, while the Economic Threshold Level is the density used to determine the probability of economic injury and the need for nematode control. It may be equal to, or less than, the economic injury level (O'Brien and Stirling, 1991).

Other approaches in modelling plant parasitic nematodes have involved exploring the "ceiling" reached by nematode populations on hosts (Dropkin, 1989). Nobling and Ferris (1986) assumed each habitat to hold a "carrying capacity" for a particular nematode, implying that the environment can support a certain size population and when this is reached the population remained more or less constant. Intraspecific and interspecific competition for a limited resource is a major density dependent factor

constraining population growth (Duncan and Ferris, 1982, 1983). In annual crops such as wheat, with relatively short growth cycles, logistic type depressions in multiplication rates are observed in the influence of P_i (initial population) on the P_f/P_i (final population/initial population) ratio (Seinhorst, 1970; Jones and Kempton, 1978; Ferris , 1985). Such models of seasonal multiplication are used for nematode management decisions. The objectives of such models is to predict final population size even though the dynamic interactions between nematode and host plant have been essentially ignored (Brown and Kerry, 1987). Measurements taken over a range of initial population densities indicate a density-dependent relationship between seasonal multiplication rate and initial population density. The maximum multiplication rate is seen at low initial densities when resources are unlimited. As initial population increases, the multiplication rate decreases owing to increasing competition between individuals, and a decreasing supply of food. At higher population densities the equilibrium level may be reached, at which the final and initial nematode populations are equal. Brown and Kerry (1987) reported that the work of Evans and Fisher (1970), Seinhorst (1970), Johnson *et al.*, (1974), Jones and Kempton (1978) and Nobling and Ferris (1986) fit this general model.

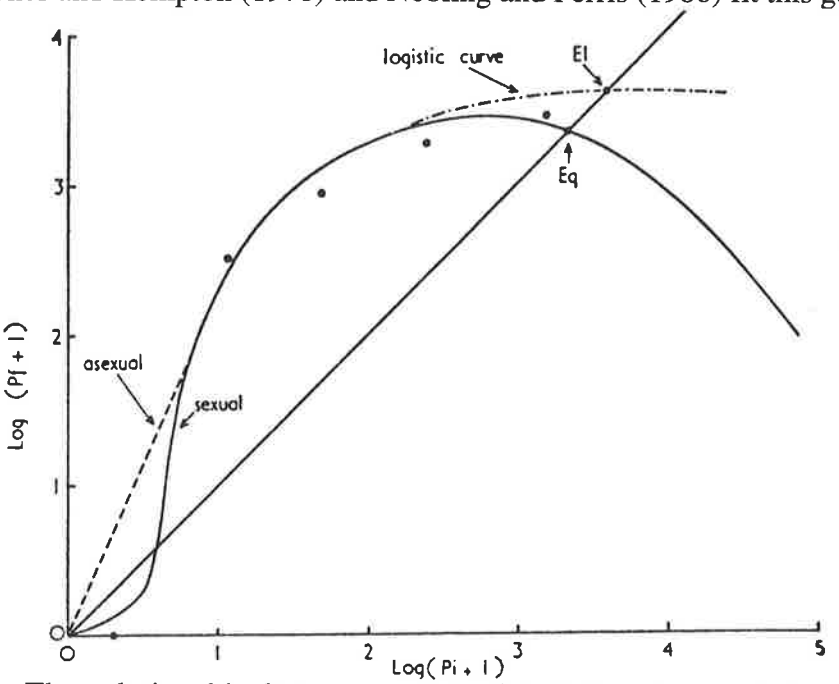


Fig. 2.2 : The relationship between preplanting (P_i) and post-harvest population densities (P_f) of asexual and sexual species. The upper, logistic curve is the one that would hold if the food supply remained constant. E1, logistic equilibrium point; E_q, observed equilibrium point. (Source: Jones and Kempton, 1978).

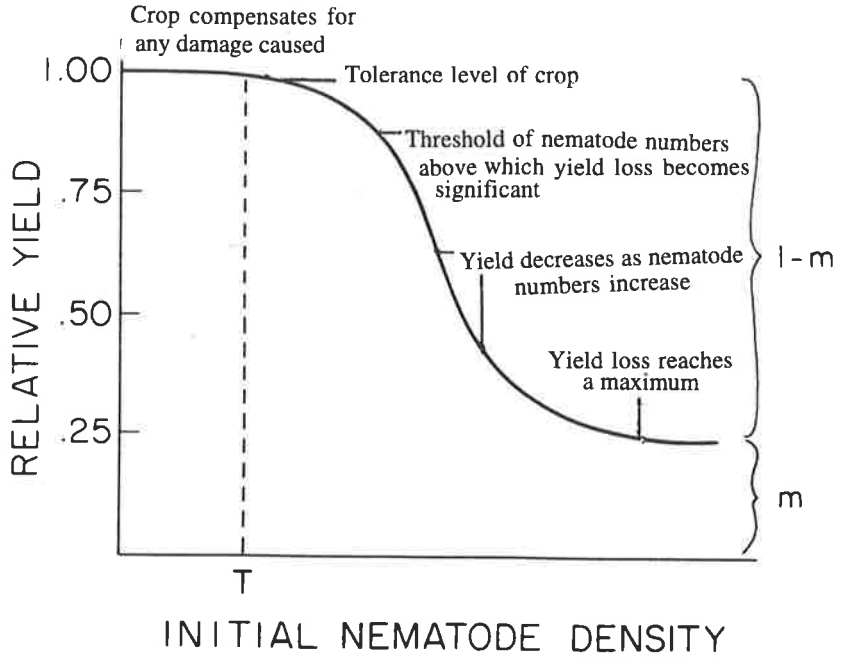


Fig. 2.3: The relationship between relative plant growth and number of nematodes; $Y=m + (1-m)Z^{Pi-T}$. For Pi>T, y=1.0 for Pi<T, where Y=relative yield, m=mimimum yield, T=tolerance limit, Z is a constant reflecting nematode damage, Pi is the initial population density. (After Seinhorst, 1965. Source: Brown and Kerry, 1987 and O'Brien and Stirling, 1991).

The above models are only useful if the information from which they are derived is accurate. This information is essential for the implementation of management practices for nematode control, whether they be chemical, cultural, biological control or the use of resistance and tolerance or a combination of approaches, commonly termed integrated control. However, the method of control will be ultimately determined by the farmer's financial and technical resources (Dropkin, 1989). Two criteria can be used to judge whether control measures are successful. First, the yield increase by plant growth and the second, the population density of the pest after harvest (Jones and Kempton, 1978). In the following sections some possible control measures of *P. thornei* will be considered.

2.9.1 Chemical Control

Nematicides not only protect the crop to which they are applied but may leave few nematodes in the soil for the next crop, resulting in residual yield increases of up to 0.5 tonnes/hectare in *P. thornei* infested sites (Thompson *et al.* , 1981). There are several problems associated with the use of nematicides. *P. thornei,* as previously mentioned, can complete several life cycles in the span of a single wheat crop so that nematicides may not be fully effective (Doyle *et al.,* 1987). Also *P. thornei* appears to be associated with heavier soil and to depths of 1.2m (Doyle *et al.,* 1987), the amount of nematicides required will be too high and expensive to be viable in most cases. Furthermore, the use of nematicides is becoming increasingly undesirable because of environmental concerns they are associated with.

Nematicides can be categorised into three major groups; fumigants, organophosphates and carbamates. Considering fumigants, Ethylene dibromide (2.7-10.8 L/ha) was found to have no effect in reducing *P. thornei* numbers or increasing yield (Doyle *et al.*, 1987). However, Methyl bromide increased wheat yields from 32-78% (Thompson, private comm. 1993; Doyle *et al.*, 1987). Van Gundy *et al.* (1974) reported a side effect of bromine toxicity induction in Mexican wheat. Work with the closely related species, *P. mediteraneaus* by Orion *et al.* (1984) found soil treated with Metham sodium resulted in 50-70% yield increase and 90% reduction of nematode populations, but its use was only viable in irrigated areas. Thompson (private comm. 1993) found that broad spectrum fumigants controlled *P. thornei* , but they proved toxic to mycorrhizal fungi and may kill much of the microbial biomass which play an important role in nitrogen supply. The fumigants Chloropicrin (220kg/ha) and Methyl-isothiocyanate liberator Dozomet (450kg/ha) were less effective than aldicarb (10kg/ha) (Thompson, private comm. 1993). The more specific nematicidal fumigant D-D (1, 3-Dichloropropene) at 187L/ha, led to yield increases on wheat plants in *P. thornei* infested soil in Mexico (Van Gundy *et al.*, 1974), and Telone 11 (1, 3-Dichloropropene and 1, 2-Dichloropropane) applied at 29.6

L/ha controlled *P. thornei* and increased grain yield in Australia (Doyle *et al.,* 1987). However, Thorne (1961) believed soil fumigation was impractical due to the distribution of *P. thornei* in the soil profile.

The most effective control in Australia is found with the granular carbamate Aldicarb (Temik®) (Thompson *et al.*, 1980a, b, 1982, 1983, 1984; Doyle *et al.*, 1987; Klein *et al.*, 1987), increasing wheat yields by 1.6 tonnes/hectare or 130% in Queensland. Application of 10kg Aldicarb /ha before the planting rain, worked into the soil the day of planting, or with 2-5kg Aldicarb /ha applied as granules in the seed row, provide the best results. However, this is uneconomic in field situations, and the best results obtained at any rate of Aldicarb was for the value of the extra grain produced to just match the cost of the nematicide (Thompson *et al.*, 1982). Other carbamates (Oxamyl, Carbosulfan or Cleothocarb) and organophosphates (Fenamiphos, Terbufos, Ethoprop and Fensulfothion) were less effective than Aldicarb (Thompson, private comm. 1993).

Further studies conducted in Queensland indicated coating seeds with the carbamates, Carbofuran and Oxamyl (0.125-0.5 kg/ha) resulted in only a slight reduction in numbers of *P. thornei* and had little effect on yield (Thompson, private comm. 1993). However, in Israel Furathiocarb (10g/kg seed) or Oxamyl (3.6g/kg seed) reduced *P.mediterraneus* populations by 65-80% and increased ear count and grain yield by 20-31% and 48% respectively (Orion and Shlevin, 1989), and were economically viable for use on low cash crops grown in marginal areas. However, in most cases nematicides are not an economically viable proposition for *P. thornei* control in cereals (Van Gundy *et al.,* 1974; Thompson, private comm. 1993).

2.9.2 Cultural Practices

Cultural practices imply the use of crop rotations, tillage practices, soil solarisation and time of planting. DiVito *et al.* (1991) found that mulching fields with polyethylene film for up to 8 weeks suppressed populations of *P. thornei* by 50%. The reduction in nematode numbers was correlated with an increase in grain yield for plots treated for 6-8 weeks. It may also be possible to control *P. thornei* by manipulation of planting dates to avoid peak activity of the nematode. Van Gundy *et al.* (1974) found delaying sowing of winter irrigated wheat by 1 month in Mexico gave maximum yields. However, in NSW Pattison (1993) suggested that wheat crops should be sown early to allow maximum root development to occur when temperatures are less favourable for *P. thornei* multiplication (April and May).

In Israel, Orion *et al.* (1984) found that biannual fallowing reduced *P. mediteraneus* populations by 90% and increased grain yield by 40-90%. In Mexico the numbers of *P. thornei* were higher in wheat-fallow-wheat rotations than those involving *Zea mays*, *Gossypium spp.* or *Glycine max* (Van Gundy *et al.*, 1974). The lowest numbers of *P. thornei* were associated with rotations which were out of wheat for two consecutive years. The host range of *P. thornei* is wide (see section 2.3), with 18 botanical families being affected. *Triticum aestivum* and *Phaseolus lunatus* are good hosts of *P. thornei*, fair hosts include *Zea mays*, *Secale cereale*, *Glycine max*, *Hordeum vulgare* and *Avena sativa,* while *Sorghum vulgare* is a poor host (Van Gundy *et al.*, 1974). Both Thompson and Clewett (1986) and Van Gundy *et al.* (1974) suggested that fields infested with *P. thornei* should never be sown with wheat in succession.

Rotations involving crops other than wheat which favour lower nematode multiplication can be devised. Thompson *et al.*(1981) suggested that Clipper barley was partially resistant to *P. thornei* and could be grown in place of the second wheat crop after a long

fallow on the Darling Downs in Queensland. Another common practice in Queensland is to alternate wheat with sorghum accompanied with long fallows. Experience on the Darling Downs suggests that current rotations from wheat to non-host crops of setaria, linseed and canary and the inefficient hosts of sorghum, sunflower and pigeon pea, are suitable to keep *P. thornei* at non damaging levels, provided wheat is grown no more frequently than once every three years (Clewett *et al.*, 1993). Diversification of cropping patterns to include susceptible crops like chickpea, mungbeans, triticale, maize and barley leave moderate to high residual populations of *P. thornei* and provide less satisfactory breaks for wheat in nematode infested fields (Clewett *et al.*, 1993). In NSW, yield of barley and sorghum was satisfactory with the yield of the next wheat crop improved, however the subsequent wheat crops yielded poorly (Doyle *et al.*, 1987). Esmenjaud *et al.* (1990) showed that *P. thornei, P. neglectus* and *P. crenatus* were most numerous on wheat after maize, with wheat monocultures having intermediate numbers. Irrigated sugarbeet decreased populations of *Pratylenchus,* with the effect sustained even on the third crop after sugar beet. In Utah, rotations with alfalfa and sugar beet for several years reduced *P. thornei* populations to negligible numbers (Thorne, 1961). Oostenbrink *et al.* (1956), found that flax, peas, potatoes and beet reduced *P. thornei* populations in Holland.

An eleven year management trial conducted on the Darling Downs at the Hermitage Research Station revealed that the top soil of zero tillage fallow systems had higher *P. thornei* populations than mechanically cultivated treatments (Thompson *et al.*, 1983). Further studies in Queensland revealed an increase in *P. thornei* numbers throughout the soil profile where stubble was retained for two years. Minimum soil disturbance in wheat fields favoured high numbers of root lesion nematodes (Klein *et al.*, 1987). In a four year continuous wheat trial on a red-brown earth, on the Darling Downs, 107 *P. neglectus* /200g topsoil were found from zero tillage plots, but only 15/200g from mechanically cultivated plots (Thompson, private comm. 1993). S. Taylor's (pers.

comm.) studies found the numbers of *P. neglectus* in Wallaroo oats were 55% lower in mechanically cultivated plots compared to reduced tillage treatments attributing to a 27% increase in yield with cultivation. However, Overnoff (1991) found *P. neglectus* densities were greater in conventional than in no-tillage systems.

Organic amendments to the soil may also offer some control for *P. thornei.* Soil samples taken at Hermitage Research Station after 18 months of a weed free fallow showed significantly fewer *P. thornei* with stubble retention than with stubble burning (Thompson, private comm. 1993). Esmenjaud *et al.* (1990) found that wheat monoculture with straw ploughed into the soil supported significantly fewer *Pratylenchus* than when straw was removed.

2.9.3 Biological Control

Successful biological control of *Pratylenchus* species is likely to be difficult due to their migratory behaviour. *Pratylenchus* spend much of their lives in roots and tend to be found in soil only when their host plants are stressed, senescing or diseased, or when their hosts have been ploughed out after harvest (Stirling, 1991). Since most eggs are laid in the root tissues and juveniles can hatch and develop to maturity without moving from roots, multiplication sometimes can proceed for several generations without nematodes being exposed to soil-borne antagonists (Stirling, 1991). Antagonists with some specificity towards the target nematode are required. The bacterial parasite *Pasteuria thornei* is a proven pathogen of *P. brachyurus* but has not been fully tested against other *Pratylenchus* species (Starr and Sayre, 1988). Although the bacterium has been found in Australia nothing is known of its role in nematode population dynamics and epidemiology (Stirling, 1991).

The fungus *Hirsutella rhossiliensis* which produces adhesive conidia was virulent to *P. penetrans* on potatoes (Timper and Brodie, 1993). The trapping fungi *Arthrobotrys dactyloides*, *A. oligospora*, *Monacrosporium ellipsosporum* and *M. cronapagum* trapped and killed most *P. penetrans* added to fungal cultures (Timper and Brodie, 1993). Pria *et al.* (1992) had similar findings in Brazil in aseptic Petri dish experiments with a selection of adhesive fungi (*Arthrobotrys musiformis*, *A. oligospora*, *A. oviformis*, *Monacrosporium eudermatum* and *M. gephyropagum*) and adhesive knob fungi (*M. ellipsosporum*, *M. parvicollis*, *M. drechsleri* and *Monacrosporium* sp.), where all of the fungi where found to be highly predacious to *Pratylenchus* sp.. In Australia, field observations by Thompson *et al.* (1980a) suggested that the nematode trapping fungus, *Arthrobotrys conoides* may limit populations of *P. thornei*. Other work by Gapasin (1986) in the Philippines found that the fungus *Paecilomyces lilacinus*, which is considered to act as an egg parasite (Stirling, 1991) reduced *Pratylenchus* sp. on corn.

2.9.4 Resistance and Tolerance

A plant is considered resistant when the ability of the nematode to feed, develop and reproduce is inhibited, but if reproduction of the nematode occurs the plant is said to be susceptible (Wallace, 1963). A plant which is infested by a high number of actively reproducing nematodes but shows little indication of injury is tolerant (Wallace, 1963). A tolerant plant is advantageous for growth and yield, but is generally associated with a high population of the nematode in the soil to infect the next susceptible crop. Breeding resistant crop varieties is therefore a priority area for the control of *P. thornei*. Such varieties would result in decreased nematode populations which would allow increased yield and would also lessen the chance of spreading the infestation to unaffected soil (Anon., 1990). Farmers would also have the benefits of more flexibility in choice of cropping sequences and control without increased production costs.

Van Gundy *et al.*(1974) tested 51 different varieties and selections for resistance and tolerance to *P. thornei*. All varieties and selections tested were susceptible to invasion and reproduction of *P. thornei,* but some showed tolerance to *P. thornei*. Thompson and Clewett (1986) assessed the tolerance of wheat cultivars using Aldicarb (5kg/ha) as a control, and identified genotypes with greater tolerance than any of the recommended varieties. The three barley varieties recommended for Queensland have more tolerance than the recommended wheats for this region. A range of tolerances to *P. thornei* exist among wheat cultivars which have been recommended for commercial sowings in Queensland. Gatcher is highly intolerant while Gamut, Hartog, Oxley and Cook are cultivars with better *P. thornei* tolerance.

O'Brien (1983) was unable to detect any *P. thornei* resistance of wheat lines in pots. However, there are several wheat lines with superior tolerance and partial resistance. Thompson and Clewett (1986) have identified possible resistance and tolerance from the commercial Queensland wheat varieties. The intolerant variety Gatcher was found to produce 1 plant in about every 3000 which was highly tolerant and also had some resistance (Thompson and Clewett, 1986). One selection (GS28) has outyielded all commercial wheats on nematode infested soils and although resistant to stem rust, is susceptible to leaf and stripe rust. Another wheat selection, QT2997 (Potam/Cook) had superior tolerance but was susceptible to a new strain of stem rust. Recently a stem rust resistant selection QT4118 (Pedigree Potam 70/4*Cook) from QT2997 was released as the new variety "Pelsart" which offers superior tolerance, but no resistance to *P. thornei* (P. Brennan, pers. comm.).

Another current program in Queensland is the screening of known tolerant wheats for resistance. At present there are no commercial wheat varieties available in Queensland with *P. thornei* resistance, although the line GS50A selected from the highly susceptible

Gatcher has been identified (J. Thompson, pers. comm.). Field tests enabling quantitative comparisons of genotypes at a range of initial *P. thornei* populations are being assessed to determine which cultivars combine superior tolerances with a high level of partial resistance. Thompson suggests that wheats with least tolerance in field trials are those which showed the highest *P. thornei* multiplication rates in O'Brien's studies (1983). It has also been suggested that high populations of *P. thornei* will limit their own further multiplication on susceptible wheat cultivars of low tolerance (Thompson, private comm. 1993), but would suffer a yield penalty. Results with intolerant cultivars such as Gatcher suggest that growing these varieties as a second wheat crop resulted in fewer *P. thornei* and more residual soil nitrate and water for the third wheat crop which yielded better than the second. Interestingly, wheat cultivars with resistance to Cereal Cyst Nematode, *H. avenae* differ in resistance to *P. thornei*. Growth of *H. avenae* resistant AUS10894 resulted in moderately high numbers of *P. thornei*, whereas *H. avenae* resistant Festiguay and *H. avenae* susceptible Halberd and Egret led to low numbers of *P. thornei* (Thompson, private comm. 1993). Alternation of *H. avenae* resistant and susceptible cultivars is a possible way of exploiting the inverse relationship between *P. neglectus* and Cereal Cyst Nematode, whilst controlling cyst nematode populations in intensive cereal producing systems (Lasserre *et al.*, 1994).

In South Australia, resistance and tolerance to *P. thornei* has yet to be adequately assessed. Until such information is obtained, the cultural practices of rotations and cultivation offer some means of partial control.

Chapter 3
General Laboratory Techniques

3.1 Nematode Inocula

Inocula of *P. thornei* were obtained from carrot cultures (Plate 3.1). This method of culturing was modified by Nicol (1991) after Moody *et al.* (1973). Originally, the carrots were inoculated with *P. thornei* isolated from Urrbrae loam at the Waite Agricultural Research Institute by Mrs. Heather Fraser, in 1991. Carrot cultures were inoculated using either sterile nematodes, individual pieces of already cultured carrot or with *P. thornei* reared on chickpea callus on White's medium (Nicol & Vanstone, 1993). Cultures were kept at 20°C in the dark for a period of between 2-3 months after which time more than 200,000 *P.thornei* could be extracted (Plate 3.1). Evidence of sufficient population development was provided by the cow-web like expression of nematodes on the interior walls of tubs. If cultures were left too long, the structural integrity of the carrot collapsed and the culture was unusable (Plate 3.2).

P. thornei were extracted from carrot cultures by cutting carrots into 0.5cm slices in a laminar flow cabinet and placing the slices in petri dishes with SDW covering the cut section. The nematodes migrated from the carrot medium into the surrounding water over a period of several hours. The water was then passed through a sintered glass filter (pore size 15µm) to reduce volume. Subsequent dilutions were made to obtain the desired density of *P. thornei.*

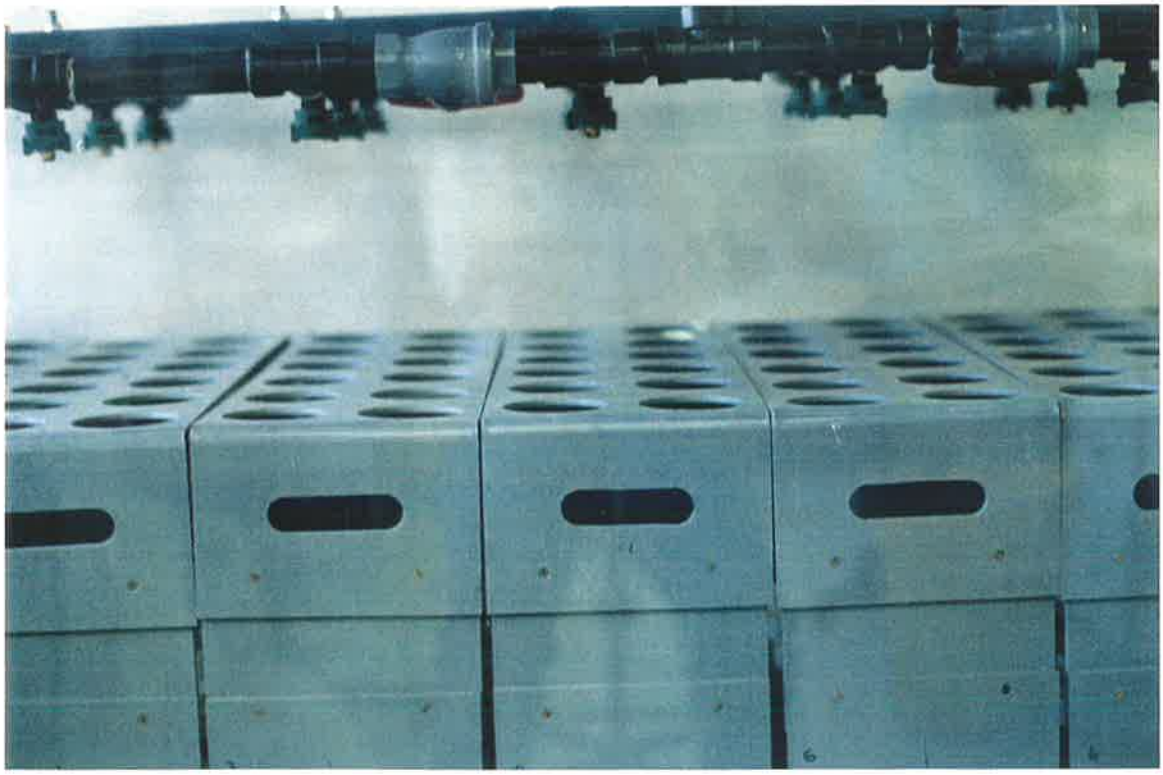
Experiments which involved the closely related species *P. neglectus* were similarly prepared from carrot culture, originally maintained by Dr. V.A. Vanstone at the Waite Agricultural Research Institute. The original source of *P. neglectus* was wheat roots collected from the South Australian cropping region, Palmer (V. Vanstone, pers. comm.).

Plate 3.1 : Representative replicate of the *P. thornei* carrot culturing technique in tubs 12 weeks after inoculation with 10 *P. thornei* per carrot piece. Note the cobweb expression of *P. thornei* on the interior of the tub walls.

Plate 3.2 : Illustration of the various stages of development of *P. thornei* using the carrot culturing technique. **Left**; reduction in the size of carrot accompanied by some evidence of cell proliferation on the exterior portions of the carrot. **Middle**; the honeycomb effect caused by the swarming of nematodes, indicating harvesting is appropriate. **Right**; rapid blackening of carrot and deterioration in structure. The nematodes previously associated with the tub walls are collected on the bottom, in a rotten liquid mass in the base of the tub.

Plate 3.3 : The mister extraction funnel system used in the root extraction of nematodes. There are four funnels displayed, each having a coarse mesh disk wrapped in Kleenex ® tissue, fitted with a funnel below. Water is projected in a fine water spray and the nematodes are collected either in a test tube (as with the two left funnels) or in the bottom of a sealed tube (as with the two right funnels).

Plate 3.4 : Section of mister chamber used for the funnel extraction technique of nematodes within roots. Each rack has twelve holes to hold funnels (see Plate 3.3). The spray jets (shown at the top of the plate) are located above the racks within the chamber and project a fine water spray every 5 minutes for 20 seconds at 30 psi over the roots.



3.2 Nematode Extraction

3.2.1 Soil

Root lesion nematodes were extracted from soil (200g composite sample) using the Whitehead tray extraction technique (Southey, 1986). Two layers of Kleenex® tissue were placed in a plastic basket (265 x 300mm) with the soil evenly distributed. The basket with soil was then placed in a Whitehead tray (270 x 310mm) with 1500ml tap water and extracted for 48 hours at room temperature. The tray with soil was gently removed and the water remaining in the Whitehead tray passed through a sintered glass filter (pore size 15µm) to a reduced volume of 20-30 ml. Nematodes in the sample were identified and then counted (Section 3.3).

3.2.2 Root

Nematodes were extracted from roots of various plants (cereal, legume or non-legumes) by misting (Southey, 1986). Roots were cut into 1.0 cm segments and spread evenly on a coarse mesh disk wrapped in Kleenex® tissue which fitted into a funnel (Plate 3.3). The funnels with disks were placed in a mister (Plate 3.4), which projected a fine water spray every 5 minutes for 20 seconds at 30 psi over the roots. The water and nematodes were collected in a test tube at the base of each funnel. After three days, the water containing the nematodes in the test tube was reduced using a sintered glass filter (pore size 15µm) under suction and the nematodes were then identified and counted (Section 3.3).

3.3 Nematode Counting

All nematodes, whether from soil or plant, were counted in a modified Doncaster (1962) counting dish (3cm diameter) with 4 concentric circles using a light microscope. A 1ml subsample from a known volume was used to estimate the numbers of *P. thornei* and *P. neglectus* where applicable. The 2 nematode species could only be distinguished in the adult stage by the position of the vulva in the female (Appendix D). *P. thornei* and *P. neglectus* in the juvenile stages were classified as *Pratylenchus* spp.

3.4 Staining Nematodes and/or Fungi

3.4.1 Nematodes Only

Nematodes inside root tissues were stained with acid fuchsin (Southey, 1986) in lactoglycerol to detect larvae, adults and eggs of *Pratylenchus*. The modified method developed by A. Taheri (unpublished) was used to clarify nematode staining against root tissue. This involved soaking about ten root segments of approximately 10cm length in 20ml of chlorine bleach (4.47% NaOCl) for 10 minutes followed by rinsing for 60 seconds in SDW. The root segments were then allowed to soak for 15 minutes in SDW, drained and added to a 3.5% solution of acid fuchsin (Southey, 1986), and boiled over a low flame for 30 seconds, and allowed to cool at room temperature. The roots were placed in 20ml glycerol with 10 drops of 5M HCl and heated to boiling. The pure glycerine and roots were then transferred to a petri dish and nematodes viewed microscopically.

3.4.2 Nematodes plus Fungi

Taheri (unpublished) developed a new technique for staining both nematodes and fungi, maintaining the resolution of both. The root segments were fixed in 4:1 FA (formalin: acetic acid) fixative over night. The root segments were rinsed several times with SDW, placed in 5% KOH and stored at room temperatures for 12-24 hours, depending on the age of the root system. The roots were then rinsed again with SDW and transferred to 0.01 % trypan blue in lactoglycerol for 1-2 hours at room temperature. They were rinsed and transferred into destaining solution (10 drops of HCl in 20 ml of glycerol) for storage before microscopic examination. The hyphae, spores and nematodes were stained blue.

3.5 Seed Sterilisation and Germination

Seeds of cereals and non-leguminous hosts were selected for uniformity of size and washed in ethanol (98%) for 5 minutes, then surface sterilised with sodium hypochlorite

(4.5%) for 10 minutes, with occasional agitation. The seeds were rinsed three times in SDW, placed in plastic petri dishes (9cm diameter) containing sterile moistened filter paper (approximately 20 seeds/ petri dish) and incubated at 20°C in the dark for 48 hours. Uniform seedlings of both cereal and non-legumes were selected on the basis of 3 roots of equal length (approximately 3cm).

3.6 Statistical Analysis

3.6.1 Statistical Designs

Depending on the aims of the experiment and the resources available various statistical designs were used (see for example Sokal and Rohlf, 1969). These designs included;

CRD (Completely Randomised Design) which can be used to compare several treatment classes or types simultaneously, where there is no indication of heterogeneity over the experimental units and the total number of experimental units (e.g. plots or tubes) is not large. The treatment classes or types are randomly allocated to the experimental units such that there is equal (or near equal) replication.

RCBD (Randomised Complete Block Design) is used where one wishes to remove the effect of heterogeneity over the total experimental units thereby increasing the precision associated with the comparison of the treatment classes or types. Each block contains as many experimental units as there are treatment classes to be compared with random allocation of the treatment classes to these units.

SPD (Split Plot Design) is used when there are two or more treatment factors and there are management or other reasons for randomly allocating one factor to sub-plots within whole plots of the second factor. Blocking may or may not be applied to the whole plots of the SPD and the concept can be extended to include more than two treatment factors if desired.

3.6.2 Analysis of Variance and Error Bars

All data were subjected to the appropriate analysis of variance (ANOVA) corresponding to the design used. This method, based on least squares, was pioneered by R. A. Fisher, and is fundamental to much of the application of statistics in biology (Sokal and

Rohlf, 1969). The variance ratio (VR observed) or F statistic is used to test the associated null hypothesis versus the alternative hypothesis (Moore and McCabe, 1989). If the null hypothesis, that all treatment classes have the same mean is rejected then it is useful to present means, in a table or simple figure, and indicate which means differ significantly from others by including the Standard Error of Difference (SED) of Tukey's Honestly Significant Difference or Range (Sokal and Rohlf, 1969). Other specific statistical tests are applied where appropriate with due reference given in the text.

3.6.3 Transformations

If there is evidence of failure of the assumptions underlying the analysis of variance the appropriate transformation is applied. Testing of assumptions is particularly achieved using plots of the residual values versus the fitted values from the ANOVA. The assumptions most frequently failing are, homogeneity of variance over the treatment classes, and normality of the residual variation (Atkinson, 1985). On the occasions when the assumptions fail either a square root ($\sqrt{x+0.5}$) or a logarithmic ($\log_e(x+1.0)$) often overcame the problem.

3.7 Classification of Soils used in Laboratory Experiments

The mechanical analysis using the hydrometer method described by Day (1965) was used to classify the three different soil compositions used in laboratory experiments involving the root lesion nematodes. The method principally involved the dispersion of soil particles over time after all aggregates were removed by chemical treatments, allowing the primary particles to be dispersed in water. In addition, the pH of the 3 soils was determined using 2 methods, 0.01M $CaCl_2$and DDW (Rayment and Higginson, 1992). The soil and pH classifications are presented in Table 3.1. Further explanation of soil texture is referred to in Table 4.1.

Table 3.1 Soil and pH classification of the soils used in Laboratory Experiments.

	% Clay	% Silt	%Sand	pH (0.01M CaCl2)	pH (DDW)
Urrbrae loam (Ul)	14	25	61	5.2	5.4
Roseworthy sand (rs)	4	1	95	7.1	8.2
Palmer sand (ps)	9	1	90	7.5	8.4

Chapter 4
Statewide Survey for *P. thornei* and *P. neglectus* in the Cereal Regions of South Australia

4.1 Introduction

Pratylenchus thornei was first recorded in South Australia by J. Fisher in 1956 (pers. comm.). Since then it has been found in the Adelaide metropolitan area (Grandison, 1972; Singh, 1984) and in paddocks sampled across the cereal growing regions (S. Taylor and V. Vanstone, pers. comm.). Commonly, *P. thornei* has been associated with the closely related species *P. neglectus* (S. Taylor and V. Vanstone, pers. comm.). However, the distribution of *P. thornei* and *P. neglectus* across the cereal regions of South Australia has not been clearly defined.

In South Australia, *P. thornei* can cause significant yield reductions of wheat both in the field (Taylor and McKay, 1993) and in aseptic laboratory studies (Nicol, 1991). Evidence from this work and the literature suggest *P. thornei* is usually found in clay based soils, both in Australia (Grandison and Wallace, 1974; Doyle et al., 1987) and overseas (Thorne, 1961; Loof, 1960; Corbett, 1970; Van Gundy *et al.*, 1974 and Fortuner, 1977).

In order to determine the damage potential of *P. thornei* on wheat in South Australia, a study of the distribution of the two species, *P. thornei* and *P. neglectus* is essential. A statewide survey of the cereal growing regions was carried out involving the assessment of over three hundred soil samples accompanied with sampling over one hundred different types of commonly cultivated plants comprising different species and cultivars. The plants were taken from the same paddock as the respective soil samples. Attempts were made to correlate the species of plant nematode with soil type.

4.2 Materials and Methods

For the purposes of the survey, the cereal growing regions in South Australia were subdivided into nine geographical regions (Fig. 4.1). Within each subdivision both soil and root samples were taken from a wide cross section of the region concerned. The survey was carried out over a period of three consecutive years. Emphasis was placed on sampling clay-based soils due to the suggested association of clay soils and *P. thornei* (Fig. 4.1).

The main objective of the survey was to determine the presence or absence of the nematode species, rather than precisely quantify the nematode population densities. The large area of the survey limited the extent of indivudal samples taken from each paddock. As a consequence the information gained should be used in a qualitative rather than a quantitative sense.

A range of farms across the state were selected by contacting district agronomists, Agricultural Bureaus and Land Care Groups. The individual farmers and paddocks were selected to give a cross section of the geographical area and crop rotational sequence.

From each individual paddock, soil and sometimes plant roots were collected. Approximately 300g of soil was collected to a depth of 20 cm across five sections of the paddock approximately 10m apart. The five soil samples collected from each paddock were combined and mixed to give a homogenous 1.5 kg sample. Where plant samples were taken, two or three plants were uprooted in each of the 10m transects where soil was obtained. Plant samples were analysed immediately while soil samples were stored at 4°C and processed within a fortnight.

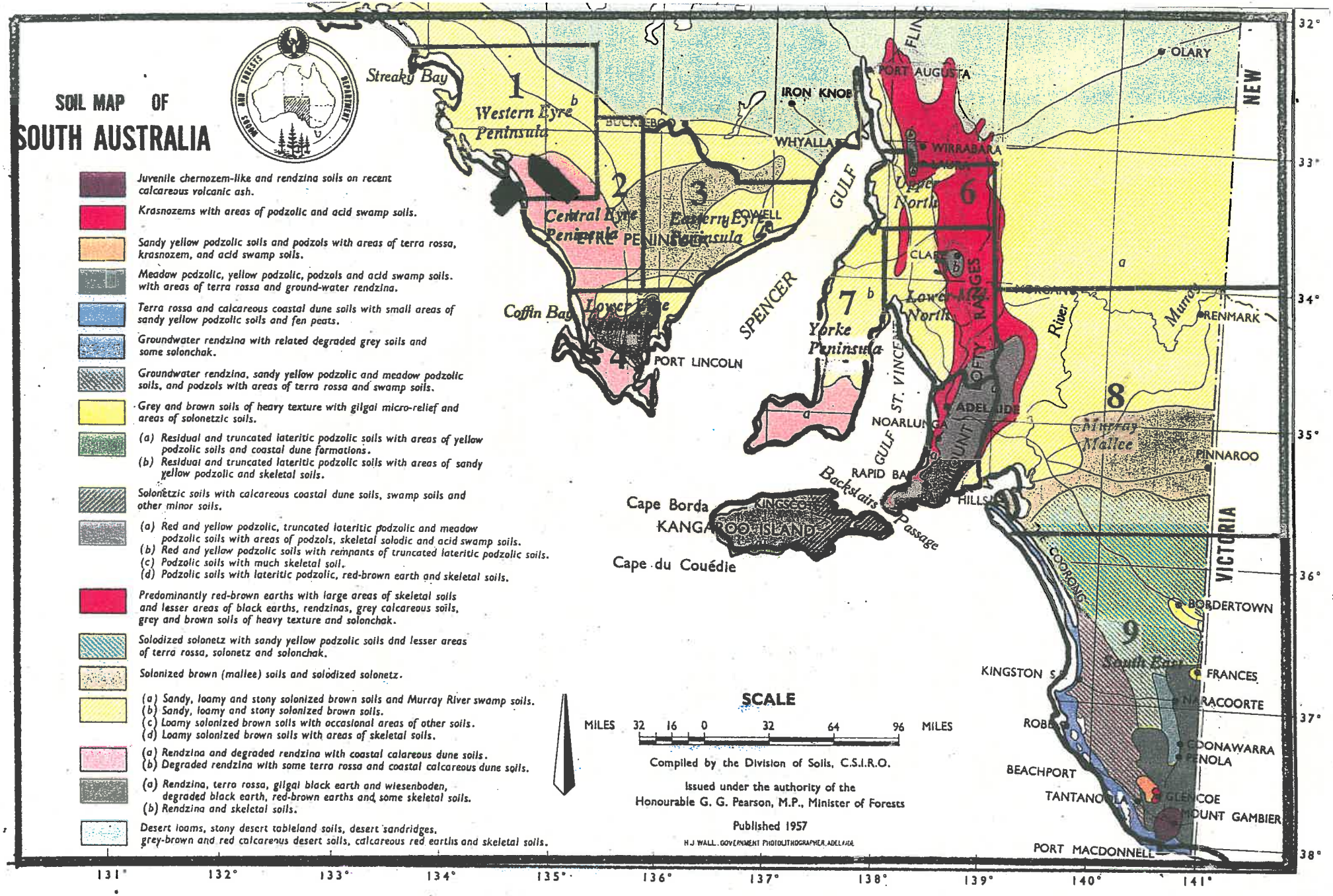


Fig. 4.1 Soil Classification of South Australia overlayed with cereal region sub-division for *Pratylenchus* soil surv

SOIL MAP OF SOUTH AUSTRALIA

- Juvenile chernozem-like and rendzina soils on recent calcareous volcanic ash.
- Krasnozems with areas of podzolic and acid swamp soils.
- Sandy yellow podzolic soils and podzols with areas of terra rossa, krasnozem, and acid swamp soils.
- Meadow podzolic, yellow podzolic, podzols and acid swamp soils. with areas of terra rossa and ground-water rendzina.
- Terra rossa and calcareous coastal dune soils with small areas of sandy yellow podzolic soils and fen peats.
- Groundwater rendzina with related degraded grey soils and some solonchak.
- Groundwater rendzina, sandy yellow podzolic and meadow podzolic soils, and podzols with areas of terra rossa and swamp soils.
- Grey and brown soils of heavy texture with gilgai micro-relief and areas of solonetzic soils.
- (a) Residual and truncated lateritic podzolic soils with areas of yellow podzolic soils and coastal dune formations.
  (b) Residual and truncated lateritic podzolic soils with areas of sandy yellow podzolic and skeletal soils.
- Solonetzic soils with calcareous coastal dune soils, swamp soils and other minor soils.
- (a) Red and yellow podzolic, truncated lateritic podzolic and meadow podzolic soils with areas of podzols, skeletal solodic and acid swamp soils.
  (b) Red and yellow podzolic soils with remnants of truncated lateritic podzolic soils.
  (c) Podzolic soils with much skeletal soil.
  (d) Podzolic soils with lateritic podzolic, red-brown earth and skeletal soils.
- Predominantly red-brown earths with large areas of skeletal soils and lesser areas of black earths, rendzinas, grey calcareous soils, grey and brown soils of heavy texture and solonchak.
- Solodized solonetz with sandy yellow podzolic soils and lesser areas of terra rossa, solonetz and solonchak.
- Solonized brown (mallee) soils and solodized solonetz.
- (a) Sandy, loamy and stony solonized brown soils and Murray River swamp soils.
  (b) Sandy, loamy and stony solonized brown soils.
  (c) Loamy solonized brown soils with occasional areas of other soils.
  (d) Loamy solonized brown soils with areas of skeletal soils.
- (a) Rendzina and degraded rendzina with coastal calcareous dune soils.
  (b) Degraded rendzina with some terra rossa and coastal calcareous dune soils.
- (a) Rendzina, terra rossa, gilgai black earth and wiesenboden, degraded black earth, red-brown earths and some skeletal soils.
  (b) Rendzina and skeletal soils.
- Desert loams, stony desert tableland soils, desert sandridges, grey-brown and red calcareous desert soils, calcareous red earths and skeletal soils.

Streaky Bay · BUCKLEBOO · IRON KNOB · WHYALLA · PORT AUGUSTA · FLINDERS RANGES · OLARY · NEW SOUTH WALES · WIRRABARA · LAURA · COWELL · EYRE PENINSULA · GULF SPENCER · Coffin Bay · PORT LINCOLN · CLARE · MORGAN · River Murray · RENMARK · GULF ST. VINCENT · MOUNT LOFTY RANGES · ADELAIDE · NOARLUNGA · RAPID BAY · HILLS · PINNAROO · Backstairs Passage · Cape Borda · KINGSCOTE · KANGAROO ISLAND · Cape du Couédie · THE COORONG · VICTORIA · BORDERTOWN · KINGSTON S.E. · FRANCES · NARACOORTE · ROBE · COONAWARRA · PENOLA · BEACHPORT · TANANOOL · GLENCOE · MOUNT GAMBIER · PORT MACDONNELL

32° 33° 34° 35° 36° 37° 38°

131° 132° 133° 134° 135° 136° 137° 138° 139° 140° 141°

SCALE

MILES 32 16 0 32 64 96 MILES

Compiled by the Division of Soils, C.S.I.R.O.

Issued under the authority of the Honourable G. G. Pearson, M.P., Minister of Forests

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N.J. WALL, GOVERNMENT PHOTOLITHOGRAPHER, ADELAIDE

The soil type was classified as sand, loam or clay on the basis of texture in the field by the 'feel method' (Brady, 1984) and the soil terminology used is explained in Table 4.1. This involved adding water to the field soil and once wet the way it 'slicked out' when pressed between the thumb and fingers indicated the amount of clay present. The slicker the wet soil the higher the clay content. Sand particles are gritty, while silt particles have a floury or talcum powder feel when dry, and are only slightly plastic or sticky when wet.

Table 4.1 : General Terms used in the classification of soil based on texture.
(sourced from Brady (1984) and Foth (1978), International Soil Science Society Standards)

Common Name	Texture	Basic Soil Texture	Diameter (mm)	No. particles/g
Sandy Soil	coarse	sands	0.2 - 2.00	720
		loamy sands		
Loamy Soil	mod.-coarse	sandy loam		
		fine sandy loam	0.02 - 0.2	46,000
	mod.	v.fine sandy loam		
		loam		
		silt loam		
	mod.-fine	sandy clay loam		
		silty clay loam		
		clay loam		
Clay Soil	fine	sandy clay		
		silty clay	0.002 - 0.02	5,776000
		clay	< 0.0002	90,260,853000

Plant samples were collected to obtain a diversity of varieties and/or cultivars of cereal, grain and pasture legumes, non-leguminous and a wide array of weed species. This gave preliminary information about the ability of plants to be hosts for *Pratylenchus* spp. The rotational sequence in each paddock was recorded.

The methodology for nematode extractions for both the soil and plant root samples are described in Section 3.2.1.

4.3 Results

The raw data from the statewide survey are presented in Appendix A. Extensive detail of the individual farmers, specific cultivars/varieties and crude estimates of nematode numbers are given for future reference. Table 4.2 summarises the distribution of *P. thornei* and *P. neglectus* by region while Table 4.3 relates nematode distribution to soil type.

Due to the lack of adult specimens, not all samples could be positively identified as *P. thornei* or *P. neglectus*. As a result only a certain percentage of soil samples in each region or soil type could be positively identified (column 4, Table 4.2 and Table 4.3). The number of samples positively identified was used to calculate the next four columns in both Table 4.2 and Table 4.3. The last column of data in both Tables was determined irrespective of positive nematode species identification, hence it was calculated on the total number of samples.

Table 4.2 : Distribution of *P. thornei* and *P. neglectus* in relation to cereal growing regions in Southern Australia.

	Region	Year of collection	Total no. samples	% tvely identified as *P. thornei* and and/or *P. neglectus*	% null *P.thornei* or *P.neglectus*	% *P.thornei* only	% *P.neglectus* only	% both *P.thornei* & *P.neglectus*	% with one or both *P.thornei* & *P.neglectus*
1	Western Eyre Peninsula	1993	54	100	0	0	80	20	**100**
2	Central Eyre Peninsula	1993	12	100	0	0	100	0	**100**
3	Eastern Eyre Peninsula	1993	42	100	0	0	100	0	**100**
4	Lower Eyre Peninsula	1993	36	100	11	0	75	14	**89**
5	Lower-Mid. North	1992	107	52	21	45	34	0	**89**
6	Upper North	1992	22	41	67	22	11	0	**73**
7	Yorke Peninsula	1994	30	97	3	0	10	87	**97**
8	Murray Mallee	1994	8	75	0	0	67	33	**100**
9	South East	1994	22	86	21	26	16	37	**82**

Table 4.3 : Distribution of *P. thornei* and *P. neglectus* in relation to soil type in the cereal areas of Southern Australia.

Soil Classification	Year of collection	Total no. samples	% tvely identified as *P. thornei* and and/or *P. neglectus*	% null *P.thornei* or *P.neglectus*	% *P.thornei* only	% *P.neglectus* only	% both *P.thornei* & *P.neglectus*	% with one or both *P.thornei* & *P.neglectus*
Clay (smallest pore size)	1992 1993 1994	97	66	11	37	34	18	**87**
Loam (moderate pore size)	1992 1993 1994	184	89	12	12	61	16	**88**
Sand (largest pore size)	1992 1993 1994	52	92	11	6	63	20	**90**

In 1992, over one third of the total samples were collected from the Lower, Mid and Upper North of the State because of the preponderance of clay based soils in this region. Nematodes in only half of these samples were positively identified due to the lack of adequate adult specimens, possibly due to the prolonged wet season. Of the samples which could be identified nematodes were either solely *P. thornei* or *P. neglectus*. In the Lower-Mid North 45% of the samples were *P. thornei* only and 34% were *P. neglectus*, while in the Upper-Mid North there were more *P. thornei* than *P. neglectus* that were positively identified, and once again there were no mixes of species within samples.

On Eyre Peninsula, 100% of samples were positively identified. All samples from Central and Eastern Eyre contained *P. neglectus* only. Both Western and Lower Eyre had a small population of mixes of both species while the majority of samples were solely *P. neglectus*. The samples taken from Regions 7, 8 and 9 (Yorke Peninsula, Murray Mallee and the South-East of South Australia) taken in 1994 could be identified to species level. On the Yorke Peninsula 97% of samples contained *Pratylenchus* spp. In the majority of samples (87%), both species were present, but in the remaining 10% only *P. neglectus* was identified. 67% of the soil samples from the Murray Mallee

contained *P. neglectus* alone, while the remainder (33%) were mixes of both species. In comparison, 37% of samples from the South-East (which extended across the Victorian border) had mixed populations, 26% had *P. thornei* only and 16% had *P. neglectus* only.

From some of the 325 soil sample sites, root samples were also collected. There were over one hundred of these root samples, taken from a wide range of plant crops including cereals, legumes (pasture/grain), non-legumes as well as common weed species (Appendix A). The crops sampled and the nematode species and numbers corresponding to each crop are presented. The qualitative nature of the information presented in Appendix A, does not, unfortunately, allow ranking of the varieties/cultivars from the field due to the many variables cited. However, the results suggest that the majority of plants used in rotation with wheat, and common weeds, need to be considered potential hosts for both *P. thornei* and *P. neglectus*.

4.4 Discussion

Pattison's work (1993) in Northern NSW on *P. thornei* in wheat concluded that the distribution of the nematode was more random in wheat fields at sowing than other nematode species in other crops. This suggested that the sampling technique used can be less intensive than those recommended for nematode populations which are aggregated. However, although nematode extractions and counts were obtained from a composite sample of at least five transects within each paddock, the numbers resulting only provide an indication of their presence or absence. To use them to quantify density could be misleading given the differing times of collection, varying management practices and the differing crop maturity in different samples.

As indicated in Appendix A, both *P. neglectus* and *P. thornei* were present in a range of different crop plants, including cereal, grain and pasture legumes as well as weed species. This supports the polyphagous feeding habits of both *P. thornei* (Table 2.1) and *P. neglectus* (Anderson and Townshend, 1976). In order to confirm this hosting ability of the range of crop plants assessed an appropriate resistance assay needs to be developed.

The results presented in Table 4.2 regarding the distribution of *Pratylenchus sp.* in South Australia indicate that approximately 90% of soils contain either *P. thornei* and/or *P. neglectus*. The Eyre Peninsula (encompassing Western, Central, Eastern and Lower sub-divisions) has a 97% chance of *Pratylenchus* sp. being present, with the majority of samples containing *P. neglectus*. There were isolated pockets of mixed populations on Western and Lower Eyre Peninsula. Unexpectedly around Minnipa and Streaky Bay mixed populations of *P. thornei* and *P. neglectus* were found where the soils were very sandy and calcareous. This does not support literature associating clay soil type with *P. thornei.*

As shown by the results, the species distribution between regions is variable. It appears that both species are present in all regions except the Eyre Peninsula, where only *P. neglectus* occurs. The distinction between proportions of species (*P. thornei* and/or *P. neglectus*) in a given region or soil type may be a reflection of the sampling year and/or previous rotation and/or management practices. However, the higher proportion of *P. thornei* in the Lower to Mid-North and the South-East may reflect the higher clay content of the soils sampled (Table 4.3, Fig. 4.1). It is also possible that other soil characteristics, such as soil pH may play an important role in species distribution.

The relation of soil type to species is summarised in Table 4.3. Irrespective of soil type, there was a 90% probability of finding *Pratylenchus* spp. The work of Wallace (1968) suggested that optimum conditions for movement of both *P. thornei* and *P. neglectus* would occur in sandy soils. The distribution of *P. neglectus* appears to be associated with optimal nematode movement, as the higher proportion of *P. neglectus* populations were found in sand. However, *P. thornei* was found more commonly in clay based soil (Table 4.3) with most of these soils being found in Lower to Mid-North and the South-East (Fig. 4.1, Appendix A). This suggests that factors other than movement determine the distribution of this species. About 20% of all samples had mixes of both species and were found across all types of soils, suggesting that soil type alone does not determine *Pratylenchus* spp. distribution. It is probable that if more samples had been taken the proportion of mixed populations would have been greater. O'Brien and Stirling (1991) noted that as the number of soil samples increased, the likelihood of finding nematodes which are present in low numbers also increased.

Therefore, *P. thornei* and *P. neglectus* is widespread in cereal growing regions. They show polyphagy on cultivated wheat , common weeds and crops grown in many rotational combinations in the cropping regions of South Australia. Because of this, the damage potential of *P. thornei* is high, but this is dependent on initial nematode density. In order to assess the hosting status of the these crops commonly used in cereal production, a resistance screening procedure needs to be developed.

Chapter 5

Multiplication of *P. thornei* and Development of a Resistance Assay for Cereal and Non-Leguminous Hosts

5.0 General Introduction

Species of *Pratylenchus* have a wide geographical distribution, and a great diversity of plants act as hosts to different species within the genus. As discussed in Chapter 2, the life cycle is dependent primarily on host and temperature. Because *Pratylenchus* are migratory endoparasites, they are attracted to roots and invade in a non-random manner, subsequently feeding on and reproducing in the cortex (Baxter and Blake, 1967). The relationship of *Pratylenchus* with its host is less complex than that of other plant parasitic nematodes such as *Meloidogyne* or *Heterodera* as no specialised feeding nurse cells develop inside root tissues (Townshend, 1990).

Little is known about the susceptibility or resistance (Section 2.9.4) of various wheat cultivars/varieties and other rotational crops to *P. thornei.* To examine these aspects it is necessary to determine the multiplication of the nematode on the various crop plants and to assess the effect of container size, soil type and time allowed for multiplication on the numbers of nematodes that result. It is beneficial to compare the multiplication of the two closely related species, *P. thornei* and *P. neglectus* , as these commonly occur as mixed populations in South Australia (Ch. 4). From such information it may be possible to design a resistance assay that will produce results in minimal time and with optimal use of resources. Such information produced in the laboratory will help in understanding multiplication and damage in the field.

In South Australia, the majority of wheat is grown in rotation with other cereal and leguminous species including grain and pasture legumes. Cereals grown in rotation with wheat include barley, triticale, rye, durum and oats. The diversification of the

cereal industry has seen alternative crops also being used in rotations, for example canola. This crop, however, is currently confined to the higher rainfall areas. The relative susceptibilities of the different rotational crops to *Pratylenchus* spp. will be a major determinant of the initial density of the nematodes in the succeeding crop.

5.1 Comparative Multiplication of *P. thornei* over time

5.1.1 Introduction

The approaches used in screening for resistance are determined by the cultivars and facilities available. In the laboratory a number of techniques have been used to screen for resistance to *Pratylenchus* spp., including paper towelling, silica sand or agar media in test tubes, petri dishes or jars (Townshend, 1990). In addition screening for resistance to *Pratylenchus* spp. may be undertaken in a greenhouse using multi-celled containers such as Styrofoam trays or plastic trays of plastic pipe containing infested soil and plants of interest. The multiplication rate of the nematodes may be assessed on the basis of the capacity of the nematode to penetrate roots, cause lesions and reproduce. It is imperative that a non-damaging initial inoculum is used in order not to affect the subsequent multiplication rate.

The multiplication of *P. thornei* on a range of cereals and non-leguminous hosts was assessed in two different experiments; one in large pots consisting of 6 replicates grown for a whole season, and the other in small tubes consisting of 10 replicates grown only for 2 months.

5.1.2 Materials and Methods

A range of wheat cultivars was selected from Queensland Wheat Research Institute, Sydney University and South Australia. Other species of cereal and non-leguminous hosts were selected from South Australia (Table 5.1). In addition, two non-leguminous

hosts, linseed and canola, were also assessed because of the diversification of the cereal industry into alternative crops, albeit on a relatively small scale and in confined geographical areas.

Urrbrae loam, collected from fields around the Waite Agricultural Research Institute (Section 3.7), was autoclaved at 180°C for 24 hours, and allowed to cool. It was then mixed to a homogeneous state and was added to a) 180 pots, 25 cm in diameter and 23 cm deep, with 4.5 cm of wood chips in the base to enable water filtration; and b) 300 tubes made from electrical conduit, 2.7 cm in diameter and 12.5 cm deep, filled with soil to approximately 7/8 of the height of the tube. Two hundred seeds of each variety/cultivar (Table 5.1) were sterilised, germinated and selected (Section 3.5). Twenty four of the selected seedlings of each variety/cultivar were chosen and four plants were planted in each of the pots. Within each pot, they were planted equidistant from each other. This gave six pots of each variety /cultivar. Selected seedlings were planted into the tubes (one seedling per tube), and approximately 2 cm layer of soil was placed over each seedling.

Table 5.1 : Varieties/cultivars tested in the comparative multiplication over time.
(**SA**; South Australian, **SUN;** Sydney University; **QLD**; Queensland University)

SPECIES	COMMON NAME	VARIETIES/CULTIVARS
Triticum aestivum	Wheat	**SA**; Halberd Festiguay, Spear, Machete, Warigal, Molineux, RAC589 **SUN** ; Sun 277, Sun 289A, Suneca **QLD** ; QT 4118, Banks, Hartog, GS50A, QT5648, Gatcher
Triticum durum	Durum wheat	Kamilaroi, Yallaroi
Secale cereale	Rye	SA Rye
Triticum secale	Triticale	Currency
Avena sp.	Oats	Marloo, Echidna, Quaker 84-187, Potoroo
Hordeum sp.	Barley	Grimmett, Skiff, Galleon
Brassica sp.	Rapeseed(Canola)	Golden Rape
Linum usitatissimum	Linseed	Glenelg

P. thornei were extracted from carrot cultures (Section 3.1). One week after planting, 1 ml of aqueous solution containing 500 *P. thornei* was evenly distributed over the soil root interface of the four seedlings. One month later the plants were re-inoculated with 1 ml of a 700 *P. thornei* /pot inoculum. Thus total inoculum was 1200 *P. thornei* per pot. Similarly, tube seedlings were inoculated with 400 *P. thornei,* in 1 ml aliquots one week after sowing. These inocula were considered a non-damaging initial density (Nicol, 1991).

Pots were arranged as a randomised complete block design (RCBD). Each pot of the 30 varieties/cultivars was considered as a single replicate, and all pots of each variety/cultivar were randomised within each of 6 blocks. Plants were grown under natural climatic conditions of the normal growing season (Plate 5.1), outside in a wire-grid bird cage. Plants were watered with tap water whenever necessary.

The tubes were also arranged as a RCBD. The 30 tubes of each variety/cultivar were randomised within each of 10 blocks. The tubes were individually embedded in a tray of soil, containing a wire grid to support the tubes (Plate 5.2). Plants were watered with tap water whenever required, so that water was not a limiting factor. Plants were placed in a controlled temperature growth room at 20°C, with a 10 hour day/14 hour night, and a light intensity of 65µ Einsteins from fluorescent light tubes.

Plants in pots were harvested after 5 months (equivalent to the end of the season in the field). A composite soil sample was obtained from eleven cores covering a uniform cross section of each pot. Each core was 20 cm deep and 1.5 cm wide, and included both roots and soil. Nematodes were extracted by the Whitehead tray method (Section 3.2.1). The number of nematodes extracted from each sub-sample was converted to *P.*

thornei per gram soil and extrapolated on a per plant basis for the 4 plants within each pot.

Plants in tubes were harvested after two months. Plants were washed out of the soil and carefully rinsed to avoid root loss. Nematodes were extracted over a period of 3 days using mister extraction (Section 3.2.1) and counted (Section 3.3). In addition, the root systems were visually assessed for the degree of root lesioning on a scale of 0 (no lesioning), 1 (low lesioning), 2 (moderate lesioning) and 3 (high lesioning).

5.1.3 Results

All data from both experiments were analysed separately as a RCBD. The analysis on the original data showed heterogeneity of variance which was removed using a logarithmic transformation ($\log_e(x+1)$). In both pots and tubes, there was a significant varietal effect, before and after logarithmic transformation (Table 5.2).

Table 5.2 : ANOVA from the pot and tube tests to compare the numbers of *P. thornei* on cereal and non-leguminous hosts.

	POT(log transformed)				TUBE (log transformed)			
	d.f.	m.s.	v.r.	Prob.	d.f.	m.s.	v.r.	Prob.
block	5	30.38			9	5.53		
block.plot								
variety	29	13.37	2.39	<0.001	29	22.91	22.17	<0.001
residual	145	5.60			261	1.034		
Total	179				299			

In both cases the variance ratio (v.r.) indicates there was very strong evidence to reject the null hypothesis that all varieties had equal numbers of *P. thornei*. Thus we concluded there is a significant difference between the varieties. The varietal effect is illustrated in Figures 5.1 and 5.2. The Tukey Honestly Significant Difference Value, or Tukey's Range ($\alpha = 0.05$) (Section 3.6) indicates the magnitude of the difference required between varieties before they can be considered statistically different.

The statistical analysis was extended with the help of orthogonal comparisons which enabled comparisons both between and within species for the pot and tube experiments (Table 5.3).

Table 5.3 : ANOVA using orthogonal comparisons to compare selected varietal combinations in both pot and tube experiments

	POT(log transformed) Prob.	TUBE(log transformed) Prob.
wheat vs. oats	<0.001	<0.001
wheat vs. barley	0.176	<0.001
wheat vs. linseed & canola	<0.001	<0.001
QLD, NSW wheat vs. SA wheat	0.280	0.319
bread wheat vs. durum wheat	0.003	<0.001

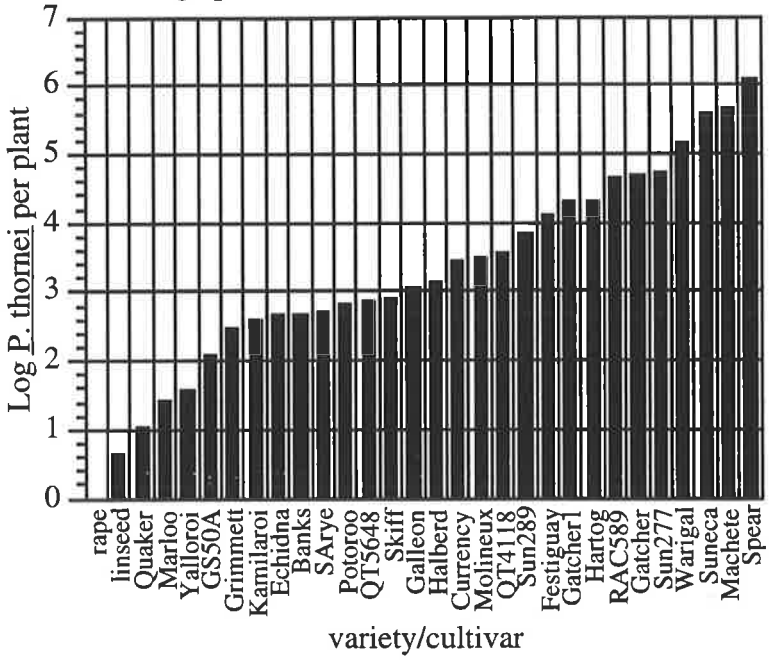
Figures 5.1 and 5.2 indicate similarities for the two experiments. Wheat varieties have a significantly greater number of *P. thornei* than oats, barley (tube only), linseed and canola. This is further supported by data in Table 5.3. Bread wheats have significantly more *P. thornei* than durum wheat, and there is no significant difference in the number of *P. thornei* when the origin of the wheat is considered.

The original ranking of root lesioning was also compared using the Friedman non-parametric test (Colquhoun, 1971) which revealed significant differences in the degree of root lesioning on the root systems of the varieties tested. Fig. 5.3 illustrates the degree of root lesioning with wheat showing much more damage than barley, triticale, rye, durum and oats, which showed little evidence of lesioning (Plates 5.3, 5.4, 5.5). The two non-leguminous hosts, linseed and canola, had negligible lesioning (Plate 5.6).

Finally, a comparison of the ranking of the varieties based on the number of *P. thornei* in pots and tubes was made using the non-parametric Spearman's Rank Correlation Test (Sokal and Rohlf, 1969). This indicated a significant t value of 5.680 ($P<0.0001$) which suggests that similar results can be obtained in a period of 2 months using small tubes as compared to 5 months using large pots. Given the reduced time needed to obtain

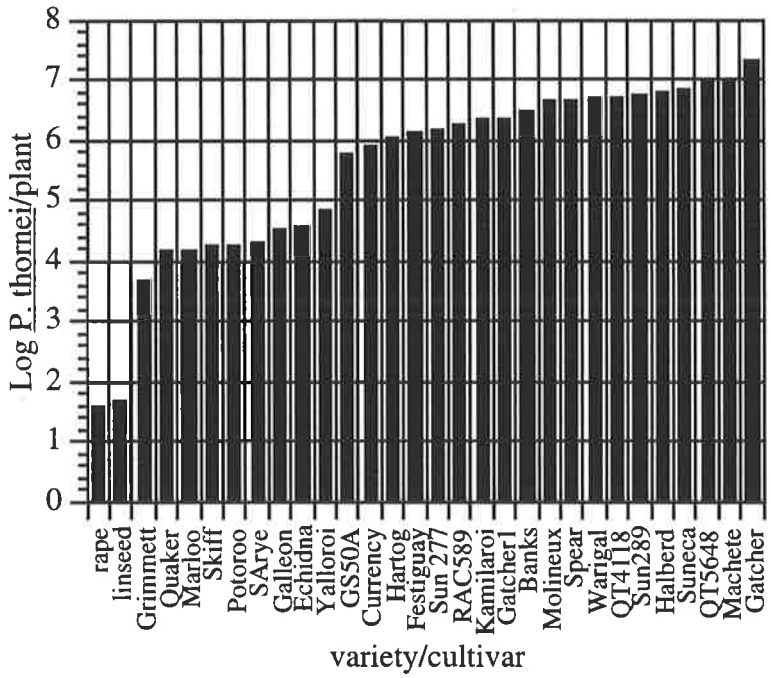
results the use of tubes can provide notable advantages when assessing the resistance of varieties.

Fig. 5.1 : Multiplication of *P. thornei* on cereal and non-leguminous hosts after 5 months in large pots in the glasshouse.



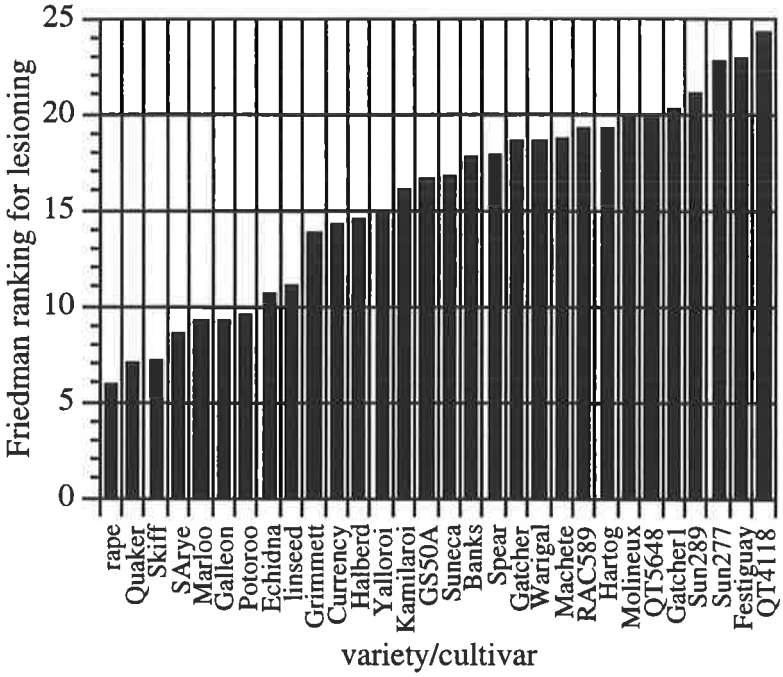
Tukey = 5.11
(i.e., means must differ by this magnitude on a log scale to be different.)

Fig. 5.2 : Multiplication of *P. thornei* on cereal and non-leguminous hosts after 2 months in small tubes at 20°C in a controlled growth room.



Tukey = 1.7
(i.e., means must differ by this magnitude on a log scale to be different.)

Fig 5.3 : Friedman's ranking of root lesioning for cereal and non-leguminous hosts grown in small tubes for 2 months at 20°C in a controlled growth room.



5.1.4 Discussion

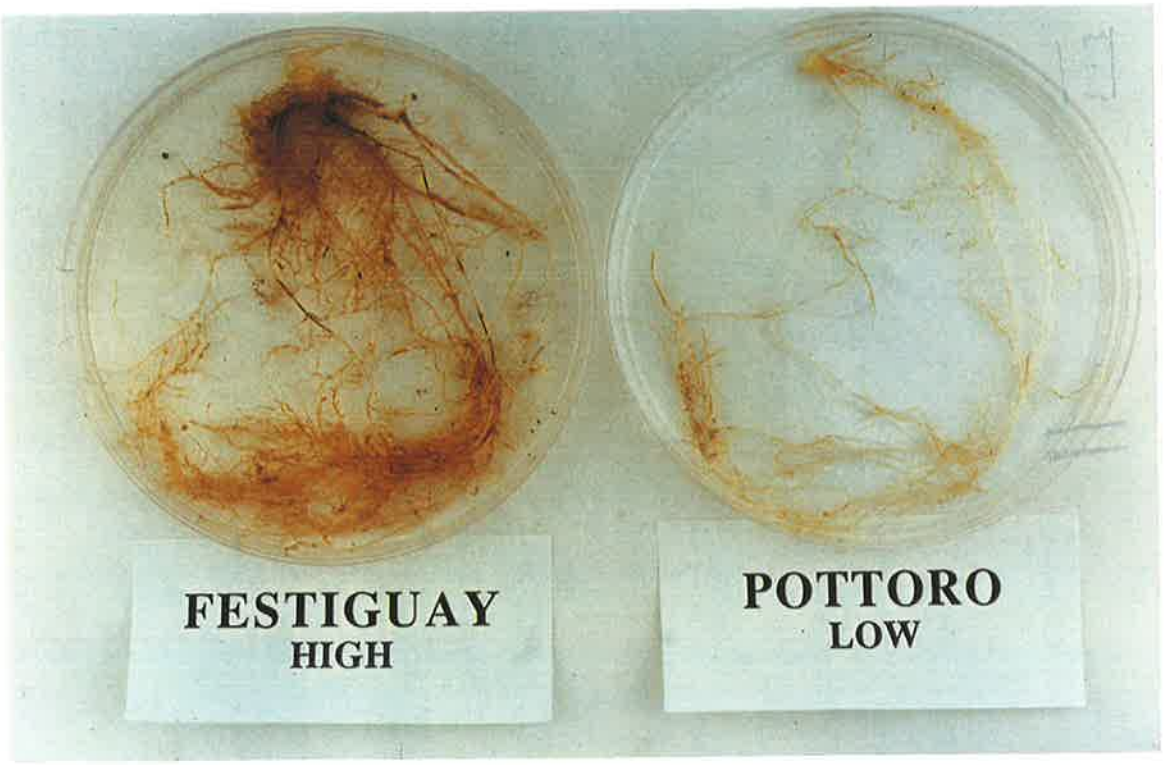
These results provide strong evidence that *P. thornei* multiplication rates differ notably on the varieties/cultivars tested. The wheat cultivars tested were moderately to highly susceptible; the triticale, rye, barley, oats and durum were moderately susceptible; while the two non-leguminous hosts linseed and canola were least susceptible i.e. they appear to have some degree of resistance. Lawn and Sayre (1992), working in highland Mexico, found significantly greater reproduction of *P. thornei* on bread wheat than on either durum or triticale. Thompson (private comm. 1993) also found that barley was associated with low nematode multiplication; in contrast, triticale encouraged *P. thornei* multiplication. Further supporting work by Thompson (private comm. 1993) on the Darling Downs suggested that wheat was associated with many residual nematodes while linseed was associated with very low numbers. Similarly, field work by

Plate 5.1 Experimental pot set-up used to assess the multiplication of *P. thornei* over a range of cereal and non leguminous hosts. Plants were grown for 5 months in a RCBD under natural climatic conditions in a glass topped enclosure. Plants were watered whenever necessary with tap water.

Plate 5.2 Experimental tube set-up used to assess the multiplication of *P. thornei* over a range of cereal and non-leguminous hosts. Plants were grown in a controlled growth room for 2 months in small polyethylene tubes embedded in a tray of soil, with a wire grid to support tubes. Light was supplied with fluorescent light tubes (65µ Einsteins) and plants watered with tap water whenever necessary.

Plate 5.3 Representative root systems of the wheat cultivar Festiguay (left) and the oat cultivar Potoroo (right) grown for 2 months in tubes at 20°C after inoculation with 400 *P. thornei* per plant. The wheat shows much greater evidence of brown cortical lesioning (high), particularly on seminal roots. In contrast the oat shows little evidence of nematode symptoms (low).

Plate 5.4 Representative root system of the wheat cultivar Machete (left) and the barley cultivar Galleon (right) grown for 2 months in tubes at 20°C after inoculation with 400 *P. thornei* per plant. The wheat shows much greater evidence of brown cortical lesioning (high), particularly on seminal roots. In contrast the barley shows little evidence of nematode symptoms (low).


FESTIGUAY
HIGH
POTTORO
LOW

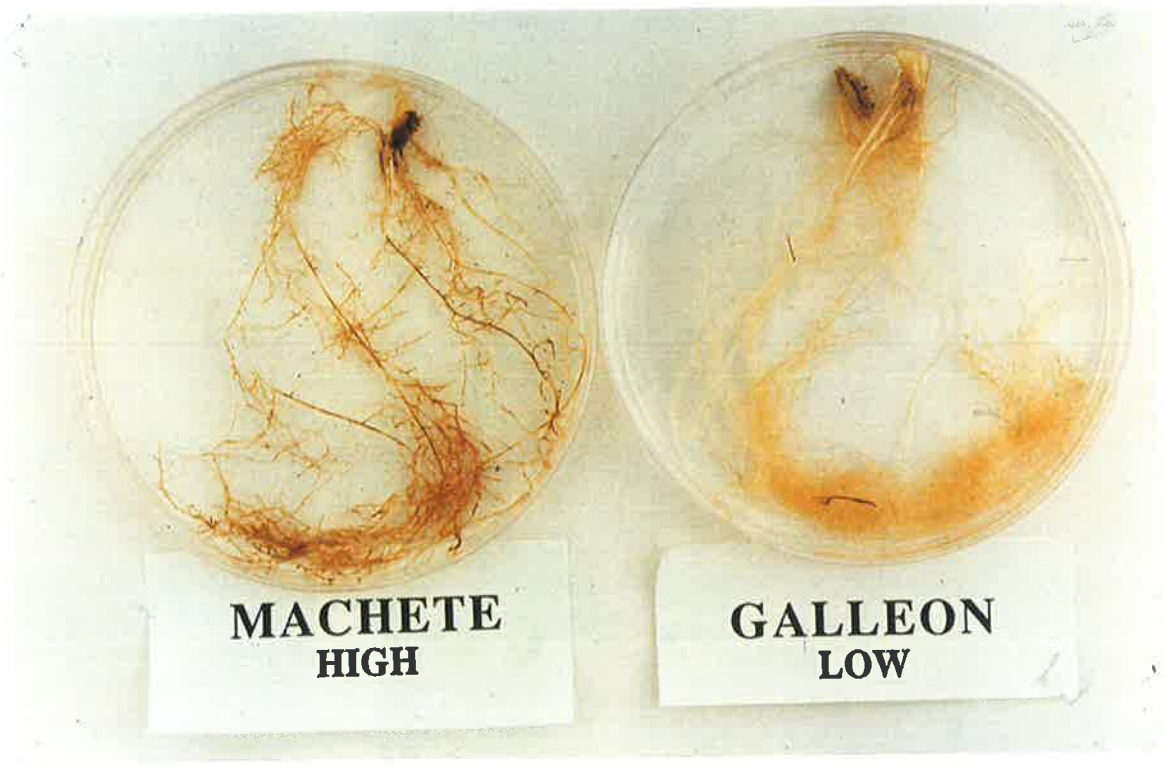

MACHETE
HIGH
GALLEON
LOW

Plate 5.5 A representative root system of the triticale cultivar Currency grown for 2 months in tubes at 20°C after inoculation with 400 *P. thornei* per plant. There is some evidence of cortical darkening on seminals, however in comparison with the wheats (Plates 5.3 and 5.4) root lesion index is low.

Plate 5.6 Representative root system of the non leguminous host Glenelg linseed grown for 2 months in tubes at 20°C after inoculation with 400 *P. thornei* per plant. There is little (low) evidence of lesioning by the nematode.


CURRENCY
LOW


LINSEED
LOW

Vanstone (1993) on the closely related species *P. neglectus* in South Australia, found that rye, triticale, barley and durum were more resistant to *P. neglectus* than the majority of wheat cultivars.

The evidence suggesting that canola (rapeseed) is partially resistant to *P. thornei* may be associated with nematode suppression by glucosinolates which are sulphur containing compounds produced in all parts of the plant (Sang *et al.*, 1984). The breakdown of glucosinolates forms various iso-thiocynates which are known to have fungal and bactericidal properties. Methyl iso-thiocyanate is a breakdown product of methyl sodium which is an effective nematicide (Lear, 1956). The work of Mojtahedi *et al.* (1991) found that amending *Meloidogyne chitwoodi* infested soil with chopped up Jupiter canola reduced the population significantly more than did amendment with wheat roots.

The number of nematodes extracted from the tubes was greater than from pots (Figs 5.1 and 5.2). Possible explanations for this are associated with the different experimental techniques used. Although plants in the pots were grown for three months longer than those in tubes, the extraction efficiency of nematodes from roots and soil in the pots may not have been as high as that used for tubes, in which nematodes were extracted from the roots alone. In addition, given the relative size distinction between pots and tubes, penetration in a larger volume of soil medium could be less effective. Multiplication is ultimately determined by the numbers of nematodes which actually penetrate the root.

The fact that statistically comparable results were derived from the two experiments suggests the assay to measure multiplication rate of *P. thornei* can be reduced effectively to two months. In addition to this, the ability to distinguish varietal

differences in the tube experiment is three times greater than with the pot experiments (Figs. 5.1 and 5.2) this once again may be attributed to the size of the assay system. The smaller the soil volume the greater and more consistent penetration of the nematode would be, associated with less variability between individual replicates within a treatment.

5.2 Relationship between *P. thornei* and *P. neglectus*

5.2.1 Introduction

As discussed in Chapter 4, *P. thornei* and *P. neglectus* occur quite commonly together in field situations on a range of soil types and different hosts in South Australia. For this reason, it is important to gain an understanding of the relative susceptibilities of similar hosts to the two species. Given that both species are considered to be potentially damaging in South Australia, control measures for both species will need to be developed and implemented.

An examination of the multiplication of *P. thornei* and *P. neglectus* on a range of cereals and non-leguminous hosts was carried out in collaboration with Research Associate, Dr Vivien Vanstone, Dept. of Plant Sciences, University of Adelaide. Several wheat varieties were selected from the Australian Winter Cereals Collection for their known resistance to Cereal Cyst Nematode (*Heterodera avenae*), another common root pathogen on cereals in South Australia. Both *Pratylenchus* species were assessed on the same range of host plants to allow comparisons to be made.

5.2.2 Materials and Methods

The plants to be tested (Table 5.4) were set up in a similar way to those in the previous section (5.1) as a RCBD with 10 blocks and 22 varieties randomly allocated to the 22 experimental units within each block. Two hundred seeds of each variety/cultivar were sterilised, germinated and selected (Section 3.5). One seedling was grown in each electrical conduit tubes (2.7 cm width x 12.5 cm height) with autoclaved Palmer sand (Section 3.7), which had been sterilised at 65°C for 45 minutes. One week after sowing, seedlings were inoculated with 400 *P. thornei* and 400 *P. neglectus* per plant in 1 ml aliquots. This was considered to be a non-damaging density as the subsequent

multiplication rate was not affected (Nicol, 1991). The nematode inocula were obtained from carrot cultures of the two respective species (Section 3.1).

Plants were placed in a controlled temperature growth room at 20°C, 12 hour day and night, provided by fluorescent light tubes (65µ Einsteins). Nematodes were extracted from plants harvested after two months using 3 day mister extraction technique (Section 3.2.2) and counted (Section 3.3).

Table 5.4: Varieties/cultivars used for comparison of susceptibility to *P. thornei* and *P. neglectus*.. (**SA;** South Australian, **SUN**; Sydney University; **QLD**; Queensland, **AWCC**; Australian Winter Cereals Collection)

SPECIES	COMMON NAME	VARIETIES/CULTIVARS
Triticum aestivum	Wheat	**SA**; Machete, RAC 589 **SUN** ; Sun 223A, Suneca **QLD** ; GS28; GS50A, Banks, Potam, Gatcher **AWCC**; AUS4918, AUS7869, AUS 10938, AUS 4930, AUS 7639, AUS 13807, AUS 10894
Secale cereale	Rye	SA Rye
Triticum secale	Triticale	Currency, Tatiara
Avena sp.	Oats	Marloo, Wallaroo
Brassica sp.	Rapeseed(Canola)	Barossa

The range of cereal hosts was selected to provide a wide cross section of different wheat genotypes from breeding programs. The Queensland wheat variety GS50A is a resistant selection from the highly susceptible and intolerant wheat cultivar, Gatcher, while the varieties GS28, Banks and Potam are known to be tolerant, but little is known about their susceptibility. The AWCC selections, AUS10938, AUS4930, AUS13807 and AUS10894, were obtained from Ms. Franky Green (SARDI) and investigated because of their known single gene resistance to *H. avenae*. The rye, triticale, oats and non-legume canola were chosen to see if previous results could be verified for both species of *Pratylenchus*.

5.2.3 Results

All data for both *P. neglectus* and *P. thornei* per plant were analysed separately as a RCBD. The original analysis showed heterogeneity of variance, so the data was log transformed $Log_e(x+1)$. A significant varietal effect was found from the analysis of variance for both *P. thornei* and *P. neglectus*, before and after log transformation (Table 5.5).

Table 5.5 : ANOVA of *P. thornei* and *P. neglectus* on cereal and non-leguminous hosts.

	log (*P. thornei* +1)				log (*P. neglectus* + 1)			
	d.f.	m.s	v.r.	Prob	d.f.	m.s.	v.r.	Prob.
block	9	1.963			9	1.3355		
block.plot stratum								
variety	21	17.172	13.93	<0.001	21	1.5506	6.68	<0.001
residual	175(14)	1.232			188(1)	0.2323		
Total	205(14)				218(1)			

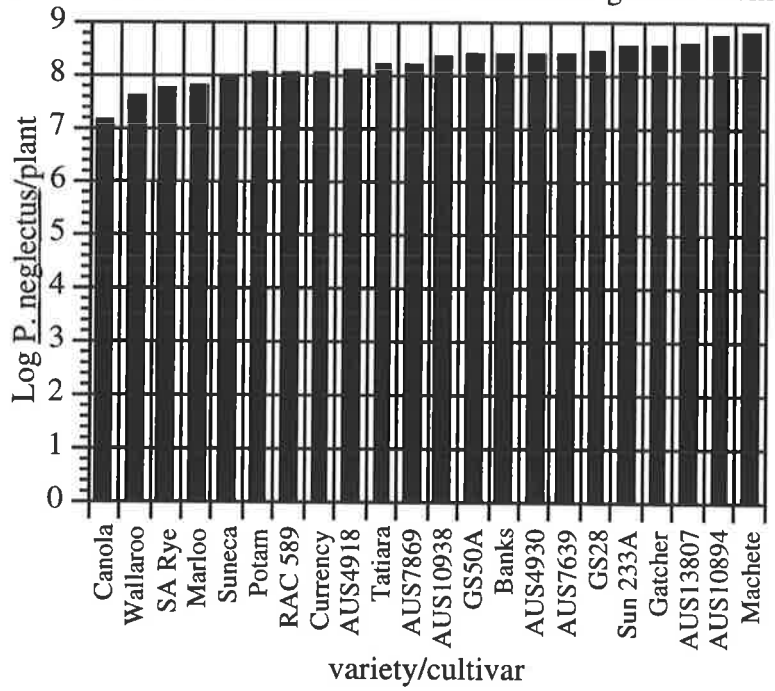
The significant varietal effect is illustrated in Figure 5.4 and Figure 5.5, with the Tukey Honestly Significant Difference Value ($\alpha = 0.05$) indicating the range required to significantly differentiate between varieties .

Further partitioning of the variety mean square with orthogonal comparisons allowed selective comparison of varieties from both *P. thornei* and *P. neglectus* are summarised in Table 5.6.

Table 5.6 : Orthogonal comparisons comparing selective combinations of varieties for both *P. thornei* and *P. neglectus*.

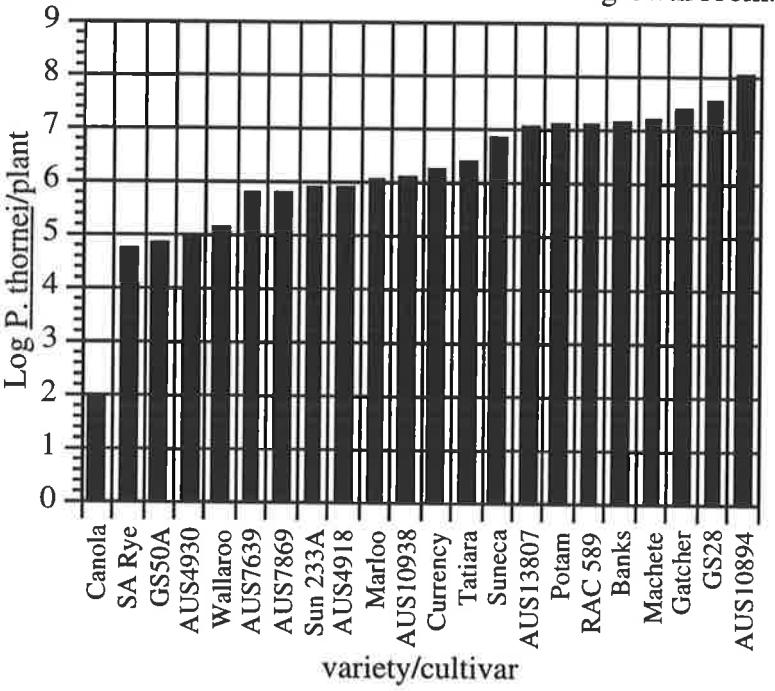
	P. thornei (log transformed) Prob.	*P. neglectus* (log transformed) Prob.
wheat vs' oats	<0.001	<0.001
wheat vs' barley	<0.001	<0.001
QLD, NSW wheats vs' SA wheat	0.380	0.911
AWCC wheat vs' SA wheat	<0.003	0.311
wheat vs' triticale	0.924	0.269

Fig. 5.4 : Multiplication of *P. neglectus* on cereal and non-leguminous hosts after 2 months in small tubes of 20°C in a controlled growth room.



Tukey = 0.76
(i.e., means must differ by this magnitude on a log scale to be different)

Fig. 5.5 : Multiplication of *P. thornei* on cereal and non-leguminous hosts after 2 months in small tubes of 20°C in a controlled growth room.



Tukey = 1.76
(i.e., means must differ by this magnitude on a log scale to be different)

From the results presented in Table 5.6 *P. thornei* and *P. neglectus* behaved similarly, both having significantly higher numbers of nematodes in wheat compared to oats and barley. However, there was no significant difference between wheat and triticale varieties. The wheats obtained from different states in Australia, were not found to differ significantly for either *Pratylenchus* species. However, the AWCC wheat lines had significantly different *P. thornei* multiplication.

The correlation between the multiplication of the two species *P. thornei* and *P. neglectus* was calculated using the non-parametrical Spearman's Rank test (Sokal and Rohlf, 1969). A t-value of 2.086 was found, which was non-significant at the 5 % level. This implies that the multiplication rates of *P. thornei* and *P. neglectus* were not dissimilar over the range of hosts tested. This is evident from Figs. 5.4 and 5.5, where for example, the AWCC variety AUS4930 is ranked the 8th most susceptible variety for *P. neglectus*, but is the 4th least susceptible for *P. thornei.* Similarly, GS50A, (the variety selected by Queensland breeders for resistance to *P. thornei*) is the 10th most susceptible variety for *P. neglectus*, but the 3rd least susceptible variety for *P. thornei.* In contrast, Canola was the least susceptible of all varieties/cultivars tested irrespective of *Pratylenchus* species.

5.2.4 Discussion

The results suggest that there is a range of susceptibility to both *P. thornei* and *P. neglectus* within the cereals and non-leguminous hosts tested. The multiplication rate of *P. neglectus* was much greater for all varieties/cultivars tested than for *P. thornei* (Figs 5.4 and 5.5). The statistical distinction between varieties for *P. neglectus* was limited by the similar range of susceptibilities (7.2 to 8.8 on the logarithmic scale) and the range required for distinction (Tukey = 0.76). The range of susceptibilities for *P. thornei* was much greater (2 to 8 on the logarithmic scale), but the range for distinction

was two fold greater (Tukey = 1.76). Nevertheless, *P. thornei* offers a much greater range of susceptibilities for breeders to work with compared to *P. neglectus*. The wheats appear to show greater susceptibilities than the rye, oat and canola, supporting previous observations for both *P. thornei* (Thompson, private comm. 1993) and *P. neglectus* (Vanstone, 1993).

A plant is termed resistant if it restricts or prevents nematode multiplication (Trudgill, 1991). From the data presented in Figs 5.4 and 5.5, a plant could be considered resistant if 400 or less nematodes per plant were recovered two months post inoculation (ie. when the initial density or less was recovered). On the logarithmic scale 6 is approximately equivalent to 400 nematodes per plant. From Figure 5.4 we see that for *P. neglectus* none of the hosts tested had nematode numbers at or below this level. In comparison, Figure 5.5 indicates that almost half of the varieties tested showed final *P. thornei* numbers that were less than 400. Caution should be taken in defining the critical cut-off for what may be termed a resistant host as it is unlikely that the initial inoculation density reflects the number of nematodes which penetrated the root. In fact, depending on the experimental conditions (soil type, host, temperature), the number of nematodes which initially penetrated the host could vary widely, but should, undoubtedly, be less than 400. The work of Phillips (1984) on screening for *G. pallida* resistance with potatoes also found that ranking clones was more reliable than absolute values for similar reasons.

The data presented suggest that within the cereals assessed there is a differential host status for the two nematode species, *P. thornei* and *P. neglectus*. In the event that both species occur in the field and require effective control with the use of resistant cereals, the probability that one cereal will provide this for both nematode species appears unlikely. The resistance of the variety GS50A, originally a re-selection from the highly

intolerant susceptible cultivar Gatcher (Thompson, private comm. 1993), is evident for the South Australian *P. thornei*, but it appears highly susceptible to *P. neglectus.* In addition, a variety of wheat (AUS 10894) recently released in Victoria on the basis of field resistance to *H. avenae* is one of the most susceptible varieties for both nematodes. However, another AWCC variety (designated AUS4930, originally from Iraq) offered promising resistance for *P. thornei* , and also carries with it a single gene for *H. avenae* resistance (F. Green, pers. comm.). This is not suprising as Tinline *et al.* (1989) suggests that regions where crop plants evolved from wild progenitors, the so called centres of origin or gene centres, are rich in plant diversity and may afford good sources of resistance.

5.3 Development of a Resistance Assay

5.3.1 Introduction

The previous two sections showed differences in multiplication of *P. thornei* in different cereal varieties under different conditions and suggested that this could form the basis for an assessment of comparative resistance between varieties. To be useful, such an assay should be as quick as possible, use the optimum number of nematodes that produce adequate separation of resistant and susceptible plants and occupy as little space as possible in the growth room or glasshouse. These factors are examined in this section.

5.3.2 Materials and Methods

Six cereal varieties ranging from resistant to highly susceptible, 2 soil types, 1 container size, 3 nematode densities and 3 harvest times were investigated.

The 6 cereal varieties were chosen deliberately to express a range of susceptibilities to *P. thornei* (Table 5.7), as previously displayed in Section 5.1 and 5.2.

Over one hundred seeds of each variety (Table 5.7) were sterilised, germinated and selected (Section 3.5). Two soil types were used. Urrbrae loam (clay based) from soil collected near the Waite Agricultural Research Institute (Section 3.7) was autoclaved at 120°C and allowed to cool. The second type was a sandy soil from Palmer in South Australia, which was heat treated at 65°C for 45 minutes. Both soil types were mixed thoroughly and sieved through a 2 mm mesh to remove organic matter and small rocks. Large electrical conduit tubes (3.7 cm x 12.5 cm) were filled to approximately 7/8 th of the tubes' height with either Palmer soil or Urrbrae loam and 108 seedlings of each variety were sown, half in each soil type, with 1 seedling per tube, and then covered

with about 2 cm of the appropriate soil type. *P. thornei* was obtained from carrot cultures (Section 3.1).

Table 5.7 : Varieties used for comparison of susceptibility to *P. thornei* in the resistance assay.
(**SA**; South Australian; **QLD**; Queensland University; **AWCC**; Australian Winter Cereals Collection)

SPECIES	COMMON NAME	VARIETY & SUSCEPTIBILITY TO *P. thornei*
Triticum aestivum	Wheat	**SA**; Machete - highly susceptible **QLD** ; GS50A - resistant **AWCC**; AUS 4930 - moderately resistant
Tritico secale	Triticale	**SA**; Currency - moderately susceptible
Avena sativa	Oats	**SA**; Wallaroo - moderately resistant
Hordeum vulgare	Barley	**SA**; Galleon - moderately resistant

Three *P. thornei* densities were used in this experiment. 100, 200 and 400 *P. thornei*/plant were added to seedlings one week after transplantation. These were considered to be non-damaging initial inocula (Nicol, 1991).

The plants were arranged as a split plot design (SPD) with 6 blocks where each block corresponded to a particular harvest time, and 6 whole plots within each block in which the 6 varieties were randomised. Within each whole plot there was a factorial arrangement of the 2 soil types and 3 *P. thornei* densities. The tubes were placed in a wire grid in a centralised position in order to avoid border effects throughout the experiment and shading effects at the latter stages of growth. The wire grid was placed on a tray of sterilised potting soil (Plates 5.2). The tubes were firmly placed in the soil at the bottom of the tray. Plants were keep moist at all times.

Plants were grown in a controlled temperature growth room at 21°C, 12 hour day, 12 hour night with light intensity 300-400µ Einsteins supplied with high pressure sodium

lamps and supplemented by fluorescent light tubes. The plants were sprayed with Dichlorvos-114 one month after inoculation due to a mite infestation. There was also evidence of powdery mildew infestation which was combated with the routine use of Bayleton (1 ml/L).

After 1 and 2 months the tubes were harvested and carefully rinsed in water to avoid root loss. Nematodes were extracted over three days using the Mister Extraction (Section 3.2.1) and counted (Section 3.3).

Plants were healthy after the first month, but by the end of the second month they were suffering an unknown problem. This was possibly a nutrient deficiency, toxicity or possibly another disease. Plants were not harvested after the third month as the results from the first two months indicated great variability, and the plants were suffering from head sterility and chlorosis by the third month.

5.3.3 Results

The original data showed heterogeneity of variance so a logarithmic transformation was applied ($\log_e(x+1)$). The results were analysed separately for each harvest, rather than including harvest time as an additional factor.

At both harvests, the number of *P. thornei*/plant significantly increased with initial nematode density (Fig. 5.6). Fewer nematodes were within the roots at harvest 1 than at harvest 2, at all densities. At harvest 1, only 5.5 - 6.5% of the inoculum had penetrated the root system, irrespective of initial density. At harvest 2, after some multiplication the percentage of nematodes within the roots compared with the initial density had risen to 17.0 - 22.5% for the 3 inoculum levels used.

Table 5.8 : ANOVA for Harvest 1 and Harvest 2 to compare the numbers of *P. thornei* on cereal and non-leguminous hosts.

	HARVEST 1 (log transformed)				HARVEST 2 (log transformed)			
	d.f.	m.s.	v.r.	Prob.	d.f.	m.s.	v.r.	Prob.
block								
block.plot.subplot		5	1.736		5	3.083		
variety	5	1.736	2.14	0.093	5	38.383	8.43	<0.001
residual	25	52.39			25	4.526		
block.plot.subplot								
soil	1	0.063	0.03	0.860	1	3.556	1.32	0.252
nem.density	2	38.61	19.26	<0.001	2	25.51	9.50	<0.001
variety.soil	5	2.365	1.18	0.322	5	6.661	2.48	0.035
variety.nem density	10	1.936	0.97	0.476	10	2.567	0.96	0.485
soil .nem density	2	0.011	0.01	0.994	2	10.95	4.08	0.019
variety.soil.nem density	10	2.522	1.26	0.260	10	2.283	0.85	0.581
Residual	143(7)	2.005			142(8)	2.685		
Total	208(7)				207(8)			

Fig. 5.6: The effect of *P. thornei* initial density on the number of nematodes extracted after one and two months in large tubes at 20°C in a controlled growth room.

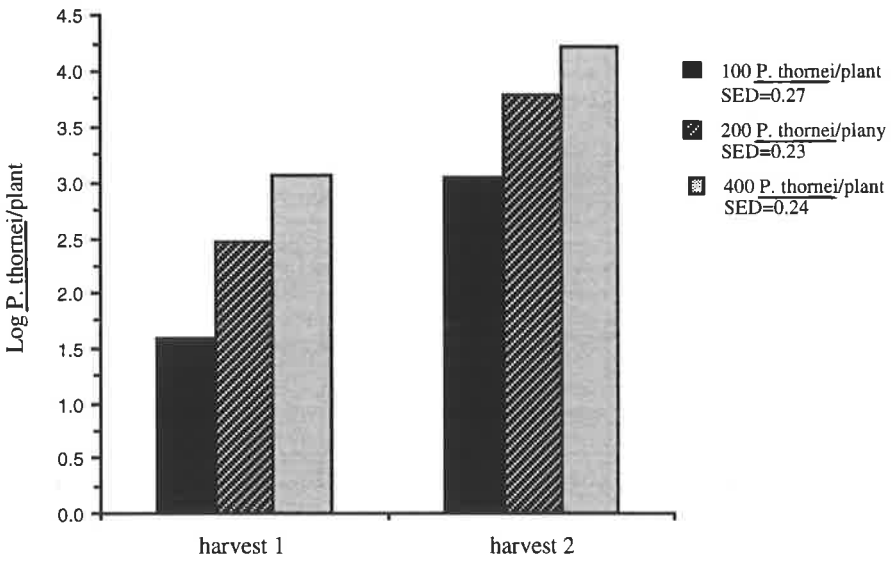


Fig. 5.7: The varietal effect on the number of nematodes extracted after 2 months in large and small tubes at 20°C in a controlled growth room.
(The data for small tubes was sourced from the same varieties from Section 5.1 (Ch 5), with the exception of Galleon which was not used in that experiment).

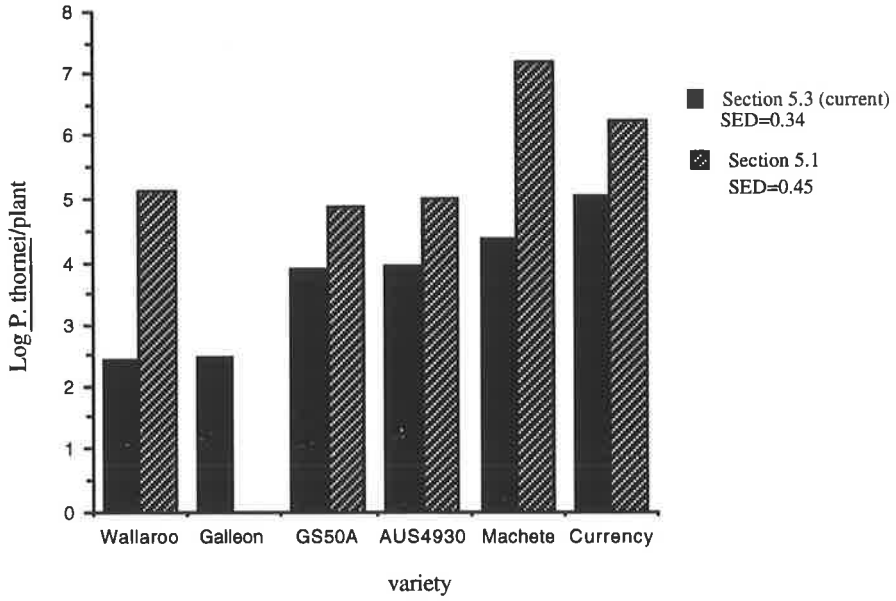


Fig. 5.8 : The interaction between soil type and variety on the number of *P. thornei* per plant after 2 months in large tubes at 20°C in a controlled growth room.

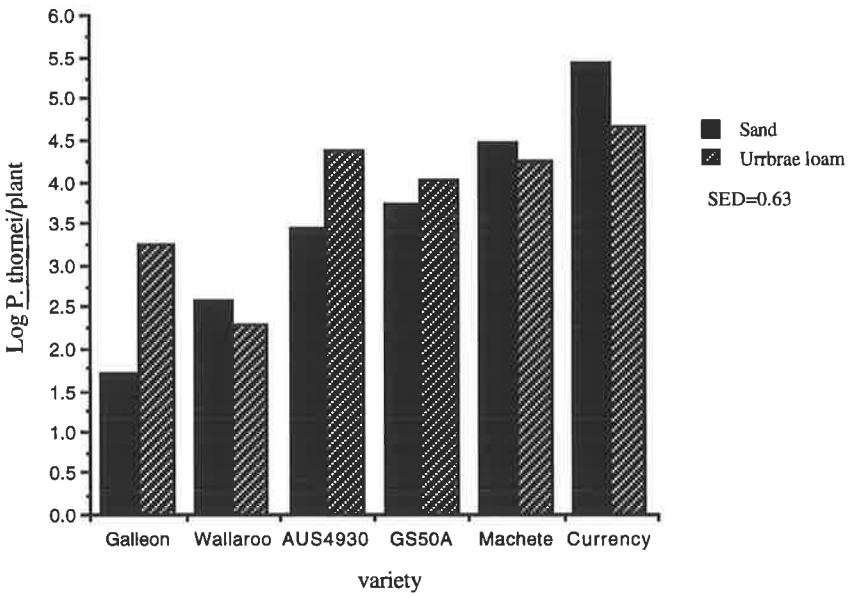
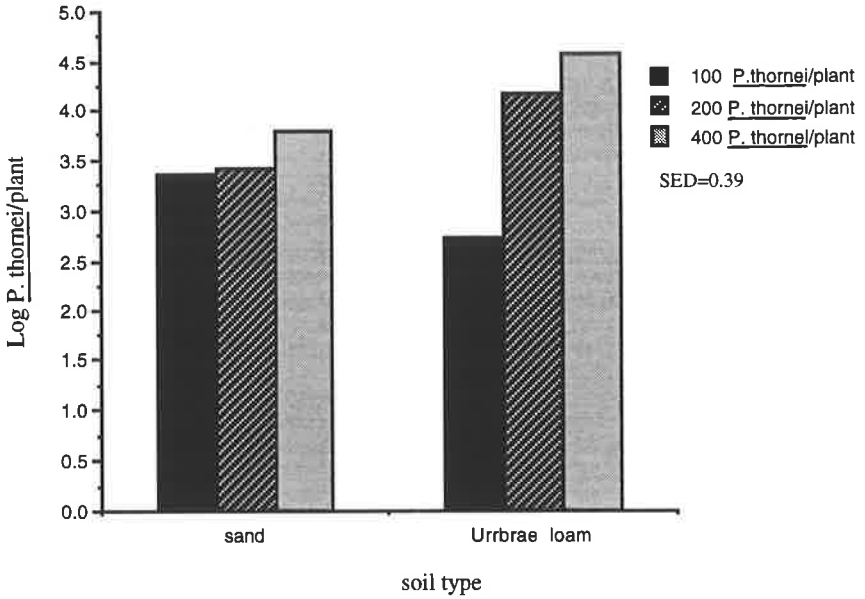


Fig. 5.9 : The interaction between nematode density and soil type on the number of *P. thornei* per plant after 2 months in large tubes at 20°C in a controlled growth room.



At harvest 2, "variety" had a significant effect on numbers of nematodes in the root systems (Fig 5.7), with Machete wheat having the most nematodes followed by the triticale variety Currency. The two wheats GS50A and AUS4930 had fewest nematodes among the wheats.

Soil type had two significant interactions, the first with variety (Fig 5.8) which (depending upon the soil type) appeared to produce variety specific results. For instance, the number of *P. thornei* in Urrbrae loam with Galleon is significantly higher than with sand, whereas the number of *P. thornei* is greater in sand than Urrbrae loam for the triticale Currency. Also, Figure 5.9 shows how nematode inoculum density was affected differently by soil type. The number of *P. thornei* was greater in Urrbrae loam for the two higher inoculum densities (200 and 400 *P. thornei* per plant); but for the lowest inoculum density of 100 *P. thornei* the number per plant was greater in sand.

5.3.4 Discussion

From the Analysis of Variance (Table 5.8) it can be seen that few of the factors tested were significant. At harvest 1, after just one month of growth, few differences were obtained between varieties examined probably because insufficient time had elapsed for nematode multiplication. The results reflected the numbers of nematodes which penetrated from the inoculum, which was small at 5.0 - 6.5%. It was surprising that soil type at harvest 1 did not affect the percentage penetration significantly as a sandy soil should provide better conditions for movement (Wallace, 1963). It may be that the Urrbrae loam had very good structure and crumb formation for movement or the containers may have been small enough to limit the contribution that movement might make to numbers penetrating.

After 2 months, the nematodes had multiplied at all initial *P. thornei* densities so that differences between levels of resistance had begun to show. The fact that no differences were apparent after 1 month shows that the minimum time to detect resistance is probably close to 2 months. In considering tube size, evidence from Section 5.1 comparing multiplication of nematodes on the same host species, with the exception of Galleon, suggested smaller tubes produced greater numbers of nematodes when the same time frame was considered (Fig. 5.7). This probably reflects greater efficiency in penetration in the smaller tubes and suggests that container size could be most important in the resistance assay. An increase in inoculum density up to 400 *P. thornei* per plant did not seem to affect the multiplication rate of the nematode on the range of hosts tested. This confirms the findings of Nicol (1991) and suggests that the initial densities used do not alter the multiplication rate of the nematode.

Finally, the best conditions for a resistance assay involved the use of small containers allowing for maximum nematode penetration, an initial inoculum density which is non-damaging (100 - 400 *P. thornei* per plant), and sufficient time between planting and harvesting (at least two months) to allow for nematode multiplication and distinction between host susceptibilities.

5.4 Modification of Resistance Assay

5.4.1 Introduction

The findings in Sections 5.2 and 5.3 indicated that a two month assay in small tubes provided a reasonable method of distinguishing the multiplication rate of *P. thornei* on a range of different cereals and non-leguminous hosts. The assay has the potential to rank not only different varieties/cultivars but also to identify lines of low susceptibility, possibly inferring some degree of resistance to *P. thornei*. However, the distinction between the susceptible and resistant varieties may be further differentiated over a longer period than two months. In addition to identifying resistant plants, the assay may also allow investigations into the mode of inheritance of resistance.

In the following section, changes in plant susceptibility to *P. thornei* over time with selected potentially resistant and highly susceptible wheat varieties were examined in large and small tubes .

5.4.2 Materials and Methods

Three wheat cultivars (two potentially resistant and one susceptible) were tested over two harvest times (two and three months) in two sizes of container (small and large tubes). The wheat cultivars were selected from the previous susceptibility rankings in Sections 5.1 - 5.3. These included GS50A *(T. aestivum)*, a wheat cultivar potentially resistant to *P. thornei*, and AUS4930. The latter (*T. vulgare nigro-graecum*) was designated by Watkins as "IRAQ 48" and was collected from wheat areas of Iraq in 1919 (M.Mackay, pers. comm.). It is a medium height, hard grained, early spring wheat. The third wheat cultivar chosen was the highly susceptible South Australian wheat, Machete (*T. aestivum*).

The plants were set up as an RCBD with eight replicates as described in previous sections (5.1 and 5.2). All seeds were sterilised, germinated and selected as in Section 3.5. One seed of each cultivar with three even seminal roots of length 3 cm was grown in electrical conduit tubes of two sizes, small (2.7 cm wide by 12.5 cm high) and large (3.7 cm wide by 12.5 cm high). Both large and small tubes were tested in order to detect possible limitations of container size due to root volume after 3 months. The tubes were filled with autoclaved Palmer soil (Section 3.7) which had been heat-treated at 65°C for 45 minutes. Seedlings were inoculated (Section 3.1) with 400 *P. thornei* per plant in 1 ml aliquots with nematodes from carrot cultures (Nicol, 1991).

Plants were placed in a controlled temperature growth room at 20°C, 12 hour day and night provided by fluorescent light tubes (65µ Einsteins). Nematodes were extracted from plants harvested after 2 and 3 months using a 3-day mister extraction (Section 3.2.2) and counted (Section 3.3).

5.4.3 Results

Data were analysed as a RCBD with the original analysis showing heterogeneity of variance so the data was log transformed ($\log_e(x+1)$) (Table 5.9).

Table 5.9 : ANOVA of resistance assay with potentially resistant and susceptible wheat over 2 harvest times.

	d.f.	m.s.	v.r.	Prob.
block	7	4.628		
block.plot				
harvest	1	0.883	0.46	0.198
tube size	1	0.191	0.10	0.753
variety	2	49.22	25.81	<0.001
harvest.tube size	1	0.0807	0.42	0.517
harvest.variety	2	7.952	4.17	0.019
tubesize.variety	2	8.786	4.16	0.013
harvest.tubesize.variety	2	0.565	0.30	0.745
residual	76(1)	1.907		
Total	94(1)			

Fig. 5.10 : The effect of tube size on the number *P. thornei* per plant extracted from three different cultivars grown at 20°C in a controlled growth room.

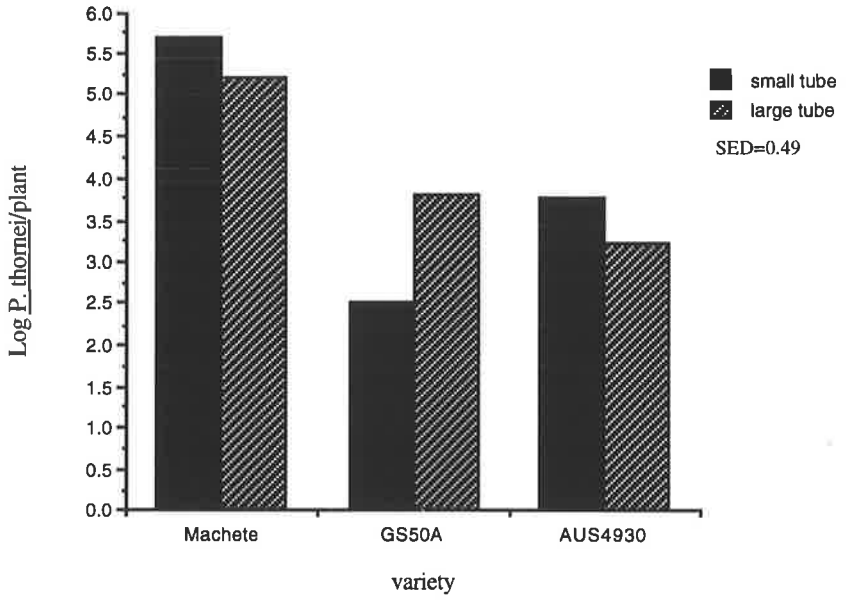
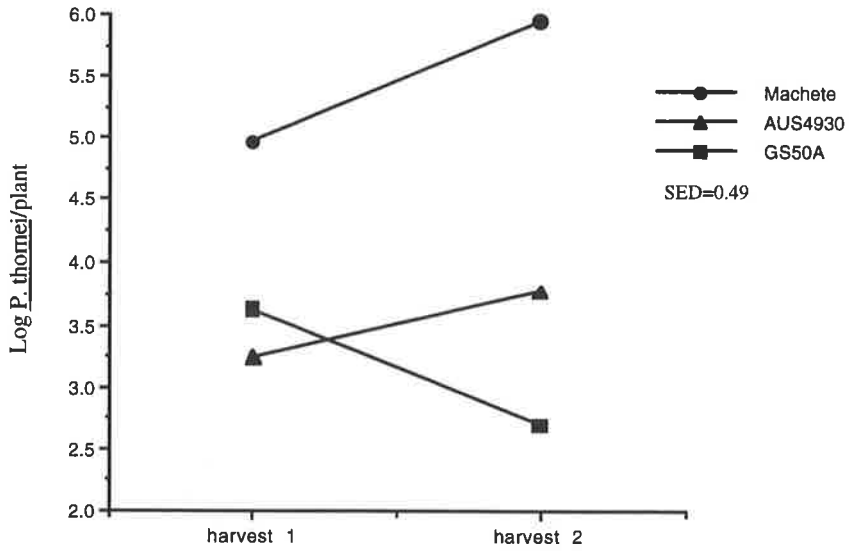


Fig. 5.11 : The effect of harvest time on the number of *P. thornei* extracted from 3 different wheat cultivars grown at 20°C in a controlled growth room.



The two way interaction between tube size and variety was significant (Fig. 5.10). From the figure it is seen that AUS4930 and Machete exhibit higher numbers in smaller than larger tubes, however the opposite occurs for GS50A.

The two harvest times, of two and three months, had a significant interaction on the number of nematodes per plant for the three varieties (Fig. 5.11). The number of nematodes per root system increased for Machete and AUS4930 over time; however GS50A showed reduction in *P. thornei* numbers over time.

5.4.4 Discussion

There was a significant difference between wheat lines tested at both harvest times irrespective of tube size. The number of nematodes recovered changed only slightly for AUS4930 whereas GS50A showed decreased numbers at the second harvest time. In comparison for the susceptible host, Machete, the numbers of *P. thornei* increased from the second to the third month. This implies that the distinction between resistance and susceptibility is increased as the time duration of the assay is extended.

The number of nematodes within the roots of the plants at both harvest times was less than in previous sections described in Chapter 5. It is possible that the inoculum from the carrot culture was less vigorous; however the ranking of the three varieties relative to each other was the same. If the assay was conducted for extended periods of time (up to three months), the volume of the root system may confound the results, due to its limited size in the tubes, both small and large.

5.5 General Discussion

The results presented here clearly indicate that cereal and non-leguminous hosts tested differ in their susceptibility to *P. thornei.* The ranking of varieties with respect to their

susceptibility to this nematode species is statistically comparable regardless of whether the plants were grown over a whole season or for only two months after inoculation. The Australian bread wheats were much more susceptible than most of the other cereals tested (barley, triticale, rye, oat and durum). The non-leguminous plants linseed and canola also offered some degree of resistance.

The relative susceptibilities of different hosts are determined primarily on the basis of nematode multiplication within the root system over a specific time period. It is important to note that the multiplication rate is highly dependent on the initial nematode density (Section 2.9). The use of a particular multiplication rate to define resistance is not a reliable method of distinguishing resistant plants. As reported by Phillips (1985), it is possible to standardise the initial density for test procedures, however the "effective" density will vary. The findings presented in this chapter support this. These variations in the effective initial density include environmental conditions which may affect invasion and development of nematodes (Phillips, 1985). This makes establishment of the ranking a more appropriate method to assess resistance than absolute numbers.

P. thornei is generally assumed to be an endoparasitic plant nematode (Dropkin, 1989). However, it should be noted that some evidence suggests that *P. thornei* may feed ectoparasitically, as well as endoparastically (Zunke, 1990). The ranking of the varieties in the present work was statistically comparable (Section 5.1), regardless of whether extraction was done from roots alone or from roots plus soil. This finding supports the view that *P. thornei* is mostly endoparasitic. Hence assessment of resistance using root systems alone seems a valid approach to the assay.

The range of susceptibility of host plants to the closely related species *P. neglectus* and *P. thornei* differed significantly, although wheats were generally more susceptible to both nematodes than oats and barley. These specific variety/cultivar differences have important implications for possible control strategies, given that mixes of both nematode species are frequently found in the cereal growing regions (Chapter 4).

The screening of a range of wheat lines identified two potential sources of resistance in wheat. The resistance in GS50A verifies the work of Thompson (private comm. 1993), while the land race variety designated AUS4930 appears to confer some degree of resistance to *P. thornei* in addition to the single gene for resistance to the cereal cyst nematode, *H. avenae.* The assay was found to give best results in small tubes over a two month time period post inoculation in a sandy soil medium using an initial *P. thornei* density which is non-damaging (i.e. up to 400 nematodes per plant).

Chapter 6
Population Dynamics and Yield Relations of *P. thornei* in the Laboratory

6.0 General Introduction

Little is known about *P. thornei* population dynamics on cereals (Section 2.8). Jones (1956) noted that the principles underlying the populations dynamics of plant parasitic nematodes can be studied in pots of various size, small field plots or microplots. Population dynamics can be defined as the changes in the number, age, class, sex ratio and behaviour of a population through time and space, determined by inherent characteristics of the individuals, and mediated by the environmental conditions of food resources and interacting biotic agents (Ferris and Wilson, 1987). An understanding of population dynamics is considered essential for the development of control measures.

Ideally, field studies should be conducted to obtain accurate information about population dynamics. However, in order to determine the effect of *P. thornei* alone with wheat in relation to populations dynamics of the nematode and the associated damage relations of the host, several aseptic laboratory experiments were conducted on wheat.

6.1 Population Dynamics and Pathogenicity of *P. thornei* on Machete at 25°C

6.1.1 Introduction

There is no information available on the multiplication of *P. thornei* on local wheat cultivars in South Australia. Pattison (1993) has examined changes in *P. thornei* multiplication on various cereal cultivars in northern NSW in different soil types and conditions. Clearly, there is a need to examine multiplication rates and damaging densities on local cultivars.

6.1.2 Materials and Methods

An attempt was made to examine the effect of different initial *P. thornei* densities on nematode population dynamics and the yielding capacity of the wheat cultivar Machete. Nine nematode densities were investigated each with eight replicates grown for a period of four months.

Three hundred seeds of the wheat cultivar Machete were selected on the basis of size uniformity and were sterilised and germinated as in Section 3.5. Soil (Urrbrae Loam) taken from the Waite Institute was autoclaved at 120°C for 1 hour and after cooling was thoroughly mixed. It was then placed into 72 perforated (4 holes) black polyethylene bags (11.5cm wide by 16cm high) over a layer (2-3cm) of wood chips to encourage drainage. Germinated seedlings of Machete were selected for uniformity (three roots each about 3cm long) and one seedling was planted in each bag.

P. thornei was extracted from carrot cultures as described in Section 3.1 and inocula of 0, 500, 1500, 2000, 3000, 4000, 6000, 10000, and 15000 nematodes were used per bag with 8 replicates per treatment. The nematodes were added to the bags as close to the seedling as possible. SDW was used for the 0 nematode treatment. Plants were arranged as a CRD in a controlled growth room at 25°C with 12 hours light, intensity 300-400 μEinsteins supplied by high pressure sodium lamps. Plants were sprayed routinely for powdery mildew with Bayleton (1ml/L).

Plants were harvested 4 months after inoculation. The soil was gently washed from the root system. Nematodes were extracted over a period of 7 days using the mister extraction (Section 3.2.2) and were counted (Section 3.3). Dry weight of shoots and roots were recorded after drying at 80°C for 7 days.

6.1.3 Results

Plants were very healthy till after heading which occurred at approximately 2 months after sowing. Unfortunately, due to unknown reasons many of the heads were found to be sterile at harvest. As a consequence only shoot dry weight only was recorded.

The data, number of *P. thornei*/ plant; number of *P. thornei*/ g plant; multiplication rate (number *P. thornei* per plant/ initial *P. thornei* density); dry weight roots (g); dry weight shoots (g); total dry weight (g)); were analysed as a CRD. Initial density had a highly significant effect on the final number of *P. thornei*/plant (Table 6.1), the numbers in the plant increasing in a linear fashion with increasing density (Fig. 6.1).

Table 6.1 : ANOVA for variables measured on Machete after 4 months

	d.f.	m.s.	v.r.	Prob.
Variable : No. *P. thornei*/plant				
Density	8	1.483E+10	19.49	<0.001
Linear	1	1.161E+11	152.67	<0.001
Quadratic	1	1.459E+08	0.19	0.663
Deviation	6	3.917E+08	0.51	0.794
Residual	53(10)	7.606E+08		
Variable : No. *P. thornei*/ g plant				
Density	8	2.561E+09	9.80	<0.001
Linear	1	1.799E+10	68.86	<0.001
Quadratic	1	7.363E+08	2.82	0.099
Deviation	6	2.937E+08	1.12	0.361
Residual	53(10)			
Variable : Multiplication rate *P. thornei*				
Density	7	73.96	1.96	0.082
Residual	46(10)	37.82		
Variable : Total dry weight (g)				
Density	8	89.49	1.04	0.421
Residual	53(10)	86.34		
Variable : Dry weight shoots (g)				
Density	8	23.389	2.48	0.023
Residual	53(10)	9.412		
Variable : Dry weight roots (g)				
Density	8	56.74	1.00	0.446
Residual	53(10)	56.68		
Total	61(10)			

Fig. 6.1: The effect of initial density of *P. thornei* on the number of nematodes extracted per plant from Machete after 4 months.

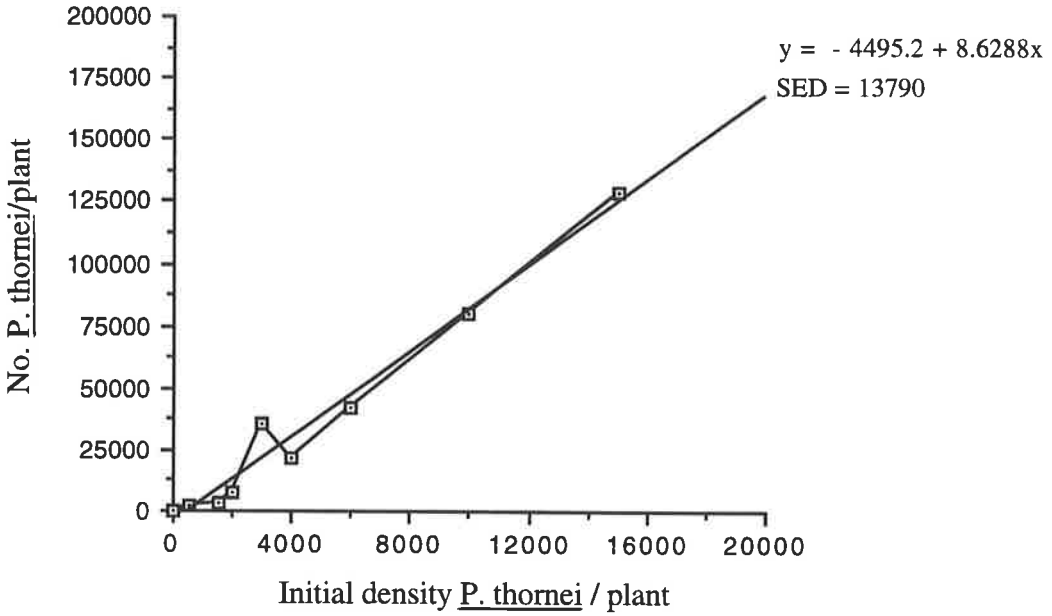


Fig. 6.2 : The effect of initial density of *P. thornei* on the nematode multiplication rate on Machete after 4 months (note : non-significant).

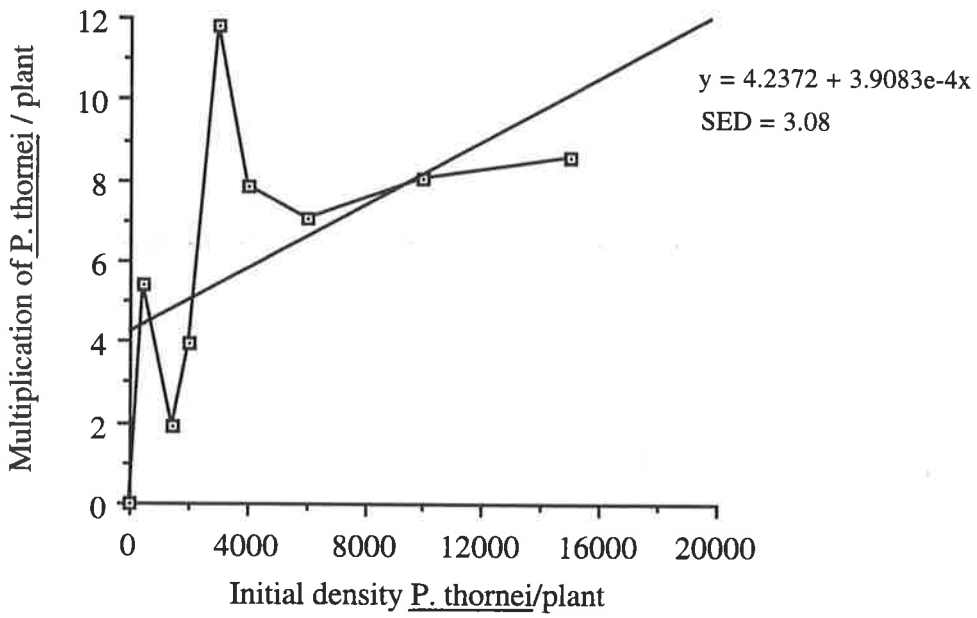


Fig. 6.3 : The effect of initial density of *P. thornei* on the number of nematodes per gram dry Machete root.

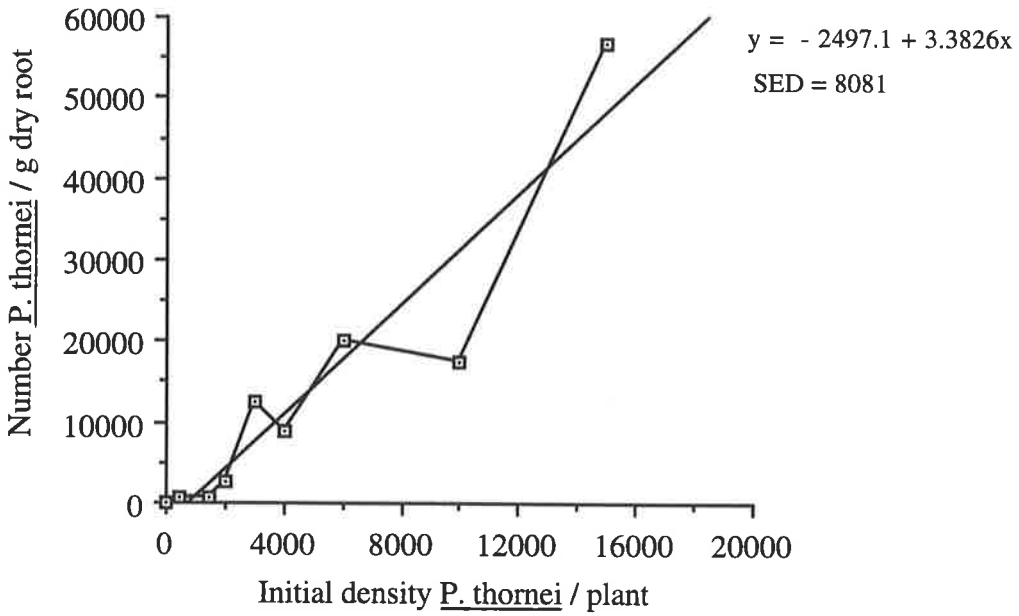
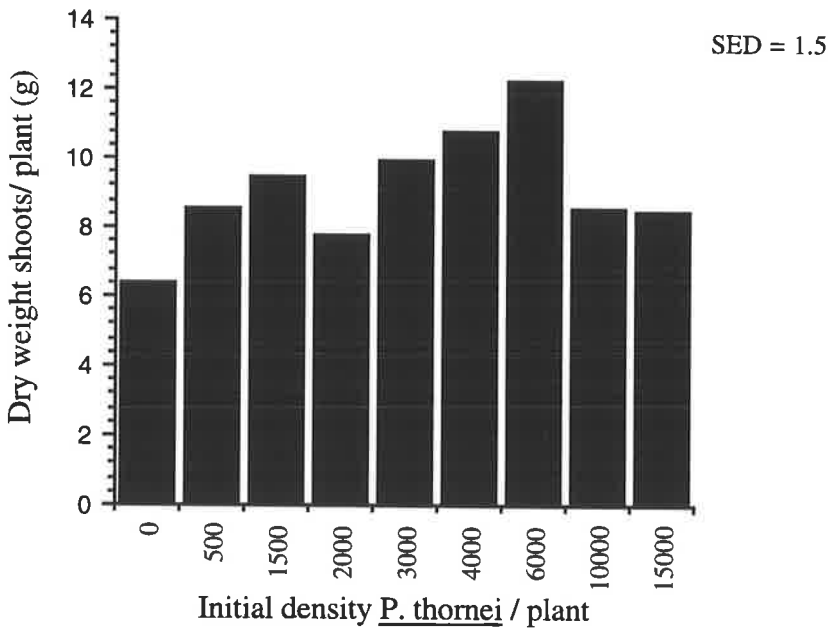


Fig. 6.4 : The effect of initial density of *P. thornei* on the dry weight of Machete shoots after 4 months.



When nematode multiplication rate was plotted against the initial density we see a gradual increase in multiplication rate as initial density increased (Fig. 6.2). Although this was non-significant ($P<0.05$) the Fig. 6.2 is still included. The number of *P. thornei* per gram of plant had a highly significant linear relationship with initial nematode density (Fig. 6.3).

Significant differences in dry weight of shoots were obtained (Table 6.1) but from Fig. 6.4 it can be seen that dry weight increased with increasing density up to 6000 *P. thornei* per plant but was lower for higher initial densities. Neither total dry weight nor dry weight of roots were affected significantly by increasing initial density (Table 6.1).

6.1.4 Discussion

Unusual effects and relationships were demonstrated in this experiment. Neither multiplication rates of the nematodes, nor the response of growth to increasing initial density of nematodes gave responses that would be normally expected. An explanation for both these unexpected results could be that initial penetration of the roots was extremely poor and although large numbers of inoculum were added only a very small percentage actually penetrated the roots. This would mean that numbers in roots were very small and that the very low initial density meant that stimulation of growth occurred. Multiplication rates normally drop rapidly as initial density increases (Brown and Kerry, 1987). Similarly, growth of plants is normally increasingly inhibited as initial density increases (Nicol, 1991). Nusbaum and Ferris (1973) suggest conditions unfavourable to the host crop can indirectly reduce the maximum rate of multiplication and the equilibrium density of the nematode compared to those found under favourable conditions.

At low levels nematode infestations are known to produce a stimulus in growth (Chitwood and Buhrer, 1946; Chitwood and Feldmesser, 1948; Chitwood and Esser, 1957 and Peter, 1961; in Wallace, 1963). Tillering was increased in oats with

Ditylenchus dipsaci (Dunning, 1954 in Wallace) and in tall fescue with *Paratylenchus projectus* (Coursen and Jenkins, 1958; in Wallace, 1963). Species of the sedentary nematode, *Heterodera* are noted for causing increases in root growth and low initial densities (Wallace, 1963).

6.2 Soil Type Relations with *P. thornei* and *P. neglectus*

6.2.1 Introduction

Given the results in the previous section, it is important to examine the penetration efficiency of the nematode. *P. thornei* and *P. neglectus* usually inhabit different soil types; *P. neglectus* is found more commonly in lighter soils while *P. thornei* more commonly inhabits heavier, clay based soils (Section 2.6.2). However, the optimal soil conditions for movement (Wallace, 1963) suggest both nematodes should move well in sandy soil.

This section has two components. The first is an experiment using *P. neglectus* instead of *P. thornei* investigating nematode penetration in relation to soil type and container size. *P. neglectus* was used because sufficient numbers of *P. thornei* were not available. The second experiment examines penetration of both *P. thornei* and *P. neglectus* in relation to soil type and the efficiency of the extraction process. This latter work was done in collaboration with Mr. Abdolhossein Taheri, Department of Plant Science, Waite Campus.

6.2.2 Materials and Methods

Machete wheat seeds were sterilised, germinated and selected as in Section 3.5. For the first experiment 2 soil types; Urrbrae Loam (Ul) a red brown earth from the Waite Institute with a high clay content, and Palmer sand (Ps) a very sandy soil from the farming region of Palmer were used (Section 3.7). The Ul was heat treated at 100°C for 1 hour and the Ps at 65°C for 45 minutes. Both soil types were mixed separately and all the Ps and half the Ul were sieved through a 2mm sieve.

For the first experiment two containers were used, large perforated (4 holes) black polyethylene bags (11.5cm wide by 16cm high), and electrical conduit tubes (3.7cm wide by 12.5cm high). Equal numbers of each type of container were filled with each soil type (Ul non-sieved, Ul sieved, Ps sieved) and 1 seedling was planted in each

container as described previously (Section 3.5). The seedlings in the tubes were inoculated with 170 *P. neglectus*/ ml of water and the plastic bags with 1500 *P. neglectus*/ ml, 1 week after sowing.

Plants were arranged as a CRD, with the bags and tubes separated due to space limitations, but soil type was randomised within the container sizes. Ten replicates of each soil type and container size were grown in a controlled temperature room at 20°C with 12 hour day length and light intensity of 65 μEinsteins. The tubes were supported by a wire grid and the bases were embedded into potting soil while the bags were placed on a metal tray. Plants were harvested 1 and 3 weeks after inoculation. Nematodes were extracted in a mister (Section 3.2.2) for 3 days and counted (Section 3.3).

In the second experiment four different soil types were used, two with a high clay content and two with a sandy composition. These were Urrbrae loam non-sieved (Ulns), Urrbrae Loam sieved (Uls), Palmer sand sieved (Ps) and Roseworthy sand (Rs) also sieved through a 2mm sieve. The four soil types were tested for penetration efficiency of the 2 nematodes, *P. thornei* and *P. neglectus.* Plants were similarly inoculated, and the inoculum density was 2000 *P. thornei* or *P. neglectus* per plant.

Plants were set up as a Split Plot with 6 replicates for each nematode species, soil type and harvest time. The plots contained either *P. thornei* or *P. neglectus*, while within each subplot the soil type was randomised. The plants were grown in 300ml plastic cups (Plate 6.1) without drainage holes and were harvested 1 week after inoculation.

The nematodes were extracted in a mister (Section 3.2.2) for a period of 4 days and counted (Section 3.3). The nematodes remaining in the root system were also counted after staining with acid fuschin (Section 3.4.1).

6.2.3 Results

The data from both experiments was analysed by ANOVA. For the first experiment, because the tubes and pots were inoculated with different nematode densities, the data was converted to percent penetration for the analysis, presented in Table 6.2.

Table 6.2 : ANOVA from the first experiment: effect of soil type and volume on the penetration efficiency of *P. neglectus*.

	d.f.	m.s.	v.r.	Prob.
container	1	0.18129	6.92	0.010
soil	2	0.48985	18.70	<.001
harvest time	1	0.10481	4.0	0.048
container.soil	2	0.10545	4.02	0.021
container.harvest time	1	0.02117	0.81	0.371
soil.harvest time	2	0.02702	1.03	0.361
container.soil.harvest time	2	0.00720	0.27	0.760
Residual	93(15)			
Total	104(15)			

There was a significant interaction effect between container and soil type. Also harvest time significantly affected penetration. The significant interaction between container and soil type arose because container type had no influence with Ps but with the Ul (sieved or non-sieved) the percentage penetration was significantly higher in tubes than in plastic bags (Fig. 6.5). There were significantly more nematodes in the roots at the 3 week harvest (Fig. 6.6), but no significant interaction between harvest time and container size or harvest time and soil type.

The ANOVA for the second experiment, analysed as a SPD, is presented in Table 6.3. The three variables analysed were total number nematodes per plant (mister extraction plus stained nematodes in roots), mister extracted nematodes only per plant, and stained nematodes in roots only per plant. All results were analysed as percentage penetration.

The ANOVA of the total nematodes showed that *P. neglectus* and *P. thornei* acted similarly regardless of soil type. Overall, the actual effectiveness of the original inoculum was low. Fig. 6.7 illustrates the significant interaction between soil type and

the total nematodes extracted from the soil. Sandy soil was by far the best medium to allow maximum penetration within the roots (up to 40%). However, the Ul was very inefficient, particularly if unsieved, with fewer nematodes (only 5%) penetrating the roots.

Table 6.3 : ANOVA from the second experiment: effect of soil type and extraction technique on penetration efficiency of *P. thornei* and *P. neglectus*.
(data analysed as a percentage of the initial inocula)

	d.f.	m.s.	v.r.	Prob.
Variable : Total Nematodes per plant				
block stratum	5	0.01568		
block stratum				
nemtype	1	0.00004	0.00	0.956
Residual	5	0.01283		
block.wplot.subplot stratum				
soil	3	0.37377	21.33	<0.001
nematype.soil	3	0.00916	0.52	0.670
Residual	29(1)	0.01752		
Total	46(1)			
Variable : Mister nematode extraction only per plant				
block stratum	5	0.002662		
block.wplot stratum				
nemtype	1	0.035100	12.06	0.018
Residual	5	0.002911		
block.wplot.subplot stratum				
soil	3	0.026381	8.84	<0.001
nematype.soil	3	0.007924	2.64	0.067
Residual	29(1)	0.002985		
Total	46(1)			
Variable : Stained nematodes in roots only per plant				
block stratum	5	0.006524		
block.wplot stratum				
nemtype	1	0.037630	6.75	0.048
Residual	5	0.005573		
block.wplot.subplot stratum				
soil	3	0.021152	24.20	<0.001
nematype.soil	3	0.023475	2.69	0.065
Residual	29(1)	0.008742		
Total	46(1)			

Fig. 6.5 : The effect of soil type and container size on the penetration of *P. neglectus* in Machete roots.

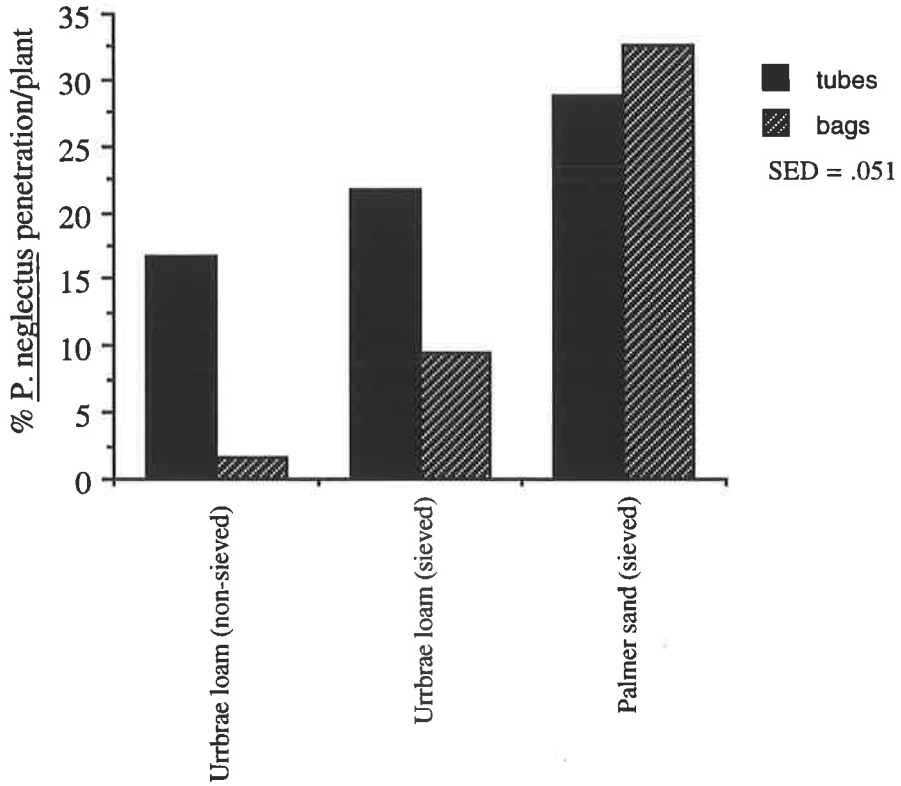


Fig. 6.6 : The effect of harvest time on the penetration of *P. neglectus* in Machete roots.

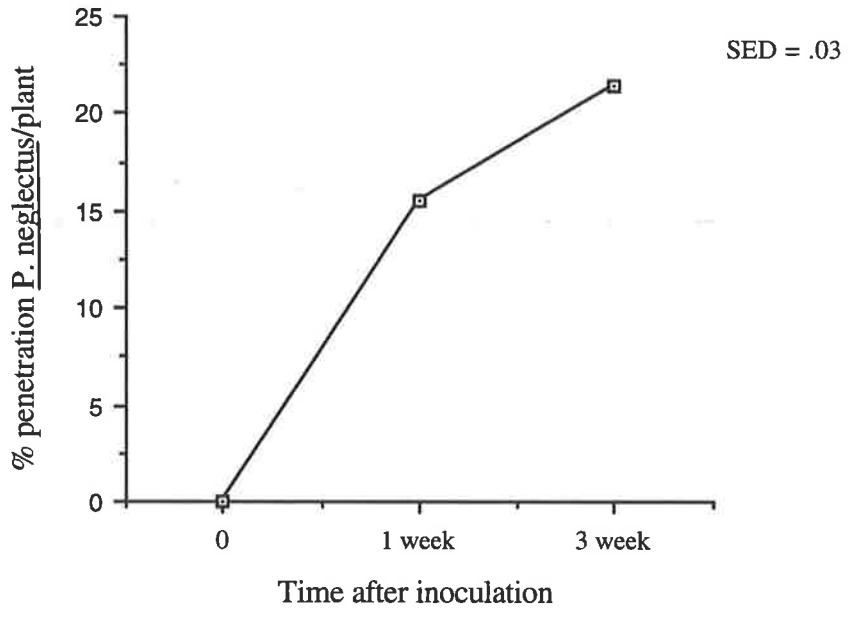


Fig. 6.7 : The effect of soil type and nematode extraction technique on the numbers of *Pratylenchus* in Machete roots.

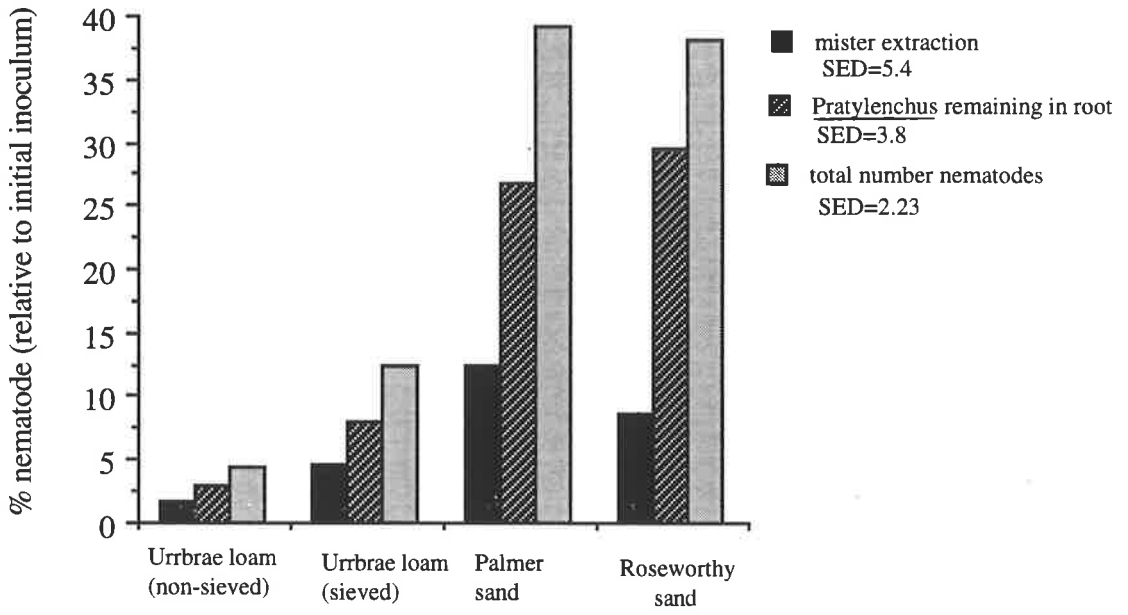
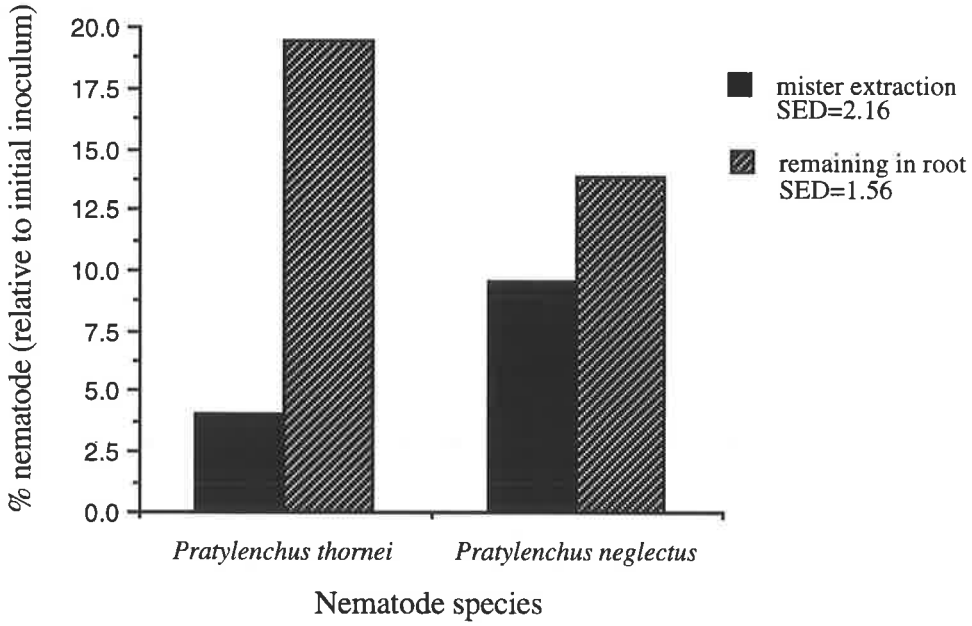


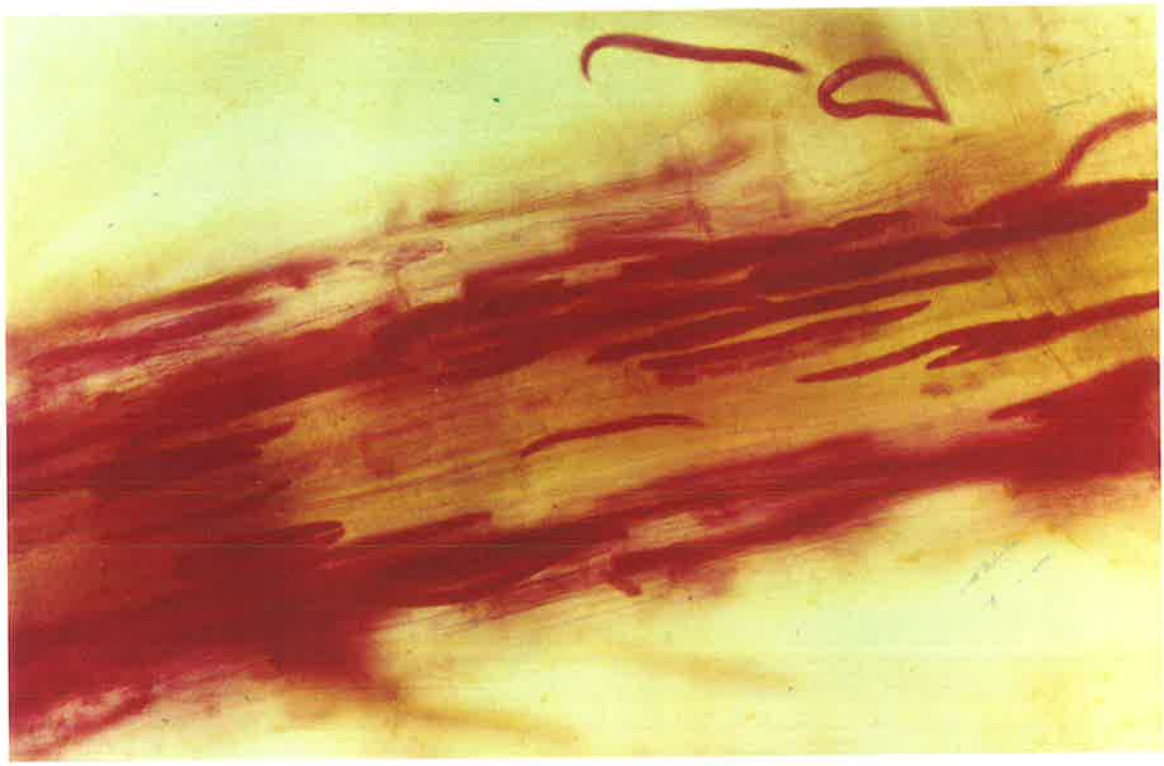
Fig 6.8 : The effect of mister extraction and staining nematodes in the remaining Machete roots on the proportion of *P. neglectus* and *P. thornei.*



The ANOVA of the total nematodes showed that *P. neglectus* and *P. thornei* acted similarly regardless of soil type. Overall, the actual effectiveness of the original

Plate 6.1 : The experimental setup for the second experiment used to assess the penetration of *P. thornei* and *P. neglectus* over a range of soil types after 3 weeks. 300ml plastic cups were arranged as a SPD and grown in a controlled growth room at 20°C. Light was supplied by flourescent light tubes (65µ Einsteins) and plants watered with tap water whenever necessary.

Plate 6.2 : A representative stained Machete wheat root system, 21 days post inoculation, initially inoculated with 2000 *P. thornei* in Urrbrae loam (non-sieved). Evidence of masses of *P. thornei* adults (represented by the dark pink area) and nematode eggs (oval shaped rods) in cortical cells of seminal root system. (1cm = 50µm)



inoculum was low. Fig. 6.7 illustrates the significant interaction between soil type and the total nematodes extracted from the soil. Sandy soil was by far the best medium to allow maximum penetration within the roots (up to 40%). However, the Ul was very inefficient, particularly if unsieved, with fewer nematodes (only 5%) penetrating the roots.

Further analysis (Table 6.3) of the two components of the total number of nematodes per plant (mister extracted and stained nematodes within the root) revealed there was a significant species effect, both with the mister extraction and the numbers of stained nematodes remaining in the root system (Fig. 6.8). From the mister extraction 42% of *P. neglectus* left the root system in 4 days, but the number of *P. thornei* leaving the root was significantly less, almost 3 times fewer (16%). However, there were significantly more *P. thornei* left inside the roots (84%) (Plate 6.2), compared with *P. neglectus* (58%).

6.2.4 Discussion

Infection depends on the movement of nematodes through soil and their possible attraction to roots. As the same cultivar of wheat was used for both experiments, the attractants did not vary, but the movement through soil did. The percentage penetration in both experiments was higher in sand (Palmer and Roseworthy) than in sieved Urrbrae loam than in unsieved Urrbrae loam, so that particle size influenced movement. Why sieving improved penetration is not clear, but it may have changed particle or crumb size or removed possible toxic materials in the organic matter.

The first experiment showed that larger containers, such as the bags, interacted with soil type to influence percentage penetration. If the nematode had difficulty in moving in the soil type then larger volumes of soil exaggerated this difficulty. The differences in percentage penetration between bags of Ul in bags and tubes was much greater than for the Ps. Thus container size can influence percentage penetration in unfavourable

soils. Nevertheless, the best penetration was in sand and so particle size was more important than container size though both may influence penetration. The best penetration would probably be obtained in small tubes in sand.

In addition, the first experiment showed that the increase in numbers in roots at the three week harvest could have been due to further penetration or to multiplication of the nematodes in the roots. At 20°C, there was probably time for any adult females which penetrated to lay eggs and for them to hatch. There is no way to distinguish between these possibilities.

The second experiment reconfirms the finding that sandy soil provided the best medium for maximum penetration of either nematode species, *P. thornei* or *P. neglectus*. However, although the data presented in this Section reveal the total number of nematodes per plant (mister plus remaining in roots) do not vary between species, the individual components vary significantly. The implications of these results need to be considered. The previous experiment (Section 5.2) assessing the multiplication of *P. thornei* and *P. neglectus* over a range of hosts and comparing and ranking respective cultivars was done principally on the basis of the number of nematodes extracted per plant. As a result the comparison of numbers is not strictly comparable. Unfortunately to stain and count all the nematodes inside root systems would be too time consuming. *P. neglectus* left the roots much faster than *P. thornei*, this may possibly be associated with differences in species mobility. It is important to understand the nematode penetration in different soil types and the extraction efficiency of the method used. If comparative data are required a standardised method is needed.

6.3 Population Dynamics and Pathogenicity of *P. thornei* on Machete at 20°C

6.3.1 Introduction

An initial attempt (Section 6.1) was made to examine the effect of *P. thornei* density on population dynamics and yielding capacity of the wheat cultivar Machete. Results suggested the initial densities used in the soil medium tested were too low to limit multiplication of the nematode and cause damage to the host.

The previous section established unsieved Urrbrae loam as a poor medium for penetration and movement by both *P. thornei* and *P. neglectus*, and that reduction in container size could overcome part of these problems. However, the use of small containers may limit growth of the plant at later stages of development and this could cause problems in understanding the relationship between the nematode and the plant. Given that soils like Urrbrae loam tend to be associated with the distribution of *P. thornei* in the field, it may be possible to use increased initial densities with this soil to overcome some of the problems associated with penetration. These aspects are examined in this section.

6.3.2 Material and Methods

Machete seeds were sterilised, germinated and selected as in Section 3.5. Urrbrae loam was autoclaved at 120°C for 1 hour, allowed to cool, mixed thoroughly, sieved through a 2mm sieve and placed in electrical conduit tubes (2.7cm wide by 12.5 cm high) (Section 5.1). One week after sowing 9 replicates of individual seedlings were inoculated with *P. thornei* densities of 0, 5000, 10000, 15000, 20000, 25000 and 30000 in 1ml of water. Plants were grown at 20°C with 12 hours light at an intensity of 65 μEinsteins and harvested 3 and 9 weeks after inoculation. Thus the design was a CRD where the seven densities of the nematode by the two times of harvest was relplicated nine times to give a total of 126 individual plants in tubes. Dry weights of shoot, root and total plants were determined by placing individual plants in alfoil trays in a drying

oven at 80°C for 7 days. Nematodes were extracted in a mister (Section 3.2.2) over 3 days and counted (Section 3.3).

6.3.3 Results

The ANOVA of all variables is presented in Table 6.4. There was a significant 2-way interaction between initial density and harvest for the number of nematodes per root system and the multiplication rate (final no. *P. thornei* per plant/ initial density of *P. thornei* infected per plant), which can be seen in Figs. 6.9 and 6.10. At harvest 1, there was little change in the numbers of nematodes per plant until higher initial densities were reached, while at harvest 2 the plants inoculated with the highest density had significantly higher numbers. A cubic relationship was found to best fit the data at both harvest times ($P<0.05$). The multiplication rate of *P. thornei* for harvest 1 showed little change with initial density, but by harvest 2, there were considerable differences. The number of nematodes per gram root was found to increase over initial density, irrespective of harvest time, although the magnitude of this increase was reduced as density increased (Fig. 6.11). A significantly higher number of nematodes was present within wheat roots at harvest 2 than harvest 1 (Fig. 6.12).

There was no significant 2-way interaction between initial *P. thornei* density and harvest for the total dry weight of the plant. Furthermore, density was not found to effect the total dry weight, however there was a significantly higher total dry weight at harvest 2 than harvest 1 (Fig. 6.13).

A break down of total dry weight of plants to weight of roots and shoots revealed a highly significant 2 way interaction of *P. thornei* initial density by harvest for both variables (Figs. 6.14, 6.15). Shoot growth was significantly higher at harvest 2 than harvest 1 for all densities, however at higher initial densities for both harvests there was a declining trend. At high initial nematode densities beyond 20,000 per plant, the roots weighed significantly less at harvest 2 than at harvest 1. At harvest 1 root growth

increased with densities up to 20000 *P. thornei* per plant, but thereafter was not seen to alter. Harvest 2 showed very little change in root growth over density. There was no evidence of damage to the root system by the nematode at both harvest times.

Table 6.4 : ANOVA for variables measured on Machete after 3 and 9 weeks.

	d.f.	m.s.	v.r.	Prob.
Variable :No. *P. thornei*/plant				
density	6	4.319E+07	18.23	<0.001
harvest	1	2.339E+08	98.76	<0.001
density . harvest	6	6.772E+06	2.86	0.013
Residual	108(4)	2.369E+08		
Total	121(4)			
Variable :No. *P. thornei*/ g plant				
density	6	5.380E+10	4.55	<0.001
harvest	1	3.377E+11	28.56	<0.001
density . harvest	6	1.834E+10	1.55	0.169
Residual	108(4)	1.182E+10		
Total	121(4)			
Variable :Multiplication rate of *P. thornei*/plant				
density	5	0.17895	12.78	<0.001
harvest	1	1.94576	138.94	<0.001
density . harvest	6	0.21555	15.39	<0.001
Residual	92(4)	0.01400		
Total	103(4)			
Variable : Total dry weight/plant (g)				
density	6	0.000589	0.45	0.846
harvest	1	1.053978	800.06	<0.001
density . harvest	6	0.000843	0.64	0.698
Residual	105(7)	0.001317		
Total	118(7)			
Variable : Dry weight shoots (g)				
density	6	0.004051	4.16	<0.001
harvest	1	1.070251	1098.26	<0.001
density . harvest	6	0.002861	2.94	0.011
Residual	110(2)	0.000974		
Total	123(2)			
Variable : Dry weight roots (g)				
density	6	0.002486	16.26	<0.001
harvest	1	0.000131	0.86	0.356
density . harvest	6	0.001643	10.75	<0.001
Residual	105(7)	0.000152		
Total	118(7)			

Fig. 6.9 : The effect of initial density of *P. thornei* and harvest time on the number of *P. thornei* extracted from Machete.

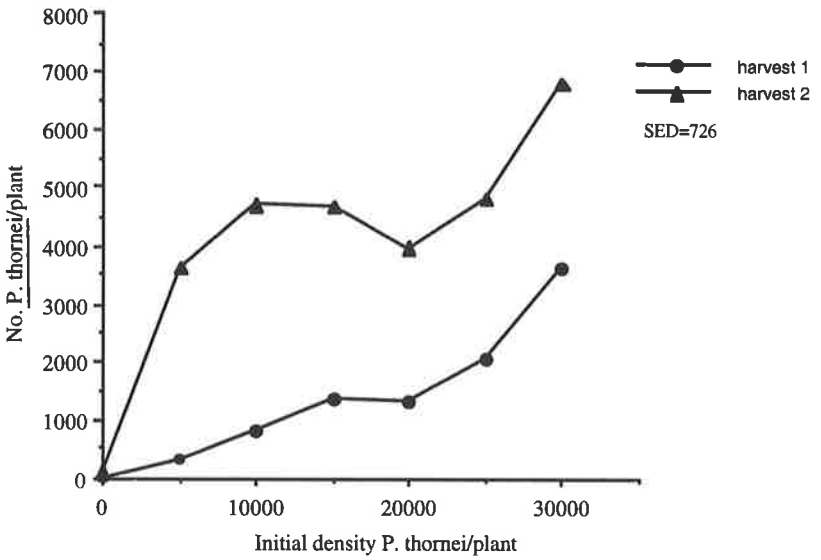


Fig. 6.10 : The effect of initial density *P. thornei* and harvest time on nematode multiplication rate on Machete.

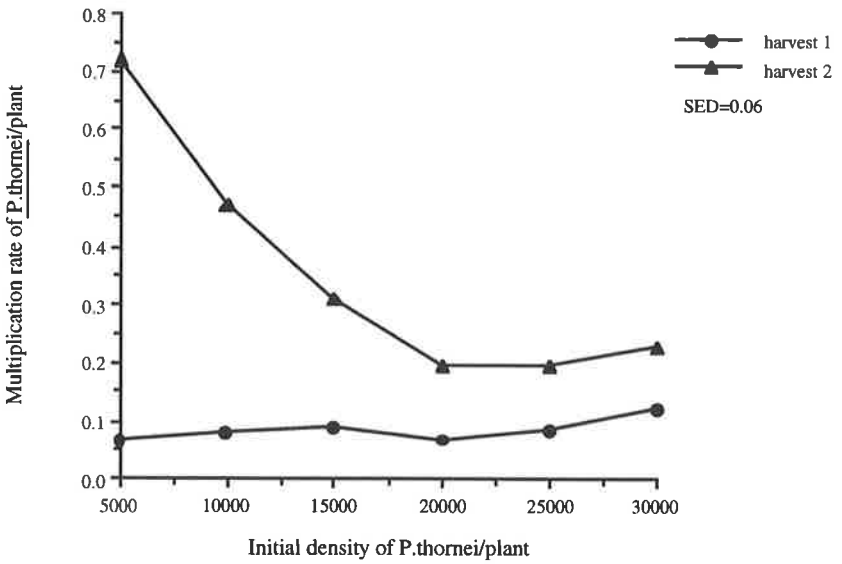


Fig. 6.11 : The effect of initial density of *P. thornei* on the number *P. thornei* per gram dry weight Machete.

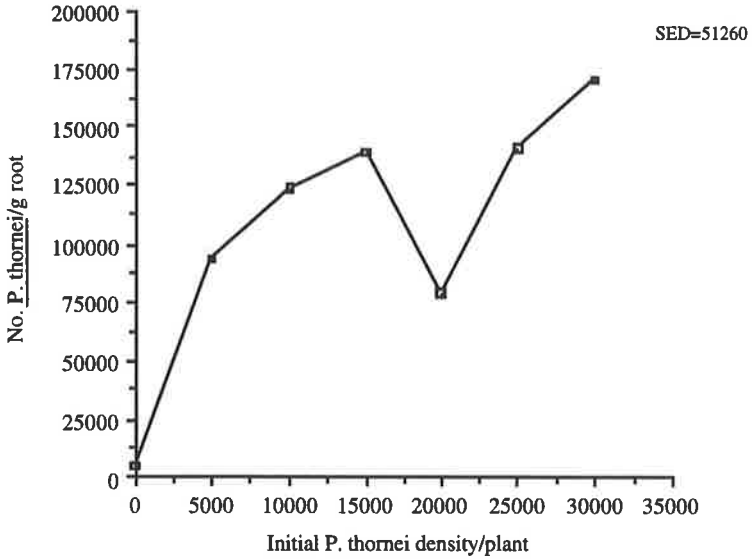


Fig. 6.12 : The effect of harvest time on the number of *P. thornei* per gram dry weight Machete.

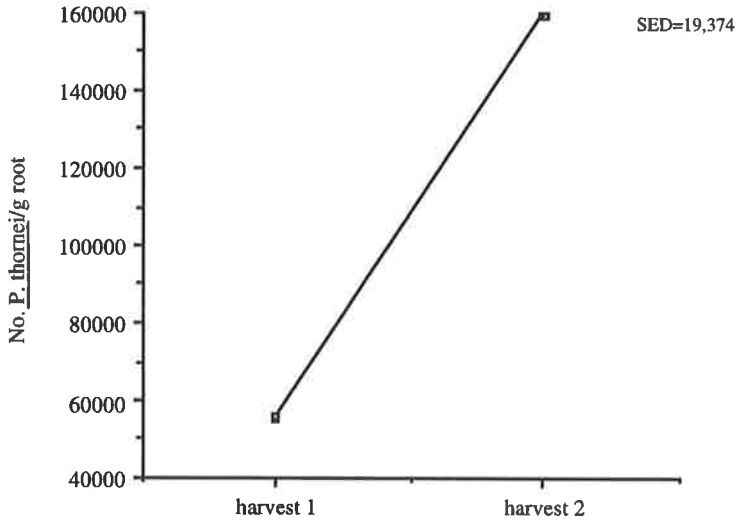


Fig. 6.13 : The effect of harvest time on the total dry weight of Machete.

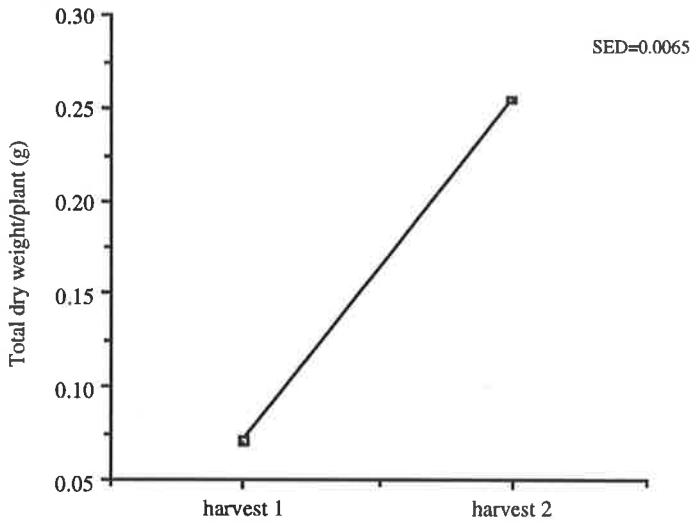


Fig. 6.14 : The effect of initial *P. thornei* density and harvest time on the dry weight shoots per Machete plant.

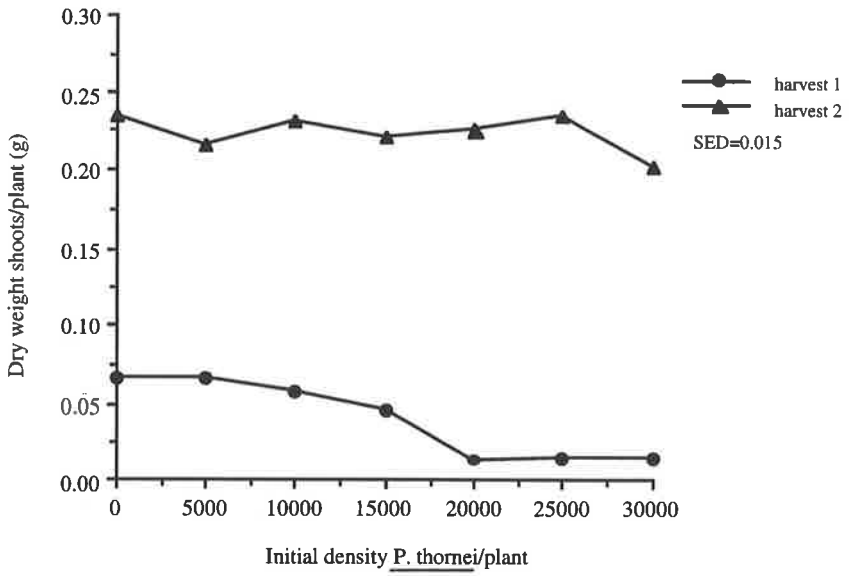
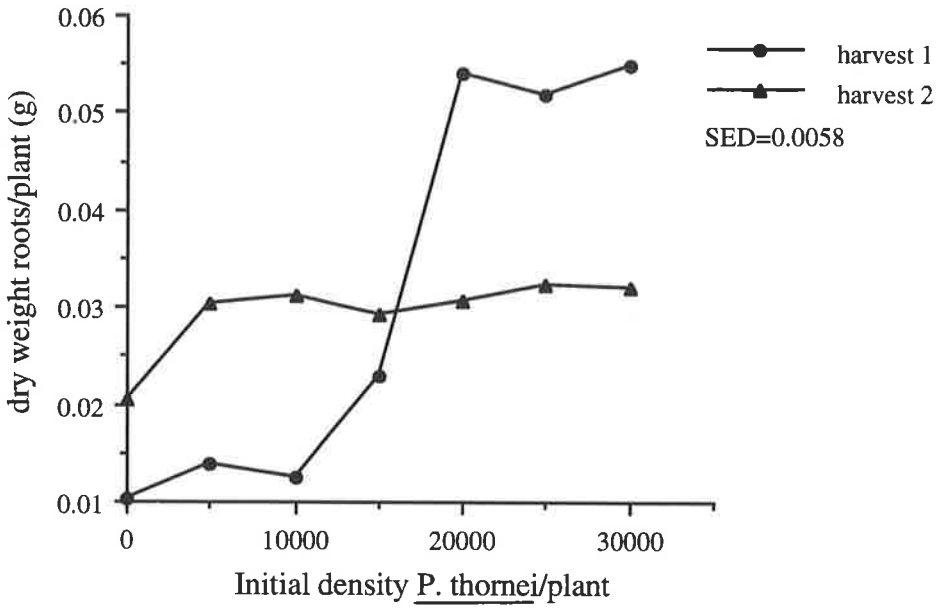


Fig. 6.15 : The effect of initial *P. thornei* density and harvest time on the dry weight roots per Machete plant.



6.3.4 Discussion

Once again it appears that *P. thornei* did not cause yield damage to Machete with regard to the growth parameters measured under the experimental conditions used here. Although from previous experiments sieved Urrbrae loam gave a penetration efficiency of 22% with *P. neglectus* in polyethylene tubes, it appeared to range from 8-12% over the densities used in this experiment. This is very low and may possibly explain why losses in yield are not found. The fact that little damage on the roots was evident also supports this.

In contrast to reductions in yield, stimulatory responses in shoot growth occurred as initial density increased. This stimulus in shoot growth confirms previous findings in Section 6.1. Stimulation of root growth appeared at harvest 1, but by the second harvest at 9 weeks this stimulus had ceased. This is in contrast to the work of Nicol (1991), where the wheat host Warigal suffered significant yield reductions with 15,000 *P. thornei* per plant by 9 weeks, also using Urrbrae loam grown in similar conditions.

P. thornei displays attributes of the models proposed for nematode population dynamics (Section 2.9). *P. thornei* populations in the plant increased with increasing initial *P. thornei* density at both harvest times. However, initial densities failed to reach the equilibrium level where final and initial nematode populations are equal (Fig. 2.3). The density dependent relation for plant parasitic nematodes with regard to multiplication rate (Brown and Kerry, 1987) holds true for *P. thornei* (Fig. 6.10). At harvest 1, there was probably little time for multiplication of *P. thornei*. However, by harvest 2 (9 weeks) the low initial densities led to the maximum multiplication rate, and as initial density increased the multiplication rate of *P. thornei* was significantly reduced, presumably due to increased competition between individuals accompanied with a decreasing food resource.

At 3 weeks initial densities of 20,000 *P. thornei* per plant caused a stimulus in growth. This is possibly a response to initial penetration. Between the two harvest times the nematodes multiplied, reduced stimulation of growth of the plant and in turn reduced nematode multiplication. In this instance, no difference was found between the growth of inoculated and control plants, but if this experiment had been conducted for a longer period this may have been changed.

6.4 Population Dynamics and Pathogenicity of *P. thornei* on Warigal at 20°C

6.4.1 Introduction

This experiment examined the effect of initial *P. thornei* density on the nematode population dynamics and the yielding capacity of wheat for the whole growing season. The previous two experiments were using the wheat cultivar Machete (Section 6.1, 6.2). Results presented in Section 6.1 suggested that the initial density did not limit the *P. thornei* multiplication rate after 4 months in polyethylene bags. Further investigations (Section 6.3) revealed that poor nematode penetration could have accounted for this. Adjusting the experimental technique in order to improve penetration efficiency, accompanied with a range of nematode densities used, indicated that the multiplication rate could be limited with 10000 *P. thornei* per plant after 9 weeks of growth in sieved Urrbrae loam. However, reduction in growth of the host, Machete was negligible in the presence of *P. thornei* . In contrast, Nicol (1991) demonstrated yield reductions on a similar wheat host, Warigal, over the same time frame and with similar soil. This experiment examined the population dynamics and yield relations of the wheat cultivar Warigal 4 months post inoculation with *P. thornei.*

6.4.2 Materials and Methods

The wheat cultivar Warigal was selected due to its known intolerance to *P. thornei* (Nicol, 1991; Taylor, pers. comm.). Warigal seeds were sterilised, germinated and selected as in Section 3.5. Sand collected from Palmer was heat treated at 65°C for 45 minutes and allowed to cool. It was mixed thoroughly and sieved through a 2mm sieve. The sand was then placed into eighty 14cm diameter pots with wood chips in the base (2-3 cm height) to enable drainage. Selected Warigal seedlings were placed centrally in the pots (1 seedling/pot). *P. thornei* was extracted from carrot cultures as described in Section 3.1. One week after sowing 8 replicates of individual seedlings were inoculated with 10 *P. thornei* densities (0, 500, 1000, 3000, 5000, 7000, 9000, 12000, 15000 and 20000) in 1ml of water. Plants were arranged as a CRD and grown

at 20°C with 12 hours light at 65µ Einstein's intensity (Plate 6.4). They were harvested 4 months after inoculation.

The following parameters of growth were recorded for individual plants: number of leaves on the main tiller of each plant, number of tillers per plant (excluding main tiller); maximum height of shoots per plant; number of seminal roots per plant; maximum length of seminal roots per plant; number of nodal roots per root system; number of seeds per main tiller head; dry weight heads per plant; dry weight shoots per plant (g); total foliar dry weight (g) (dry weight fertile heads per plant + dry weight shoots per plant); dry weight roots per plant (g); total dry weight per plant (g) (total foliar dry weight + dry weight roots per plant); days to anthesis; number of nematodes per root system; number of nematodes per gram of root; nematode multiplication rate (number of nematodes per root system/ nematode initial inoculum density). Dry weight of leaves and roots were determined by placing individual plants in alfoil trays in a drying oven at 80°C for 7 days. Nematodes were extracted in a mister (Section 3.2.2) over 3 days and counted (Section 3.3). Plants were also checked for any evidence of lesioning on the root systems.

Nicol (1991), similarly conducted an experiment examining the pathogenicity of *P. thornei* on Warigal in small electrical conduit tubes (2.7cm width x 12.5cm height) using 2 nematode densities 1000 and 15000 *P. thornei* per plant harvesting plants at 5 and 9 weeks. Eleven comparable quantitative characters were examined in this experiment.

6.4.3 Results

All results were analysed as a CRD, however because of the number of variables, only the F probabilities from the ANOVA are given (Table 6.5). Simple linear and polynomial regressions were fitted to the data where the initial *P. thornei* density was

found to significantly affect the variable. In addition Table 6.5 gives some reference to the comparable variables examined by Nicol (1991).

The initial density of *P. thornei* had no significant effect on the number of tillers per plant (excluding main tiller), maximum height (cm) of shoots per plant, number of seminal roots per plant, maximum length of seminal roots per plant, dry weight heads per plant (g) and total foliar dry weight (g). Density of *P. thornei* also had no significant effect on the anthesis date of Warigal.

Table 6.5 : Summary ANOVA of the significance of the growth variables measured (in addition to comparable parameters previously examined by Nicol, 1991).

Growth Variable Measured	F prob.	polynomial relationship	Fig.	Nicol 1991
Number leaves on the main tiller of each plant	<0.001	linear	6.16	Y
Number tillers per plant (excluding main tiller)	0.064	-	-	Y
Maximum height shoots per plant(cm)	0.295	-	-	Y
Number seminal roots per plant	0.085	-	-	Y
Maximum length seminal roots per plant(cm)	0.783	-	-	Y
Number nodal roots per root system	0.002	cubic	6.17	Y
Number grains per main tiller head	<0.001	cubic	6.18	N
Number heads per plant	0.056	linear	6.19	N
Dry weight heads per plant(g)	0.134	-	-	N
Dry weight shoots per plant(g)	0.059	linear	6.20	Y
Total foliar dry weight(g)	0.099	-	-	N
Dry weight roots per plant(g)	0.029	quartic/linear	6.21	Y
Total dry weight per plant(g)	0.070	quartic	6.22	Y
Days to anthesis	0.427	-	-	N
Number *P. thornei* per root system	<0.001	cubic	6.23	Y
Number *P. thornei* per g dry root	<0.001	linear	6.24	Y
P. thornei multiplication	<0.001	quartic	6.25	N

Y = previous variable examined
N = previous variable not examined
- = not applicable

The number of leaves on the main tiller of each plant was found to decline significantly with greater than 5000 *P. thornei* per plant (Fig 6.16), with a 30% reduction in leaf number at 20000 *P. thornei* per plant. The number of nodal roots was significantly affected (<0.002) by *P. thornei* density as illustrated in Figure 6.17. This significance was due to a 17% peak in growth stimulation at 3000 *P. thornei* per plant followed by a

gradual decline in the number of nodal roots per plant to a 17% reduction (from 11 nodals to 9) at the highest *P. thornei* density.

Fig. 6.16 : The effect of initial *P. thornei* density on the number of leaves on the main tiller of each Warigal plant.

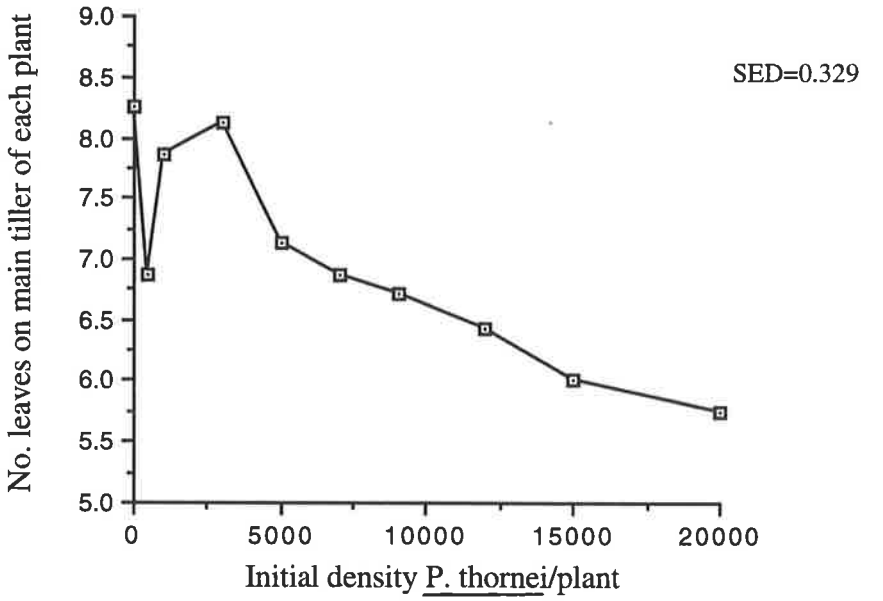


Fig. 6.17 : The effect of initial *P. thornei* density on the number of nodal roots per Warigal plant.

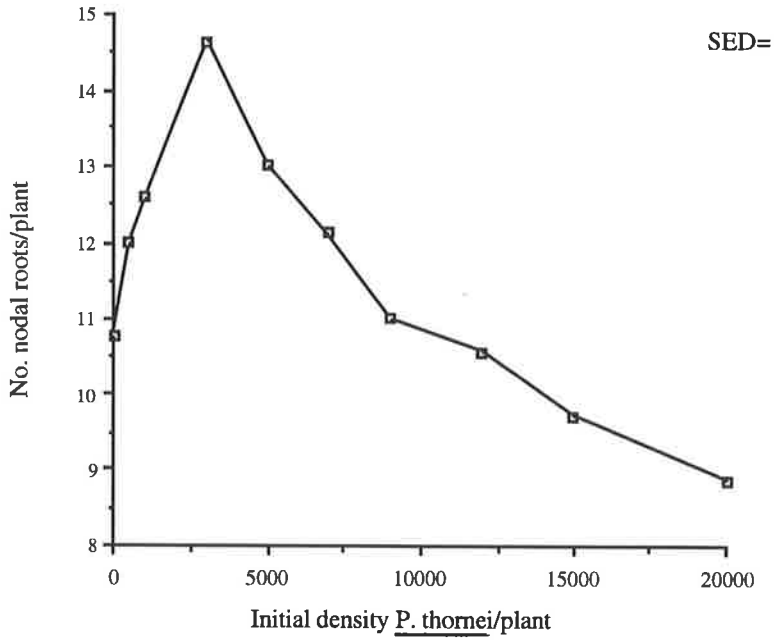


Fig. 6.18 : The effect of initial *P. thornei* density on the number of seeds on the main Warigal tiller.

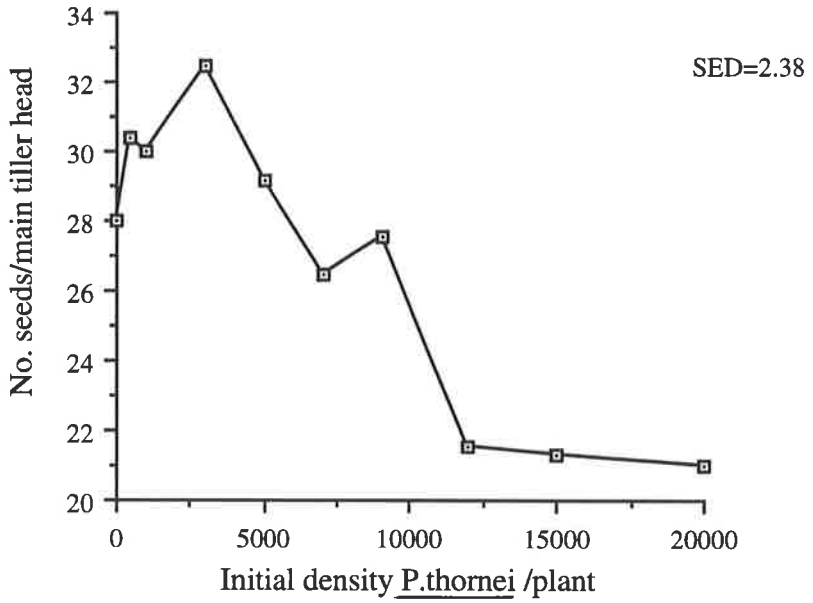


Fig. 6.19 : The effect of initial *P. thornei* density on the number of heads on each Warigal plant.

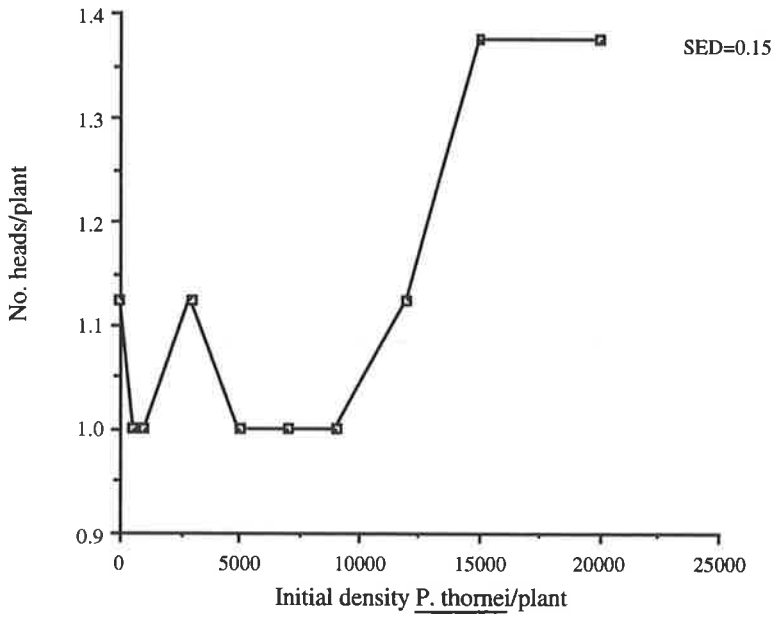


Fig. 6.20 : The effect of initial *P. thornei* density on the dry weight of shoots per Warigal plant.

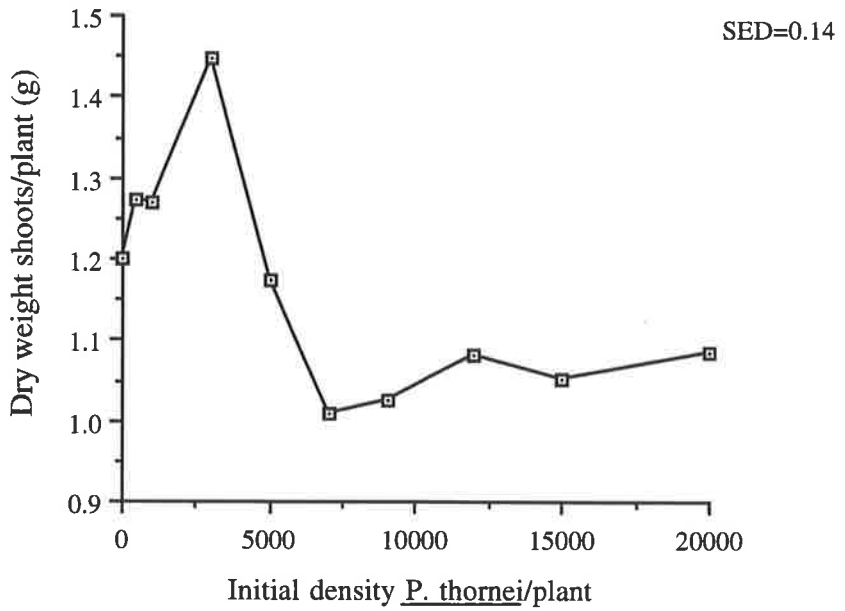


Fig. 6.21 : The effect of initial *P. thornei* density on the dry weight of roots per Warigal plant.

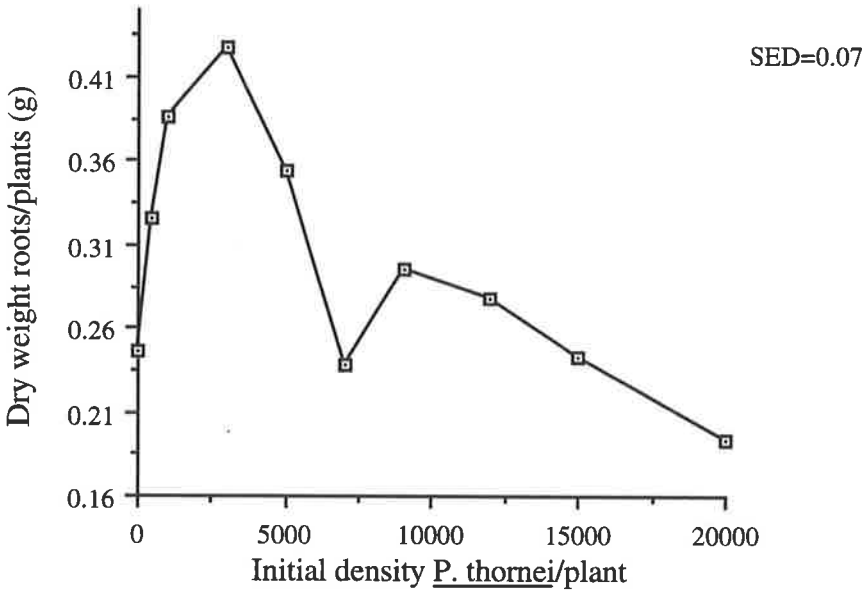


Fig. 6.22 : The effect of initial *P. thornei* density on the total dry weight per Warigal plant.

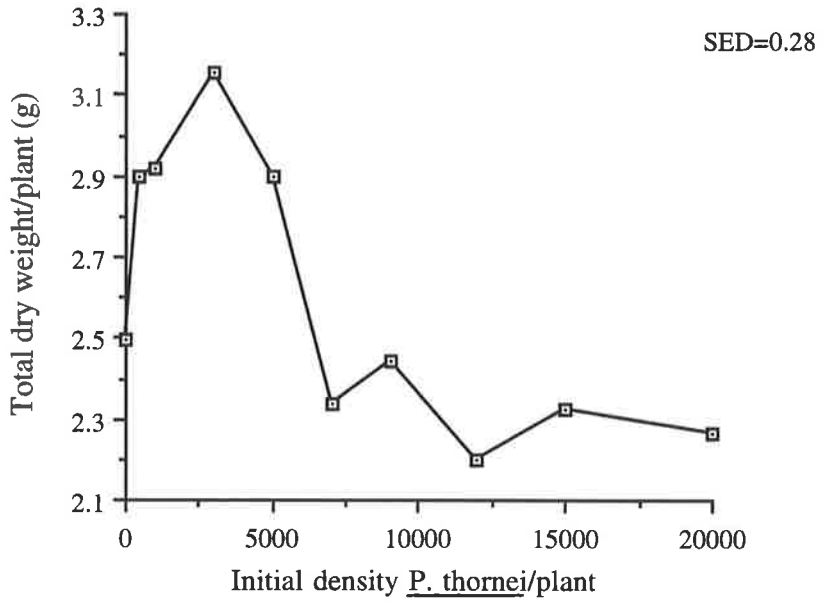


Fig. 6.23 : The effect of initial *P. thornei* density on the number of nematodes extracted per gram Warigal.

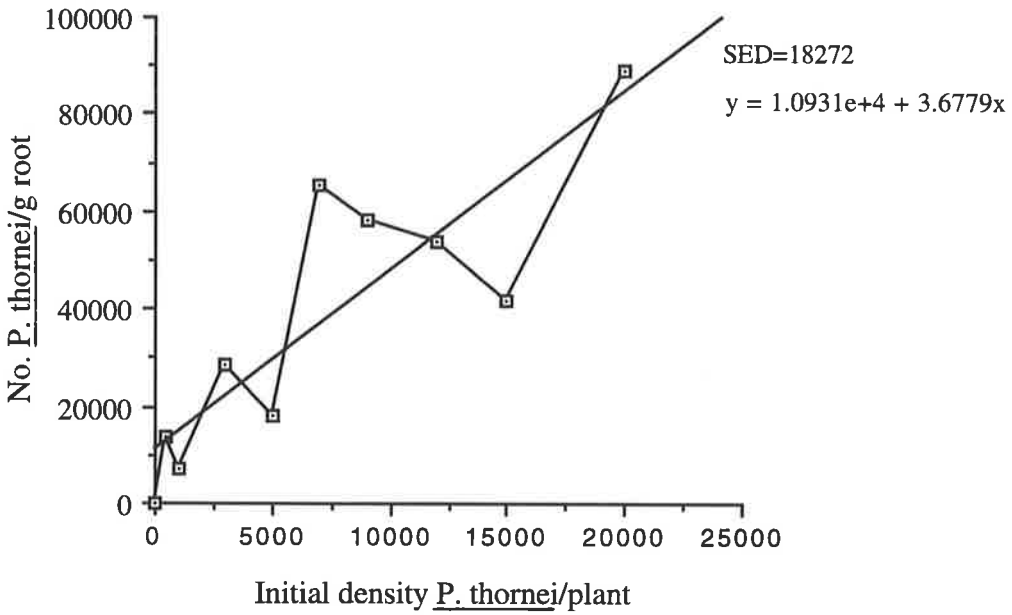


Fig. 6.24 : The effect of initial *P. thornei* density on the number of *P. thornei* per gram Warigal.

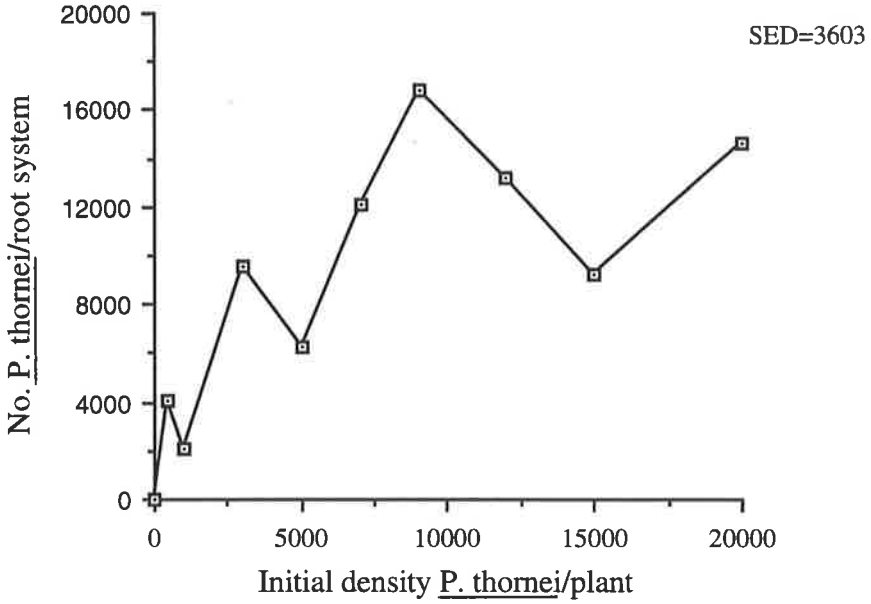
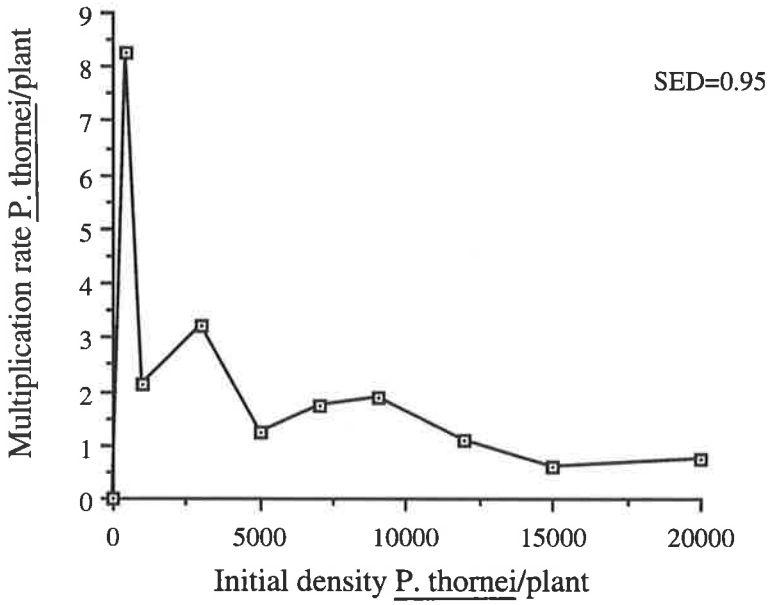


Fig. 6.25 : The effect of initial *P. thornei* density on the multiplication of *P. thornei* per Warigal plant.



Changes in the number of seeds per main tiller head was highly significant and the number of heads per plant was marginally significant. The number of seeds (Fig. 6.18) was significantly reduced from 28 to 21 seeds per head, a 25% reduction by densities at

and greater than 12000 *P. thornei* per plant. In contrast, the number of heads was increased at densities greater than 12000 *P. thornei* per plant (Fig. 6.19).

Dry weight of the shoots per plant (g) was found to be marginally significant (Fig. 6.20). Similarly, the dry weight of the roots per plant (g) was found to be highly significantly affected by increasing *P. thornei* density (Fig. 6.21). Both roots and shoots display similar trends at low initial densities with a stimulus in growth up until 3000 *P. thornei* per plant with the stimulus greater with roots (43%) than shoots (17%). This is also reflected in changes with total dry weight (Fig. 6.22) although this was non-significant. At *P. thornei* densities above 7000 per plant there was a reduction in growth between 10-16% in the shoots (Plate 6.4). However, with the roots, growth was stimulated until *P. thornei* densities beyond 12000 per plant were reached, with plant growth reduced by 22% only at the highest density of 20000 *P. thornei* per plant (Plate 6.5).

A highly significant effect on the number of *P. thornei* per root system, per dry gram of plant and *P. thornei* multiplication is illustrated in Figs. 6.23, 6.24 and 6.25. As evident from Figs. 6.23 and 6.24, an almost linear relationship exists with the number of *P. thornei* extracted per root system and per gram root in relation to initial density up until the initial density of 7000 *P. thornei* per plant.

The population dynamics of the nematode are further illustrated in Fig. 6.25, where the multiplication rate (number of *P. thornei* extracted from the root system divided by the initial density of *P. thornei*) in relation to initial nematode density is shown. Very low initial densities were associated with a high nematode multiplication of 8 times, but at 1000 *P. thornei* per plant there was a severe restriction in multiplication to 2 times.

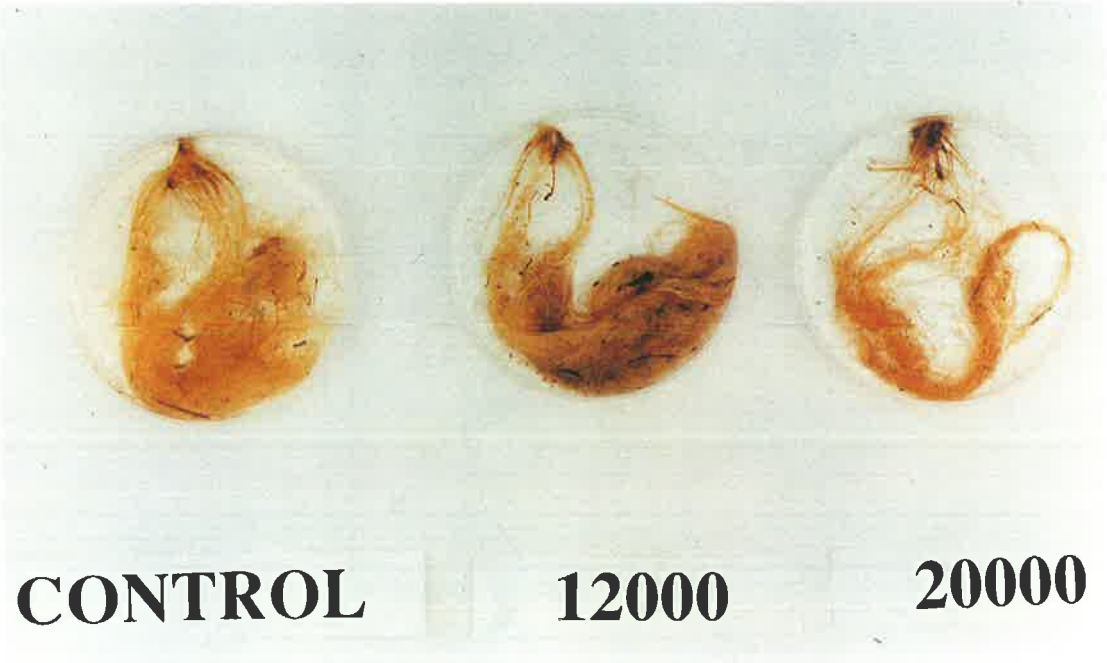
Qualitative observation of the root system for evidence of lesioning was found to be variable between replicates. Evidence of damage was rarely seen at densities up to

Plate 6.3 : The experimental set up of the pots used to assess the relation between initial density and growth of the wheat cultivar Warigal. Plants within pots at various initial *P. thornei* densities were arranged as a CRD and grown in a controlled growth room at 20°C. Light was supplied by fluorescent light tubes (65µ Einsteins) and plants watered with tap water whenever necessary.

Plate 6.4 : Representative replicates of the wheat cultivar, Warigal over a range of initial *P. thornei* densities after 4 months. At low initial densities (< 5000 *P. thornei*/plant) a stimulus in shoot fresh weight can be seen. The higher initial densities were associated with a corresponding decrease in shoot dry weight. The maximum height per plant showed a trend of decreasing with increasing initial density, however this was not significant.

Plate 6.5 : Representative replicates of the wheat cultivar Warigal for 0 nematodes (left), 12,000 *P. thornei*/plant (middle) and the highest initial density, 20,000 *P. thornei*/plant (right). A highly stimulatory response to growth was seen up to 12,000 *P. thornei*/plant but a reduction in root growth is found with the highest initial density. This reduction is highly attributable to a loss of the finer branching lateral roots.


CONTROL
1000
4000
7000
12000
15000
20000


CONTROL
12000
20000

3000 *P. thornei* per plant. This damage may be absent due to the compensatory stimulation of root growth (Fig. 6.21). Densities above and beyond 5000 *P. thornei* per plant were associated with some dark brown necrotic lesioning, predominantly on the seminal root system. Most replicates of the high densities (above 15000 *P. thornei* per plant) demonstrated extensive lesioning and a lack of fine lateral root growth, as illustrated in Plate 6.5.

6.4.4 Discussion

The results clearly demonstrate that *P. thornei* can significantly affect the growth of the host Warigal. In addition, the population dynamics demonstrated here agree with models previously documented for other plant parasitic nematodes (Section 2.9). The results, although conducted in the laboratory with unrestricted water, suggest that *P. thornei* is able to multiply by a factor of 8 during the growing season at low initial densities (500 *P. thornei* per plant). Higher densities reduce nematode multiplication, with 2.12 times multiplication at 1000 *P. thornei* per plant, and only 1.1 at 12000 *P. thornei* per plant. Densities beyond 15000 *P. thornei* per plant inhibit multiplication, so that *P. thornei* is unable to replace its initial population.

Considering the relation between initial and final populations, *P. thornei* was found to increase in a linear fashion reaching an equilibrium density at the initial density of 12000 *P. thornei* per plant. This equilibrium can be attributed to increasing competition between individuals and decreasing food supply (Jones and Kempton, 1978). However, the root dry weight is unaffected except at the highest nematode population densities, implying that a reduction in nematode numbers at high densities is associated with excess nematodes for the available food. Nicol (1991) noted similar trends, with a reduction in root dry weight and multiplication rate with 15000 *P. thornei* per plant, however this effect was only significant at 25°C as opposed to 20°C. Nicol (1991) further suggested the damage at 25°C on Warigal may be greater possibly

because it is more favourable for nematode development than either 15 or 20°C. In addition the growth of the wheat host is less vigorous at 25°C compared with 20°C.

The literature suggests that several foliar measurements are affected in the presence of *P. thornei* (Section 2.4). The evidence presented here and also by Nicol (1991) found neither the number of tillers per plant or the height of the plant were affected by *P. thornei,* although there was a suggestion for a reduction in height. Nicol (1991) found a stimulatory response to the number of nodals at low initial nematode densities, however the fact that nodal roots of wheat plants are greatly reduced in the presence of high *P. thornei* densities could be expected to have large effects on the final grain yield of wheat, particularly in drought conditions. However, although the number of seeds per wheat head was reduced with high populations of *P. thornei,* the number of heads per plant was found to increase, suggesting the developmental physiology of the wheat plant was acting in a compensatory manner, with the overall total foliar weight per plant unaffected by initial density.

As with many of the other experiments conducted in this section, stimulation of growth for almost all growth variables occurs with the lower initial densities of the nematode (Chitwood and Buhrer, 1946; Chitwood and Feldmesser, 1948; Chitwood and Esser, 1957 and Peter, 1961; in Wallace, 1963). This is a common occurrence with many plant parasitic nematodes. Although both the root and shoot dry weights were significantly affected the total dry weight was not. It is possible that the degree of variability, expressed as the SED in this instance, was too great between individual replicates to distinguish significant differences. Unfortunately, this variability is common with nematodes, particularly with the migratory forms, due to the differences in initial penetration between individual replicates which ultimately affect the subsequent multiplication and damage caused to the host.

6.5 General Discussion

The four experimental sections covered in the Chapter have involved the use of different container size and, in most cases different soil types. The nematode inoculum was derived from the same method, however different cultures were used for each experiment and may have varied with respect to fitness thereby affecting pathogenicity. Because of the above variables extreme care should be taken when comparing results from these experiments. An example of different results is between Section 6.1, where a linear relationship was seen between multiplication rate and initial nematode density (Fig. 6.2), and the last experiment (Fig. 6.25). Possible explanations can be presumed to be associated with the different soil type, container size and possible intolerances of the two wheat cultivars, Machete and Warigal. This infers the need for a greater understanding of the relation between the nematode, host and the surrounding environment.

Nevertheless the results presented clearly show that *P. thornei* can affect many growth variables and also displays population dynamic models typical of those documented for other nematode species (Ch 2.9). In most cases with roots and shoots, *P. thornei* was found to cause a stimulatory effect on growth at low initial nematode densities. This effect in general was found to decline with both increasing *P. thornei* density and time. The population dynamics of the nematode were found to vary between experiments, probably due to the different experimental technique used.

Chapter 7

Field Population Dynamics and Yield Relations of *P. thornei*

7.1 Introduction

The only Australian field population study of *P. thornei* was carried out by Pattison (1993) on wheat in New South Wales . *P. thornei* had little multiplication between June and September due to low temperatures. Nematode multiplication occurred toward maturity of the crop but was dependent on environmental conditions.

As Van Gundy (1972) observed, predictions of disease potential are based upon knowledge of pathogen population dynamics and techniques for estimating economic thresholds (Section 2.8). Accuracy of predictions depends largely upon the reliability of the nematode assay data on which they are based. Wide variation in soil distribution of nematodes creates serious sampling problems (Barker and Nusbaum, 1971).

Meaningful population studies are possible in pots and small field plots. However, large plots are generally inappropriate because populations are usually patchy and the multiplication rate decreases as the population density increases (Section 2.8). The average multiplication rate for a field as a whole may give little indication of what is happening in its parts (Jones and Kempton, 1978). Assay procedures are often too insensitive to measure populations prior to planting. Also the infectivity of the nematodes present is usually unknown. Thus, refinement of the sampling and assay techniques may well play a major role in determination of economic thresholds (Nusbaum and Ferris, 1973).

The most convenient time scale to determine population dynamics in annual crops is one year, when P_i is the "initial" population density at planting and P_f is the "final" population one year later (preferably at planting time in the following year to avoid

problems of aggregation) (Jones and Kempton, 1978). *Pratylenchus* is capable of multiplying for several generations during a single season, therefore they spread only from plant to plant due to their small size and relative immobility. *P. thornei* is found randomly distributed at sowing time, but distributions of nematodes become aggregated following multiplication and development in the wheat crop (Pattison, 1993). Sampling plans should be custom made for particular situations, since the relationship between the number of sampling cores and the relative error changes in response to many factors, including nematode species, density, field size, crop and soil type (McSorley and Parrado, 1982). In addition, all techniques of extraction from soil yield only a fraction of the nematodes present. Soils differ and the techniques suitable for a light soil may be inadequate for a heavy soil. It is important, therefore, to estimate the population of nematodes by the consistent use of a particular technique. Determination of the population of *P. thornei* usually involves collection of soil cores from the upper 15-20 cm of the profile where roots are present. Eastwood *et al.* (1994) found more than 50% of the total *P. thornei* population below 30 cm in Victoria, while Peck *et al.* (1993) found that in order to accurately quantify *P. thornei* populations on the Darling Downs of Queensland, soils needed to be sampled to a depth of 120 cm. In the Barossa Valley of South Australia on clay based soils, S. Taylor (pers. comm.) found 78% of *P. thornei* in the top 20 cm of the profile. Similarly, 80% of the closely related species, *P. neglectus*, were found in the top 15 cm of a sandy soil on the Eyre Peninsula (S. Taylor, pers. comm.).

Understanding of population dynamics and crop yield reductions in South Australia is required in order to design possible management strategies to control *P. thornei* populations and keep them below the damaging threshold. *P. thornei* is known to limit yield of wheat (Section 2.8). There has been no examination of the population dynamics of this nematode and the related yield loss in wheat under South Australian conditions. The work described in this chapter is an attempt to provide that information.

7.2 Materials and Methods

A study of the population dynamics of *P. thornei* was conducted over a two year period on a field trial site at Tanunda, in the Barossa Valley (80 km north of Adelaide) in South Australia. Originally two field sites were chosen. However, while the Mallala site (also in the mid-north of South Australia) was sown for two years, it suffered a mouse plague in 1993 and a severe drought in 1994. The Tanunda field site was selected because the soil had high populations of *P. thornei*, there was low incidence of root diseases (*Rhizoctonia spp.*, *Gaeumannomyces graminis* and *Heterodera avenae*, refer to Table 7.2) and the paddock was generally clean (weed free) with a known cropping history.

7.2.1 General Method

The aim of the field trial was to determine the nematode density at which damage occurred. The first year of the trial included sowing 12 host species with differing degrees of susceptibility selected from work done in Chapter 5. These varieties were grown in replicated field plots in order to establish a range of initial densities for the second year of the trial. The cultivars were selected from the information presented in Section 5.1. The initial density (P_i year 1) was determined in each plot at the start of the first season and subsequent grain yields in each plot were measured. The yield in each plot was then related to the initial density (P_i year 1).

Having established a range of initial *P. thornei* densities, in the second year one of three wheat cultivars was sown into randomly selected plots across the whole trial area. Once again *P. thornei* initial densities were determined in each plot, designated as (P_f year 1) or as (P_i year 2) and the final grain yields in each plot were determined. In addition the following year the (P_f year 2) or (P_i year 3) was also determined.

The multiplication rate (P_i year 2 / P_i year 1) could then be related to the range of host species sown in the first year, and the relationship between P_i year 2 and the three wheat

hosts considered for the second year. Furthermore, the P_f year 2 or P_i year 3 was determined at the beginning of the following season, thereby also enabling the relationships between multiplication rate (P_i year 3 / P_i year 2) and P_i year 2 to be examined.

7.2.2 Sampling Device

The method of sampling is extremely important to ensure the accuracy of the populations extracted. The majority of *P. thornei* at Tanunda were located in the top 20 cm of the soil profile (S. Taylor, pers. comm.). Hence this layer of soil was sampled. A modified soil coring device was used based on the model described by Thompson *et al.* (1988), illustrated in Fig. 7.1. Each core was taken by pushing the sampling device 20cm into the ground, rotating the handle 180°, lifting the device out of the soil and removing the soil by hand into a collection bag.

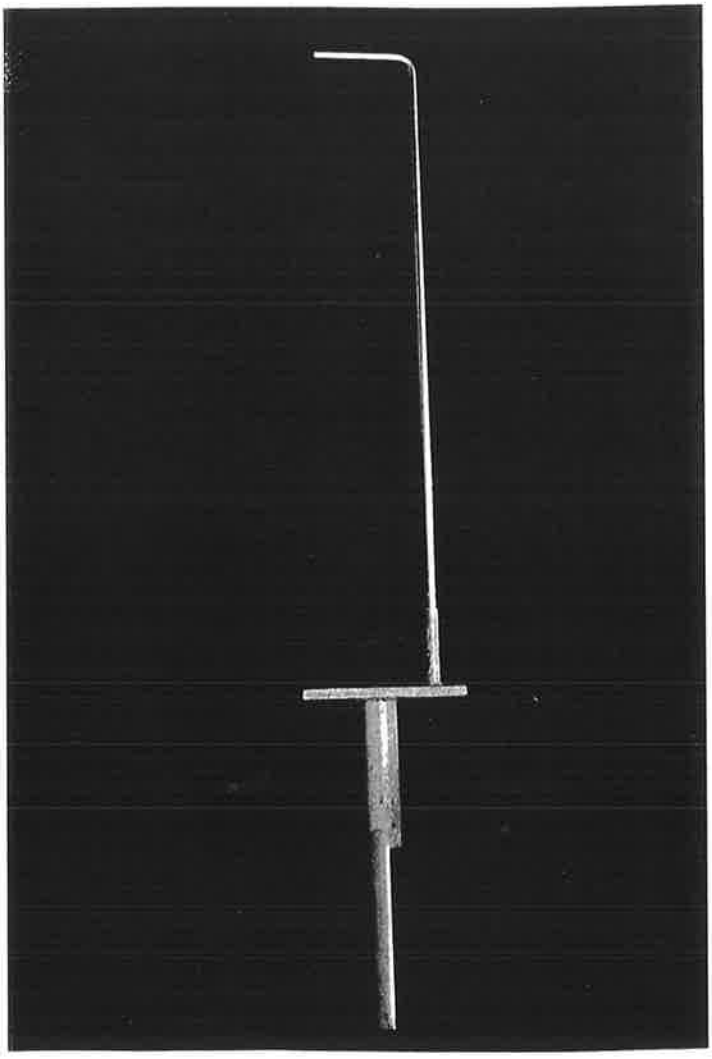


Fig. 7.1 The sampling device used to take soil cores from the two year trial conducted at Tanunda (1cm=6.5cm).

7.2.3 Field Trial Layout

The trial area was 80 m x 48 m, comprising 192 plots including 62 border plots. Each plot was 2 m x 10 m, with the sample area being 1.5 m x 8 m. The border plots in both years were sown with the wheat cultivar Janz.

The trial was set up as a randomised complete block design (RCBD) in the first year. Ten replicates of each of the 12 varieties, plus a fallow treatment, were sown (Table 7.1, Plate 7.1, Fig. 7.2).

Table 7.1 : The varieties, sowing rates and nematode susceptibilities used in the two year field trial at Tanunda.

no.	variety	sowing rate g/15m^2	no seeds/m^2	sowing rate kg/ha	nematode susceptibility
1st Year					
1.	Glenelg (Linseed)	67.5	-	45	low
2.	Barossa (Canola)	7.5	-	5	low
3.	Grimmett (barley)	115.8	200	77	low-mod.
4.	Echidna(oat)	150	250	100	low-mod.
5.	Yalloroi(durum)	130.5	200	87	low-mod.
6.	Currency (triticale)	138	200	92	mod.
7.	Tahara (triticale)	145.6	200	97	mod.
8.	GS50A (wheat)	123.6	200	82	mod.
9.	Molineux (wheat)	100.4	200	67	mod.
10.	Machete (wheat)	111.3	200	74	high
11.	Spear (wheat)	117.6	200	78	high
12.	Warigal (wheat)	118.7	200	79	high
13.	Fallow				
B=border Janz wheat		120	200	79	unknown

no.	variety	sowing rate g/15m^2	no seeds/m^2	sowing rate kg/ha	nematode susceptibility
2nd Year					
1.	Warigal (wheat)	118.7	200	79	high
2.	AUS4930 (wheat)	95	200	79	low
3.	GS50A (wheat)	123.6	200	82	low
B=border Janz wheat		120	200	79	unknown

Plate 7.1: The field trial layout at Tanunda in the first year of the experiment, taken 5 months after sowing. Plots (2 x 10m) of the 13 different treatments were arranged as a RCBD.

Plate 7.2 : A representative picture of a lesioned wheat root system taken 5 months after growth. In this case the cultivar is Spear, showing dark brown lesioning on both the main seminal roots, extending to the finer lateral branches (6X magnification).

First Year (1993)

	1	2	3	4	5	6	7	8
1	B	B	B	B	B	B	B	B
2	B	10	11	12	8	10	8	B
3	B	11	8	9	7	6	4	B
4	B	12	2	10	9	9	5	B
5	B	3	4	7	6	10	7	B
6	B	6	5	5	13	4	6	B
7	B	1	6	13	11	7	1	B
8	B	13	3	6	1	13	2	B
9	B	9	13	2	3	2	11	B
10	B	2	10	12	5	3	12	B
11	B	5	9	13	10	12	11	B
12	B	4	7	5	2	9	4	B
13	B	7	11	4	4	11	3	B
14	B	8	2	9	2	8	5	B
15	B	13	4	3	11	1	6	B
16	B	7	1	11	12	5	10	B
17	B	12	12	2	5	6	13	B
18	B	5	8	6	7	9	9	B
19	B	1	11	10	1	3	7	B
20	B	6	3	7	3	12	1	B
21	B	10	1	1	13	13	2	B
22	B	3	4	8	8	10	8	B
23	B	9	8	12	4	B	B	B
24	B	B	B	B	B	B	B	B

Second Year (1994)

	1	2	3	4	5	6	7	8
1	B	B	B	B	B	B	B	B
2	B	3	3	2	1	2	2	B
3	B	2	1	2	2	3	1	B
4	B	1	1	3	2	1	2	B
5	B	2	3	1	1	2	3	B
6	B	1	1	1	1	2	1	B
7	B	3	3	1	1	1	3	B
8	B	1	2	2	1	1	3	B
9	B	3	1	1	1	2	1	B
10	B	1	1	1	1	1	3	B
11	B	3	1	1	1	1	2	B
12	B	3	1	1	1	1	1	B
13	B	3	1	1	3	3	2	B
14	B	2	3	1	1	1	1	B
15	B	1	1	1	3	2	1	B
16	B	1	1	1	1	2	1	B
17	B	1	3	2	2	2	1	B
18	B	1	1	3	2	3	1	B
19	B	1	1	1	2	1	1	B
20	B	1	3	2	3	2	2	B
21	B	1	1	1	1	1	3	B
22	B	1	1	3	3	3	1	B
23	B	3	2	2	2	B	B	B
24	B	B	B	B	B	B	B	B

Fig 7.2 : Layout of Tanunda field trial for the 2 consecutive years. (refer to Table 7.1 for numerical explanation of cultivars).

The following year, 3 wheat varieties were sown across the previous year's trial. The 3 varieties were allocated to individual plots on the basis of the previous year's rotation and initial nematode density, in order to give an even representation of both. Thirty plots each of AUS4930 (Section 5.2), the variety thought to be resistant to *P. thornei*, and the *P. thornei* resistant selection from Queensland, GS50A, plus 70 plots of a known intolerant, susceptible South Australian cultivar, Warigal, were sown (Table 7.1). The layout of the varieties over the two years is illustrated in Fig. 7.2.

The various treatments applied to both trial sites over the two year duration of the trial are summarised in Table 7.2. The rainfall and temperature for Tanunda is presented in Fig. 7.4 and 7.5.

Table 7.2 : The various treatments applied to the Tanunda field trial site

Treatments	**1993** **(13 varietal treatments)**	**1994** **(3 varietal treatments)**
Heterodera avenae (CCN) *Rhizoctonia*	0 0	0 0
sowing date	2/6/93	16/6/94
fertiliser application	130 kg/ha DAP at sowing	130kg/ha DAP at sowing
spray regime	**Grass (12/8/93)** Hoegrass® (1.5L/ha); all except oats. Glean® ; oats only. **Broadleaf (24/8/93)** Mecoban® (2.8L/ha); wheat, barley, oats. Buctril MA ® (1.4 L/ha); Linseed only Lontrel ® (300ml/ha); Canola only **Broadleaf + Grass (24/8/93)** Roundup® (150ml/ha); Pathways.	**Grass (4/8/93)** Puma S ® (2.0L/ha); all plants. **Broadleaf (14/8/93)** Mecoban® (2.8L/ha); all plants. Starane ® (500ml/ha); all plants. **Broadleaf + Grass(24/11/93)** Roundup® (150ml/ha); Goal® (125ml/ha) Pathways.
Initial *P. thornei* density sampled	P_i year 1 taken 17/8/93	P_i year 2 taken 20/6/94 P_i year 3 taken 6/5/95
Root systems sampled pre-harvest	6/11/93	9/11/94
Trial harvested	4/12/93	17/12/94

Fig. 7.4: Monthly rainfall for Tanunda during 1993 and 1994, in comparison to the 25 year mean. (source: South Australian Bureau of Meteorology)

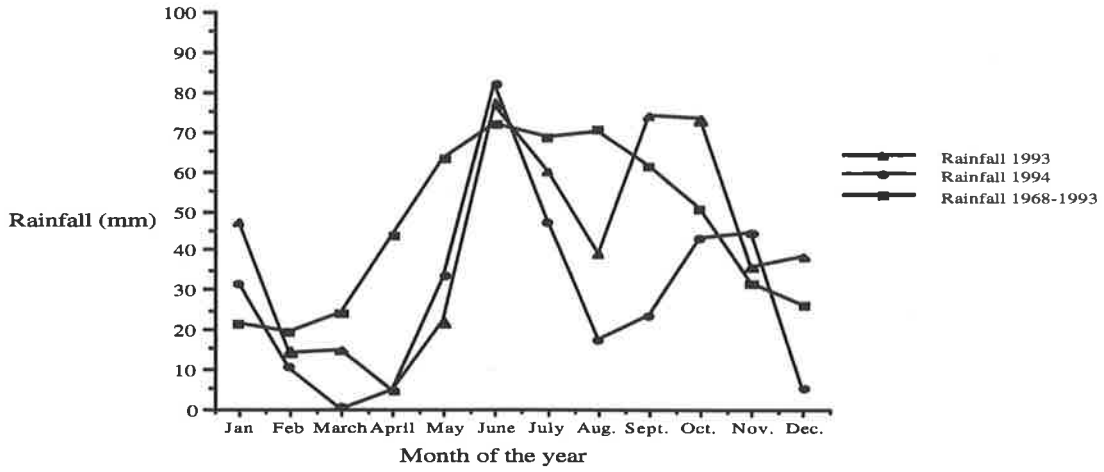
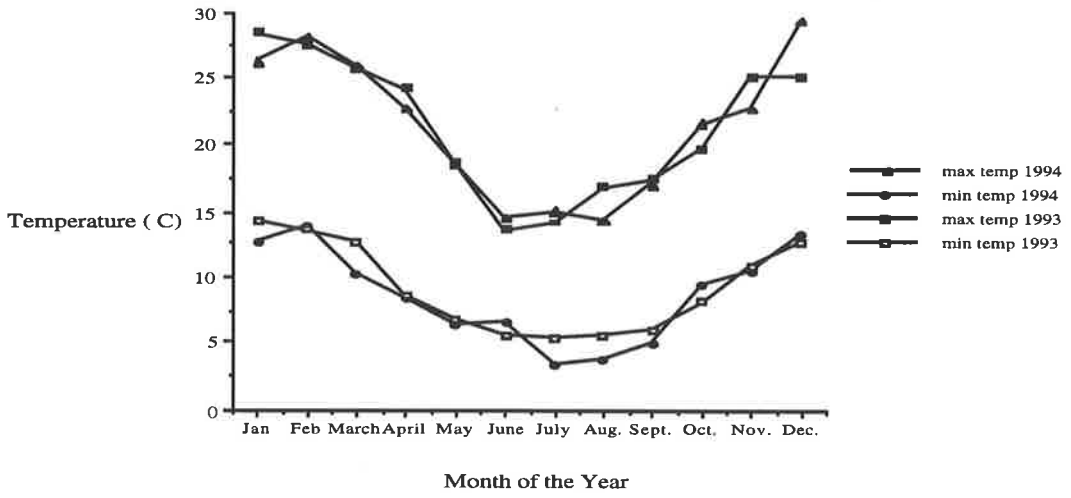


Fig. 7.5: Monthly minimum and maximum temperatures at Tanunda for 1993 and 1994. (source: South Australian Bureau of Meteorology)



7.2.4 Sampling Methodology for Initial Nematode Density

Determination of initial densities in each of the field plots was made at the beginning of each year (1993 P_i year 1, 1994 P_i year 2 and 1995 P_i year 3). Initial investigations to determine an appropriate sampling methodology were carried out.

Before the first crop was sown in April 1993, eight individual soil cores approximately 1m apart were taken to a depth of 20 cm using a soil corer (Fig. 7.1). Six plots across

the whole trial area were sampled. The total number of soil samples was 48. The number of *P. thornei* per 200 g of soil from each sample was determined using the Whitehead extraction technique (Section 3.2.1) over two days.

The heterogeneity of the samples from the 48 cores was analysed using Hierarchical Analysis of Variance. The analysis gave total, within group (plot) and among group (plot and core) variation. The plot of residuals versus fitted values indicated that a logarithmic transformation was required. The analysis indicated that within plot variation exceeded the between plot variation. Approximately 40% of the variation could be accounted for between the 6 plots, while the remaining 60% was accounted for within individual plots. The inference from this was that sampling 8 cores within a plot (8 m x 1.5 m) was not sufficient to give a reasonable estimate of the number of *P. thornei* per plot. Hence it was concluded that more cores per plot were necessary.

Due to limitations in time and man-power and the fact that 130 individual plots were required to be sampled, the number of cores were almost doubled from 8 to 14 per plot to improve the reliability of the count for each plot. This sampling method is illustrated in Figure 7.3. Cores were taken between the third and fourth, seventh and eighth seeding rows in each plot. The 14 cores sampled from each plot were then combined because the analysis of individual cores from a plot would yield little extra information, as the treatment effect was the major variable assessed. One 200g representative sample was taken from the total pooled soil samples to extract nematodes, using the Whitehead tray method (Section 3.2.1) over two days. The same plots were sampled three years consecutively with sampling as close as possible to previous coring sites.

The cores for estimation of initial densities were taken as early as possible, just after the break of the growing season (Table 7.2). Soil was collected when the soil was wet enough to allow sampling, as the heavy clay based soil texture prevented sampling when

dry. Before each coring, the corer was covered with talcum powder to enable easy removal of the sticky soil.

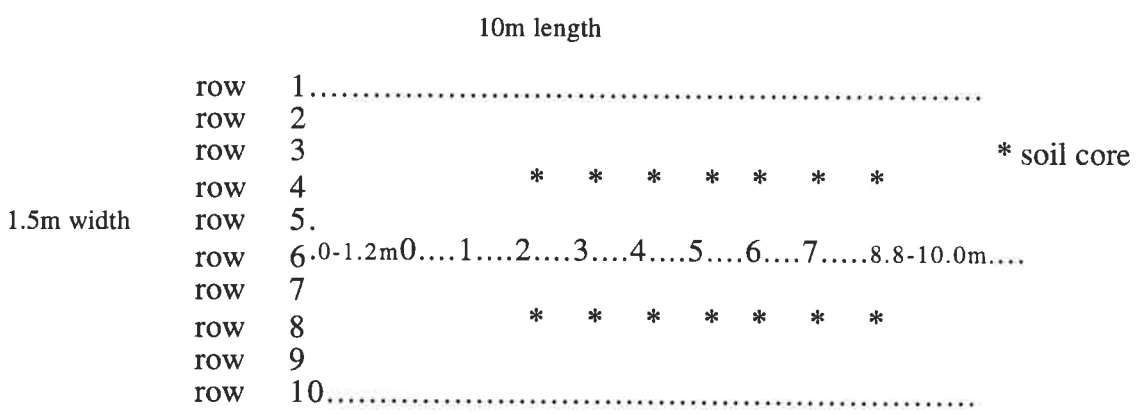


Fig. 7.3 : Diagrammatic representation of the position of 14 individual sampling cores within a plot.

The sampling method used in the first year was reassessed in the second year of sampling to confirm its reliability. From twenty-seven randomly selected plots, 14 paired cores from each plot were collected and combined to produce 2 composite samples for each plot. A 200 g representative sub-sample was taken from each sample and nematodes were extracted using the Whitehead tray extraction technique. Spearman's Ranking of Correlation Coefficient was calculated which indicated the pairs of samples were highly correlated with a t value of 5.0. This indicated sampling was consistent between each pair, and confirmed taking 14 cores from each plot was a reliable method of estimating density of nematodes.

7.2.5 Plant and Nematode Characters Measured

7.2.5.1 Plant Parameters Sampled

Plant Samples Plants of different varieties were collected in both years of the trial, during the later parts of the growing season (Table 7.2). The plots sampled were determined on the basis of the initial *P. thornei* densities calculated at the start of the season. In the first year, 3 plants were randomly sampled from both the highest and lowest initial density plots for all 12 varietal treatments tested (Table 7.1). During the

second year, 3 plants of the three different cereal varieties (AUS4930, GS50A and Warigal) were sampled from the 5 highest and lowest initial densities.

Whole plants were dug out of the soil, the root systems were washed gently in water, and any symptoms of nematode damage were noted before the wet weight of the plants were recorded. The plant samples from each plot were combined and nematodes extracted using the Mister technique (Section 3.22). They were then counted and their numbers were recorded (Section 3.3).

Quantitative Growth Characters In the second year of the trial three plant growth characters were measured for each of the 5 highest and lowest initial density plots for the three different varieties. These characters were: maximum height of plants (cm), length of individual heads (cm) and number of tillers (including primary tillers). Ten individual plants per plot were selected visually to give a representative sample of each of the characters to be recorded. A plot was considered a single replicate.

7.2.5.2 Nematode Variables Sampled

At the start of each season (1993, 1994 and 1995) the initial density of *P. thornei* was determined using the sampling methodology described in Section 7.2.4.

7.3 Results

7.3.1 *Plant Parameters Sampled*

(Refer to Materials and Methods Section 7.2.5.1).

Plant Samples

The data for samples of roots from both years were analysed separately using a RCBD. The two variables, *P. thornei* per plant and *P. thornei* per gram of fresh root, were investigated. The original analyses indicated heterogeneity of variance, so the data was log transformed, Log_e (x+1), for both variables and years.

Table 7.3: ANOVA for the plant samples taken over the 2 years of the field trial.

	d.f..	m.s..	v.r..	Prob.
Year 1: 1993				
Variable : Log (*P. thornei*/plant + 1)				
Rep.	1	4.459		
Rep. Variety Stratum				
Variety	11	4.7431	2.39	0.082
Residual	11	1.982		
Total	23			
Variable : Log. (*P. thornei*/ g root + 1)				
Rep.	1	4.810		
Rep. Variety Stratum				
Variety	11	4.194	2.16	0.109
Residual	11	1.945		
Total	23			
Year 2: 1994				
Variable : Log (*P. thornei*/plant + 1)				
Rep.	4	3.339		
Rep. Variety Stratum				
Variety	2	0.120	0.05	0.949
Initial Density	1	22.288	9.71	0.006
Variety x Initial Density	2	8.202	3.57	0.048
Residual	19(1)	2.295		
Total	28(1)			
Variable : Log (*P. thornei*/ g root + 1)				
Rep.	4	4.034		
Rep. Variety Stratum				
Variety	2	2.303	0.92	0.417
Initial Density	1	26.637	10.63	0.004
Variety x Initial Density	2	7.666	3.06	0.072
Residual	18(2)	2.505		
Total	27(2)			

From the 1993 ANOVA (Table 7.3) there was no significant difference between varieties. Although non significant, the ranking of the 12 varieties from the first year is illustrated in Fig. 7.6. The ranking did not change irrespective of the units used (log *P. thornei* / plant + 1 or Log *P. thornei* / g root + 1).

Visually, most of the wheat root systems showed lesioning and cortical degradation on nodal, lateral and seminal roots (Plates 7.2, 7.3). The size of the wheat root systems at high initial densities was much reduced. This was particularly noticeable with Spear (Plate 7.3), Machete and Warigal, three of the common South Australian wheat cultivars.

In contrast there was no visual difference in the size or degree of lesioning of the root systems of the two non-leguminous hosts, linseed and canola. The triticale, barley and oats showed some root lesioning, but little difference in the size of the root systems was seen at different initial densities (Plates 7.4, 7.5).

The second year of the trial revealed a significant effect of variety by initial *P. thornei* density. This is illustrated for log *P. thornei* per plant in Fig. 7.7. The numbers of nematodes between individual varieties were not statistically different. However, with Warigal more *P. thornei* were extracted from the roots at high than at low initial densities. There is a similar trend for GS50A and AUS4930 although this is non-significant. Once again similar results were found for log *P. thornei* / g plant.

7.3.2 Quantitative Growth Characters
(Refer to Materials and Methods Section 7.2.5.1).

Table 7.4 gives the ANOVA of plant growth characters measured for the three varieties (AUS4930, GS50A and Warigal) during the second year of the trial. The data was analysed as a CRD. None of the variables showed significant interaction between variety and nematode density. However, in all cases a varietal effect was evident for height of plant (Fig. 7.8), head length per plant and the number tillers per plant (Fig. 7.9). In all cases the unadapted landrace AUS4930 had significantly greater growth than either GS50A or Warigal.

Fig. 7.6 : The number of *P. thornei* extracted pre harvest from the cultivars/varieties used in the first year of the Tanunda Field Trial (note: non significant).

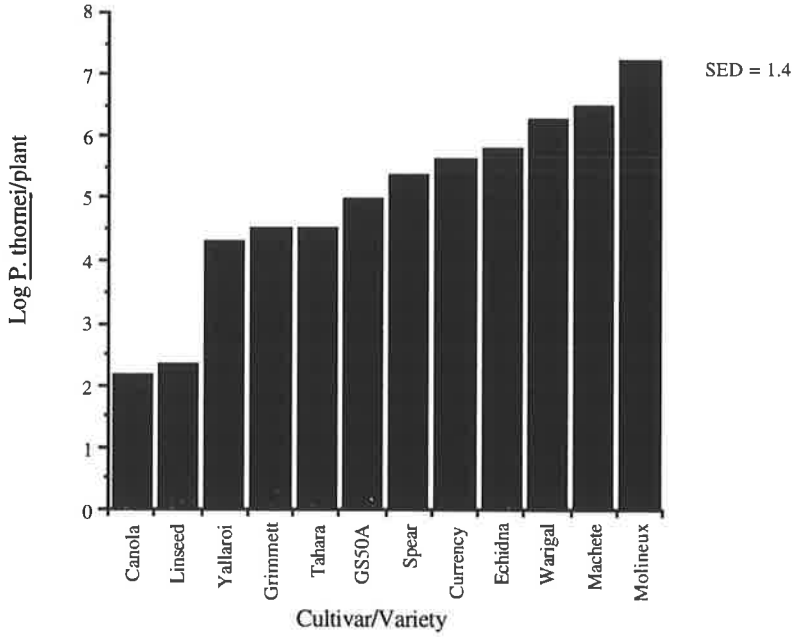


Fig. 7.7 : The interaction between variety and initial density on the numbers of *P. thornei* extracted from the 3 wheat cultivars pre harvest during the second year of the Tanunda Field Trial.

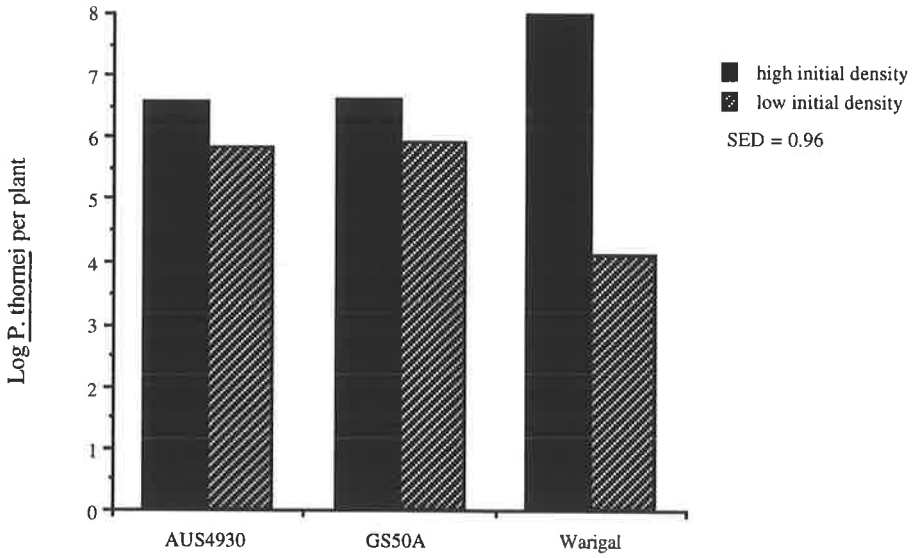


Fig. 7.8 : The significant varietal differences in wheat cultivar height measured pre harvest during the second year of the Tanunda Field Trial.

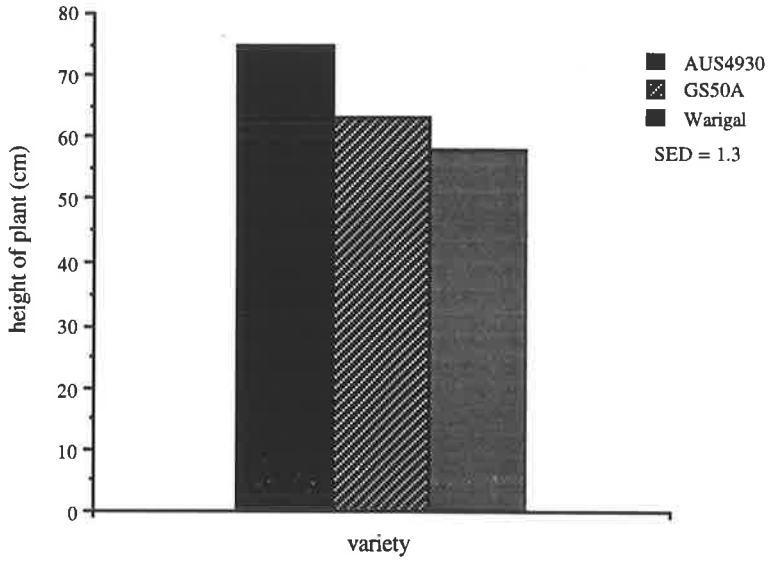


Fig. 7.9 : The significant varietal effect on the number of heads and number of tillers per plant for the 3 wheat cultivars measured pre harvest during the second year of the Tanunda Field Trial.

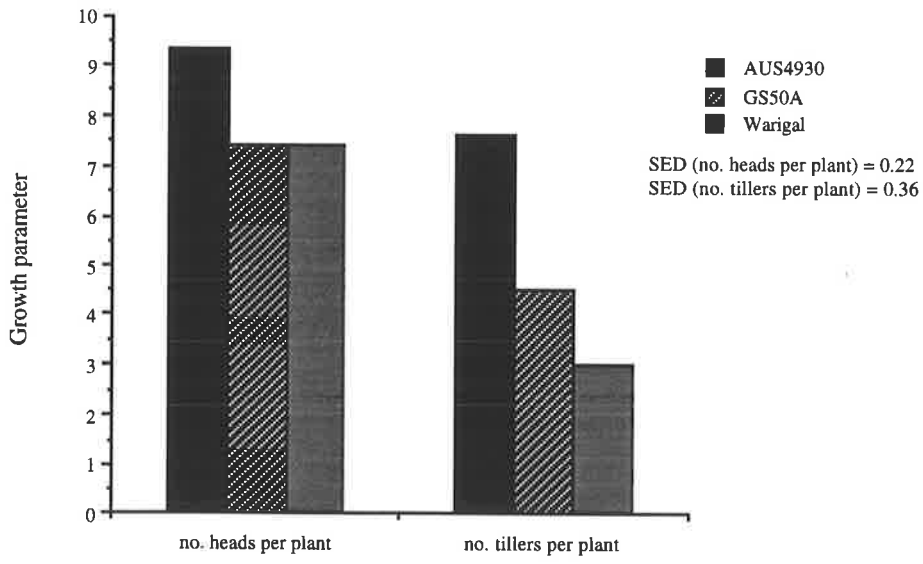


Plate 7.3 : Three representative Spear wheat roots from both low initial density plot (30 *P. thornei* per 200g OD soil) and the high initial density plot (1506 *P. thornei* per 200g OD soil), taken from the first year of the field trial after 5 months of growth. Evidence of more robust tillering capacity and root density is evident with the lower initial nematode density. However, severe restriction of both seminal and lateral root systems is seen with high *P. thornei* densities.

Plate 7.4 : Three representative Currency triticale roots from both low initial density plot (12 *P. thornei* per 200g OD soil) and the high initial density plot (535 *P. thornei* per 200g OD soil), taken from the first year of the field trial after 5 months of growth. The growth of the root system shows no apparent difference with low or high initial nematode populations.

Plate 7.5 : Three representative Yallaroi durum wheat roots from both low initial density plot (27 *P. thornei* per 200g OD soil) and the high initial density plot (2938 *P. thornei* per 200g OD soil), taken from the first year of the field trial after 5 months of growth. The growth of the root system shows no apparent difference with low or high initial nematode populations.

LOW
INITIAL DENSITY

HIGH
INITIAL DENSITY

SPEAR WHEAT

LOW
INITIAL DENSITY

HIGH
INITIAL DENSITY

CURRENCY TRITICALE

LOW
INITIAL DENSITY

HIGH
INITIAL DENSITY

YALLAROI DURUM

Large differences were observed (visual assessment) between the varieties in the second year of the field trial. Warigal suffered extreme cortical degradation and lesioning at high initial densities of *P. thornei* (Plate 7.6). Plate 7.9 shows the extensive cortical lesioning associated with reduction of the main root system (seminal and nodal roots) and also the lack of the finer lateral branching on the seminal root system. The *P. thornei* resistant wheat cultivar GS50A showed some lesioning and reduced root volume (Plate 7.7), but not to the extent of Warigal. In contrast, AUS4930 (Plate 7.8), also thought to be resistant to *P. thornei,* showed little evidence of nematode attack or reduced size at either low or high densities.

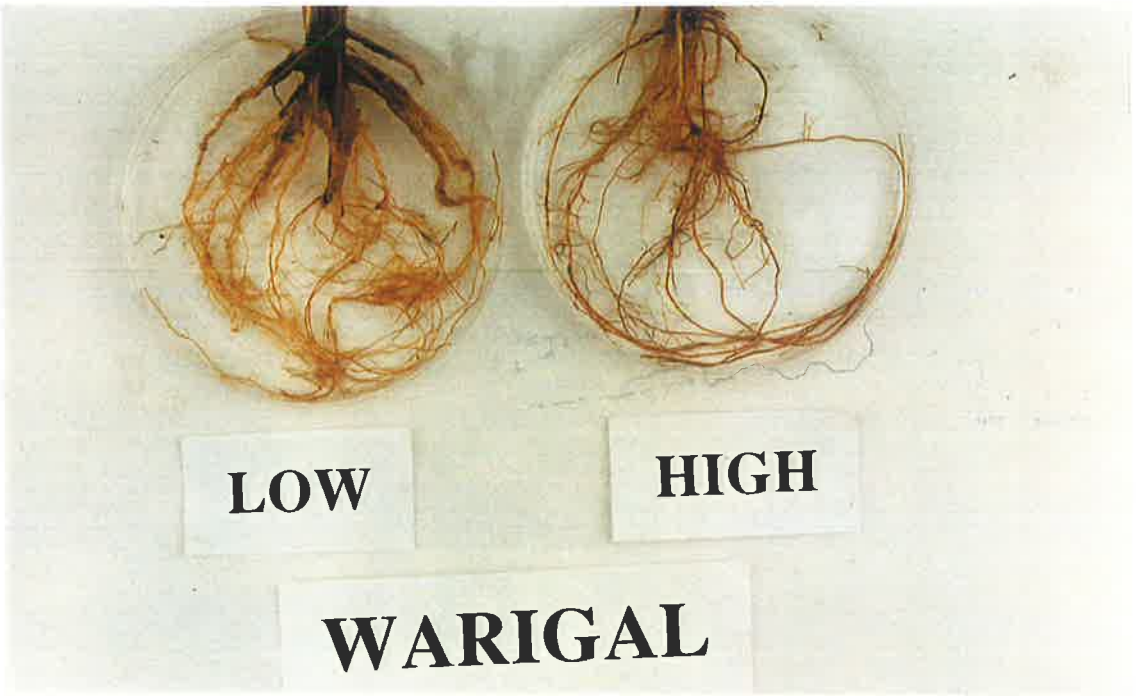
Table 7.4: ANOVA of three plant growth characters measured on AUS4930, GS50A and Warigal in the second year of the field trial at Tanunda.

	d.f.	m.s.	v.r..	Prob.
Year 2: 1994				
Variable : Plant Height (cm)				
Plot				
Variety	2	7683.08	88.64	<0.001
Density	1	66.74	0.77	0.389
Variety.Density	2	29.28	0.34	0.717
Residual	24	86.68		
Plot.Plant	270	35.45		
Total	299			
Variable : Head Length per plant (cm)				
Plot				
Variety	2	132.2036	55.27	<0.001
Density	1	1.3736	0.57	0.456
Variety.Density	2	0.7906	0.33	0.722
Residual	24	2.3921		
Plot.Plant	270	0.8059		
Total	299			
Variable : Number tillers per plant				
Plot				
Variety	2	554.54	85.01	<0.001
Density	1	1.47	0.23	0.639
Variety.Density	2	8.49	1.30	0.291
Residual	24	6.52		
Plot.Plant	270	3.38		
Total	299			

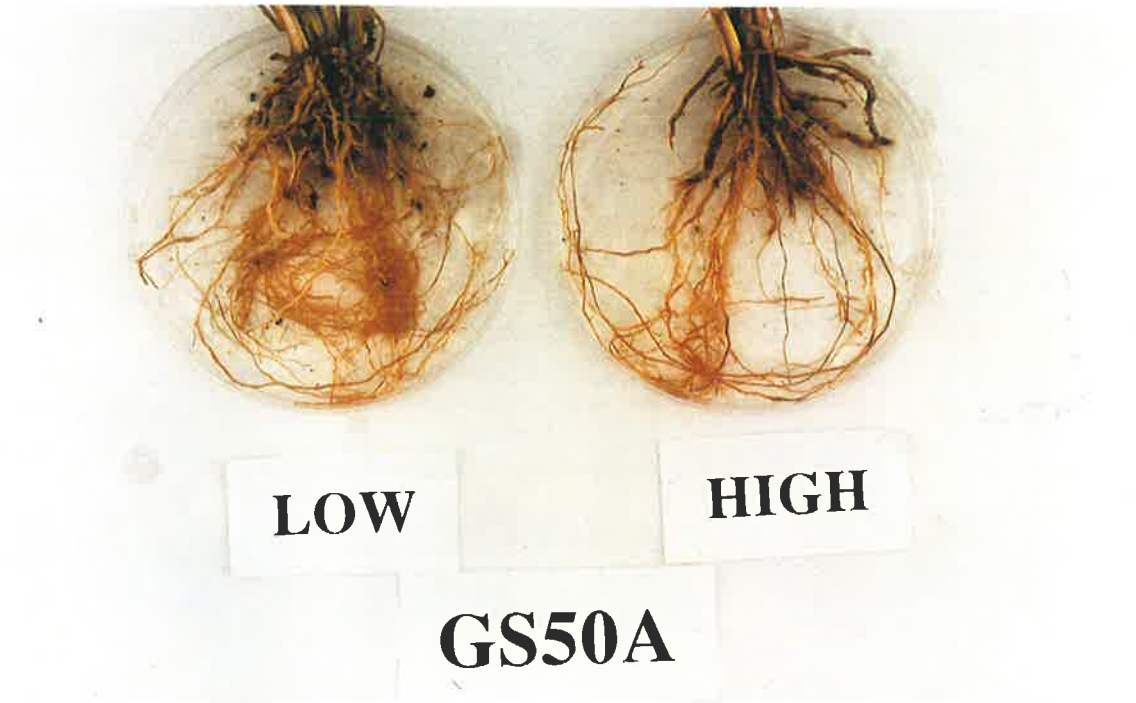
Plate 7.6 : A representative Warigal wheat plant from both a low initial density plot (39 *P. thornei* per 200g OD soil) and a high initial density plot (9044 *P. thornei* per 200g OD soil), taken from the second year of the field trial after 5 months of growth. With high nematode populations there is evidence of extensive dark brown cortical lesioning accompanied by cortical degradation on the seminal root system. Reduction of the finer lateral branches at high nematode densities is also evident.

Plate 7.7 : A representative GS50A wheat plant from both a low initial density plot (61 *P. thornei* per 200g OD soil) and a high initial density plot (9416 *P. thornei* per 200g OD soil), taken from the second year of the field trial after 5 months of growth. With high nematode populations there is evidence of extensive dark brown cortical lesioning accompanied by cortical degradation on the seminal root system. Reduction of the finer lateral branches at high nematode densities is also evident.

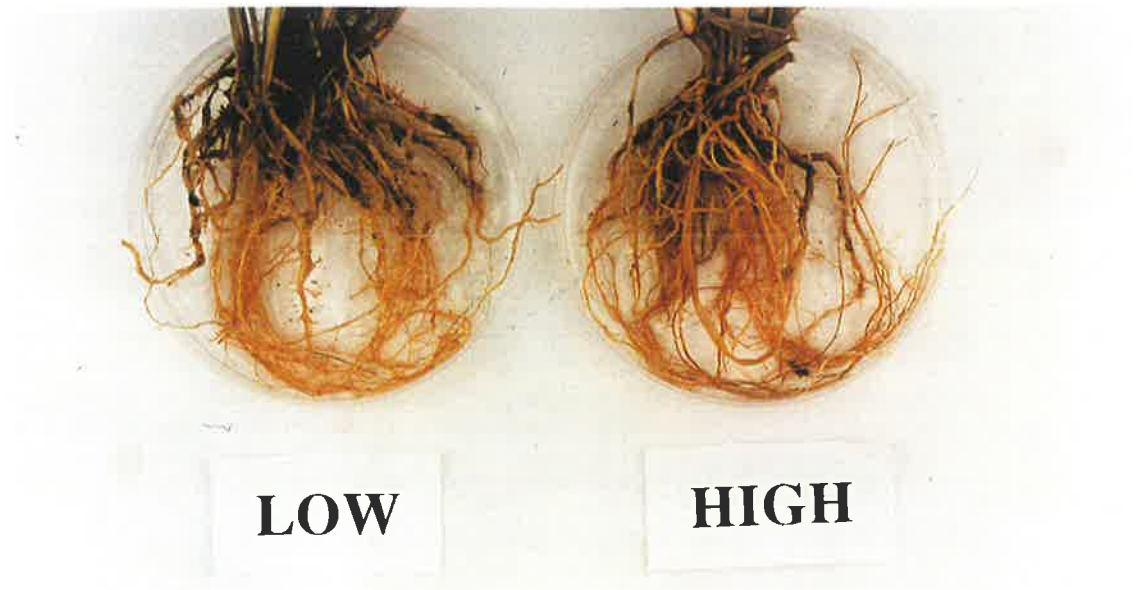
Plate 7.8 : A representative AUS4930 wheat plant from both a low initial density plot (11 *P. thornei* per 200g OD soil) and a high initial density plot (2450 *P. thornei* per 200g OD soil), taken from the second year of the field trial after 5 months of growth. The plants show no visual evidence of lesioning or apparent differences with root growth at different initial nematode densities.



WARIGAL



GS50A



AUS 4930

Plates 7.9 : Three representative Warigal wheat roots from low (39 *P. thornei* per 200g OD soil, TOP), moderate (4752 *P. thornei* per 200g OD soil. MIDDLE), and high (9044 *P. thornei* per 200g OD soil, BOTTOM) initial density plots taken from the second year of the field trial after 5 months of growth. The degree of root lesioning on both seminal and lateral root as well as the reduction in both the seminal and finer lateral root system was accentuated by increasing nematode density. The loss of grain yield between low and high plots was 27%.

7.3.2 Nematode Variables Sampled

(Refer to Materials and Methods Section 7.2.5.2).

1993

The initial density from the first year of the field trial (P_i year 1) for each individual plot was regressed against the yields of the respective cultivars (Table 7.5). Machete and Spear were the only two varieties which indicated a significant linear relationship between initial density and yield (Fig 7.10), with increasing nematode density correlated with a decrease in the yield of wheat.

Table 7.5: Regression analyses between initial *P. thornei* density and yield of various wheat cultivars used in the 2 year field trial at Tanunda.

	d.f.	m.s.	v.r.
Year 1: 1993			
Variety : Spear			
Response variable : Yield			
Fitted terms : constant, P_i year 1			
Regression	1	1.2714	5.4
Residual	8	0.2356	
Total	9	0.3507	
$F_{1,8}$ tables 5.32 < v.r. = implies linear relationship is just significant			
Variety : Machete			
Response variable : Yield			
Fitted terms : constant, P_i year 1			
Regression	1	0.82470	36.16
Residual	7	0.02281	
Total	9	0.12304	
$F_{1,7}$ tables 5.59 < v.r. = implies linear relationship is strongly significant			
Year 2: 1994			
Variety : Warigal			
Response variable : Yield			
Fitted terms : constant, P_i year 2			
Regression	1	0.44833	5.49
Residual	65	0.08164	
Total	66	0.08719	
$F_{1,65}$ tables 4.00 < v.r. = implies linear relationship is just significant			

The visual differences between plots of Machete are illustrated in Plates 7.10, with denser growth and heading at low initial *P. thornei* density relative to the high nematode density plot.

1994

An ANOVA was conducted comparing the yields of the 3 wheat varieties used in 1994 trials in order to determine whether the previous rotational crops used in 1993 had any influence on the results for the second year. For each of the three 1994 wheat varieties there was no indication that the 1993 rotation treatments had an effect on the observed yield. Given this result, a regression analysis was conducted between initial *P. thornei* density for the second year of the trial (P_i year 2) against the individual plot yields for AUS4930, GS50A and Warigal (Table 7.5). Neither of the resistant wheat yields had a statistically significant relation with the initial *P. thornei* density. Warigal, like Spear and Machete in 1993, had a significant linear relationship with yield decreasing as initial nematode density increased (Fig. 7.11).

A further regression analysis of initial *P. thornei* density for 1993 (P_i year 1) against the initial *P. thornei* density for 1994 (P_i year 2 or P_f year 1) was carried out for all 3 varieties (AUS4930, GS50A and Warigal) used in the second year of the trial (Table 7.6). Both the resistant varieties AUS4930 and GS50A were found to have a significant linear relationship between initial and final *P.thornei* density (Fig. 7.12). However, a quadratic relationship was found to be more appropriate for Warigal than a linear (Fig.7.12). It could be that the larger number of observations for Warigal results in increased power when testing the quadratic model versus the linear. Thus the relationship may be quadratic but for AUS4930 and GS50A where there is a much smaller number of observations the linear relationship is not demonstratably inferior to the quadratic.

The relationship between the initial *P. thornei* density in the 1994 (P_i year 2) and the multiplication rate (P_i year 2 / P_i year 1) was examined (Fig. 7.13). At low initial

nematode densities, the multiplication rate is high, but higher initial *P. thornei* densities are associated with reduced multiplication. No statistical analysis was conducted to test association as the Y axis (multiplication rate) is highly dependent on the X axis (P_i year 2).

Fig. 7.10 : The relationship between the initial density of *P. thornei* and the grain yield of the two wheat cultivars, Machete and Spear, grown in the first year of the field trial.

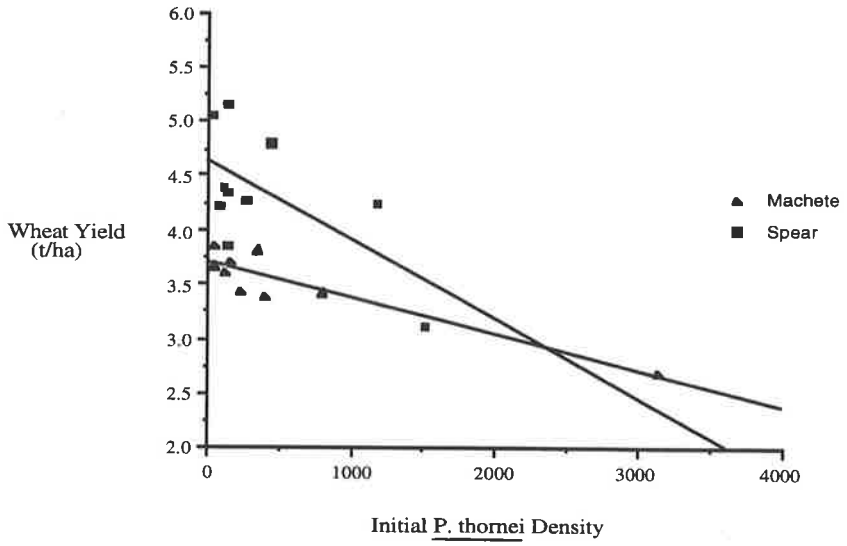


Fig. 7.11 : The relationship between the initial density of *P. thornei* and the grain yield of the wheat cultivar Warigal grown in the second year of the field trial.

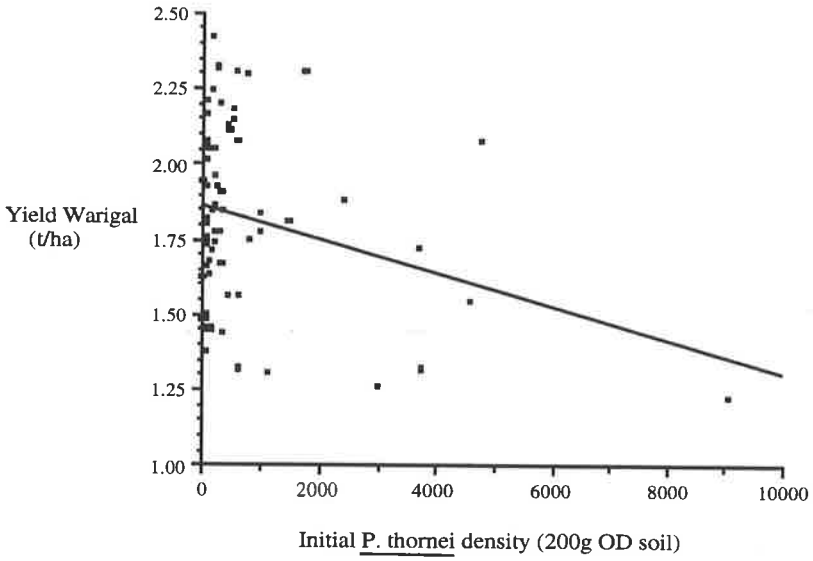


Fig. 7.12 : The relationship between the initial and final density of *P. thornei* under three different wheat hosts grown in the second year of the field trial.

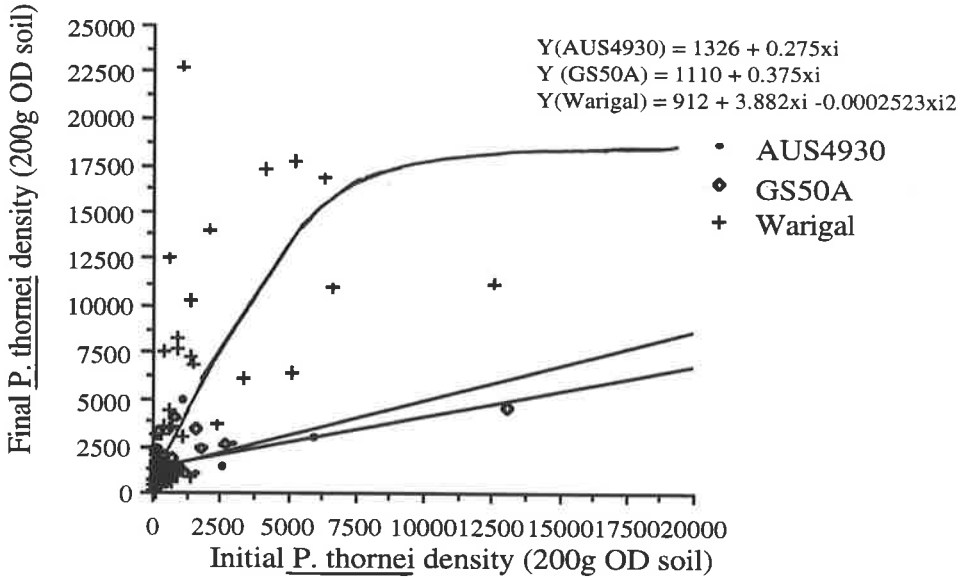
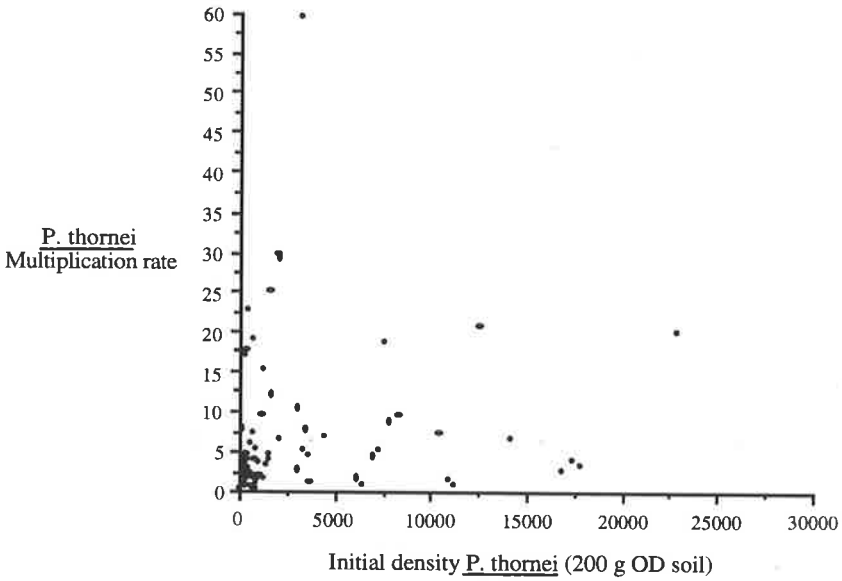


Fig. 7.13 : The relationship between the initial nematode density and the multiplication rate of *P. thornei* under the wheat cultivar Warigal, grown in the second year of the field trial.



Plates 7.10 : A low initial density plot (42 *P. thornei* per 200g OD soil, BOTTOM), and a high initial density plot (3127 *P. thornei* per 200g OD soil, TOP) of Machete taken from the first year of the field trial after 5 months of growth. The plot with few nematodes (BOTTOM) shows evidence of denser tillering and head production in comparison to the plot with many nematodes (TOP), which in comparison is less dense and relatively unthrifty.

Table 7.6: Regression analyses between 1993 initial *P. thornei* density (P_i year 1) and 1994 year initial *P. thornei* density (P_i year 2) from the second year of the Tanunda field trial.

	d.f.	m.s.	v.r.
Year 2: 1994			
Variety : AUS4930			
Response variable : P_i year 2			
Fitted terms : constant, P_i year 1			
Regression	1	12278014	14.66
Residual	27	836982	
Total	28	1245591	
$F_{1,27}$ tables 4.21 < v.r. implies significant linear relationship			
Variety : GS50A			
Response variable : P_i year 2			
Fitted terms : constant, P_i year 1			
Regression	1	5991132	6.78
Residual	26	883906	
Total	27	1073062	
$F_{1,26}$ tables 4.23 < v.r. implies significant linear relationship			
Variety : Warigal			
Response variable : P_i year 2			
Fitted terms : constant, P_i year 1			
Regression	1	6.747E+08	42.30
Residual	68	1.595E+07	
Total	69	2.550E+07	
$F_{1,68}$ tables 4.00 < v.r. implies significant linear relationship			
Response variable : P_i year 2			
Fitted terms : constant, P_i year 1, $(P_i \text{ year } 1)^2$			
Regression	2	4.817E+08	40.50
Residual	67	1.188E+07	
Total	69	2.550E+07	
$F_{1,67}$ tables 4.00 < v.r. = implies significant quadratic relationship			

Variance Ratio was calculated to determine which model is more appropriate (linear or quadratic), i.e. to test the null hypothesis B_2=0.
$F_{1,67}$ tables 4.00 < v.r. calculated (24.04), therefore implying the quadratic model more appropriate.

7.4 Discussion

The results presented here confirm that *P. thornei* is not just damaging to wheat in aseptic laboratory tests (Nicol, 1991, Ch 6), but also in the cereal growing regions in South Australia. The manipulation of *P. thornei* numbers in the field using a range of cereals and non-leguminous hosts was successful in producing a range of initial *P. thornei*

densities. These were used to examine the damage relations on wheat as well as the population dynamics of the nematode. Elston *et al.* (1991) used a range of resistant and susceptible potato genotypes to manipulate population densities of *Globodera pallida* in a field in Scotland. Use of biological means to create a range of initial densities was found to provide a useful alternative to the application of chemicals (nematicides) or addition of a range of densities prepared from cultured inoculum. There was no indication of separate effects of the 13 rotations on yield performance in 1994.

Although the primary aim of the first year (1993) of the field trial was to manipulate nematode numbers, *P. thornei* was found to reduce yields significantly for two commonly cultivated susceptible wheats, Spear (38% reduction with 1506 *P. thornei* /200g OD soil) and Machete (27% reduction with 3127 *P. thornei* /200g OD soil). It is possible that similar trends may have been expressed for some of the other cereals investigated, however the naturally occurring range of initial densities may not have been high enough to make this apparent and as there were only 10 pairs of values for each regression. That is, application of the regression where there was only 10 plots for each variety plus relying on the natural range of initial densities present was conducive to providing a powerful statistical test of the 12 varieties used(Appendix B).

The second year (1994) of the trial, showed that the South Australian wheat Warigal is highly susceptible to and intolerant of *P. thornei* with yield losses of 3% at low initial *P. thornei* densities (1000 / 200g OD soil) and 27% at higher initial nematode densities (9000 *P. thornei* / 200 OD soil). This regression was more powerful than that for Machete and Spear in 1993, as it was based on 70 individual regression points. Thus while Machete and Spear suggested a linear relation it would appear that the true relationship may be quadratic but to establish this we need considerable observations given the large experimental error associated with field data.

Thompson (1993) suggested that *P. thornei* populations exceeding 500 nematodes / 200g OD soil in the upper layer of the soil in Queensland was the economic threshold for intolerant wheats. Work by Taylor and McKay (1993) in South Australia with the use of the nematicide Temik® showed that populations of 750 *P. thornei* / 200g OD soil resulted in yield losses of up to 70%. Initial *P. thornei* populations of 500-1000 *P. thornei* / 200g OD soil in New South Wales indicated potential nematode problems could occur, while populations greater than 1000 / 200g OD soil posed a serious economic threat (Pattison, 1993).

The initial *P. thornei* densities and the associated significant yield loss of the wheat cultivars Warigal, Spear and Machete from the two year Tanunda field trial indicate economic thresholds which differ from those previously forecasted. With respect to Tanunda trial area over the two year period, the density threshold for economic damage was much greater with Warigal in the second year of the trial than either Machete or Spear in the first year. This may reflect differences in the tolerances of the varieties or may be associated with the seasonal differences (possibly rainfall) at the trial site between the two years investigated. However, work by Pattison (1993) suggests the economic threshold for damage in dry seasons will be lower, given that nematode damage to the roots may interfere with water uptake.

It is important to note that sampling nematode populations in the soil is associated with an array of potential sources of variation arising from soil collection, transport and storage, extraction and counting (Dropkin, 1989). With the technique used in this field experiment, most soil cores are obtained from the upper regions of the soil surface (0-20cm) where both soil and roots are present. Cores are usually combined, mixed and subsamples taken for extraction and counting. Dropkin (1989) notes that the actual count determined is only 20-25% of the "true mean" of the population. In combination with the above factors involved with the sampling, the numbers of plant parasitic nematodes in the field situation are determined by the carrying capacity of the environment or the available

resources (Ferris and Wilson, 1987). Resource availability refers to the abundance and state of the current or previous food resource (determined primarily by its physiology, activity and health), its distribution within the soil matrix (both horizontal and vertical) and its host status to the nematode.

The experimental situation, size of the wheat field, variability in soil type, topography, cropping history and the man power available will determine what sampling technique is utilised. As previously discussed in northern NSW the distribution of *P. thornei* is more evenly dispersed than for other nematode species in other crops (Pattison, 1993), which allows sampling procedures to be less intensive than those recommended for aggregated (or patchy) nematode species in intense agricultural or horticultural crops. Pattison (1993) also suggested aggregation of *P. thornei* was close to uniform at the time of sowing, but became aggregated later in the season. This supports the method of sampling used here.

Although samples were taken as close to the start of the cropping season as possible when *P. thornei* populations should be uniformly distributed, particular parts of the trial paddock at Tanunda showed aggregations of nematodes. These were not associated with different rotational combinations as the cropping history of the paddock was known. They must be attributable to some form of environmental and/or edaphic influence, although Pattison (1993) could not correlate such aggregations to a plant or edaphic factor. This phenomenon is not uncommon (Jones and Kempton, 1978), and many species of plant parasitic nematodes may have an overall light infestation interspersed with a few patches of high density. Use of average population densities may suggest that the multiplication is small, but this may be misleading. Estimates of densities from such field trials as Tanunda generally have proportionally high variance compared to their means, confirming the patchy distribution (Wallace, 1963). Here, the frequency distribution of *P. thornei* initial densities is always skewed to the left, with the lower counts more numerous than high, and the bell-shaped distribution consistent with normal

distributions seldom if ever seen. Given that field populations of nematodes are not universally uniform, the method of sampling and interpretation of data is of paramount importance. As a result of the skewed initial *P. thornei* densities found in both years of the Tanunda trial, the subsequent regressions of density and yield for both years are not strong. This is so particularly for the first year, where the regression was only based on 10 data values. In the second year, where there were over 60 data points for Warigal and 30 each for AUS4930 and GS50A, the statistics are more reliable, however it could be that the statistical outcome has more to do with the number of data pairs than a fundamental difference between Warigal and the other two varieties.

Crop rotation is known to be the single most useful practice of nematode control and is effective if few hosts are grown often. That is, the ability of specific susceptible crops to multiply *P. thornei* will determine the number of resistant varieties which need to be grown in succession in order to reduce nematode populations to below the economic threshold for damage. More care should be taken to quantify differences in *P. thornei* populations between alternative crop species under field conditions (Thompson, private comm. 1993). Although the primary aim of the first year of the trial at Tanunda was to manipulate a range of initial *P. thornei* densities, the use of 12 different rotational crops offered some information about the relative susceptibilities of these crops. Even though non-significant, there was a trend for the wheats to multiply greater numbers of *P. thornei* than the triticale, durum and non-leguminous hosts. Work by Lawn and Sayre (1992) in Mexico similarly found greater *P. thornei* multiplication on bread wheat than either durum or triticale varieties tested. The closely related species, *P. pratensis* was increased more by wheat and less by oats and barley, and least by rye (Oostenbrink *et al.*, 1956). Thompson (private comm. 1993) found in Queensland field populations of *P. thornei* on linseed were much smaller than on wheat. In NSW, Pattison (1993) tested a selection of cereals and found a range of resistance to *P. thornei.* The barleys Skiff and Grimmett did not increase nematode populations, and the durum Kamilaroi had excellent resistance.

The ranking of varieties from the first year of the field trial (Fig. 7.6) showed a ranking of susceptibility to *P. thornei* consistent with that observed in screening techniques used in the laboratory (Ch. 5). Similar studies with the potato cyst nematode *G. pallida* on potatoes showed that pot tests could be directly correlated to field performance (Phillips, 1984). Once the relationship between performance of the standards in the pots and the field is known, it should be possible to predict the effectiveness of a variety in the field from pot tests (Phillips, 1985). Phillips (1984) also found that when estimating the resistance of a partially resistant potato clone, it was preferable to rank clones consistently in order of resistance rather than use absolute values on nematode multiplication rates. Resistant standards should be included because without reference to these the results of pot tests may be misleading (Phillips and Trudgill, 1985). Pot tests have advantages over field trials as they are more convenient, less effort, the growth conditions can be standardised, the inoculum and other environmental factors are easily manipulated for proper replication, and initial and final numbers can be more accurately measured than in the field (Phillips and Trudgill, 1985). It is thus more likely that statistically significant results can be obtained from laboratory studies. However, laboratory results should always be verified by field evaluation.

There is little information on the relation between population density and the increase in numbers of *Pratylenchus* spp. (Wallace, 1963). The opportunity to measure the initial *P. thornei* density over a two year time frame in the Tanunda field trial allowed investigation of the field population dynamics of the nematode. The relationships between initial and final density and the initial density and subsequent multiplication rate of *P. thornei* were demonstrated for the three wheat cultivars assessed in the second year of the trial, and conform to the models previously described for other plant parasitic nematodes (Section 2.9).

Both tolerance and resistance are linked to the population curve relating initial and final densities of nematodes (Fisher, 1993). The results of the field trial showed that in a susceptible cultivar such as Warigal, the multiplication rate is high (up to 20-30 times) at low initial densities, but dropped rapidly reaching unity, at which time the population merely replaced itself, as initial density increased. This level is commonly referred to as "equilibrium density". With *P. thornei*, the equilibrium density and associated multiplication rate depend on external conditions as well as the inherent characters of the nematodes and host plant (Seinhorst, 1967). Unfortunately, the influence of these may not necessarily be related and a change in conditions may result in an increase of one and a decrease of the other or in increase or decrease of both (Seinhorst, 1967).

In this trial the equilibrium density of *P. thornei* on the susceptible host Warigal occured at an initial nematode density of about 10,000 / 200g OD soil. Pattison (1993), found the equilibrium density for initial nematode density was approximately 2000 / 200g OD soil, with populations above this leading to a decline in nematodes present in the soil at harvest. Seinhorst's (1967) work in the Netherlands found *P. crenatus* on cereals reached a maximum equilibrium density of 2400 nematodes / 200g soil. At Tanunda, the equilibrium density for *P. thornei* with Warigal was well above examples previously documented. However, Seinhorst (1967) showed that the equilibrium density of *P. crenatus* was consistently different in different fields with the same cropping sequences during seven sequential years of observation. This suggests that in order to gain an understanding of population dynamics of a nematode several seasons of data are required so that future predictions are based on averages.

As previously discussed (Section 2.8), control of nematodes is primarily about control of crop damage. The damage caused by root feeding nematodes is proportional to their population density (Seinhorst, 1965). In order to control nematodes, information about the damage threshold and the associated strategies to decrease populations to below densities threatening the next susceptible crop are necessary. In the trial described here,

the distribution of the data points was concentrated at the lower region of initial *P. thornei* densities and there was limited data to fit the models described in Section 2.8 or by Elston *et al.* (1991). Instead of log linear, exponential and inverse linear models being applied only linear and lower order polynomials were considered. In the Tanunda trial, the Warigal yield loss in relation to the initial *P. thornei* density was defined by the simple linear regression equation; Yield Reduction = 1.8617-0.0000556 x (initial *P. thornei* density). For example, if 10% yield loss was considered economically unacceptable (2.42t/ha to 2.19t/ha), then from the regression *P. thornei* populations need to be less than 5,905 / 200g OD soil to avoid such a yield penalty.

Most nematologists use resistant plants in order to maintain nematode populations below the economic threshold for damage. As discussed (Section 2.9), at present there are no varieties known to be resistant to *P. thornei* within the *Gramineae* or the *Leguminosae*, the two most common plant families associated with rotational wheat growing in South Australia. However, the response of the two putative resistant wheats tested at Tanunda suggests they have potential. Unlike Warigal, which was found to have a quadratic relationship between initial and final *P. thornei* densities, both AUS4930 and GS50A had simple linear regressions. From the regression equations, it can be seen the rate of population increase over the range of initial densities between 0 - 15,000 *P. thornei*/200g OD soil is 0.375 for GS50A and 0.2755 for AUS4930 compared with 3.882 for Warigal. Considering the hypothetical example above, populations at the damaging density would be effectively reduced with both GS50A and AUS4930 from 5905 to 3324 for GS50A and 2953 for AUS4930. This further suggests that both AUS4930 and GS50A have field resistance to *P. thornei* . As with *Heterodera avenae* in South Australia (Fisher and Hancock, 1991), the resistance for *P. thornei* will not avoid damage entirely. Use of resistance will not eradicate the nematode, which will remain a potential threat should susceptible cultivars be grown too often.

The trends observed in the field population dynamics are similar to those described by many nematologists (Section 2.8). At low initial densities of the nematode, multiplication can be up to 30 times. As the initial density increases, a corresponding decrease in multiplication rate is observed (Fig. 7.13). Multiplication rate is affected by factors such as soil temperature, soil type, soil moisture as well as initial density of the nematode (Phillips, 1985). Thus, although a resistant variety may lower nematode populations, due to the polycyclic nature of *P. thornei*, associated with very high multiplication at low initial densities, the effectiveness of this resistance is less where it is used to control monocylic nematodes. As a consequence studies such as these are imperative in order to forecast the effectiveness of the resistance.

Even though the data presented is not statistically strong, it illustrates how mathematical relationships can be used to predict the effect of *P. thornei* on wheat yield. As with the extensive work by Elston *et al.* (1991) with potato cyst nematode, combining such models with one for estimating the population dynamics of nematodes would provide a powerful means of developing integrated control strategies utilising resistant cultivars for the management of *Pratylenchus*.

AUS4930 and GS50A gave evidence of field resistance from the initial and final densities measured over the second year of the field trial. However, plants sampled relatively late (at grain filling in November) in the growing season (with numbers of *P. thornei* in the roots counted) produced atypical results (Fig. 7.7). At that time, there was no significant difference in nematode numbers between varieties. The highly susceptible wheat Warigal had similar numbers of nematodes to the resistant wheats. It is possible that the resistance mechanism in AUS4930 and GS50A may act very late in the growth of the wheat plant. Again, the seed populations may not have been completely homozygous for *P. thornei* resistance, particularly with the landrace AUS4930. Further, the initial densities between the different plots sampled for the three varieties may not have been truly comparable, hence confounding the corresponding multiplication rate measured. In

addition, although most roots were collected, the heavy soil type precluded effective sampling of the whole root system, particularly by this late (dry) stage in the season . The data also suggested that Warigal had significantly more nematodes in the roots in the high initial density plots relative to the low initial density plots sampled which would question the theory of reducing multiplication rates over a range of initial nematode densities. The confusion caused by such data further supports the findings of Jones and Kempton (1978), who suggest assessing population changes at the start of the season and again one year later.

Most of the symptoms produced by *P. thornei* were comparable with those described by other workers (Section 2.4), although like most field work they were not found to be a statistically significant difference between few and many nematodes. Van Gundy *et al.* (1974) also found suggestions of stunting, reduced tillering and occasionally reduced head length. It was of interest that the resistant wheat, AUS4930 had much greater tillering capacity and head length. This may be of agronomic advantage for breeding than the current commercial varieties, although the excessive height may make it more prone to lodging.

The histopathological symptoms produced by *P. thornei* are similar to those previously reported (Section 2.3.2). Cortical degradation was indicated by the visual appearance of lesions on the root system, and was more extensive on susceptible wheats such as Warigal, Spear and Machete than AUS4930 and GS50A. Barley, triticale and non leguminous hosts showed less cortical degradation. This was similarly found in laboratory screening work (Chapter 5). As reported by Baxter and Blake (1968), segments of the cortex were found to slough off and expose the stele. Microscopic investigation similarly showed, *P. thornei* distributed in the cortical cells of the root system, with adults and eggs usually situated in the cortex parallel to the long axis of the root. Plants grown in the high initial density plots, particularly the wheats highly susceptible to *P. thornei*, had a severe degree of cortical degradation. Work by Simmond

and Sallans (1933) and Sallans (1942) showed loss of seminal roots significantly reduced the grain yield with the loss of the nodal roots eccentuating this damage. As a consequence, the damage to wheat caused by *P. thornei* during relatively dry season would be expected to be greater than in wet seasons.

In conclusion, the evidence indicates that *P. thornei* reduces yield on wheat in the field. Most of the commonly cultivated South Australian wheats appear highly susceptible while AUS4930 and GS50A suggested resistant wheats in the field did show resistance. Studies on the population dynamics of *P. thornei* associated yield with nematode multiplication relationships, give the first opportunity to understand the field behaviour of *P. thornei* . These results provide some preliminary field evidence of the control management opportunities which may exist using rotational combinations.

Chapter 8
Plant Genetic Control and Possible Mechanisms of *P. thornei* Resistance in Cereals

8.0 General Introduction

Plant resistance has been identified as the major priority in attempts to control nematodes, over chemical, biological, cultural and regulatory control (Roberts, 1990). Resistant varieties maximise and stabilise yields through their effects on nematode population dynamics (Cook, 1974). A plant is resistant when the ability of the nematode to feed, develop and reproduce is inhibited (Wallace, 1963).

Breeding plants with disease resistance relies upon genetic variation in the host plant population exposed to the parasite (Tinline *et al.*, 1989). These interactions are unique and the inheritance of the resistance relates to a specific pathosystem. Plant resistance has been found and developed for highly specialised plant parasitic nematodes such as *Globodera*, *Heterodera*, *Meloidogyne*, *Rotylenchus*, *Tylenchus* and *Ditylenchus* which (with the exception of *Ditylenchus*) have a sedentary endoparasitic relationship with their host (Roberts, 1990). Nematodes which feed ectoparasitically or are migratory endoparasites are less likely to induce resistance due to the transient nature of their feeding. However, several plant species including tea (*Camellia sinerisis L.*), coffee (*Coffea liberica L.*) and tobacco (*Nicotiana tabacum* L.) have resistance to *Pratylenchus* (Dropkin, 1989).

In commercial agriculture, single genes for resistance are a priority, as most plant breeders have problems breeding successfully with multiple gene controlled resistance. In addition to the mode of inheritance of the resistance, the mechanism of the resistance process also determines the field applicability of this source of resistance (Cook, 1974).

With sedentary nematodes in particular there are numerous reported cases (Howard and Cotten, 1978 and Cook and Evans, 1987) which support Flor's gene for gene hypothesis (Flor, 1956), i.e., for each gene controlling the response in the host plant there is a specific and complementary gene controlling pathogenicity in the pathogen.

This chapter is divided into two sections. The first describes a preliminary investigation of the mechanism of the resistance and the second an attempt to determine the genetics of *P. thornei* resistance in the wheat variety, AUS4930.

8.1 Initial Penetration of Resistant and Susceptible Hosts

8.1.1 Introduction

The ways that plants defend themselves against attack by nematodes are varied. Each way is usually specific for a given pathogen-host relationship and depends upon the activation of a coordinated multicomponent defence mechanism (Dropkin, 1989). Dropkin (1989) refers to four possible modes of plant resistance: barriers to attraction, reduced rate of nematode growth in an incompatible host, hypersensitivity response of plant cells penetrated by nematodes, and/or inhibition of growth of sedentary females.

As previously described from the laboratory screening assay (Section 5.2, 5.3) and also from the field data (Ch. 7), the wheat genome (*Triticum aestivum*) offers a range of susceptibilities to *P. thornei* . The mechanisms for resistance to *P. thornei* are unknown. This experiment was an initial attempt to determine whether reduced initial penetration with identified resistance lines was a major factor contributing to host resistance. The highly susceptible wheat cultivar Machete and two resistant wheat lines GS50A (selection from Queensland) and AUS4930 (from the Australian Winter Cereals Collection, originally from Iraq) were used. Information on the mechanism of resistance is of paramount importance, particularly in South Australia, because if the

resistance acts post-penetration the invasion sites will be open to many soil borne fungi. Fungal associations may cause greater damage to the plant than either pathogen alone.

8.1.2 Materials and Methods

Seed of the three wheats, Machete, GS50A and AUS4930 was sterilised, germinated and selected as in Section 3.5. Sand collected from Palmer was heat treated at 65°C for 45 minutes and allowed to cool. It was mixed thoroughly and sieved. One seed of each cultivar with 3 seminal roots of 3cm length was grown in electrical conduit tubes of 2 sizes, small (2.7cm wide by 12.5cm high) and large (3.7cm wide by 12.5 cm high). *P. thornei* was extracted from carrot cultures. One week after planting, using a truncated pipette, 1ml of an inoculum containing 400 *P. thornei* was added to each seedling.

Plants were placed in a controlled temperature growth room at 20°C, with a 12 hour day and night provided by fluorescent light tubes (65µ Einstein's). There were seven replicates of each variety and tube size for each harvest time. The small tubes were harvested at 2, 6 and 9 days post inoculation and large tubes only at the last harvest of 9 days. Nematodes were extracted using the 3 day mister extraction technique (Section 3.2.2) and counted (Section 3.3).

8.1.3 Results

Data was analysed as a RCBD with two analyses conducted. Data for both analyses was converted to percent *P. thornei* penetration of the initial nematode inoculum density. The first ANOVA (Table 8.1) gave a comparison of the differences in variety and harvest for the small tubes, and the second (Table 8.2) a comparison between small and large tubes at the 3rd harvest time.

No significant variety effect was found (Table 8.1, 8.2), but in small tubes there was a significant increase in penetration between day 2 and 6 post inoculation (Fig. 8.1). The large tubes had significantly less nematode penetration by day 9 than the small tubes (Fig. 8.2).

Fig 8.1 : The effect of harvest time in relation to *P. thornei* penetration on wheat grown at 20°C in a controlled growth room in small tubes.

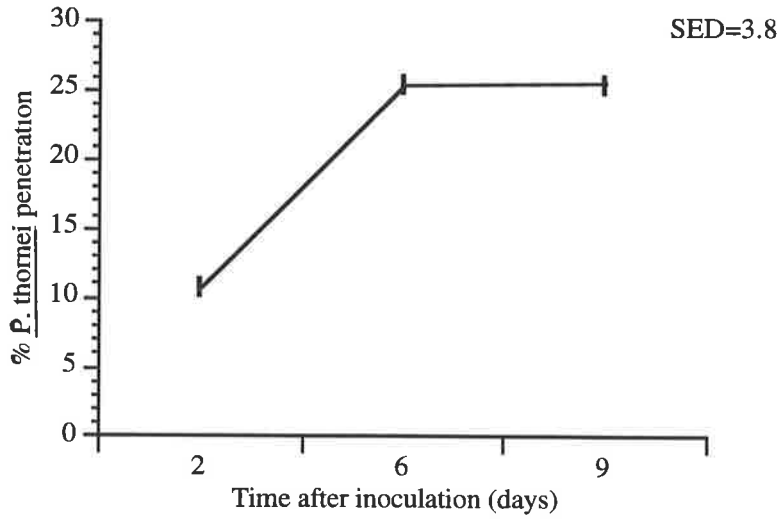


Fig. 8.2 : The effect of tube size on the penetration of *P. thornei* after 9 days on wheat grown at 20°C in a controlled growth room.

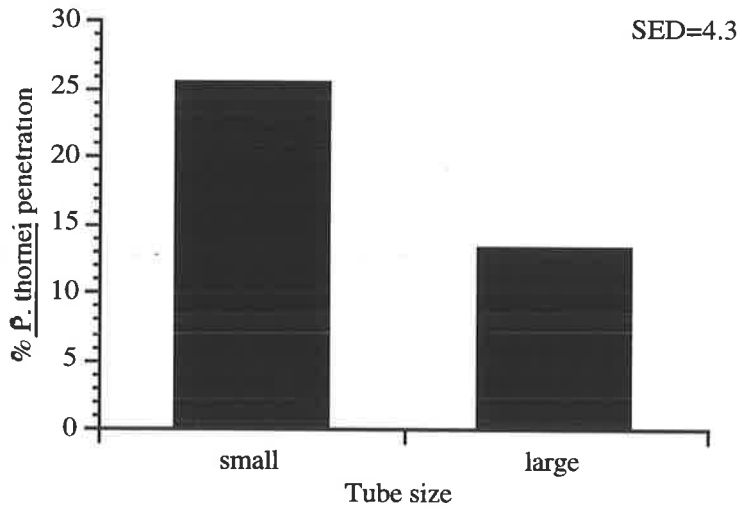


Table 8.1 : ANOVA of *P. thornei* penetration in small tubes over the 3 harvest times.

	d.f.	m.s.	v.r.	Prob.
variety	2	230.9	1.53	0.225
harvest	2	1513.2	10.05	<0.001
variety.harvest	4	176.9	1.17	0.332
Residual	53(1)	150.5		
Total	61(1)			

Table 8.2 : ANOVA of *P. thornei* penetration after nine days in both small and large tubes.

	d.f.	m.s.	v.r.	Prob.
variety	2	417.5	2.17	0.129
harvest	1	1566.5	8.15	0.007
variety.harvest	2	192.9	1.00	0.337
Residual	36	192.2		
Total	41			

8.1.4 Discussion

As the percentage penetration did not differ significantly between varieties, the mechanism involved in *P. thornei* resistance in the two wheat cultivars AUS4930 and GS50A act in some manner after invasion of the nematode. There are some cases where juveniles fail to penetrate resistant plants or do so in reduced numbers, but most documented resistance operates after invasion and results in the juveniles being unable to complete their development and reproduce (Howard and Cotten, 1978).

The use of large and small polyethylene conduit tubes confirms (see Section 5.3) that the smaller the volume of soil medium the greater the penetration of the nematodes.

The implications for the applicability of the post penetration mode of resistance in the field should be carefully considered. Invasion by the nematode, whether or not followed by a later resistance defence mechanism, may influence susceptibility to soil borne pathogens, whether they be primary or secondary invaders. A better understanding of the histopathology and migratory habits of *P. thornei* throughout the

duration of the life cycle on both resistant and susceptible hosts is needed, if the chance of disease complexes involving nematodes and fungi are to be assessed.

8.2 Inheritance of *P. thornei* resistance in the wheat cultivar, AUS4930

8.2.1 Introduction

The inheritance of plant resistance to *P thornei* may be simple, due to a single gene (dominant or recessive), or complex, controlled by many genes. Heritable resistance to nematodes has been incorporated into many important crops, including cereals, forages, vegetables, fruits, ornamentals, tobacco, cotton and soybeans (Bingefors, 1982). There are three main sources of resistance; wild plant species, induced mutants usually produced by irradiation and plant regeneration from organs, tissues or cells producing somaclonal variants (Fassuliotis, 1987). Since plant parasitic nematodes are soilborne, the spread of new virulence genes should be relatively slow in comparison with airborne pathogens, and therefore resistant varieties should remain effective for relatively long periods (Cook, 1974).

Although much of the research for sources of resistance has concentrated on nematodes with a highly specialised relationship with the host, thirty two crops have been screened for resistance to various species of *Pratylenchus* (Townshend, 1990). With regard to *Pratylenchus* species in cereals, resistance of maize both to *P. zeae* and *P. brachyurus* is due to two dominant genes with an additive effect (Sawazaki *et al.*, 1991). The *P. thornei* resistance in wheat selection GS50A from Queensland is thought to be controlled by a single dominant gene (J. Thompson, pers. comm.). There is a complex mode of inheritance for *P. penetrans* resistance in *Medicago sativa* L. (Christie and Townshend, 1992).

In a breeding program where a promising wheat line has been identified for resistance to an economic pathogen, the inheritance of resistance is usually investigated by comparing the F2 segregation ratios observed from crosses between the susceptible and resistant parents against hypothesised ratios. In this way many monogenic, dominant modes of inheritance have been verified, which have adhered to Flor's gene for gene hypothesis (Flor, 1956). An attempt was made to investigate the genetics of *P. thornei* resistance of AUS4930, since it was identified as resistant both in the laboratory (Section 5.2) and the field (Ch 7). This variety was also chosen as it carries a single gene for *H. avenae* resistance (F. Green, pers. comm.), in addition to *P. thornei* resistance.

8.2.2 Materials and Methods

Glasshouse crosses were made using the susceptible wheat cultivar Schomburgk as the parent in combination with the resistant wheat AUS4930. Seed of the homozygous parent Schomburgk was obtained from the Waite Agricultural Research Institute wheat breeder, Dr A. Rathjen, and of AUS4930 from the Australian Winter Cereals Collection. The F1 plants were grown for seed propagation and allowed to self fertilise and the F2 seeds were collected.

All plants were grown in 8 inch pots with a nutrient rich soil mix allowing for maximum tillering capacity. Careful synchronisation of flowering times was sought because AUS4930 was an unadapted landrace originally from Iraq while Schomburgk was a locally adapted spring wheat. This synchronisation was achieved by continued weekly plantings of AUS4930 and Schomburgk over three months in Spring (Sept.-Nov.). Crossing was usually attempted mid-morning as the pollen appeared more receptive at that time. Once crossing had taken place, the emasculated heads were bagged to avoid contamination problems with drifting pollen.

An inheritance of resistance assay was conducted, adapted from the technique established in Chapter 5. This involved 30 selected parental seeds of both Schomburgk and AUS4930, 30 F1 seed (Schomburgk * AUS4930) and 100 of the F2 progeny (F1 seed selfed). All seeds were sterilised, germinated and selected as in Section 3.5. Each seed with 3 seminal roots of 3cm length was grown in electrical conduit tubes (2.7cm wide x 12.5cm height). The tubes were filled with Palmer soil (Section 3.7) which had been heat treated at 65°C for 45 minutes. Seedlings were inoculated 1 week post sowing with 400 *P. thornei* per plant in 1ml aliquots using nematodes from carrot cultures (Nicol, 1991, Section 3.1).

Plants were placed in a controlled temperature growth room (Plate 8.2) at 20°C, with 12 hour day and night provided by fluorescent tubes (65µ Einstein's). The tubes were individually embedded in a tray of soil, with a wire grid to support the tubes. Plants were watered with tap water whenever required, so that water was not a limiting factor. Plants were harvested after 2 months and nematodes were extracted using the 3-day mister technique (Section 3.2.2) and then counted (Section 3.3.). In addition, dry weight of roots of plants was determined by placing individual plants in alfoil trays in a drying oven at 80°C for 7 days.

8.2.3 Results

The results (number of nematodes per plant and root dry weight per plant (g)) were analysed as a CRD and summarised in Table 8.3. The F1, F2 and parents were significantly different from each other (Fig. 8.3) with the resistant parent AUS4930 having significantly less nematodes than the susceptible parent. The F1 cross was almost significantly different from AUS4930 but not from Schomburgk. Further ANOVA (Table 8.3), comparing individual combinations (Schomburgk and AUS4930,

AUS4930 and F1, Schomburgk and F1) confirmed that Schomburgk had significantly more *P. thornei* in its roots than the resistant parent AUS4930 (Fig. 8.3). There was almost significantly less *P. thornei* per AUS4930 plant compared with the F1 cross. However, when the susceptible parent was compared against the F1 there was no significant difference between numbers of nematodes in their root systems. This information suggests that resistance with AUS4930 is possibly homozygous recessively inherited, which means it will only occur when the genotype is homozygous.

In addition to analysing the number of nematodes per plant, the dry weight of roots(g) was also considered as a response variable when comparisons were made between individual combinations of the parents and F1 populations (Table 8.3). This revealed that the dry weight of roots did not significantly differ between the parents or F1 population. For this reason, dry weight of roots was not used as a covariate.

Table 8.3 : ANOVA comparing the number of *P. thornei* and dry weight of roots of the parental lines, F1 and F2 populations. (The first ANOVA compares the parents, F1 and F2 while the next three are individual comparisons)

(g)	Variable : no. *P. thornei* per plant				Variable : Dry weight roots per plant			
	d.f.	m.s.	v.r.	Prob.	d.f.	m.s.	v.r.	Prob.
Parents, F1, F2								
variety	3	3.68E+06	2.63	0.05	3	.0008542	1.28	0.281
Residual	165(21)	1.40E+06			174(12)	.006648		
Total	168(21)				177(12)			
Schomburgk and AUS4930								
variety	1	3404495	4.39	0.04	1	.004717	1.05	0.311
Residual	53(5)	774890			52(6)	.004569		
Total	54(5)				53(6)			
AUS4930 and F1								
variety	1	2849800	3.65	0.06	1	.017874	3.07	0.09
Residual	53(5)	780011			53(5)	.005823		
Total	54(5)				54(5)			
Schomburgk and F1								
variety	1	24580	0.02	0.882	1	.004223	0.72	0.40
Residual	50(8)	1101264			49(9)	.005863		
Total	51(8)				50(9)			

Further investigations of the F2 data using the Chi-square test (X^2), were initiated to determine the possible number of genes involved in controlling the *P. thornei* resistance

in AUS4930. In order to separate the F2 data into resistant or susceptible, a cut off point for resistance is required to distinguish resistant F2 progeny from susceptible F2 progeny. The value of 1216 *P. thornei* per plant was selected for this purpose, being the mid-value between the mean values for the resistant and susceptible parents tested. Because the distribution of the numbers of *P. thornei* per plant was not distinctly separated between the two parents but showed considerable overlap (Fig. 8.4), the classification of the F2 progenies into parental type was dependent on the threshold chosen. That is, the use of 1216 *P. thornei* per plant to distinguish resistance from susceptibility appears sensible but it must be appreciated that the frequencies obtained are highly dependent on the choice of the threshold. The information presented suggests the AUS4930 resistance to *P. thornei* is controlled by a recessive allele. In order to test this the expected segregation ratios in the F2 were investigated for a single recessive locus and two independent recessive loci.

Fig. 8.3 : Multiplication of *P. thornei* on susceptible, resistant, F1 and F2 progeny wheat plants grown for 2 months at 20°C in a controlled growth room.
(**a**: no significant difference; **b**: significantly different from **a**; **a'**: almost (P<0.05) significantly different from **b** but not from **a**.)

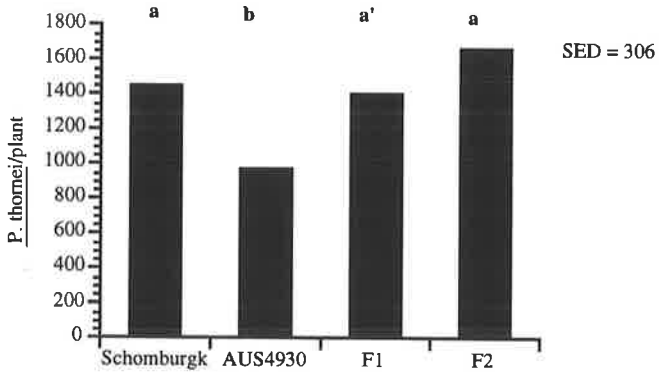
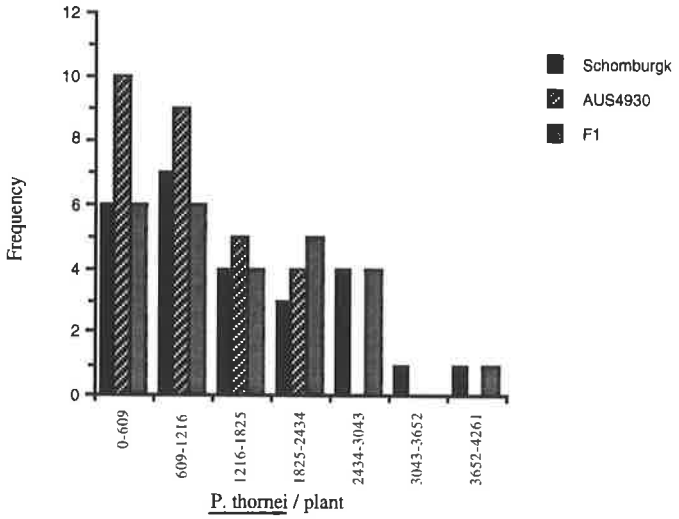


Fig. 8.4 : Frequency distributions of the number *P. thornei* per plant of parents and F1 progenies from plants grown for 2 months in a controlled growth room.



Hypothesis 1 : Resistance is controlled by a single recessive loci. As a result a 3 (susceptible) : 1 (resistant) ratio would be expected in the F2 progeny.

Parents	AA (susceptible)	X	aa (resistant)
	↓		
F1	Aa (susceptible)		
	↓		
F2 progeny	AA, aA, Aa, aa		
Expected F2 ratio	3 (susceptible) : 1 (resistant)		
Expected F2 plants	66 (susceptible) : 22 (resistant)		
Observed F2 plants	46 (susceptible) : 42 (resistant)		

$X^2 = 24.24 > X^2$ tables (3.84)

Conclusion : significant departure from Hypothesis 1

Hypothesis 2 : Resistance is controlled recessively by two independent loci. As a result a 9 (susceptible) : 7 (resistant) ratio would be expected in the F2 progeny
(This resistance can be present if either of the 2 independent loci are present in the homozygous recessive form).

Parents AAA'A' (susceptible) X aaa'a' (resistant)

↓

F1 AaA'a' (susceptible)

↓

F2 progeny F1 gametes

		AA'	Aa'	aA'	aa'
	AA'	AAA'A'	AAa'A'	aAA'A'	aAa'A'
F1 gametes	Aa'	AAA'a'	AAa'a'	aAA'a'	aAa'a'
	aA'	AaA'A'	Aaa'A'	aaA'A'	aaa'A'
	aa'	AaA'a'	Aaa'a'	aaA'a'	aaa'a'

Expected F2 ratio 9 (susceptible) : 7 (resistant)
Expected F2 plants 49 (susceptible) : 39 (resistant)
Observed F2 plants 46 (susceptible) : 42 (resistant)
$X^2 = 0.57 < X^2$ tables (3.84)

Conclusion : no significant departure from Hypothesis 2. Thus it would appear there are two independent recessive loci in AUS4930 either of which results in resistance to *P. thornei* in the homozygous form.

8.2.4 Discussion

Cook (1974) noted that the usefulness of plant resistance to nematodes is highly dependent on the genetic mechanism involved. From the statistical analysis, the resistance found in AUS4930 to *P. thornei* appears to be controlled by two independent recessive genes either of which results in resistance when present in the homozygous resistant form. The presence of two independent loci, either producing resistance in the one genotype, would seem unlikely from the evolutionary point of view, but not impossible. However, it should be remembered that Schomburgk

(susceptible parent) and AUS4930 (resistant parent) did not divide distinctly into two types. In theory, if both resistant and susceptible genotypes were pooled we should find support for a 1:1 ratio. However, as demonstrated in Fig. 8.5, the numbers of *P. thornei* counted in individual root systems suggested that some of the Schomburgk plants were resistant and similarly some of the AUS4930 were highly susceptible (i.e. the seed used was variable). The overlapping distribution of parents equally could be expected to affect the F2 progeny. Because of this, using 1216 *P. thornei* per plant as the cut off point to determine the number of resistant and number of susceptible F2 plants may be misleading and explain why significant departure from a single gene hypothesis is seen with the F2 populations examined.

There are two major possible explanations for such an overlap of parental genotypes. The assay technique used may have given false results. Although 400 *P. thornei* were inoculated to each plant post germination, negligible penetration occurred with some replicates, and hence there was minimal multiplication when harvested two months later. The data from Chapter 5 showed much less variation between replicates where only 10 replicates were used, but in this experiment 30 parents and F1 and 100 F2 were assessed. Christie and Townshend (1992) found their assay technique for counting the numbers of root lesion nematode also produced rather variable results with large standard errors. They pointed out that for a breeding program to be successful techniques which would give more precise results would be essential. Nelson *et al.* (1985) suggested using root weight as an indicator of resistance rather than nematode numbers for measuring resistance of *Pratylenchus spp.* to the genus *Prunus*. However, this approach was not supported here with *P. thornei* and wheat as the root dry weight did not significantly differ between varieties.

The second possibility is that the original parental seed was not "true to type" for *P. thornei* resistance, given that crossing was performed with several plants of both parents. This hypothesis is quite plausible for AUS4930 as the seed (although multiplied by the Australian Winter Cereals Collection) was originally a landrace from Iraq and the genetic diversity is expected to be quite high. Results from field sampling of the resistant AUS4930 (Ch 7) late in the growing season showed some plants with high nematode populations comparable to the susceptible wheat Warigal. In addition, the heads showed morphological differences, with and without awns and with colours varying between white and dark cream, further supporting lack of genetic uniformity. It is also possible that Schomburgk, although homozygous for the disease and quality traits it was purposely breed for, may carry some heterozygosity within loci for *P. thornei* resistance. A possible way to test such a hypothesis could be to obtain several single heads of both parents and re-run the assay. If seed from some individual heads produced highly variable results while others were uniform this would suggest variation in the parental type. If there was a similar high variability in seed from all heads this would implicate problems with the assay technique.

It is important to note that if the F2 segregation had been more reliable, the confirmation of the F2 ratios could have been verified by analysis of the F3 families. As a result segregation for resistance and susceptibility would have been enhanced providing a more precise confirmation of the numbers of genes involved in AUS4930 resistance. If some reselection of both resistant and susceptible parents were carried out and the above experiment was repeated with reliable parental separation the investigation of the F3 populations would be highly desirable.

Although the attempt to determine the number of genes involved in AUS4930 resistance to *P. thornei* was not conclusive, the finding of the apparently recessive

nature of resistance and that the mechanism of *P. thornei* resistance in both GS50A and AUS4930 acted after penetration should be carefully considered. Nematode invasion is likely to predispose the plant to infection by other soil borne pathogens (Lawn and Sayre, 1992) . As a consequence, the mechanism of resistance has implications for the exploitation by soil fungi. It is possible that similar numbers of *P. thornei* invade the cortex of resistant and susceptible plants throughout the life of the plant, and the number of entry sites may not differ, but the corresponding multiplication may. An understanding of the migratory and histopathological behaviour of *P. thornei* is of paramount importance, particularly for South Australia which has numerous soil borne fungi (many considered to be secondary invaders). Current evidence suggests that *P. thornei* is a non-random invader, attracted to pre-invasion sites (Baxter and Blake, 1967). The reasons for this behaviour are poorly understood.

It is obvious from the literature and the above work that little information exists with regard to understanding the mechanisms and genetic control of *P. thornei* resistance in wheat. Further selection to improve the purity of *P. thornei* resistance of GS50A and AUS4930 is essential.

Chapter 9
Nematode/Fungal Interactions

9.1 Introduction

Under aseptic laboratory conditions, *P. thornei* alone can cause damage to cereals (Nicol, 1991, Ch. 6). In the field plants are rarely ever subject to the influence of only one potential pathogen. This is especially true of soil borne pathogens (Powell, 1971). In field situations world-wide, *P. thornei* is also known to contribute to yield reductions of cereals (Section 2.8).

The first report of a nematode-fungus interaction was made by Atkinson in 1892 where increased disease severity was found with *Fusarium* wilt on cotton grown in soil coinfected with *Meloidogyne* sp. Species of *Pratylenchus* appear to be the dominant form of nematode involved in interactions with *Verticillium* wilt fungus (Powell, 1971). Usually either pathogen is capable of causing disease but damage is much greater when both are present together. In addition to 'wilt' complexes with *Pratylenchus* , there are numerous reports of *Pratylenchus* and associated complexes with 'soil-borne' fungi (Section 2.5). Early work was conducted on root rot of winter wheat in Canada, where *P. neglectus* was found to interact with *Rhizoctonia solani* resulting in significant reductions in wheat growth (Mountain, 1954; Benedict and Mountain, 1956). Since this initial work, numerous soil borne fungal associations with *Pratylenchus* species have been documented (Powell, 1971).

In South Australia, soil borne fungi are numerous and commonly present in cereal growing regions (A. Rathjen, pers. comm.). Taheri *et al.* (1994) found that in South Australia wheat roots and the nematode *P. neglectus* were commonly associated with *Fusarium oxysporum*, *F. acuminatum*, *F. equiseti*, *Microdochium bolleyi*, *Gaemannomyces graminis*, *Bipolaris sorokiniana*, *Pythium irregulare*, *Pyrenochaeta*

terrestris, Phoma sp. and *Ulocladium atrum* . As previously discussed (Ch. 4), the two *Pratylenchus* species, *P. neglectus* and *P. thornei* are commonly found in southern Australia and frequently together. Preliminary evidence suggests fungal associations with *P. neglectus* are responsible for increased damage to wheat (A. Taheri, pers. comm.).

Due to the frequency of soil fungi in South Australia and similar widespread distribution of *Pratylenchus*, preliminary investigations were undertaken to investigate any interaction of two soil borne fungi, *Microdochium bolleyi* and *F. acuminatum* with the two nematode species, *P. thornei* and *P. neglectus.* These fungi were selected as they are two of the most common soil borne fungi in South Australia (A. Taheri, pers. comm.), and are both considered to be secondary pathogens. This work was undertaken in collaboration with Mr. Abdolhossein Taheri, Department of Plant Science, Waite Campus.

9.2 Materials and Methods

Machete seeds were sterilised, germinated and selected as in Section 3.5. Sandy soil (Ch. 3.7) from wheat fields near Roseworthy Campus of the University of Adelaide was steam pasteurised at 70°C for 40 minutes, then aerated for 72 hours and sieved through a 2mm sieve.

Fungal inoculum of *M. bolleyi* and *F. acuminatum* was prepared on millet seed that had been placed in plastic autoclave bags and autoclaved at 120°C for one hour on each of three consecutive days. The fungal inoculum from PDA medium was added to each plastic autoclave bag containing sterilised millet seed (0.5kg), grown for four weeks at 25°C and then air dried in a laminar flow cabinet for one week prior to use. 300ml plastic cups (as in Plate 6.2), with no drainage, were filled with 420g air dried soil.

Fungal inoculum of *F. acuminatum* or *M. bolleyi* was added to the soil at 1% w/w. One layer of fungus was added after 150g of soil was in the cup and a second another 150g soil. The remaining 120g of soil was added to fill the cup. Pregerminated Machete seeds were sown in each cup at a depth of 1.5cm (1 seed per cup).

P. thornei and *P. neglectus* were extracted from carrot cultures as described in Section 3.1. The nematodes were added in a volume of 1ml using a truncated pipette, at the densities of 0, 2000, 6000 and 12000 per plant, as close to the seedling as possible. Sterile distilled water was added for the 0 nematode treatment.

The experiment was set up as a split plot with 6 replicates. There were 2 harvest times (7 and 10 weeks post inoculation) were randomised to the two whole plots within each of the six replicates (blocks). Two nematode types (*P. neglectus* and *P. thornei*) at 4 different initial densities, and two fungi (*M. bolleyi* and *F. acuminatum*) were randomised to the 24 subplots within each whole plot. Plants were grown in a controlled temperature room at 23°C with a 12 hour day length and light intensity of 65μEinsteins. This temperature was selected as it is optimal for both fungal and nematode species.

Plants were harvested 7 and 10 weeks post inoculation. The soil was gently washed from the root system. Nematodes were extracted over a period of 4 days using mister extraction (Section 3.1), was counted (Section 3.3). At each harvest time, the root lesions were scored from 0-5 (0=healthy roots and 5=complete lesioning of whole root system) and the number of tillers per plant (excluding main tiller) were counted. Dry weights of shoots and roots were recorded after drying at 80°C for 7 days. A 2cm root segment from each treatment was sampled and fixed in FAA preservative for staining nematodes and fungus (Section 3.4.2).

9.3 Results

The data (no. nematodes/plant; multiplication rate (no. nematodes per plant/ initial nematode density); dry weight roots (g); dry weight shoots (g); total dry weight (g); root lesioning/plant and number tillers/plant) were analysed as a SPA. Where the original analysis showed heterogeneity of variance the data was either log transformed, $\log_e(x+1)$, or square root transformed (sqrt x+0.5).

There are two significant 3-way interactions for the square root transformation of the number of nematodes per plant. The first of these is a 3-way interaction between harvest time, nematode species and initial nematode density. In general, as the initial nematode density increases the number of nematodes per plant also increases, however at harvest 2, *P. thornei* shows little increase at the highest density whereas *P. neglectus* exhibits much higher numbers at the highest density (Fig. 9.1). The second is a 3-way interaction between harvest time, nematode and fungal species. Plants with *F. acuminatum* had greater number of both *P. thornei* and *P. neglectus* at harvest 1, but by harvest 2 there was an increase in the number of *P. thornei* with or without fungi but no difference between treatments. Similarly the number of *P. neglectus* increased, but the highest number of nematodes was in the absence of fungi (Fig. 9.2).

A 3-way interaction between harvest, nematode species and fungus for the multiplication rate of nematodes per plant is illustrated in Fig. 9.3. This has the same pattern as the numbers of nematodes per plant. From the analysis we also note that as initial density increases the multiplication rate of the nematode decreases (Fig. 9.4). There are two significant 2-way interactions for shoot dry weight per plant(g). First nematode density had little effect at harvest 1 but much greater effect at harvest 2 (Fig. 9.5). The significant 2-way interaction between harvest time and fungus showed both

fungi increased the shoot dry weight however the increase at harvest 2 was much greater than at harvest 1 (Fig. 9.6, Plate 9.1 and 9.2).

Table 9.1 : ANOVA variables examining the effect of fungus, nematode density and species over two harvest times on the wheat cultivar Machete.

	d.f.	m.s.	v.r.	Prob.	
Variable : Square root (No. nematodes/plant + 1)					
block stratum	5	4958			
block.wplot stratum					
harvest	1	107248	19.20	<0.007	
Residual	5	5587			
block.wplot.subplot stratum					
nemtype	1	8054	3.01	0.085	
fungus	2	341	0.13	0.880	
nemden	3	152367	56.90	<0.001	
harvest.nemtype	1	1274	0.48	0.491	
harvest.fungus	2	18273	6.82	0.001	
nemtype.fungus	2	2587	0.97	0.383	
harvest.nemden	3	4495	1.68	0.190	
nemtype.nemden	3	7421	2.77	0.065	
fungus.nemden	6	1491	0.56	0.694	
harvest.nemtype.fungus	2	9524	3.56	0.031	Fig. 9.2
harvest.nemtype.nemden	3	9878	3.69	0.027	Fig. 9.1
harvest.fungus.nemden	6	3708	1.38	0.241	
nemtype.fungus.nemden	6	1179	0.44	0.779	
Residual	168(6)	2678			
Total	277(10)				
Variable :Multiplication rate of nematodes/plant					
block stratum	5	9.536			
block.wplot stratum					
harvest	1	362.824	61.06	<0.001	
Residual	5	5.942			
block.wplot.subplot stratum					
nemtype	1	14.338	1.53	0.218	
fungus	2	0.164	0.02	0.983	
nemden	3	28.257	3.02	0.052	Fig 9.4
harvest.nemtype	1	2.755	0.29	0.588	
harvest.fungus	2	40.209	4.29	0.015	
nemtype.fungus	2	10.006	1.07	0.346	
harvest.nemden	3	3.034	0.32	0.724	
nemtype.nemden	3	9.625	1.03	0.360	
fungus.nemden	6	7.939	0.85	0.497	
harvest.nemtype.fungus	2	30.784	3.29	0.040	Fig. 9.3
harvest.nemtype.nemden	3	14.890	1.59	0.207	
harvest.fungus.nemden	6	5.129	0.55	0.701	
nemtype fungus.nemden	6	4.434	0.47	0.755	
Residual	168(6)	9.364			
Total	209(6)				

	d.f.	m.s.	v.r.	Prob.	
Variable : Dry weight shoots/plant (g)					
block stratum	5	0.16079			
block.wplot stratum					
harvest	1	16.72968	309.24	<0.001	
Residual	5	0.05410			
block.wplot.subplot stratum					
nemtype	1	0.05338	3.58	0.060	
fungus	2	3.46281	232.26	<0.001	
nemden	3	0.13482	9.04	<0.001	
harvest.nemtype	1	0.01371	0.92	0.339	
harvest.fungus	2	0.13916	9.33	<0.001	Fig. 9.6
nemtype.fungus	2	0.00091	0.06	0.941	
harvest.nemden	3	0.04765	3.20	0.024	Fig. 9.5
nemtype.nemden	3	0.01257	0.84	0.472	
fungus.nemden	6	0.02007	1.35	0.237	
harvest.nemtype.fungus	2	0.01426	0.96	0.386	
harvest.nemtype.nemden	3	0.00603	0.40	0.750	
harvest.fungus.nemden	6	0.01082	0.73	0.629	
nemtype.fungus.nemden	6	0.01657	1.11	0.356	
Residual	232(4)	0.01491			
Total	283(4)				
Variable : Dry weight roots/plant (g)					
block stratum	5	0.39057			
block.wplot stratum					
harvest	1	0.85594	5.00	0.076	
Residual	5	0.17121			
block.wplot.subplot stratum					
nemtype	1	0.38964	5.04	0.026	
fungus	2	1.27373	16.47	<0.001	
nemden	3	0.57571	7.44	<0.001	
harvest.nemtype	1	0.35423	4.58	0.033	Fig. 9.8
harvest.fungus	2	1.15116	14.88	<0.001	
nemtype.fungus	2	0.35648	4.61	0.011	
harvest.nemden	3	0.10483	1.36	0.257	
nemtype.nemden	3	0.03355	0.43	0.729	
fungus.nemden	6	0.44199	5.71	<0.001	
harvest.nemtype.fungus	2	0.15205	1.97	0.142	
harvest.nemtype.nemden	3	0.11318	1.46	0.225	
harvest.fungus.nemden	6	0.33807	4.37	<0.001	Fig. 9.7
nemtype.fungus.nemden	6	0.06211	0.80	0.569	
Residual	221(15)	0.07734			
Total	272(15)				

	d.f.	m.s.	v.r.	Prob.	
Variable : Total dry weight/plant (g)					
block stratum	5	0.9900			
block.wplot stratum					
harvest	1	9.8572	27.43	0.003	
Residual	5	0.3593			
block.wplot.subplot stratum					
nemtype	1	0.1791	1.57	0.212	
fungus	2	5.9711	52.32	<0.001	
nemden	3	1.3248	11.61	<0.001	
harvest.nemtype	1	0.5932	5.20	0.024	Fig. 9.11
harvest.fungus	2	1.2051	10.56	<0.001	
nemtype.fungus	2	0.3859	3.38	0.036	Fig. 9.10
harvest.nemden	3	0.0870	0.76	0.516	
nemtype.nemden	3	0.0219	0.19	0.902	
fungus.nemden	6	0.6785	5.95	<0.001	
harvest.nemtype.fungus	2	0.2720	2.38	0.095	
harvest.nemtype.nemden	3	0.1401	1.23	0.300	
harvest.fungus.nemden	6	0.3506	3.07	0.007	Fig. 9.9
nemtype.fungus.nemden	6	0.1231	1.08	0.376	
Residual	218(18)	0.1141			
Total	269(18)				
Variable : Root lesioning/plant					
block stratum	5	0.4421			
block.wplot stratum					
harvest	1	14.8640	333.97	<0.001	
Residual	5	0.0445			
block.wplot.subplot stratum					
nemtype	1	0.2770	1.24	0.267	
fungus	2	9.0179	40.27	<0.001	
nemden	3	53.5480	239.15	<0.001	
harvest.nemtype	1	0.2353	1.05	0.306	
harvest.fungus	2	1.9730	8.81	<0.001	
nemtype.fungus	2	0.1255	0.56	0.572	
harvest.nemden	3	1.2496	5.58	0.001	Fig. 9.14
nemtype.nemden	3	0.1131	0.51	0.679	
fungus.nemden	6	0.7717	3.45	0.003	Fig. 9.13
harvest.nemtype.fungus	2	1.2498	5.58	0.004	Fig. 9.12
harvest.nemtype.nemden	3	0.4590	2.05	0.108	
harvest.fungus.nemden	6	0.3789	1.69	0.124	
nemtype.fungus.nemden	6	0.1761	0.79	0.581	
Residual	226(10)	0.2239			
Total	277(10)				

	d.f.	m.s.	v.r.	Prob.	
Variable : No. tillers/plant					
block stratum	5	8.4661			
block.wplot stratum					
harvest	1	2.0387	2.62	0.167	
Residual	5	0.7792			
block.wplot.subplot stratum					
nemtype	1	0.0772	0.14	0.711	
fungus	2	62.6843	111.63	0.001	Fig. 9.15
nemden	3	0.8534	1.52	0.210	
harvest.nemtype	1	0.3516	0.63	0.430	
harvest.fungus	2	0.9928	1.77	0.173	
nemtype.fungus	2	1.5705	2.80	0.063	
harvest.nemden	3	0.2344	0.42	0.741	
nemtype.nemden	3	0.5900	1.05	0.371	
fungus.nemden	6	0.8815	1.57	0.157	
harvest.nemtype.fungus	2	0.6053	1.08	0.342	
harvest.nemtype.nemden	3	0.3339	0.59	0.619	
harvest.fungus.nemden	6	0.3202	0.57	0.754	
nemtype.fungus.nemden	6	0.3188	0.57	0.756	
Residual	228(8)	0.5615			
Total	279(8)				

The 3-way interaction between harvest time, fungus and initial nematode density on the dry weight of root per plant (g) is illustrated in Fig. 9.7. There was a general trend at all harvest times and fungal treatments for a decrease in root weight with increasing initial nematode density. Unlike shoot dry weight, the dry weight of root systems between harvest times did not change except that *M. bolleyi* caused more root growth than was found in the control or with *F. acuminatum* at harvest 1. The two-way interaction between nematode species and harvest time revealed that *P. neglectus* significantly reduced the root dry weight of Machete at harvest 1, while both *P. neglectus* and *P. thornei* reduced root dry weight at harvest 2 (Fig 9.8). The root dry weight per plant was less at harvest 2 than harvest 1, unlike the shoot dry weight per plant.

Fig. 9.1 : Effect of interaction between harvest, nematode species and initial density on the number of nematodes extracted from Machete wheat root systems.

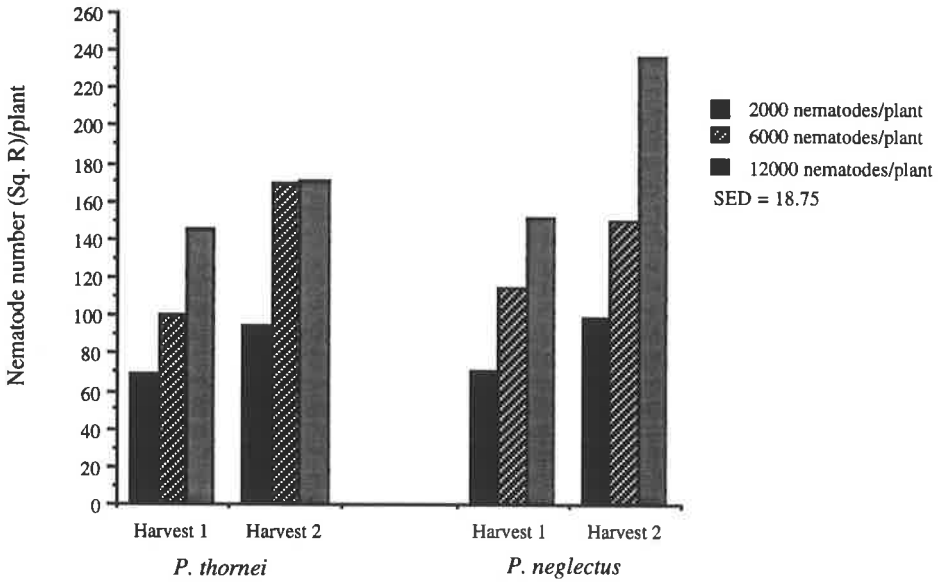


Fig. 9.2 : Effect of interaction between harvest, nematode and fungal species on the number of nematodes extracted from Machete wheat root systems.

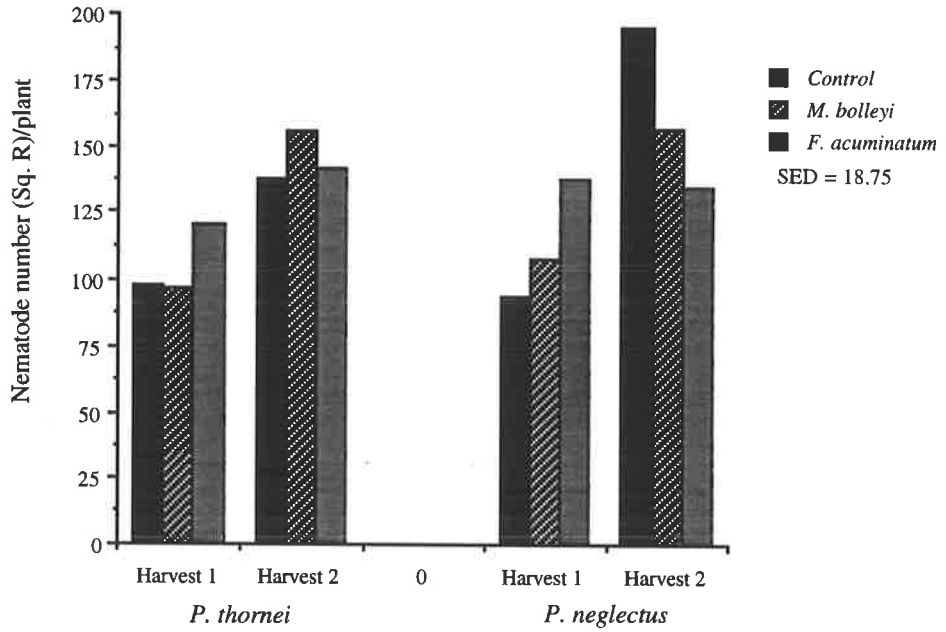


Fig. 9.3 : Effect of interaction between harvest, nematode and fungal species on the nematode multiplication rate per Machete wheat root system.

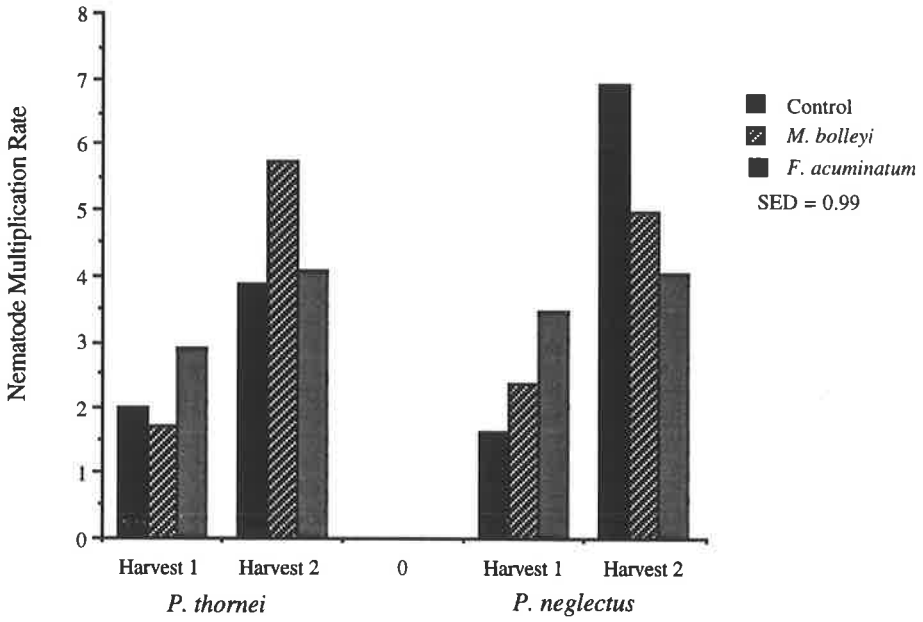


Fig. 9.4 : Effect of initial nematode density on the nematode multiplication rate per Machete wheat root system.

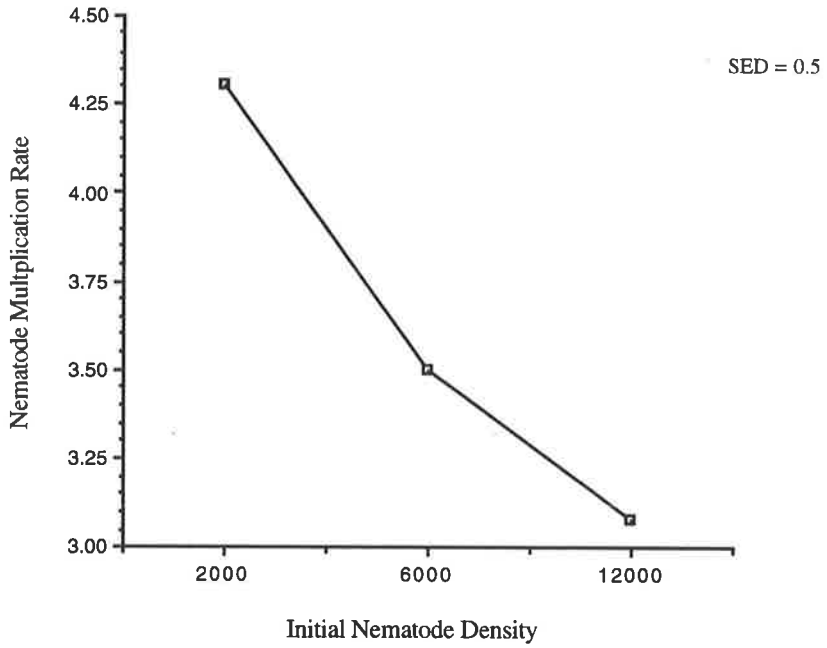


Fig. 9.5 : Effect of harvest time and initial nematode density on the shoot dry weight of Machete.

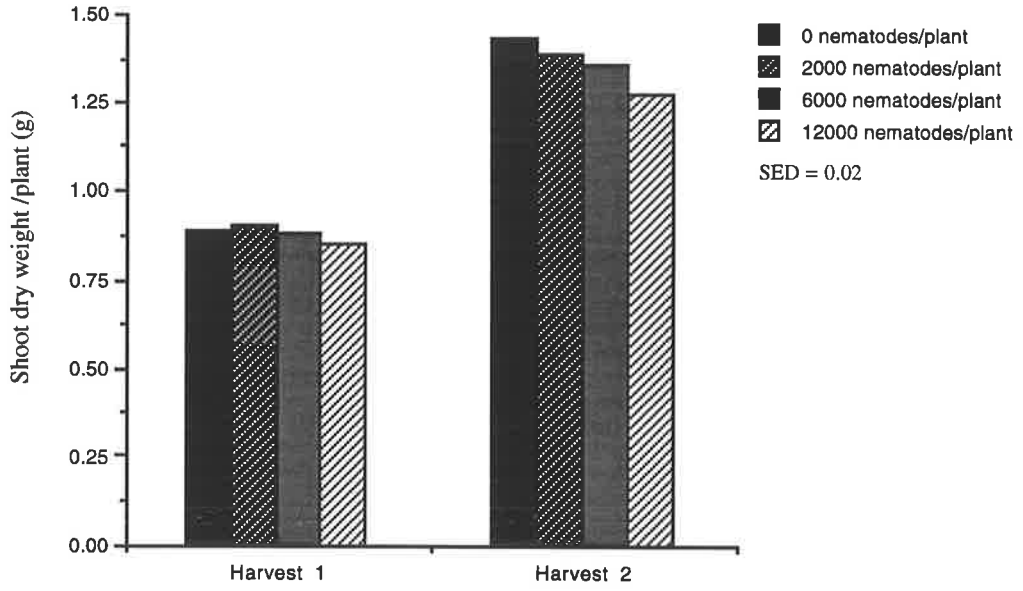


Fig. 9.6 : Effect of harvest time and fungal species on the shoot dry weight of Machete.

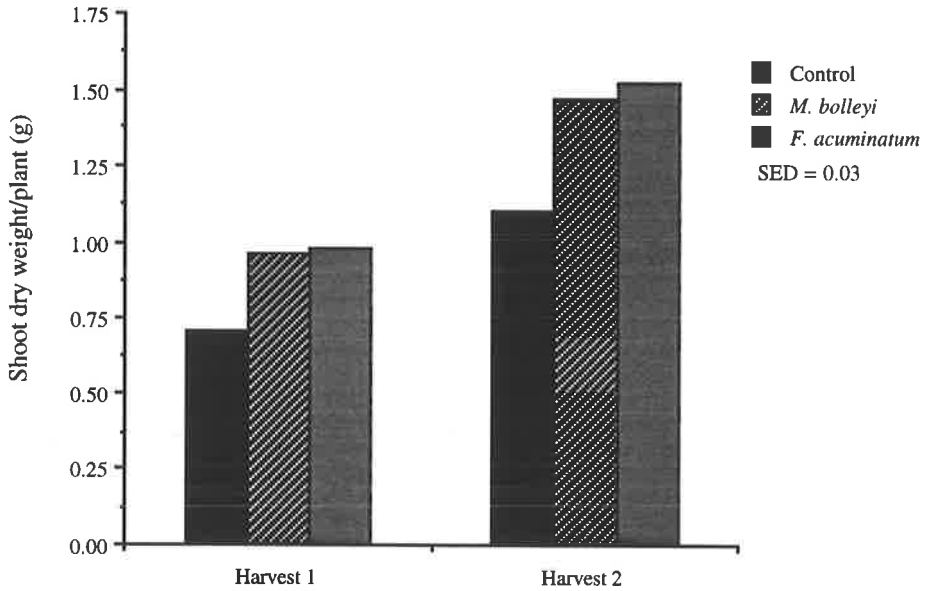


Fig. 9.7 : Effect of interaction between harvest, nematode species and initial nematode density on the root dry weight per Machete wheat root system.

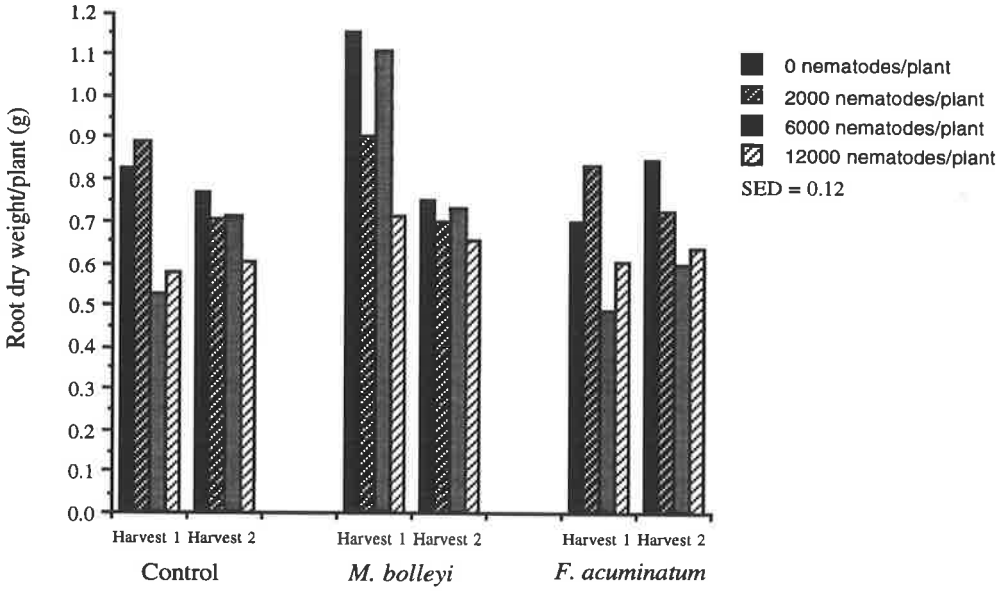


Fig. 9.8 : Effect of interaction between harvest and nematode species on the root dry weight per Machete wheat root system.

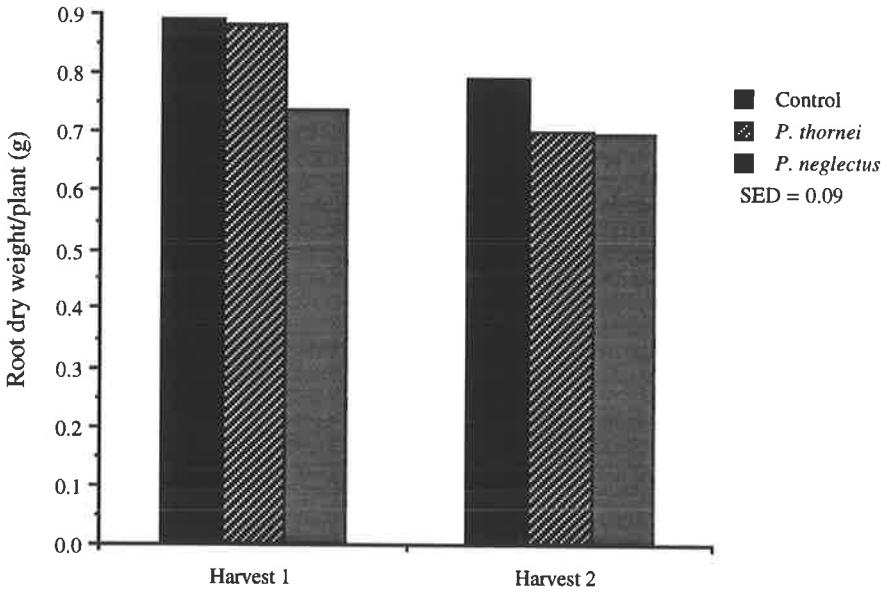


Fig. 9.9 : Effect of interaction between harvest, nematode species and initial nematode density on the total dry weight per Machete wheat root system.

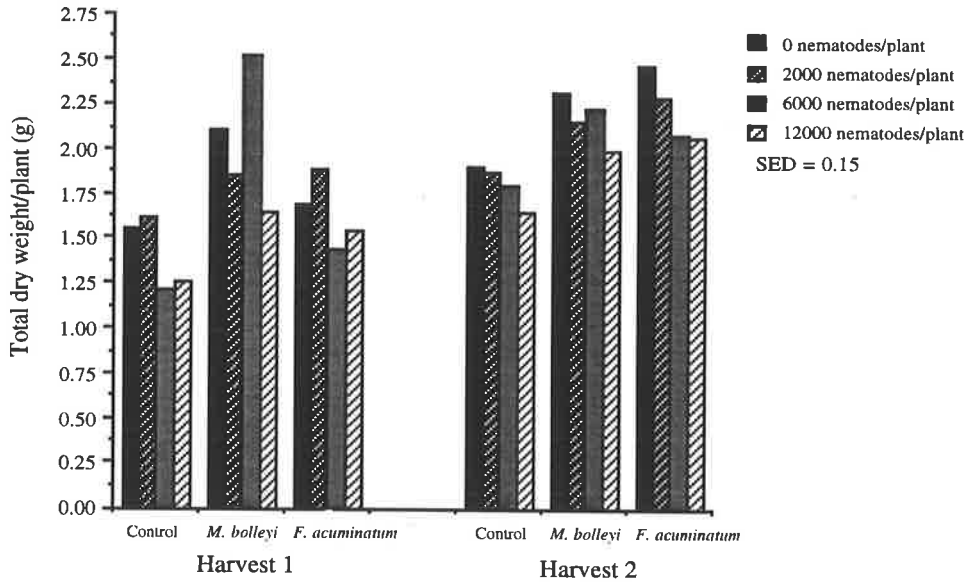


Fig. 9.10 : Effect of interaction between nematode and fungal species on the total dry weight per Machete wheat root system.

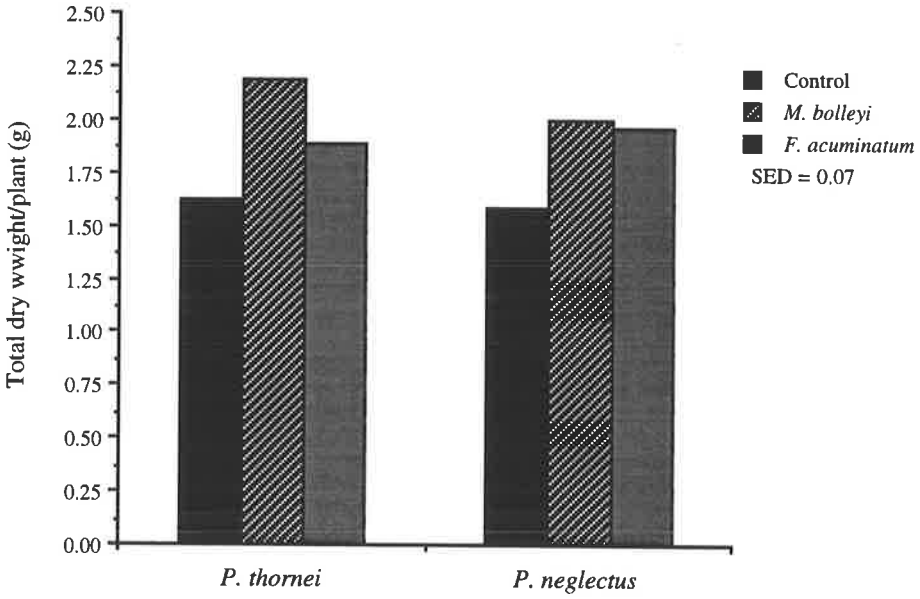


Fig. 9.11 : Effect of interaction between time of harvest and nematode species on the total dry weight per Machete wheat root system.

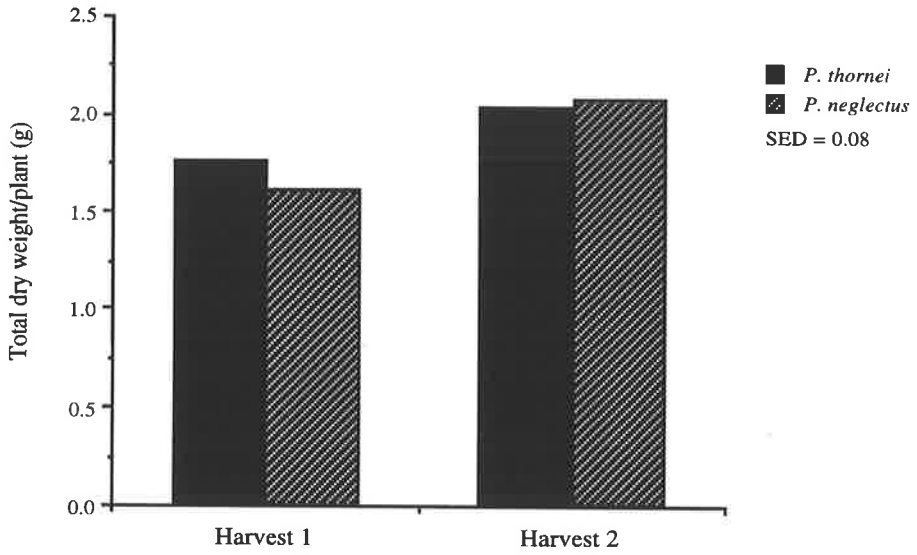


Fig. 9.12 : Effect of interaction between harvest, nematode and fungal species on the degree of root lesioning per Machete wheat root system.

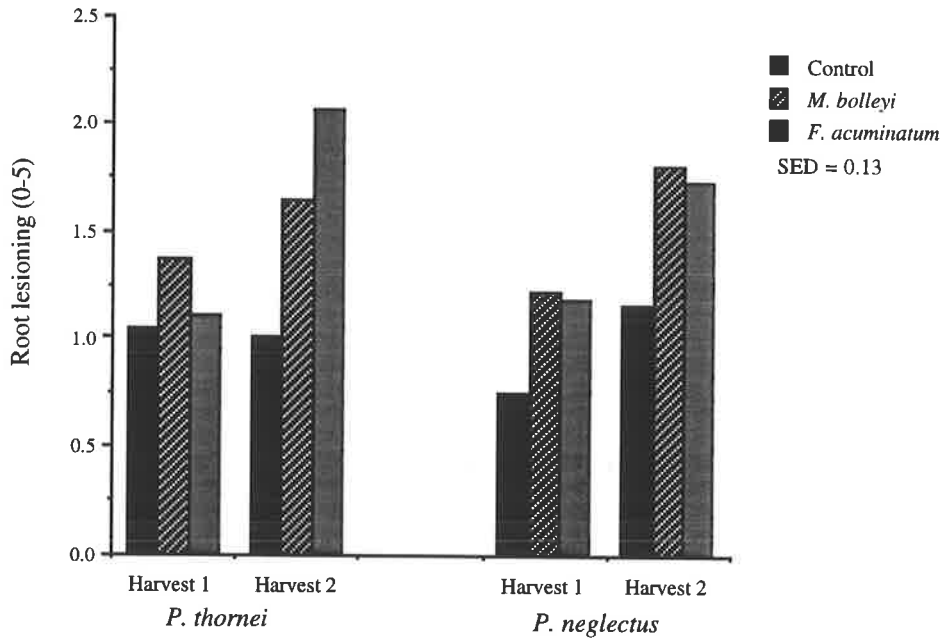


Fig. 9.13 : Effect of interaction between fungal species and nematode density on the degree of root lesioning per Machete wheat root system.

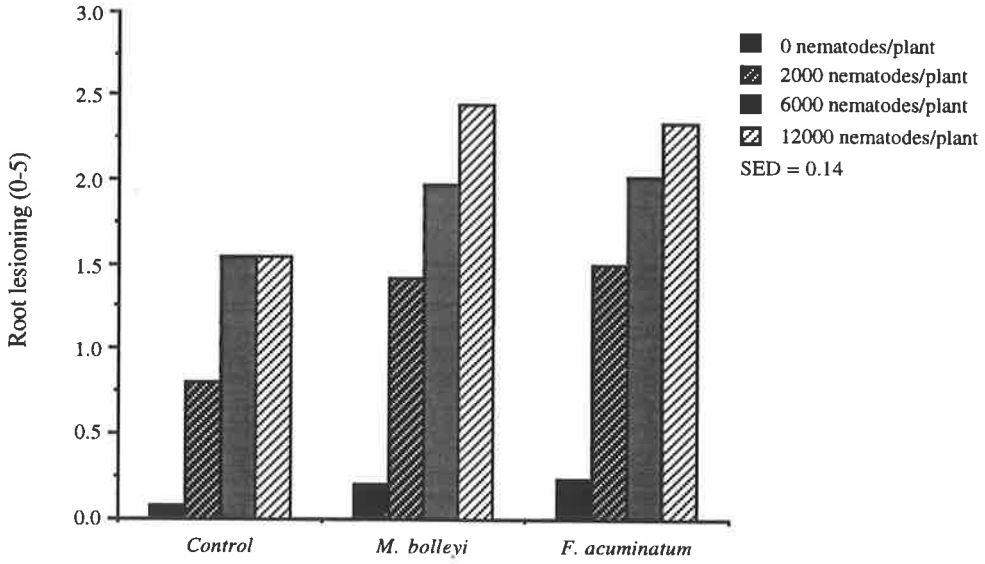


Fig. 9.14 : Effect of interaction between harvest and nematode density on the degree of root lesioning per Machete wheat root system.

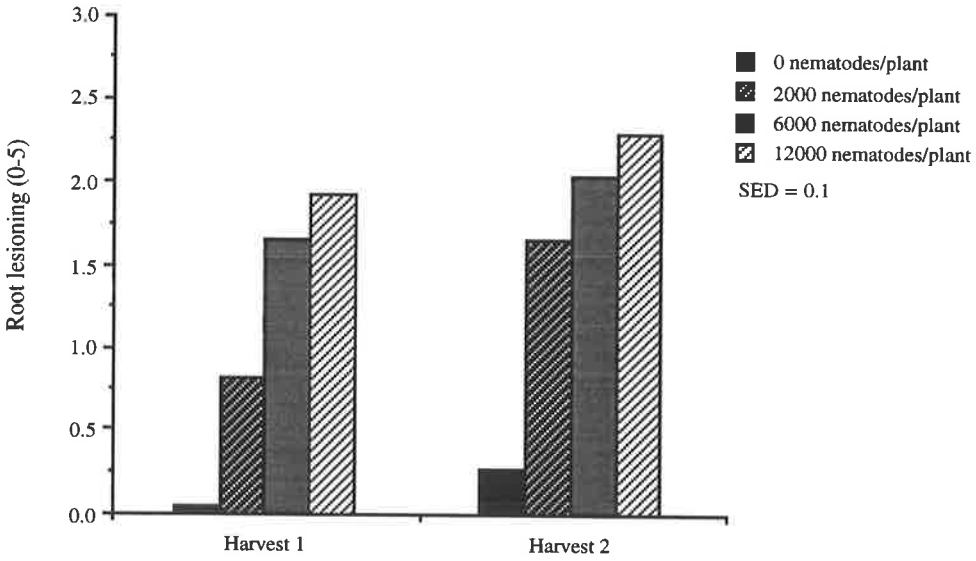
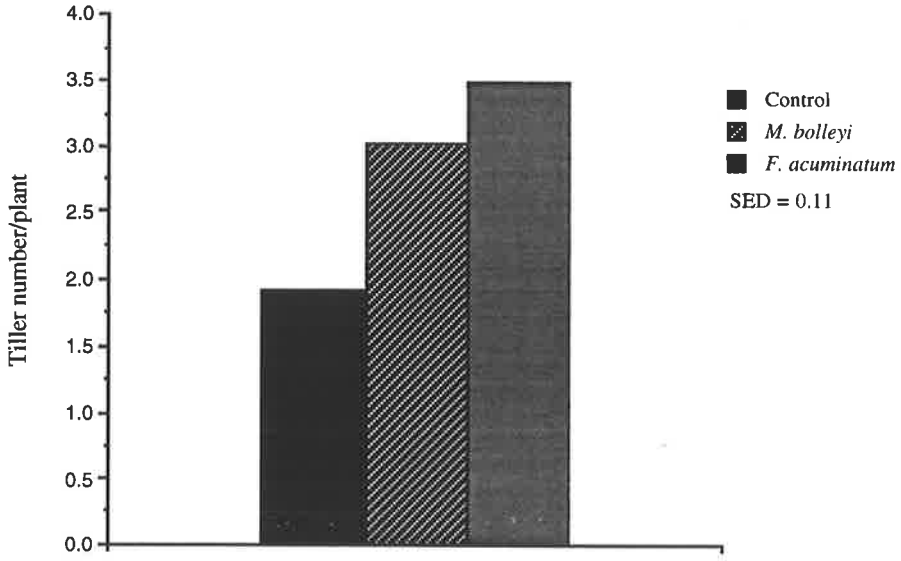


Fig. 9.15 : Effect of fungal inoculum on the number of tillers per Machete wheat root systems.



The significant 3-way interaction between harvest time, fungus and initial nematode density for the variable total dry weight per plant (g) is illustrated in Fig. 9.9. *P. thornei* and *P. neglectus,* with and without associated fungi, caused significant reductions in total dry weight per plant at high initial densities for both harvest times. The presence of *M. bolleyi* and *F. acuminatum* led to an increase in total dry weight relative to the nematode alone (Fig. 9.9), irrespective of nematode species (Fig. 9.10).

A significant 2-way interaction between nematode species and harvest revealed the total dry weight with both nematode species was greater at harvest 2 than harvest 1 (Fig. 9.11). It was also noted that nematode species responded differently at harvest 1, with *P. neglectus* giving lower total dry weight than *P. thornei*, whereas the opposite occurred at harvest 2 (Fig. 9.11).

Plate 9.1 : Representative Warigal plants from the three nematode fungus treatments; 2,000 *P. thornei* + *M. bolleyi* (left), *M. bolleyi* alone (middle) and control plants (right). The plants were grown for 10 weeks at 20°C in a controlled growth room. The presence of fungi with or without nematodes caused a stimulation of shoot growth.

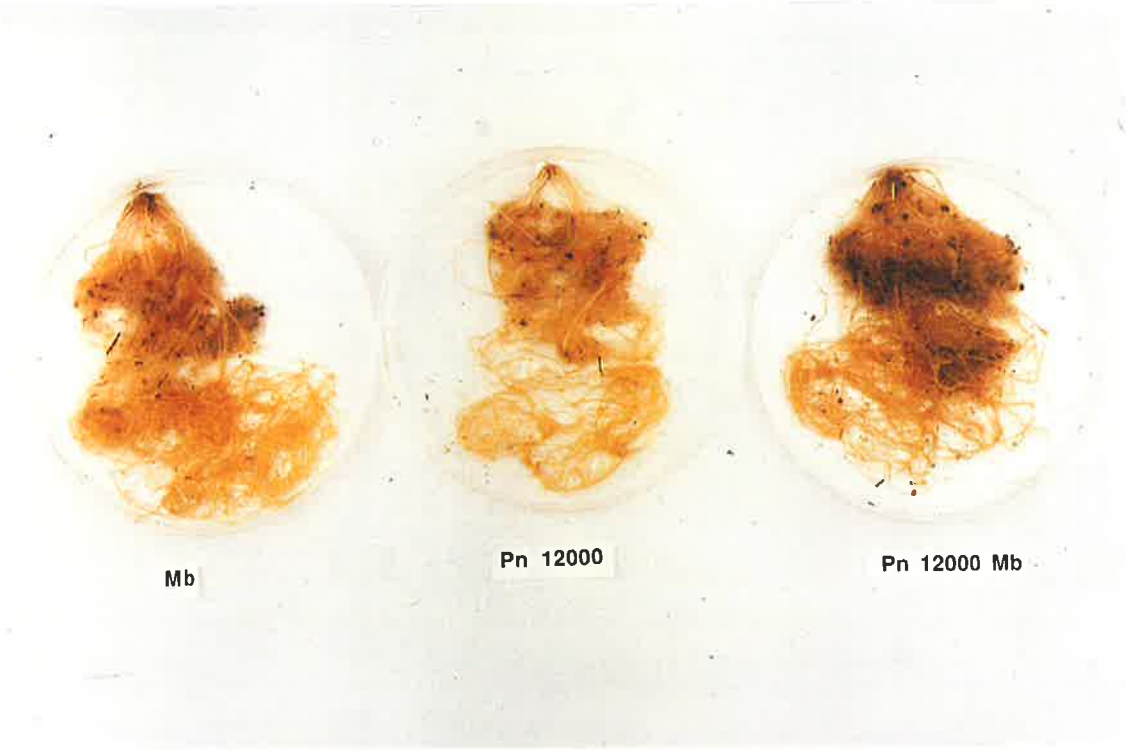
Plate 9.2 : Representative Warigal plants from the three nematode fungus treatments; 2,000 *P. thornei* + *F. acuminatum* (left), *F. acuminatum* alone (middle) and control plants (right) Plants were grown for 10 weeks at 20°C in a controlled growth room. The presence of fungi with or without nematodes caused a stimulation of shoot growth.


2000Pt*Mb
Mb
Control


2000Pn*Fa
Fa
Control

Plate 9.3 : Representative Warigal replicates from the three treatments, *M. bolleyi* (left), 12,000 *P. neglectus* (middle) and 12,000 *P. neglectus* + *M. bolleyi* (right). Plants were grown for 10 weeks at 20°C in a controlled growth room. In the presence of nematodes with fungi the degree of root lesioning was much greater than either nematode or fungi alone as indicated by the dark cortical lesions.

Plate 9.4: Representative Warigal replicates from the three treatments, control with no nematodes (left), 6,000 *P. thornei* + *F. acuminatun* (middle) and 12,000 *P. thornei* + *F. acuminatun* (right). Plants were grown for 10 weeks at 20°C in a controlled growth room. In the presence of nematodes with fungi the degree of root lesioning was much greater than either nematode or fungi alone as indicated by the dark cortical lesions.


Mb
Pn 12000
Pn 12000 Mb

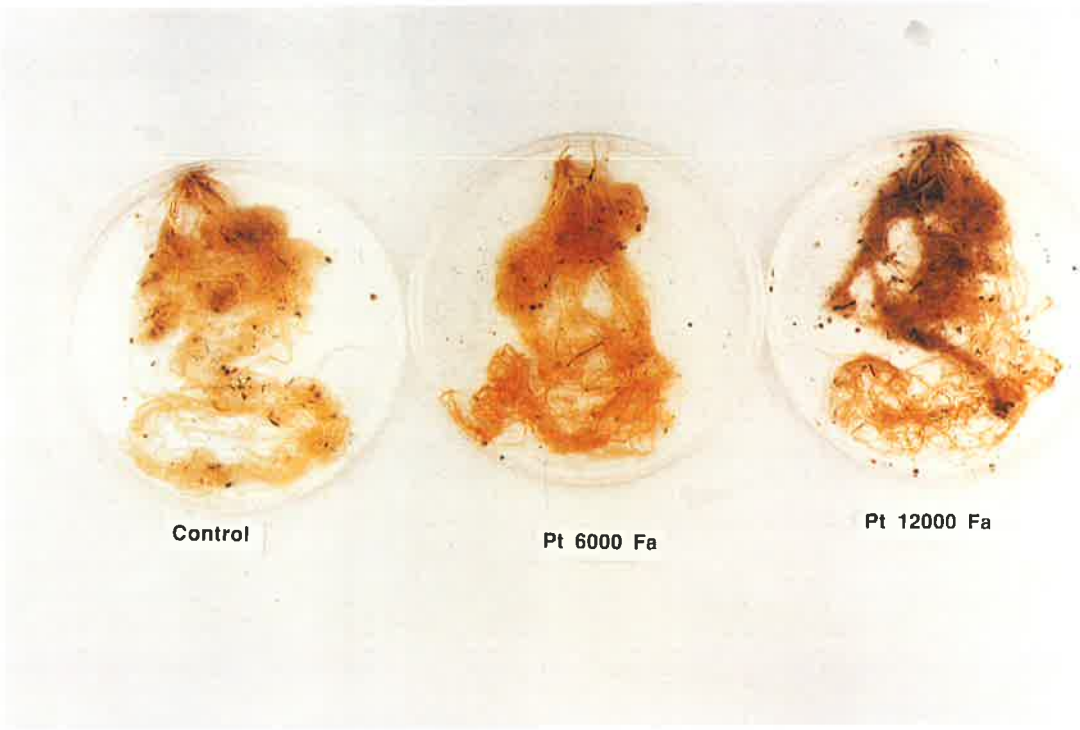
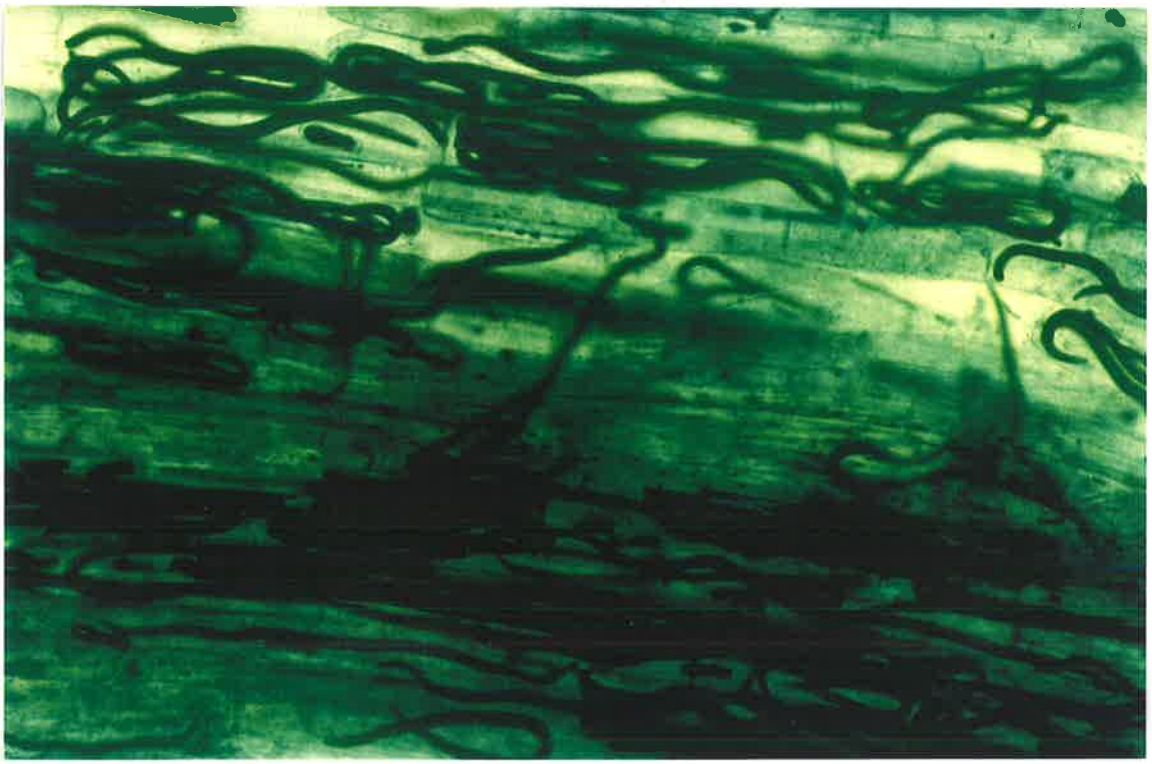

Control
Pt 6000 Fa
Pt 12000 Fa

Plate 9.5 : Stained Machete root system 10 weeks after growth in 300ml cups, initially inoculated with 12,000 *P. thornei* and *M. bolleyi*. Evidence of masses of *P. thornei* (dark green) and eggs (oval shaped rods) in the cortical cells of the seminal root system. Nematodes were not seen to penetrate the stele. (1cm=50μ).

Plate 9.6 : Stained Machete root system 10 weeks after growth in 300ml cups, initially inoculated with 6,000 *P. neglectus* and *M. bolleyi.* Evidence of extensive cortical degradation resulting in the loss of outer cortical layers. Nematodes were not seen to penetrate the stele. (1cm = 50μ).



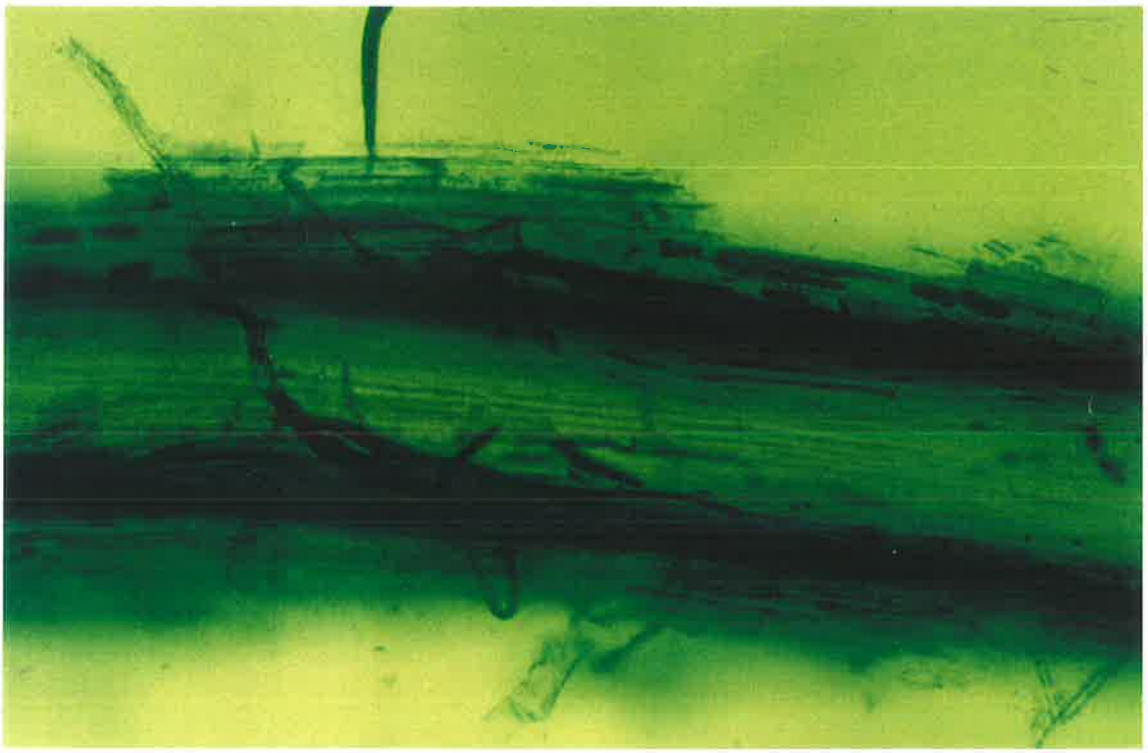
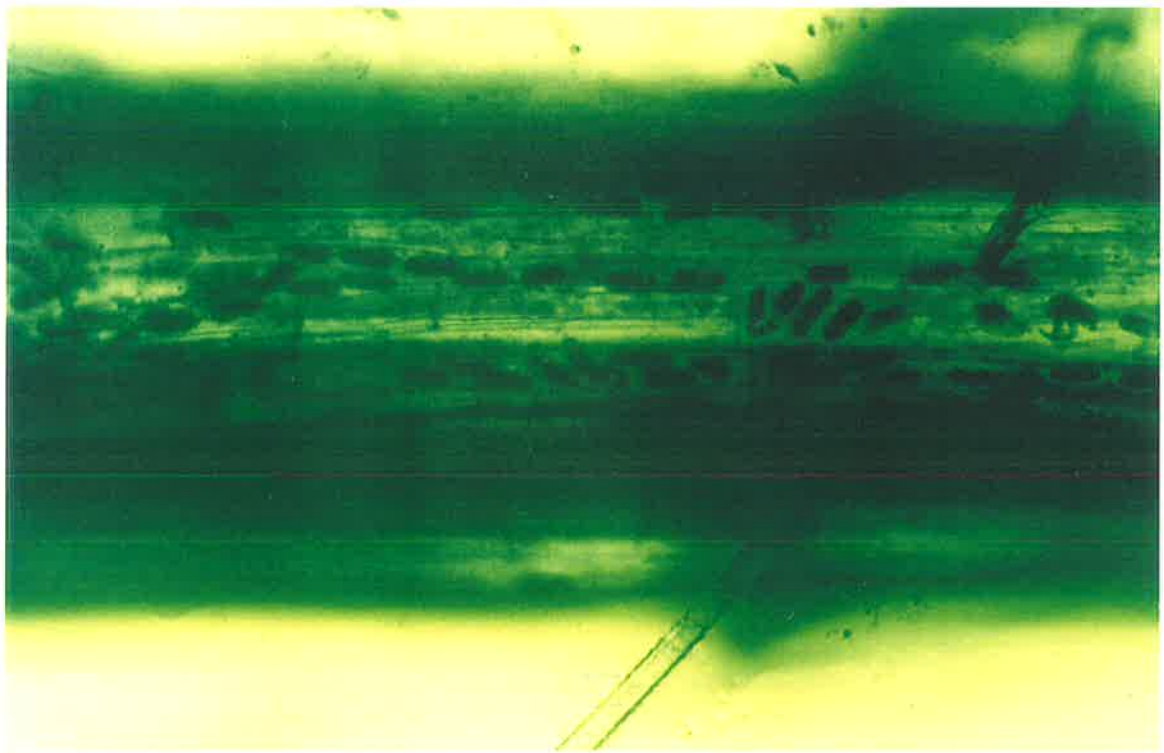


Plate 9.7 : Stained Machete root system 10 weeks after growth in 300ml cups, initially inoculated with 2,000 *P. neglectus* and *M. bolleyi*. A cortical lesion can be seen developing by the dark green regions associated with several nematodes and masses of *P. thornei* eggs lying parallel to the axis of the root. (1cm=50µ).

Plate 9.8 : Stained Machete root system 10 weeks after growth in 300ml cups, initially inoculated with 6,000 *P. neglectus* and *M. bolleyi*. Within both seminal and lateral roots the deposition of eggs (oval shaped rods) in rows distributed among the cortical cells was commonly seen, particularly in regions where many nematodes were present. (1cm=50µ).



Presence of either *M. bolleyi* or *F. acuminatum* increased root lesioning with both *P. thornei* and *P. neglectus*, with greater lesioning over time (Fig. 9.12, Plate 9.3) except *F. acuminatum* for *P. thornei*. A significant 2-way interaction between initial nematode density and fungal species showed increasing initial density resulted in a corresponding increase in the severity of root lesioning except for the highest density of nematodes for the control (Fig. 9.13, Plate 9.4). The degree of lesioning increased with time and increasing initial nematode density (Fig 9.14), however 2000 nematodes per plant were much more similar to higher densities at harvest 2. Tillering was significantly increased when the nematode was present with fungi, and more so with *F. acuminatum* (Fig. 9.15, Plate 9.1 and 9.2).

When Machete wheat roots were stained at harvest 2, fungal spores as well as nematodes were seen inside the cortex of the root. The high initial nematode densities were associated with many nematodes usually found in groups along the cortex (Plate 9.5). Cortical degradation was evident, whatever the initial nematode densities but was particularly severe at high initial densities (Plate 9.6). It was more severe when both fungi and nematodes were present, and did not occur in roots with fungi alone. Fungi appeared to be more uniformly distributed within the roots than nematodes. Nematodes were found in both the seminal and lateral roots but were not present in all parts of the root. Lesion formation was commonly associated with nematode proliferation (Plate 9.7). Adult nematodes were often seen associated with a line of eggs in the cortical tissue (Plate 9.8).

9.4 Discussion

Infection by one pathogen alters the host response and subsequent infection by another pathogen (Powell, 1971). The results reported in this chapter suggest that the

interaction of *M. bolleyi* and *F. acuminatum* with the root lesion nematodes *P. thornei* and *P. neglectus* does affect the way the host (in this case Machete wheat) responds.

Plant parasitic nematodes are primary plant pathogens and capable of causing important plant diseases (Prot, 1993). This is verified in this experiment, where both *P. thornei* and *P. neglectus* caused significant reductions in total dry weight at high initial densities, suggesting that both nematodes are pathogenic on wheat in their own right. All root parasitic nematodes cause mechanical injuries as they penetrate within or feed on root tissues, providing ready avenues for the entry of other pathogens (Prot, 1993). However, mechanical wounding does not always promote fungal penetration within root tissues. There is strong evidence, particularly with the sedentary nematodes such as root knot nematodes, that physiological and/or biochemical changes predispose their host to fungal pathogens (Prot, 1993). However, the precise physiological and/or biochemical changes induced or produced by the nematodes which predispose their hosts to fungal pathogens or directly enhance the invasion and development of pathogenic fungi in host tissues are not known (Prot, 1993).

The inoculum density of each pathogen relative to the other was found to affect the expression of the disease complex. Because plant parasitic nematodes reproduce only in the presence of a living host, organisms such as fungi, particularly those with a similar histopathology to the nematode, are likely to be most important. Some soil fungi which are not normally parasitic on plants become parasitic on roots infected with nematodes (Powell, 1971; Powell *et al.*, 1971). In general, synergistic interactions, nematodes provide an opportunity for fungal pathogens to show their greater pathogenic capabilities (Hasan, 1993).

Both the fungi (*M. bolleyi* and *F. acuminatum*) and nematodes (*P. thornei* and *P. neglectus*) tested here have similar ecological niches. *M. bolleyi* has been found in cortical and epidermal cells (Spiegel and Schönbeck, 1991) and can be isolated in high frequency from cereal roots (Sprague, 1948; Murray and Gadd, 1981). It is considered to be non-pathogenic (Liljeroth and Bäath, 1989) and is largely restricted to the invasion of naturally senescing cortices of cereal grasses (Kirk and Deacon, 1987). However, it was suggested to be the primary root rot fungal coloniser on cereals in the eastern prairies of Canada (Steirz and Bernier, 1989). *F. acuminatum* has similar pathology to *M . bolleyi* , penetrating epidermal cells, but hyphae are rarely observed to enter wounds directly (Stutz *et al.* , 1985). Both fungi are found in high levels in late July and August in South Australian cropping regions (Vanstone, 1991), and *M. bolleyi* is known to be a late coloniser of barley roots (Liljeroth and Bäath, 1989). Evidence from field work suggests higher numbers and multiplication of *P. thornei* and presumably *P. neglectus* in the latter parts of the growing season (Pattison, 1993), primarily determined by favourable environmental conditions.

In this experiment, *P. thornei* and *P. neglectus* appeared to increase in number and multiplication rate in the presence of fungi after 7 weeks. This increase also occurred in egg plant and tomato infected with *Verticillium* and *P. penetrans* (Mountain, 1954). Increased reproduction of *Pratylenchus* occurred in the presence of *Verticillium dahliae* on peppermint (Faulkner and Skotland, 1965). The interaction with *Verticillum* could be explained by the migration of the endoparasitic nematode to fresh feeding sites, and also the growth promoting substances produced in response to fungus-host-nematode interactions (Faulkner and Skotland, 1965; Faulkner and Bolander, 1969). In the experiment described here increased nematode numbers and multiplication at low initial densities may be explained by the similar niches and histopathology of both nematodes and fungi.

However, after 10 weeks of combined exposure with nematode and fungus, the number and multiplication of *P. neglectus* significantly declined relative to the control, but *P. thornei* numbers increased in combination with *M. bolleyi* . Decreased multiplication rate of the nematode in combination with the fungus relative to the nematode alone may be associated with the damage sustained to the host.

The traditional concept of specific aetiology stipulates that a single agent operating under prescribed conditions is the cause of a given disease (Powell, 1971). In contrast, the doctrine of predisposition has contributed to a much better understanding of disease development (Powell, 1971), particularly in the field situation. This is of extreme importance, particularly with regard to plant resistance to parasitic nematodes and their fungal associates. Nematodes are known to break resistance to fungal infection in crop cultivars and it is logical that the contrary may occur, that fungal pathogens might also be involved in reducing resistance of cultivars to nematode infection (Hasan, 1993). This is an important consideration and warrants further investigation. The control of *P. thornei* and *P. neglectus* on cereals is currently being investigated to allow breeding of resistant cereal lines. It will be important to identify the mechanisms of resistance in order to avoid resistance break down by the interacting fungal organisms in the soil system. If the form of plant resistance precludes the nematodes from penetrating the plant, then there is little concern, but if the resistance acts after penetration (as it does with a number of sedentary nematodes (Hasan, 1993) then the breeding strategy may need to incorporate fungal resistance(s) as well. Unfortunately, little is known about varieties with resistance to either nematode or the mechanisms behind the resistance.

Nematode numbers were found to increase with increasing nematode density while the multiplication rate declined. This is consistent with population changes associated with

other migratory endoparasitic nematodes (Section 2.8). In this experiment, increases in nematode number and density increased the severity of root lesioning, with the degree of severity increasing with time. Lesioning was worse at high initial densities (6000 and 12000 nematodes per plant) with both *M. bolleyi* and *F. acuminatum* . Taheri *et al.* (1994) found similar increased root lesioning with the closely related species *P. neglectus* and with common root rotting fungi.

The measured growth parameters of Machete were all found to be significantly affected by both *P. thornei* and *P. neglectus*. In general, increasing nematode density, with or without a fungal combination, was found to decrease the growth parameters of root, shoot and the summation of these. However, there was some evidence that at low initial densities (2000 nematodes per plant) plant growth was stimulated particularly up to 7 weeks. This was also reported by Nicol (1991) and in Ch. 6. However, because *P. thornei* is a migratory nematode and multiplication occurs continuously throughout the growing season in the presence of a host it is likely that damaging densities would override this stimulatory effect with time, as appeared to have happened by week 10.

Shoot dry weight was significantly higher in plants inoculated with fungi in combination with nematodes at both harvest times. This was most strongly correlated with an increased tillering capacity of Machete. *F. acuminatum* did not significantly affect root dry weight, but *M. bolleyi* significantly stimulated root growth at the first harvest. However, by 10 weeks there was no difference between the control, *F. acuminatum* or *M. bolleyi*.

Nematode species with closely related biology and feeding habits may present differences in their ability to predispose a plant to infection by the same fungus (Prot, 1993). This appears to be the case here, where *P. neglectus* limited root growth of

Machete earlier than did *P. thornei*. It is possible that *P. neglectus* invaded roots earlier in greater numbers than *P. thornei* or that the plant cells were more damaged by *P. neglectus* saliva. As discussed in Chapter 6, *P. neglectus* left roots faster than *P. thornei*, also suggesting possible differences in biology. However, by the second harvest after 10 weeks, there was no distinction between the root dry weight of plants with either *P. thornei* or *P. neglectus*. Regions of extensive cortical degradation were commonly associated with many nematodes and eggs lying parallel to the long axis of the root nematode. This observation has been commonly made in the work of Orion *et al.*, (1979) and Baxter and Blake (1968).

Overall, the total dry weight was stimulated in the presence of fungi with or without nematodes at both harvest times. The magnitude of nematode multiplication was reduced by both fungi relative to the control over the two harvest periods of 7 and 10 weeks. It is possible that the apparent stimulation of growth was an initial response to damage, namely an attempt by the plant to compensate for damage. The fact that the degree of root lesioning was increased in the presence of fungi and nematodes supports this. If the experiment was conducted for longer than 10 weeks significant growth reductions would probably have occurred when either nematode was present with *M. bolleyi* or *F. acuminatum*.

This work clearly demonstrates that there is an interaction between both nematode species and root rotting fungal species investigated. The fact that South Australian cropping regions contain over 19 different species of fungi (Taheri *et al.*, 1994) suggests that further investigations are warranted into the role of the root rotting fungi on the damage caused by either nematode.

Chapter 10
Final Discussion and Conclusions

The work described here shows that the root lesion nematode, *P. thornei* can be considered an economically important pathogen on wheat in South Australia. The damage caused by the nematode in the field is a function of the initial density in the soil and the influence of the environment on the host and the nematode. The geographical distribution of both *P. thornei* and *P. neglectus* is widespread in South Australia with a 90% chance of finding one or both nematodes in any soil sampled from the cropping regions. *P. thornei* has a tendency to be found in clay soils, while the closely related species *P. neglectus* is more commonly found in soils of a sandy composition. However, this distribution is not definitive, as both nematodes were found in all soil types.

Plants of commonly cultivated South Australian legumes (grain/pasture), cereals and weed species were sampled from the field, and contained *P. thornei* and/or *P. neglectus*. This was expected given that *P. thornei* is reported to be polyphagous and able to develop on a wide range of botanical families. Aseptic laboratory tests screening a number of cereals and non-leguminous hosts for their ability to support multiplication of *P. thornei* indicated a range of susceptibilities within genera of the *Gramineae*. In general terms, wheat is considered to be a 'good' host supporting high *P. thornei* multiplication. Triticale, rye, barley, oats and durum are 'moderate' hosts with moderately low to moderately high *P. thornei* multiplication, while the non-leguminous hosts of linseed and canola are 'poor or non-hosts' with negligible nematode multiplication and possible resistance. Similarly, *P. neglectus* multiplied more on wheat than on either rye or oat cultivars. Although both species of the root lesion nematode were found to have similar reactions to hosts between plant genera, some varieties/cultivars within plant genera (particularly the *Gramineae*) supported very different rates of multiplication for the two species. The variety AUS4930, which is

resistant to CCN, was one of the least susceptible wheats tested for *P. thornei,* but the most susceptible for *P. neglectus.*

An assay for screening cereal and non-leguminous hosts to *P. thornei* was developed and found more rapid, accurate and cost effective over a two month period in a controlled growth room as opposed to five months in an exposed glasshouse. The optimal conditions for this two month assay included growing plants in small polyethylene tubes with sandy soil in a growth room at 20°C, and inoculating with a non-damaging initial inoculum density of 400 *P. thornei* per plant one week after sowing seedlings. Even though experimental conditions for each assay were relatively standardised, care should be taken with the interpretation of results when comparing between assays because plants were inoculated with different inoculum cultures, albeit prepared by the same method. As a consequence, the most reliable interpretation of results was found from ranking the varieties rather than considering actual numbers. Further understanding of the variability in inoculum cultures is necessary.

The population dynamics and field relations of *P. thornei* on wheat were examined both in the laboratory and in the field. Laboratory studies to assess host damage in an enclosed manipulated environment using only the plant and the nematode were inconclusive. Repeated experiments using slightly different experimental techniques to investigate yield relations with *P. thornei* and wheat differed in outcome, reinforcing the need for a greater understanding of the relationship between the nematode and the host. Smaller container size and sandier soils were associated with higher *P. thornei* penetration of wheat roots. In almost all experiments, data was obtained by extraction of nematodes from roots over a defined time period. Estimates of the rate of extraction over four days showed that *P. neglectus* left roots significantly faster than *P. thornei*, even though similar numbers of nematodes were in the root systems. This further stresses the notion that care should be taken in interpreting data when comparing similar experiments for the two species of *Pratylenchus*. It is important to have a better understanding of the

ecology of *P. thornei*. Studies with other *Pratylenchus* spp. have demonstrated the nematode spends time evenly between the root system and in the surrounding soil environment. The question of possible ectoparasitism and the associated damage to root tissue are important considerations, neither of which were addressed here. However the comparable ranking of a range of cereal and non-leguminous hosts was found irrespective of whether nematodes were extracted from roots or soil in combination with roots supports the methodology used in this work.

The laboratory studies with wheat and *P. thornei* suggested that low initial densities, particularly at the early stages of growth up to five weeks after inoculation, result in stimulation of a number of plant growth variables. However, at higher initial densities, particularly over longer time periods, evidence of significant reduction of many growth variables was found. The significant reduction of nodal roots and number of leaves of the main tillers of the wheat plant at high initial densities (15-20,000 *P. thornei* per plant) would severely affect final grain yield. This affect would be worse if water were limited in the latter parts of the season. The nematode population dynamics found in the laboratory confirmed the basic population studies reported in Ch 2.9. In summary, with low initial population densities, *P. thornei* multiplication was high, however where the initial density was high multiplication was severely restricted. In all laboratory experiments, the nematode failed to reach "equilibrium density", which was probably due to non-limiting availability of food and the short duration of experiments.

Field population dynamics of *P. thornei* were assessed over two years in the Barossa Valley at Tanunda and showed that the nematode could severely restrict the yield of wheat in South Australia, depending on the initial density present. Manipulation of field populations was successfully achieved by the use of different cereal and non-leguminous hosts of varying susceptibility to *P. thornei*. The variation in initial nematode density identified across a given paddock suggest it may be possible to conduct similar studies without such manipulation. The ranking of susceptibility of varieties in relation to their

multiplication, based on soil numbers at the start of the first season and again at the opening of the following season, was not significant but revealed similar trends to the laboratory assay. This supports the value of laboratory-based studies which are conducted in a confined environment avoiding many of the unknown field variables. However, when plants were sampled during the growing season and nematodes were extracted from roots alone, the results inconsistent to those where the soil extracts were taken to establish initial densities at the start of the first season and final densities at the beginning of the second season. This may be due to confounding effects of initial nematode density on subsequent multiplication. Hence, sampling soil with roots at the start of the season was found to be a more accurate way of assessing initial nematode densities.

The initial density associated with damage was seasonally variable. In the first year, grain yield of Spear and Machete were reduced by 38 and 27% at high initial densities of 1506 and 3127 nematodes per 200g OD soil. However, in the second year of the trial, a reasonably dry year by comparison, the closely associated cultivar Warigal was found to be reduced by 27% at much higher initial *P. thornei* densities (9000 nematodes per 200g OD soil). It is possible that the tolerances of the wheat varieties used over the two years were different, but the majority of the variation was probably seasonal over the two years of the trial. Such population shifts over different seasons with initial nematode density responsible for economic damage are reported by other nematologists. As a consequence and in order to use correct information to predict future nematode populations, estimate possible crop damage and suggest management strategies, data on nematode numbers need to be averaged over several years and locations. The levels found at Tanunda over the two year trial are generally higher than those in *P. thornei* studies from NSW and Queensland.

The two varieties selected by laboratory screening for possible resistance to *P. thornei* were assessed for their resistance in the field. Both GS50A and AUS4930 had good

field resistance with a multiplication rate of less than one over a season. However, Warigal, a commonly grown South Australian wheat variety was found to have a multiplication rate of 3.88 over the season and also was intolerant. This supports the ranking of host efficiency of wheat cultivars from laboratory screening (Ch. 5), therefore making, Warigal, Machete and Spear, less favourable wheats to cultivate.

Visually, the tillering capacity and plant height of these intolerant, susceptible wheat varieties showed a trend to be reduced at higher initial nematode densities, but this was non-significant. All wheat varieties assessed, except AUS4930, had characteristic root lesioning, which was accentuated at higher initial densities. Lesioning was seen only during the later parts of the season after head formation and was worst at grain filling. High nematode densities were generally associated with a reduction in nodal root development and a reduction in finer laterals on both the seminal and nodal root system on susceptible hosts. This late season effect of *P. thornei* on the host might be a consequence of the biology of the nematode. Work in NSW suggests that low soil temperatures early in the season do not favour nematode multiplication, but as the temperatures rises later in the season (around October) were associated with considerable multiplication. As mentioned the wheat AUS4930, showed field resistance, and superior field tolerance to *P. thornei* as well as carrying a single gene for Cereal Cyst Nematode resistance.

The potential *P. thornei* resistance of AUS4930 demonstrated by the field trial warranted investigation of the resistance mechanisms and genetics of resistance. Studies revealed that the resistance in both GS50A and AUS4930 occurs post penetration, because similar numbers of *P. thornei* entered susceptible and presumed resistant hosts. The implications of nematode resistance acting post penetration is of concern for the South Australian cereal growing regions, given the high number of primary and secondary root rotting fungi which are prolific in most cropping regions which may have synergistic interactions with *Pratylenchus*. The segregation of F2 populations from the cross AUS4930 and the

susceptible wheat Schomburgk suggested that the *P. thornei* resistance was controlled by either of two independent recessive genes. However, the data should be interpreted with considerable caution due to the overlapping distribution of both parents. This is not surprising given that AUS4930 is a landrace variety, obtained originally from wetter regions of Iraq and was expected to show some variability. It was, surprising that the commonly grown wheat cultivar Schomburgk had some seed which grew into plants with a high degree of resistance. This could suggest that if some re-selection of intolerant, susceptible commonly cultivated wheats occurred, resistance to *P. thornei* may be obtained directly from highly adapted agronomically favourable current varieties. This was the case with the cultivar GS50A in Queensland, which was re-selected from the highly susceptible, intolerant wheat variety Gatcher (J. Thompson, pers. comm.). In order to clarify the results, further re-selection of both resistant and susceptible varieties is necessary to obtain isogenic lines, followed by a repeated genetic study.

Possible association of the nematodes *P. thornei* and *P. neglectus* with root rotting fungi was examined in a preliminary study. The fungi *Fusarium acuminatum* and *Microdochium bolleyi* were selected for this study due to their similar biology, being late colonisers and cortical invaders similar to *Pratylenchus*. There was a significant correlation between both nematode species in association with the two fungal species, with a combination of both organisms causing greater damage than when either species of nematode or fungus is present alone. Again, in these experiments, both nematodes were found to be damaging alone, re-confirming the nematodes as pathogenic in their own right. As noted in laboratory studies, looking at wheat yield in relation to increasing nematode density, plant growth was stimulated at low densities, both with and without fungi. At high initial densities, and with time, the nematodes alone and in combination with fungi were found to damage a number of plant variables measured. This initial investigation requires further studies on the association of other fungi with the nematode and their possible role in breakdown of nematode resistant varieties are required.

Commonly, both *P. thornei* and *P. neglectus* occur together in the cropping regions of South Australia. They appear morphologically similar to the field nematologist and are difficult for the non-taxonomist to identify using light microscopy. Both nematodes have been described from other countries but no such descriptions are available for Australia. Morphological studies from both field and culture specimens in South Australia show that both nematodes from local populations are comparable to previous descriptions from overseas. The two nematodes are distinguished primarily by the position of the vulva using the light microscope. This study revealed that the percentages used for this distinction may overlap between species, so additional characters should be used to identify species correctly. Use of high power and scanning electron microscopy can allow precise identification of the two species because there are differences in several morphological characters. In addition to the morphological studies, preliminary investigations were undertaken to identify possible molecular differences between the two nematode species. DNA was extracted from both species and digests carried out to identify Restriction Fragment Length Polymorphisms. The digests were probed with enzyme-digested labelled *P. thornei.* Repetitive band differences were identified between the two species indicating the potential of using DNA markers to allow species delineation.

In conclusion further investigations are required on several levels. On the biological level, a more adequate understanding is needed of the behaviour of the polycyclic pathogen and its migration between roots and soil throughout the growing season. The genetics of *P. thornei* resistance in AUS4930 need to be carefully reassessed, as most plant breeders require single gene resistance for successful breeding programs. Further, the mechanism of the *P. thornei* resistance is of paramount importance, particularly in South Australia due to the possible fungal associations with the nematode.

P. thornei resistance appears evident in the *T. aestivum* species, but because of the polycyclic nature, polyphagous host range and the population dynamics of *Pratylenchus*,

it is unlikely that rotation with one resistant crop will control the nematode below the threshold for economic damage. The potential resistance of the non-leguminous hosts of linseed and canola needs to be further assessed, but their adaptability and use in South Australia may be limited by environmental constraints. Canola may have great potential as a rotational crop, given that the isothiocyanates it contains may act as a soil biofumigant. Hence, further research to identify other sources of resistance within other cereal or rotational crops used in conjunction with cereals (grain and pasture legumes) is required. Finally, due to the presence of many mixed populations of the two species, and the suggested economic importance of the other species *P. neglectus,* an integrated control program will be necessary to manage both species of root lesion nematode.

Appendix A: Experimental Data for the Statewide Distribution Survey of *P. thornei* and *P. neglectus* in the cereal growing regions.

Explanation about data;

Counts of nematodes in root samples

Column	H	Root *P.thornei*
	I	Root *P.neglectus*
	J	*Pratylenchus sp.* root total

The values have a ranking from 0, low, moderate (mod) and high and refer to the number of nematodes in the root system of the crop sampled (Column G).

High > 40,000 nematodes / 3 plants.
Moderate 10,000 - 40,000 nematodes / 3 plants.
Low < 10,000 nematodes / 3 plants.

If a plant has a low, mod. or high ranking this implies some hosting ability. The ranking however, does not refer to hosting ability but rather simplified the counting procedure in the laboratory.

Counts of nematodes in soil samples

Column	K	Soil *P.thornei*
	L	Soil *P.neglectus*
	M	*Pratylenchus sp.* soil total

The numbers are all quantitative and refer to the number of nematodes per 200 g oven dry soil. However, because extensive sampling was not performed the numbers should be considered as presence or absence rather than quantitative.

Abbreviations used in the data table

RBE	= Red Brown Earth (red clay soil)
-	= missing data

APPENDIX A Experimental Data for the Statewide Survey for P. thornei and P. neglectus in the cereal growing regions

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	NO.	REGION	LOCATION	DATE	FARMER	SOIL TYPE	CURRENT CROP	ROOT P.thornei	ROOT P.neglectus	ROOT Prat.sp TOTAL	SOIL P.thornei	SOIL P.neglectus	TOTAL Prat. sp SOIL
2	1	5 Lower North	Two Wells	Oct-92	John Sharpe	–	Durum	–	–	low	–	–	–
3	2	5 Lower North	Two Wells	Oct-92	John Sharpe	RBE	Wheat(Durum)	–	–	–	–	–	14
4	3	5 Lower North	Two Wells	Oct-92	John Sharpe	–	Wheat(Durum)	–	–	–	–	–	–
5	4	5 Lower North	Two Wells	Oct-92	John Sharpe	grey clay	Faba Beans	–	–	low	–	–	10
6	5	5 Lower North	Two Wells	Oct-92	Malcom Wilson	sandy loam	Faba Beans	–	–	low	–	–	16
7	6	5 Lower North	Two Wells	Oct-92	Malcom Wilson	RBE	Durum (Yalloroi)	–	–	0.00	–	–	80
8	7	5 Lower North	Two Wells	Oct-92	Malcom Wilson	RBE	Triticale	–	–	0.00	–	–	13
9	8	5 Lower North	Two Wells	Oct-92	Malcom Wilson	RBE	Triticale(Currency)	–	–	0.00	–	–	5
10	9	5 Lower North	Mallalla	Oct-92	Peter March	loam	Durum (Yallaroi)	–	–	low	–	–	20
11	10	5 Lower North	Mallalla	Oct-92	Peter March	RBE	Wheat(Machete)	–	–	low	50	0	50
12	10	5 Lower North	Mallalla	Oct-92	Peter March	sandy loam	Pasture(leg/med/ryegrass)	–	–	–	12	0	12
13	11	5 Lower North	Mallalla	Oct-92	Peter March	clay loam	Roseclover	–	–	low	–	–	60
14	12	5 Lower North	Mallalla	Oct-92	Peter March	loam	Medic(Paraponto)	–	–	low	50	0	50
15	13	5 Lower North	Mallalla	Oct-92	Peter Irish	sand	Barley (Galleon)	–	–	low	–	–	4
16	14	5 Lower North	Mallalla	Oct-92	Peter Irish	loam	Oats(Pottoroo)	–	–	low	10	0	10
17	15	5 Lower North	Mallalla	Oct-92	Peter Irish	sandy loam	Pasture (Barley Grass)	–	–	low	12	0	12
18	16	5 Lower North	Mallalla	Oct-92	Peter Irish	sandy loam	Pasture(Hanalord Medic)	–	–	low	–	–	–
19	16	5 Lower North	Mallalla	Oct-92	Peter Irish	sandy loam	Pasture(Barley Grass)	–	–	low	–	–	–
20	17	5 Lower North	Red Banks	Oct-92	John Blacket	RBE	Barley (Galleon)	–	–	low	–	–	13
21	18	5 Lower North	Red Banks	Oct-92	John Blacked	RBE	Peas(Alma)	–	–	low	–	–	3
22	19	5 Lower North	Red Banks	Oct-92	John Blacked	loam	Wheat(Dagger)	–	–	low	–	–	40
23	20	5 Lower North	Red Banks	Oct-92	John Blacked	loam	Faba Beans (Fiord)	–	–	0.00	–	–	40
24	21	5 Lower North	Stockport	Oct-92	Ian Rohde	RBE	Wheat(Machete)	–	–	low	10	0	10
25	22	5 Lower North	Stockport	Oct-92	Ian Rohde	–	Peas(Alma)	–	–	0.00	–	–	–
26	23	5 Lower North	Stockport	Oct-92	Ian Rohde	clay loam	Pasture	–	–	0.00	51	0	51
27	24	5 Lower North	Stockport	Oct-92	Ian Rohde	RBE	Chickpea(Dooen)	–	–	low	0	0	0
28	25	5 Lower North	Tarlee	Oct-92	Tony Clarke	RBE	Wheat(Machete)	–	–	low	8	0	8
29	26	5 Lower North	Tarlee	Oct-92	Tony Clarke	RBE	Triticale(Tahara)	–	–	low	64	0	64
30	27	5 Lower North	Tarlee	Oct-92	Tony Clarke	RBE	Chickpea(Amethyst)	–	–	low	12	0	12
31	28	5 Lower North	Tarlee	Oct-92	Tony Clarke	RBE	Wild Oats	–	–	0.00	472	0	472
32	28	5 Lower North	Tarlee	Oct-92	Tony Clarke	RBE	Peas(Alma)	–	–	low	472	0	472
33	29	5 Lower North	Tarlee	Oct-92	Graham Hill	RBE	Durum(Yallaroi)	–	–	low	–	–	2
34	30	5 Lower North	Tarlee	Oct-92	Graham Hill	RBE	Chickpea(Amethyst)	–	–	low	72	0	72
35	30	5 Lower North	Tarlee	Oct-92	Graham Hill	RBE	Barrel Medic	–	–	low	72	0	72
36	30	5 Lower North	Tarlee	Oct-92	Graham Hill	RBE	Dinninup Clover	–	–	low	72	0	72
37	30	5 Lower North	Tarlee	Oct-92	Graham Hill	RBE	Ryegrass	–	–	low	72	0	72
38	31	5 Lower North	Tarlee	Oct-92	Graham Hill	RBE	Wheat(Dagger)	–	–	0.00	–	–	8
39	32	5 Lower North	Tarlee	Oct-92	Graham Hill	RBE	Pasture(Barley/clover)	–	–	–	–	–	3
40	33	5 Lower North	Stockport	Oct-92	Dean Branson	clay loam	Wheat (Machete)	–	–	0.00	0	0	0
41	34	5 Lower North	Stockport	Oct-92	Dean Branson	sand	Durum(Yallaroi)	–	–	0.00	0	0	0
42	35	5 Lower North	Stockport	Oct-92	Dean Branson	grey clay	Chickpea	–	–	0.00	0	0	0
43	36	5 Lower North	Stockport	Oct-92	Dean Branson	clay loam	Wheat (Machete)	–	–	low	–	–	6
44	37	5 Lower North	Stockport	Oct-92	Brian Koch	sandy loam	Wheat(Machete)	–	–	low	–	–	72
45	38	5 Lower North	Stockport	Oct-92	Brian Koch	sand	Wheat(Molineux)	–	–	low	–	–	2
46	39	5 Lower North	Stockport	Oct-92	Brian Koch	sandy loam	Peas(Alma)	–	–	low	0	0	0
47	40	5 Lower North	Stockport	Oct-92	Brian Koch	RBE	Vetch (Blanchefleur)	–	–	low	–	–	8
48	41	5 Lower North	Stockport	Oct-92	Syd Nairn	sandy loam	Durum(Yalloroi)	–	–	0.00	0	0	0
49	42	5 Lower North	Stockport	Oct-92	Syd Nairn	–	Oats(Marloo)	–	–	low	–	–	–
50	42	5 Lower North	Stockport	Oct-92	Syd Nairn	–	Clover(Paradana)	–	–	low	–	–	–

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51	43	5 Lower North	Stockport	Oct-92	Syd Naim	sand	Wheat(Machete)	–	–	0.00	0	0	0
52	44	5 Lower North	Stockport	Oct-92	Syd Naim	clay loam	Peas(Dunn)	–	–	0.00	6	0	6
53	45	5 Lower North	Kapunda	Oct-92	Neil Weichet	RBE	Wheat (Spear)	–	–	0.00	0	0	0
54	46	5 Lower North	Kapunda	Oct-92	Gary Schmidt	RBE	Peas(Alma)	–	–	0.00	–	–	3
55	47	5 Lower North	Kapunda	Oct-92	Gary Schmidt	RBE	Wheat(Molineux)	–	–	0.00	–	–	14
56	48	5 Lower North	Kapunda	Oct-92	Jamie Lewis	–	Barley(Clipper)	–	–	low	–	–	–
57	49	5 Lower North	Kapunda	Oct-92	Peter Ryan	RBE	Wheat(Janz)	–	–	0.00	–	–	5
58	50	5 Lower North	Kapunda	Oct-92	Peter Ryan	–	Wheat (Blade)	–	–	low	–	–	–
59	51	5 Lower North	Kapunda	Oct-92	Peter Ryan	RBE	Lupins(Gungarra)	–	–	0.00	–	–	2
60	52	5 Lower North	Kapunda	Oct-92	Mick Ryan	grey clay	Wheat(Raven)	–	–	0.00	22	0	22
61	52	5 Lower North	Kapunda	Oct-92	Mick Ryan	sand	Wheat(Raven)	–	–	low	80	0	80
62	53	5 Lower North	Kapunda	Oct-92	Mick Ryan	RBE	Wheat (Raven)	–	–	low	2	0	2
63	54	5 Lower North	Kapunda	Oct-92	Robert Tilley	RBE	Wheat (Spear)	–	–	low	24	0	24
64	54	5 Lower North	Kapunda	Oct-92	Robert Tilley	clay loam	Wheat (Warigal)	–	–	low	14	0	14
65	55	6 Upper North	Jamestown	Oct-92	Trevor Crawford	RBE	Barley(Schooner)	–	–	low	–	–	16
66	56	6 Upper North	Jamestown	Oct-92	Robin Hall	–	Pasture	–	–	low	–	–	–
67	57	6 Upper North	Jamestown	Oct-92	LT Coopper	RBE	Pasture	–	–	0.00	0	0	0
68	58	6 Upper North	Jamestown	Oct-92	Micheal Clarke	RBE	Vetch(Blanchefleur)	–	–	low	–	–	3
69	59	6 Upper North	Jamestown	Oct-92	Trevor Ellis	RBE	Pasture(Lucerne trotector)	–	–	–	–	–	2
70	60	6 Upper North	Jamestown	Oct-92	Trevor Ellis	RBE	Pasture Trial	–	–	–	0	0	0
71	61	6 Upper North	Jamestown	Oct-92	Trevor Ellis	RBE	Wheat(Machete)	–	–	low	–	–	2
72	62	6 Upper North	Jamestown	Oct-92	John McArthy	RBE	Barley(Galleon)	–	–	low	–	–	3
73	63	6 Upper North	Jamestown	Oct-92	Chris Bretag	RBE	Oats(Bandicoot)	–	–	low	0	3	3
74	64	6 Upper North	Jamestown	Oct-92	Tom Cootes	–	Durum(Yallaroi)	–	–	low	–	–	–
75	65	6 Upper North	Jamestown	Oct-92	Neville Gibb	RBE	Lupins(Gungarru)	–	–	0.00	0	0	0
76	67	6 Upper North	Jamestown	Oct-92	Ken Porter	RBE	Pasture(Grass/Clover)	–	–	–	–	–	50
77	68	6 Upper North	Jamestown	Oct-92	Gary Bratke	RBE	Wheat(Spear)	–	–	low	–	–	26
78	69	6 Upper North	Jamestown	Oct-92	Martin Clarke	RBE	Lupins(Danja)	–	–	low	0	0	0
79	70	6 Upper North	Jamestown	Oct-92	Martin Clarke	clay loam	Lupins	–	–	low	0	0	0
80	70	6 Upper North	Jamestown	Oct-92	Martin Clarke	sand	Wheat	–	–	low	32	0	32
81	71	6 Upper North	Jamestown	Oct-92	Steve Jacka	clay loam	Pasture (Hykon Clover)	–	–	low	–	–	16
82	72	6 Upper North	Jamestown	Oct-92	Alan Morgan	RBE	Wheat(Spear)	–	–	low	–	–	2
83	73	6 Upper North	Jamestown	Oct-92	Barry Clapp	RBE	Lupins(Danja)	–	–	0.00	0	0	0
84	74	6 Upper North	Jamestown	Oct-92	Malcolm Sparks	RBE	Wheat(Spear)	–	–	low	–	–	51
85	75	6 Upper North	Jamestown	Oct-92	Martin Clarke	RBE	Chickpea(Dooen)	–	–	low	5	0	5
86	76	5 Lower North	Saddleworth	Oct-92	Micheal Miller	RBE	Barley(Schooner)	–	–	low	–	–	24
87	77	5 Lower North	Saddleworth	Oct-92	Micheal Miller	RBE	Peas(Alma)	–	–	low	–	–	20
88	78	5 Lower North	Saddleworth	Oct-92	Micheal Miller	RBE	Barley(Schooner)	–	–	low	–	–	8
89	79	5 Lower North	Saddleworth	Oct-92	Micheal Miller	clay loam	Wheat(Meering)	–	–	low	–	–	44
90	80	5 Lower North	Saddleworth	Oct-92	Grantley McEvoy	RBE	Vetch(Blanchefleur)	–	–	low	–	–	4
91	80	5 Lower North	Saddleworth	Oct-92	Grantley McEvoy	grey clay	Vetch(Blanchefleur)	–	–	low	–	–	51
92	81	5 Lower North	Saddleworth	Oct-92	Grantley McEvoy	RBE	Lupins(Gungarra)	–	–	0.00	10	0	10
93	82	5 Lower North	Saddleworth	Oct-92	Grantley McEvoy	–	Wheat(Machete)	–	–	low			
94	83	5 Lower North	Saddleworth	Oct-92	Grantley McEvoy	RBE	Barely(Galleon)	–	–	low	–	–	10
95	84	5 Lower North	Saddleworth	Oct-92	Andrew Plueckhahn	RBE	Wheat(Janz)	–	–	low	36	0	36
96	85	5 Lower North	Saddleworth	Oct-92	Andrew Plueckhahn	clay loam	Peas(Alma)	–	–	low	0	26	26
97	86	5 Lower North	Saddleworth	Oct-92	Andrew Plueckhahn	clay loam	Barely(Skiff)	–	–	low	–	–	8
98	87	5 Lower North	Saddleworth	Oct-92	Andrew Plueckhahn	RBE	Chickpea(Samsen)	–	–	–	10	6	16
99	88	5 Lower North	Saddleworth	Oct-92	Greg Schmaal	clay loam	Peas(Alma)	–	–	0.00	–	–	6
100	89	5 Lower North	Saddleworth	Oct-92	Greg Schmaal	sand	Barley(Galleon)	–	–	0.00	0	0	0

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101	90	5 Lower North	Saddleworth	Oct-92	Greg Schmaal	RBE	Barley(Franklin)	–	–	0.00	–	–	22
102	91	5 Lower North	Saddleworth	Oct-92	Greg Schmaal	clay loam	Wheat(Kiata)	–	–	0.00	–	–	8
103	92	5 Lower North	Point Pass	Nov-92	Ron Milde	clay loam	Wheat(Machete)	–	–	low	0	42	42
104	93a	5 Lower North	Point Pass	Nov-92	Ron Milde	sandy loam	Barley(Galleon)	–	–	low	0	44	44
105	93b	5 Lower North	Point Pass	Nov-92	Ron Milde	sandy loam	Wheat(Spear)	–	–	low	0	0	0
106	94	5 Lower North	Point Pass	Nov-92	Ron Milde	RBE	Wheat(Machete)	–	–	low	0	24	24
107	95	5 Lower North	Point Pass	Nov-92	Ron Milde	sandy loam	Wheat(Machete)	–	–	low	–	–	6
108	96	5 Lower North	Point Pass	Nov-92	John Farley	sandy loam	Wheat(Dagger)	–	–	low	0	6	6
109	97	5 Lower North	Point Pass	Nov-92	John Farley	clay loam	Wheat(Machete)	–	–	low	0	74	74
110	98	5 Lower North	Point Pass	Nov-92	John Farley	sandy loam	Wheat(Machete)	–	–	low	0	11	11
111	99	5 Lower North	Point Pass	Nov-92	John Farley	–	Peas(Alma)	–	–	low	–	–	–
112	100	5 Lower North	Point Pass	Nov-92	Bob Leditschke	sandy loam	Wheat(Molineux)	–	–	low	0	0	0
113	101	5 Lower North	Point Pass	Nov-92	Bob Leditschke	sand	Ryegrass	–	–	low	0	45	45
114	101	5 Lower North	Point Pass	Nov-92	Bob Leditschke	sand	Wheat(Spear)	–	–	low	0	45	45
115	102	5 Lower North	Point Pass	Nov-92	Bob Leditschke	sandy loam	Wheat(Machete)	–	–	low	–	–	5
116	103	5 Lower North	Point Pass	Nov-92	Bob Leditschke	sand	Pasture(Barley Grass)	–	–	low	0	34	34
117	104	5 Lower North	Point Pass	Nov-92	Ross Schutz	sand	Wheat(Molineux)	–	–	low	–	–	2
118	105	5 Lower North	Point Pass	Nov-92	Ross Schutz	sandy loam	Wheat(Molineux)	–	–	low	0	300	300
119	105	5 Lower North	Point Pass	Nov-92	Ross Schutz	sandy loam	Wheat(Spear)	–	–	low	0	300	300
120	105	5 Lower North	Point Pass	Nov-92	Ross Schutz	sandy loam	Wheat(Machete)	–	–	low	0	300	300
121	106	5 Lower North	Point Pass	Nov-92	Ross Schutz	sandy loam	Wheat(Spear)	–	–	low	0	0	0
122	107	5 Lower North	Point Pass	Nov-92	John Farley	sand	Wheat(Spear)	–	–	low	0	5	5
123	108	5 Lower North	Point Pass	Nov-92	Geoff Schutz	sand	Wheat(Spear)	–	–	low	0	112	112
124	109	5 Lower North	Point Pass	Nov-92	Goeff Shcutz	sandy loam	Wheat(Spear)	–	–	–	–	–	3
125	110	5 Lower North	Point Pass	Nov-92	Geoff Schutz	sandy loam	Pasture(Barley grass/clover)	–	–	–	–	–	3
126	111	5 Lower North	Point Pass	Nov-92	Malcom Schutz	sand	Wheat(Machete)	–	–	low	0	66	66
127	112	5 Lower North	Point Pass	Nov-92	Malcolm Schutz	clay loam	Wheat(Machete)	–	–	low	0	4	4
128	113	5 Lower North	Point Pass	Nov-92	Malcolm Scutz	sandy loam	Peas(Dunn)	–	–	low	0	12	12
129	114	5 Lower North	Point Pass	Nov-92	Malcolm Schutz	sand	Wheat(Aroona)	–	–	low	0	6	6
130	115	3 Eastern Eyre	Kimba	Nov-93	Richard Parsons	RBE	Pasture (medic)	0	low	low	0	486	486
131	116	3 Eastern Eyre	Kimba	Nov-93	Richard Parsons	RBE	Peas (Alma)	0	low	low	0	74	74
132	117	3 Eastern Eyre	Kimba	Nov-93	Richard Pasrons	RBE	Wheat (Machete)	-	-	-	0	5300	5300
133	118	3 Eastern Eyre	Kimba	Nov-93	Peter Beinke	grey clay	Wheat (Janz)	-	-	-	0	3815	3815
134	119	3 Eastern Eyre	Kimba	Nov-93	Peter Beinke	grey clay	Pasture(Barley Grass)	0	low	low	0	650	650
135	119	3 Eastern Eyre	Kimba	Nov-93	Peter Beinke	grey clay	Pasture (Wimmera Ryegrass	0	low	low	0	650	650
136	119	3 Eastern Eyre	Kimba	Nov-93	Peter beinke	grey clay	Pasture(Harbinger Medic)	0	low	low	0	650	650
137	120	3 Eastern Eyre	Kimba	Nov-93	Peter Beinke	loam	Wheat (Spear)	-	-	-	0	650	650
138	121	3 Eastern Eyre	Kimba	Nov-93	Allen Sampson	RBE	Pasture(Caliph Medic)	0	low	low	0	1000	1000
139	121	3 Eastern Eyre	Kimba	Nov-93	Allen Sampson	RBE	Pasture(Harbinger Medic)	0	low	low	0	1000	1000
140	122	3 Eastern Eyre	Kimba	Nov-93	Allen Sampson	clay loam	Barley (Galleon)	-	-	-	0	662	662
141	123	3 Eastern Eyre	Kimba	Nov-93	Rod Lienert	RBE	Wheat (Machete)	0	mod	mod	0	3130	3130
142	124	3 Eastern Eyre	Kimba	Nov-93	Rod Leinert	RBE	Wheat (Machete)	-	-	-	0	992	992
143	125	3 Eastern Eyre	Kimba	Nov-93	Dean Williams	RBE	Wheat (Spear)	-	-	-	0	1280	1280
144	126	3 Eastern Eyre	Kimba	Nov-93	Dean Williams	RBE	Wheat (Spear)	-	-	-	0	97	97
145	127	3 Eastern Eyre	Cleve	Nov-93	Roger Neild	clay loam	Wheat (Spear)	-	-	-	0	38	38
146	128	3 Eastern Eyre	Cleve	Nov-93	Roger Neild	sandy loam	Oats (Marloo)	-	-	-	0	70	70
147	129	3 Eastern Eyre	Cleve	Nov-93	Roger Neild	RBE	Clover (Paradana Balancia)	-	-	-	0	66	66
148	130	3 Eastern Eyre	Cleve	Nov-93	Roger Neild	RBE	Pasture	-	-	-	0	5	5
149	131	1 Western Eyre	Smoky Bay	Apr-93	Bill Blumson	sand	Oats (Wallaroo)	-	-	-	0	292	292
150	132	1 Western Eyre	Smoky Bay	Apr-93	Bill Blumson	sand	-	-	-	-	0	140	140

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151	133	1 Western Eyre	Smoky Bay	Apr-93	Bill Blumson	sand	Pasture	-	-	-	0	312	312
152	134	1 Western Eyre	Smoky bay	Apr-93	Bill Blumson	sand	Barley (Galleon)	-	-	-	0	347	347
153	135	1 Western Eyre	Smoky Bay	Apr-93	Bill Blumson	sand	Pasture	-	-	-	0	227	227
154	136	1 Western Eyre	Smoky Bay	Apr-93	Bill Blumson	sand	Pasture	-	-	-	0	17	17
155	137	1 Western Eyre	Smoky Bay	Apr-93	Bill Blumson	sand	Pasture	-	-	-	0	86	86
156	138	1 Western Eyre	Smoky Bay	Apr-93	Bill Blumson	sand	Wheat (Schomburgk)	-	-	-	0	836	836
157	139	1 Western Eyre	Smoky Bay	Jul-93	Bill Blumson	sand	Pasture	-	-	-	0	890	890
158	140	1 Western Eyre	Smoky Bay	Jul-93	Bill Blumson	sand	Pasture	-	-	-	0	1465	1465
159	141	1 Western Eyre	Smoky bay	Jul-93	Bill Blumson	sand	Pasture	-	-	-	0	1140	1140
160	142	1 Western Eyre	Smoky Bay	Jul-93	Bill Blumson	sand	Pasture	-	-	-	0	280	280
161	143	1 Western Eyre	Smoky Bay	Jul-93	Bill Blumson	sand	Pasture	-	-	-	0	2660	2660
162	144	9 South East	Bordertown	Jul-93	Stephen Hole	sandy loam	Pasture(clover)	-	-	-	0	198	198
163	145	9 South East	Bordertown	Jul-93	Stephen Hole	sand	Pasture	-	-	-	0	0	0
164	146	9 South East	Bordertown	Jul-93	Stephen Hole	gey clay	Pasture	-	-	-	0	65	65
165	147	8 Murray Mallee	Cambrai	Jul-93	Norm Weinke	sand	Barley	-	-	-	0	25	25
166	148	3 Eastern Eyre	Kimba	Oct-93	Dean Williams	RBE	Medic (Parabinga)	0	low	low	0	1215	1215
167	149	3 Eastern Eyre	Kimba	Oct-93	Dean Williams	RBE	Wheat (Spear)	0	low	low	0	2690	2690
168	150	3 Eastern Eyre	Kimba	Oct-93	Dean Williams	RBE	Wheat (Machete)	0	low	low	0	1104	1104
169	151	3 Eastern Eyre	Kimba	Oct-93	Dean Williams	RBE	Wheat (Machete)	0	low	low	0	3500	3500
170	152	1 Western Eyre	Smoky Bay	Oct-93	Bill Blumson	sand	Wheat (Sunfield)	-	-	-	0	192	192
171	153	1 Western Eyre	Smoky bay	Oct-93	Bill Blumson	sand	-	-	-	-	0	1800	1800
172	154	1 Western Eyre	Strreaky bay	Oct-93	Don Greig	sand	Wheat (Janz)	0	low	low	0	40	40
173	155	1 Western Eyre	Minnipa	Oct-93	Minnipa RC	clay loam	Medic (Parabinga)	0	low	low	0	4000	4000
174	56/24	1 Western Eyre	Minnipa	Oct-93	Bruce Heddle	clay loam	Canola	0	high	high	0	5000	5000
175	157	1 Western Eyre	Streaky Bay	Oct-93	Don Greig	clay loam	Oats	0	high	high	250	250	500
176	158	1 Western Eyre	Minnipa	Oct-93	Minnipa RC	clay loam	Chickpea	0	mod	mod	0	84	84
177	59/24	1 Western Eyre	Minnipa	Oct-93	Bruce Heddle	clay loam	Indian Mustard	0	high	high	0	986	986
178	60/24	1 Western Eyre	Minnipa	Oct-93	Bruce Heddle	clay loam	Canola (Narendra)	0	high	high	0	3135	3135
179	161	3 Eastern Eyre	Cleve	Nov-93	MV Bammann	clay loam	Wheat(Kite)	-	-	-	0	61	61
180	162	3 Eastern Eyre	Cleve	Nov-93	MV Bammann	clay loam	Pasture	-	-	-	0	68	68
181	163	3 Eastern Eyre	Cleve	Nov-93	MV Bammann	clay loam	Barley (Galleon)	-	-	-	0	156	156
182	164	3 Eastern Eyre	Cleve	Nov-93	Tim Kraehe	clay loam	Pasture	-	-	-	0	465	465
183	165	3 Eastern Eyre	Cleve	Nov-93	Tim Kraehe	clay loam	Wheat (Machete/Janz)	-	-	-	0	86	86
184	166	3 Eastern Eyre	Cleve	Nov-93	Tim Kraehe	clay loam	Wheat (Machete)	-	-	-	0	32	32
185	167	3 Eastern Eyre	Cleve	Nov-93	Tim Kraehe	clay loam	Lupins(Merrit)	0	low	low	0	95	95
186	167	3 Eastern Eyre	Cleve	Nov-93	Tim Kraehe	clay loam	Brome Grass	0	low	low	0	95	95
187	168	3 Eastern Eyre	Cleve	Nov-93	Dennis Fiegart	clay loam	Wheat (Machete)	-	-	-	0	212	212
188	169	3 Eastern Eyre	Cleve	Nov-93	John Ranford	clay loam	Barley (Galleon)	-	-	-	0	460	460
189	170	3 Eastern Eyre	Cleve	Nov-93	John Ranford	clay loam	Wheat (Janz)	-	-	-	0	415	415
190	171	3 Eastern Eyre	Cleve	Nov-93	John Ranford	sandy loam	asture (Parabinga/Harbinge	-	-	-	0	375	375
191	172	3 Eastern Eyre	Cowell	Nov-93	Williams	sandy loam	Wheat(Machete)	-	-	-	0	1050	1050
192	173	3 Eastern Eyre	Cowell	Nov-93	Williams	sandy loam	Wheat(Excalibur)	0	low	low	0	11	11
193	174	3 Eastern Eyre	Cowell	Nov-93	Geoff Piggot	RBE	Pasture	-	-	-	0	374	374
194	175	3 Eastern Eyre	Cowell	Nov-93	Geoff Piggot	sandy loam	Pasture	-	-	-	0	45	45
195	176	3 Eastern Eyre	Cowell	Nov-93	Geoff Piggot	sandy loam	Lupins/Wheat	-	-	-	0	9	9
196	177	3 Eastern Eyre	Cowell	Nov-93	Roger Story	sandy loam	Wheat(Janz)	-	-	-	0	20	20
197	178	3 Eastern Eyre	Cowell	Nov-93	Roger Story	sandy loam	Wheat(Machete)	-	-	-	0	250	250
198	179	3 Eastern Eyre	Cowell	Nov-93	Roger Story	sandy loam	Barley(Schooner)	-	-	-	0	64	64
199	180	3 Eastern Eyre	Cowell	Nov-93	Roger Norris	clay loam	Wheat(Schomburgk)	-	-	-	0	20	20
200	181	3 Eastern Eyre	Cowell	Nov-93	Roger Norris	clay loam	Wheat(Schomburgk)	0	low	low	0	25	25

APPENDIX A Experimental Data for the Statewide Survey for P. thornei and P. neglectus in the cereal growing regions

	A	B	C	D	E	F	G	H	I	J	K	L	M
201	182	3 Eastern Eyre	Cowell	Nov-93	Roger Norris	clay loam	Wheat(Machete)	-	-	-	0	38	38
202	183	4 Lower Eyre	Kapinnie	Nov-93	AD & ML Ness	sandy loam	Barley(Schooner)	-	-	-	0	0	0
203	184	4 Lower Eyre	Kapinnie	Nov-93	AD & ML Ness	sandy loam	Lupins(Gungarru)	-	-	-	0	14	14
204	185	4 Lower Eyre	Kapinnie	Nov-93	AD & ML Ness	sandy loam	Wheat(Schomburgk)	0	low	low	0	0	0
205	186	4 Lower Eyre	Kapinnie	Nov-93	AD & ML Ness	sandy loam	Canola(Eureka)	0	low	low	0	0	0
206	187	4 Lower Eyre	Cummins	Nov-93	Dean Fuss	sandy loam	Wheat(Yaralinka)	0	low	low	0	49	49
207	188	4 Lower Eyre	Cummins	Nov-93	Dean Fuss	clay loam	Barley(Chebec)	-	-	-	0	36	36
208	189	4 Lower Eyre	Cummins	Nov-93	Gordon Modra	clay loam	Wheat(Spear)	0	low	low	0	1550	1550
209	190	4 Lower Eyre	Cummins	Nov-93	Gordon Modra	clay loam	Wheat(Schomburgk)	0	low	low	0	80	80
210	191	4 Lower Eyre	Ungarra	Nov-93	Reece Gale	clay loam	Wheat(Janz)	0	mod	mod	0	498	498
211	192	4 Lower Eyre	Ungarra	Nov-93	Reece Gale	clay loam	Pasture	-	-	-	0	42	42
212	193	4 Lower Eyre	Ungarra	Nov-93	Reece Gale	clay loam	Wheat(Janz)	-	-	-	0	130	130
213	194	4 Lower Eyre	Ungarra	Nov-93	Reece Gale	clay loam	Wheat(Janz)	-	-	-	0	167	167
214	195	4 Lower Eyre	Greenpatch	Nov-93	Phil Hyde	sandy loam	Pasture(Trikala subclover)	0	low	low	0	28	28
215	195	4 Lower Eyre	Greenpatch	Nov-93	Phil Hyde	sandy loam	Pasture(Halum Ryegrass)	0	low	low	0	28	28
216	196	4 Lower Eyre	Greenpatch	Nov-93	Phil Hyde	sandy loam	Pasture(capeweed)	0	low	low	0	81	81
217	196	4 Lower Eyre	Greenpatch	Nov-93	Phil Hyde	sandy loam	Pasture(Barley Grass)	0	0	0	0	81	81
218	197	4 Lower Eyre	Greenpatch	Nov-93	Phil Hyde	sandy loam	Vetch(Popany)	0	low	low	0	12	12
219	197	4 Lower Eyre	Greenpatch	Nov-93	Phil Hyde	sandy loam	Wimmera Ryegrass	0	low	low	0	12	12
220	198	4 Lower Eyre	Wanilla	Nov-93	Rob McFarlane	sandy loam	Oats(Echidna)	-	-	-	0	32	32
221	199	4 Lower Eyre	Wanilla	Nov-93	Peter Myers	sandy loam	Barley(Schooner)	-	-	-	0	0	0
222	200	4 Lower Eyre	Cummins	Nov-93	Hilton Trigg	RBE	Barely(Schooner)	-	-	-	0	75	75
223	201	4 Lower Eyre	Cummins	Nov-93	Hilton Trigg	clay loam	Wheat(Yaralinka)	-	-	-	3491	1164	4655
224	202	4 Lower Eyre	Greenpatch	Nov-93	Jack Borlase	clay loam	Pasture	-	-	-	0	108	108
225	203	4 Lower Eyre	Greenpatch	Nov-93	Jack Borlase	clay loam	Barley(O'Conner)	0	low	low	50	50	100
226	204	4 Lower Eyre	Koppio	Nov-93	Brenton Growden	sandy loam	Wheat(Aroona)	-	-	-	0	0	0
227	205	4 Lower Eyre	Koppio	Nov-93	Brenton Growden	sandy loam	Pasture	-	-	-	0	0	0
228	206	4 Lower Eyre	Pt. Lincoln	Nov-93	Ashley Flint	sandy loam	Fallow	-	-	-	0	26	26
229	207	4 Lower Eyre	Tumby Bay	Nov-93	Peter Swaffer	clay loam	Wheat(Janz)	-	-	-	0	114	114
230	208	4 Lower Eyre	Tumby Bay	Nov-93	Peter Swaffer	clay loam	Wheat(Aroona)	-	-	-	0	21	21
231	209	4 Lower Eyre	Yeelana	Nov-93	Peter Glover	RBE	Pasture	-	-	-	40	40	80
232	210	4 Lower Eyre	Yeelana	Nov-93	Peter Glover	RBE	Barley(Schooner)	-	-	-	200	200	400
233	211	4 Lower Eyre	Yeelana	Nov-93	Peter Glover	RBE	Wheat(Janz)	-	-	-	0	419	419
234	212	4 Lower Eyre	Yeelana	Nov-93	David Smith	RBE	Peas(Dundale)	0	low	low	0	115	115
235	213	4 Lower Eyre	Yeelana	Nov-93	David Smith	RBE	Wheat(Aroona)	low	low	low	115	41	156
236	214	4 Lower Eyre	Cummins	Nov-93	Laurence Jerricho	sandy loam	Barley	-	-	-	0	80	80
237	215	4 Lower Eyre	Koppio	Nov-93	Les Schneider	clay loam	Wheat(H99E)	0	low	low	0	90	90
238	216	2 Central Eyre	Kyancutta	Nov-93	Peter O'Brein	sandy loam	Barley(Galleon)	-	-	-	0	74	74
239	217	2 Central Eyre	Kyancutta	Nov-93	Peter O'Brein	sandy loam	Pasture (Onion Weed)	0	low	low	0	54	54
240	218	2 Central Eyre	Wudinna	Nov-93	Ken Scholz	sandy loam	Wheat(Machete)	-	-	-	0	1105	1105
241	219	2 Central Eyre	Wudinna	Nov-93	Ken Scholz	sandy loam	Lupins(Gungarru) + Oats	-	-	-	0	44	44
242	220	2 Central Eyre	Warramboo	Nov-93	Brad Schwark	sandy loam	Pasture	-	-	-	0	270	270
243	221	2 Central Eyre	Warramboo	Nov-93	Brad Schwark	sandy loam	Pasture	-	-	-	0	155	155
244	221(b	2 Central Eyre	Lock	Nov-93	Andrew Polkinghorne	calcareous sand	Pasture	-	-	-	0	76	76
245	222	2 Central Eyre	Lock	Nov-93	Andrew Polkinghorne	calcareous sand	Pasture	-	-	-	0	405	405
246	223	2 Central Eyre	Lock	Nov-93	Trevor Pierce	clay loam	Barley(Chebec)	-	-	-	0	185	185
247	224	2 Central Eyre	Lock	Nov-93	Trevor Pierce	clay loam	Pasture	-	-	-	0	248	248
248	225	2 Central Eyre	Tooligie Hill	Nov-93	David Habner	sandy laom	Wheat(Excalibur)	-	-	-	0	293	293
249	226	2 Central Eyre	Tooligie Hill	Nov-93	David Habner	sandy loam	Pasture(Harbinger)	-	-	-	0	524	524
250	227	1 Western Eyre	Wudinna	Nov-93	Roger Scholz	sandy loam	Oats(Wallaroo)	0	mod	mod	0	476	476

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	A	B	C	D	E	F	G	H	I	J	K	L	M
251	228	1 Western Eyre	Wudinna	Nov-93	Roger Scholz	sandy loam	Pasture(Ryegrass)	0	mod	mod	0	417	417
252	229	1 Western Eyre	Wudinna	Nov-93	Roger Scholz	sandy loam	Oats(Wallaroo)	-	-	-	0	660	660
253	230	1 Western Eyre	Wudinna	Nov-93	Roger Scholz	sandy loam	Barely(Galleon)	0	low	low	0	265	265
254	231	1 Western Eyre	Wudinna	Nov-93	Roger Scholz	sandy loam	Wheat(Machete)	-	-	-	0	720	720
255	232	1 Western Eyre	Wudinna	Nov-93	Roger Scholz	sandy loam	Wheat(Machete)	0	high	high	0	4080	4080
256	233	1 Western Eyre	Wudinna	Nov-93	Roger Scholz	sandy loam	Wheat(Spear)	0	high	high	0	1515	1515
257	234	1 Western Eyre	Wudinna	Nov-93	Roger Scholz	sandy loam	Medic(Harbinger)	0	low	low	0	1170	1170
258	235	1 Western Eyre	Kyancutta	Nov-93	Kevin Schopp	sandy loam	Rye(SA)	0	low	low	0	58	58
259	236	1 Western Eyre	Kyancutta	Nov-93	Kevin Scopp	sandy loam	Oats (Wallaroo/Pottoroo)	0	low	low	0	516	516
260	237	1 Western Eyre	Kyancutta	Nov-93	Kevin Scopp	sandy loam	Barley(Galleon)	0	low	low	0	780	780
261	238	1 Western Eyre	Kyancutta	Nov-93	Kevin Schopp	sandy loam	Barley(Chebec)	0	high	high	0	1812	1812
262	239	1 Western Eyre	Kyancutta	Nov-93	Kevin Schopp	sandy loam	Wheat(Machete)	0	low	low	0	565	565
263	240	1 Western Eyre	Straky Bay	Nov-93	Don Greig	calcareous sand	Barley(Galleon)	-	-	-	212	648	860
264	241	1 Western Eyre	Streaky Bay	Nov-93	Don Greig	calcareous sand	Pasture	-	-	-	0	460	460
265	242	1 Western Eyre	Streaky Bay	Nov-93	Don Greig	calcareous sand	Wheat(Molineux)	-	-	-	855	2566	3421
266	243	1 Western Eyre	Streaky Bay	Nov-93	Don Greig	calcareous sand	Wheal(Molineux)	-	-	-	30	270	300
267	244	1 Western Eyre	Streaky Bay	Nov-93	Don Greig	calcaerous sand	Pasture	-	-	-	333	1665	1998
268	245	1 Western Eyre	Streaky Bay	Nov-93	Don Greig	calcareous sand	Pasture	-	-	-	184	551	735
269	246	1 Western Eyre	Streaky Bay	Nov-93	Don Greig	calcareous sand	Oats(Potoroo)	-	-	-	0	47	47
270	247	1 Western Eyre	Streaky Bay	Nov-93	Don Greig	calcareous sand	Pasture	-	-	-	100	40	140
271	248	1 Western Eyre	Streaky Bay	Nov-93	Bruce Heddle	clay loam	Wheat(Schomburgk)	-	-	-	0	252	252
272	249	1 Western Eyre	Minnipa	Nov-93	Bruce Heddle	clay loam	Pasture (Harbinger)	0	low	low	1500	1500	3000
273	251	1 Western Eyre	Minnipa	Nov-93	Bruce Heddle	clay loam	Pasture(Barley Grass)	-	-	-	250	926	1176
274	252	1 Western Eyre	Minnipa	Nov-93	Bruce Heddle	clay loam	Wheat(Schomburgk)	low	low	low	4500	4500	9000
275	253	1 Western Eyre	Minippa	Nov-93	Bruce Heddle	clay loam	Wheat(BT Schomburgk)	0	low	low	0	425	425
276	254	1 Western Eyre	Minnipa	Nov-93	Bruce Heddle	sandy loam	Pasture(Ryegrass)	mod	mod	mod	0	456	456
277	255	1 Western Eyre	Minnipa	Nov-93	Bruce Heddle	sandy loam	asture(Parabinger/Harbinge	low	low	low	357	1071	1428
278	256	7 Yorke	Maitland	Nov-94	Roger Johns	sandy loam	Pasture(Harbinger)	mod	mod	mod	6	50	56
279	257	7 Yorke	Maitland	Nov-94	Roger Johns	clay loam	Medic(Mogul)	low	low	low	26	27	53
280	258	7 Yorke	Maitland	Nov-94	Roger Johns	RBE	Medic(Kaliph/Burr)	-	-	-	11	11	22
281	259	7 Yorke	Maitland	Nov-94	Brendon Maloney	RBE	Chickpeas(Kaniva)	0	high	high	18	18	36
282	259	7 Yorke	Maitland	Nov-94	Brendon Maloney	RBE	Lentil(5588)	0	low	low	18	18	36
283	259	7 Yorke	Maitland	Nov-94	Brendon Maloney	clay loam	Lentil(Aldinga)	0	low	low	12	12	24
284	260	7 Yorke	Arthurton	Nov-94	Lange's Trial	clay loam	Linola (Eyre)	0	0	0	0	0	0
285	261	7 Yorke	Maitland	Nov-94	Clinton Kitto	clay loam	Barley(Franklin)	-	-	-	0	0	42
286	262	7 Yorke	Maitland	Nov-94	Trevor Polkinghorne	clay loam	Canola(Dunkeld)	mod	mod	mod	120	69	189
287	262	7 Yorke	Maitland	Nov-94	Trevor Polkinghorne	clay loam	Mustard (Ebony)	low	low	low	105	52	157
288	263	7 Yorke	Tiparra	Nov-94	Rex Davey	RBE	Peas (Alma)	-	-	-	0	10	10
289	264	7 Yorke	Yorke Town	Nov-94	Leith Daniel	silty sand	weed (Miguonette cut leaf	low	low	low	-	-	-
290	264	7 Yorke	Yorke Town	Nov-94	Leith Daniel	sand	Medic(Serena)	low	low	low	-	-	-
291	265	7 Yorke	Warooka	Nov-94	J. Koennecke	sand	Medic(Paraponto)	0	mod	mod	6	18	24
292	266	7 Yorke	Warooka	Nov-94	J. Koennecke	sand	Barley(Franklin)	0	mod	mod	0	43	43
293	267	7 Yorke	Minlaton	Nov-94	Richard Jermein	clay loam	Chickpeas(Desivic)	0	low	low	12	48	60
294	267	7 Yorke	Minlaton	Nov-94	Richard Jermein	clay loam	Lathurus(new legume)	0	0	0	12	50	62
295	268	7 Yorke	Curramulka	Nov-94	Gordon Stone	sandy loam	Pasture	-	-	-	0	70	70
296	269	7 Yorke	Sandilands	Nov-94	Rex Kakoschke	clay loam	Medic(Santiago)	0	high	high	16	72	88
297	269	7 Yorke	Sandilands	Nov-94	Rex Kakoschke	clay loam	Medic(Sephi)	0	high	high	16	72	88
298	269	7 Yorke	Sandilands	Nov-94	Rex Kakoschke	clay loam	Medic(Sapo)	0	high	high	16	72	88
299	269	7 Yorke	Sandilands	Nov-94	Rex Kakoschke	clay loam	Medic(Parabinga)	0	high	high	12	51	63
300	270	7 Yorke	Maitland	Nov-94	Alex Brown	clay loam	Coriander	0	v.low	v.low	40	16	56

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	A	B	C	D	E	F	G	H	I	J	K	L	M
301	271	7 Yorke	Sandilands	Nov-94	Paul Schulze	sandy loam	Faba Beans (Icarus)	0	mod	mod	31	42	73
302	272	7 Yorke	Sandilands	Nov-94	Paul Schulze	sandy loam	Canola(Rainbow)	0	low	low	14	70	84
303	273	7 Yorke	Maitland	Nov-94	Dean Polkinghorne	clay loam	Medic (Santiago)	0	low	low	30	50	80
304	274	7 Yorke	Cunliffe	Nov-94	Rod Davies	sandy loam	Medic (Kaliph)	0	low	low	128	84	212
305	275	7 Yorke	Kaintain Corner	Nov-94	Neil Harrop	clay loam	Peas /Chickpeas	-	-	-	72	192	264
306	276	7 Yorke	Kaintain Corner	Nov-94	Neil Pontifex	clay loam	Canola/Linola	0	low	low	20	10	30
307	277	7 Yorke	Kaintain Corner	Nov-94	Neil Pontifes	sandy loam	Caonla(Rainbow)	0	low	low	30	10	40
308	278	9 South East	Padthaway	Nov-94	Wally Elsden	clay loam	Coriander	-	-	-	0	0	0
309	279	9 South East	Apsley(Victoria)	Nov-94	Chris Honner	sandy loam	Oats(Corbar)	-	-	-	0	0	0
310	280	9 South East	Apsley(Victoria)	Nov-94	Chris Honner	clay loam	Barley(Schooner)	-	-	-	0	0	0
311	281	9 South East	Wolseley	Nov-94	Eric Pietch	clay loam	Wheat(Janz)	-	-	-	350	0	350
312	282	9 South East	Wolseley	Nov-94	Eric Pietch	clay loam	Wheat(Janz)	-	-	-	350	0	350
313	283	9 South East	Mundulla	Nov-94	David Lowe	clay loam	Lupins(Gungarru)	low	low	low	128	0	128
314	284	9 South East	Mundulla	Nov-94	David Lowe	clay loam	Wheat(Janz)	-	-	-	0	100	100
315	285	9 South East	Mundulla	Nov-94	David Lowe	clay loam	Wheat(Janz)	-	-	-	0	170	170
316	286	9 South East	Wolseley	Nov-94	Bruce Ballinger	grey clay	Wheat(Janz)	-	-	-	150	150	300
317	287	9 South East	Wolseley	Nov-94	Jim McLellan	grey clay	Barley (Chebec)	-	-	-	120	40	160
318	288	9 South East	Kaniva(Victoria)	Nov-94	A & E Dyer	grey clay	Fenigreek	low	low	low	0	0	0
319	289	9 South East	Bordertown	Nov-94	Gerald Hillier	sandy loam	Barley (Schooner)	-	-	-	24	0	24
320	290	9 South East	Bordertown	Nov-94	Michael Llyode	sandy loam	Wheat(Tatiara)	-	-	-	0	0	0
321	291	9 South East	Kaniva (Victoria)	Nov-94	A & E Dyer	clay loam	Durum (Yallaroi)	-	-	-	50	10	60
322	292	9 South East	Kaniva (Victoria)	Nov-94	A & E Dyer	clay loam	Wheat(Janz)	-	-	-	0	0	0
323	293	8 Murray Mallee	Coonmandook	Nov-94	Steve Murray	sandy loam	Canola(Rainbow)	0	mod	mod	0	600	600
324	294	8 Murray Mallee	Coonmandook	Nov-94	Steve Murray	sandy loam	Wheat(Janz)	0	low	low	0	160	160
325	295	8 Murray Mallee	Yurgo	Nov-94	A. Kreig	sandy loam	Triticale(Tahara)	low	low	low	0	0	0
326	296	8 Murray Mallee	Yurgo	Nov-94	A. Kreig	sandy loam	Barley(Schooner)	-	-	-	0	12	12
327	297	8 Murray Mallee	Coonmandook	Nov-94	Marcus Kleinig	sand	Lupins	-	-	-	0	0	0
328	298	8 Murray Mallee	Coonmandook	Nov-94	Garry Hansen	sand	Canola(Rainbow)	-	-	-	0	450	450
329	299	8 Murray Mallee	Yumali	Nov-94	Nigel Day	sand	Wheat(Barunga)	low	high	high	100	350	450
330	300	8 Murray Mallee	KiKi	Nov-94	Craig Watts	sand	Barley(Yagan)	low	low	low	0	0	0
331	301	9 South East	Bordertown	Nov-94	R & M Hunt	sandy loam	Chinese Cabbage	low	low	low	0	0	0
332	302	9 South East	Bordertown	Nov-94	R & M Hunt	clay loam	Sugar Peas no14	low	low	low	50	0	50
333	303	9 South East	Bordertown	Nov-94	R & M Hunt	sandy loam	Commercial Onions	0	0	0	0	0	0
334	304	9 South East	Mundulla	Nov-93	Chris Leach	sandy loam	Beans (Icarus)	low	low	low	0	15	15
335	305	9 South East	Mundulla	Nov-93	Chris Leach	sandy loam	Coriander	0	low	low	5	20	25
336	306	9 South East	Mundulla	Nov-93	Chris Leach	sandy loam	Wheat(Meering)	0	low	low	0	0	0
337	307	9 South East	Buckingham	Nov-93	Neville Weisse	sandy loam	Canola (Dunkeld)	-	-	-	0	0	0

Appendix B: Experimental Data for the Field Population Dynamics and Yield Relations of *P. thornei* on cereals for the 2 year trial at Tanunda.

Explanation about columns:

Column	Abbreviation	Explanation
A	Plot No.	Plot Number (from 1...130)
B	V93	Variety grown in 1993
C	Rep93	Replicate number (there were 10 reps for each variety)
D	YTH93	Yield of individual plot in 1993 (tonnes per hectare)
E	V94	Variety grown in 1994
F	YTH94	Yield of individual plot in 1994 (tonnes per hectare)
G	ID93	Initial *P. thornei* density 1993 (per 200g OD soil)
H	ID94	Initial *P. thornei* density 1994 (per 200g OD soil)
I	ID95	Initial *P. thornei* density 1995 (per 200g OD soil)
J	MR93	*P. thornei* Multiplication rate over the 1993 season
K	MR94	*P. thornei* Multiplication rate over the 1994 season
*		Missing data

PLOT NO.	V93	REP 93	YTH93	V94	YTH94	ID93	ID94	ID95	MR93	MR94
1	Machete	1	3.61	GS50A	1.65	124.18	995.84	1276.58	8.02	1.28
2	Spear	1	4.24	AUS4930	1.35	1285.71	5920.88	2955.63	4.61	0.5
3	Warigal	1	4.42	Warigal	1.91	101.1	419.45	3418.61	4.15	8.15
4	Grimmett	1	3.27	AUS4930	1.47	40.66	202.78	609.89	4.99	3.01
5	Currency	1	*	Warigal	1.76	57.14	55.56	30.87	0.97	0.56
6	Linseed	1	*	GS50A	1.18	87.91	84.72	598.78	0.96	7.07
7	Fallow	1		Warigal	1.38	42.86	52.78	65.43	1.23	1.24
8	Molineux	1	4.66	GS50A	1.46	87.91	429.17	1996.35	4.88	4.65
9	Canola	1	0.57	Warigal	1.66	18.68	54.17	207.41	2.9	3.83
10	Yallaroi	1	3.42	GS50A	1.55	85.71	152.78	635.82	1.78	4.16
11	Echidna	1	4	GS50A	1.42	35.16	543.06	493.84	15.44	0.91
12	Tahara	1	3.83	GS50A	1.61	108.79	166.67	346.92	1.53	2.08
13	GS50A	1	3.11	AUS4930	1.13	36.26	256.95	1039.53	7.09	4.05
14	Fallow	2		Warigal	1.45	20.88	211.11	503.72	10.11	2.39
15	Tahara	2	4.58	Warigal	2.05	23.08	81.95	265.44	3.55	3.24
16	Warigal	2	4.52	Warigal	2.16	21.98	81.95	492.61	3.73	6.01
17	Yallaroi	2	4.3	Warigal	*	65.93	23.61	67.9	0.36	2.88
18	Linseed	2	0.79	Warigal	1.81	26.37	31.94	53.09	1.21	1.66
19	Currency	2	3.62	Warigal	2.06	38.46	40.28	181.49	1.05	4.51
20	Machete	2	3.42	Warigal	2.3	882.42	1088.9	3022.3	1.23	2.78
21	Grimmett	2	3.57	Warigal	*	534.07	1369.46	733.35	2.56	0.54
22	Molineux	2	3.88	GS50A	*	405.49	438.89	1358.06	1.08	3.09
23	Spear	2	4.8	GS50A	1.42	478.02	1177.79	1088.92	2.46	0.92
24	GS50A	2	2.92	Warigal	1.74	306.59	313.89	2111.17	1.02	6.73
25	Canola	2	0.34	Warigal	1.44	150.55	500	938.3	3.32	1.88
26	Echidna	2	3.97	GS50A	1.39	25.27	288.89	1902.52	11.43	6.59
27	Yallaroi	3	4.51	Warigal	1.62	29.67	12.5	98.77	0.42	7.9
28	Currency	3	3.23	GS50A	1.35	21.98	81.95	283.96	3.73	3.47
29	Grimmett	3	*	AUS4930	1.3	60.44	15.28	907.43	0.25	59.4
30	Canola	3	0.4	Warigal	1.71	75.82	173.61	469.15	2.29	2.7
31	Machete	3	3.84	Warigal	1.77	47.25	311.11	1481.52	6.58	4.76
32	Molineux	3	3.66	Warigal	1.85	51.65	480.56	1083.98	9.3	2.26
33	Tahara	3	3.63	Warigal	1.74	7.69	79.17	1228.43	10.29	15.52
34	Spear	3	4.27	Warigal	1.32	284.62	5200.04	17719	18.27	3.41
35	Canola	3	0.46	GS50A	1.14	128.57	*	2555.62	*	*
36	Echidna	3	4.02	Warigal	1.87	81.32	300	203.71	3.69	0.68
37	Linseed	3	0.77	Warigal	1.85	165.93	184.72	232.1	1.11	1.26
38	Warigal	3	4.24	GS50A	1.65	131.87	369.45	834.59	2.8	2.26
39	GS50A	3	3.02	Warigal	2.05	51.65	270.84	966.69	5.24	3.57
40	Spear	4	4.22	Warigal	*	98.9	118.06	1133.36	1.19	9.6
41	Grimmett	4	3.28	GS50A	*	58.24	143.06	1334.6	2.46	9.33
42	Linseed	4	0.52	Warigal	1.68	112.09	140.28	1712.39	1.25	12.21
43	Echidna	4	3.85	Warigal	2.07	282.42	843.06	888.91	2.99	1.05
44	GS50A	4	2.72	AUS4930	1.29	71.43	195.83	750.64	2.74	3.83
45	Warigal	4	3.72	AUS4930	1.38	123.08	1105.56	4930.99	8.98	4.46
46	Molineux	4	3.66	AUS4930	1.55	303.3	1587.51	1061.76	5.23	0.67
47	Machete	4	3.82	GS50A	1.61	376.92	1605.57	3371.69	4.26	2.1
48	Tahara	4	4.44	Warigal	2.15	35.16	720.84	532.11	20.5	0.74
49	Yallaroi	4	4.74	Warigal	1.8	73.63	80.56	609.89	1.09	7.57
50	Fallow	4		Warigal	1.5	30.77	93.06	375.32	3.02	4.03
51	Currency	4	4.27	AUS4930	1.32	98.9	38.89	277.79	0.39	7.14
52	Fallow	5		Warigal	1.48	35.16	11.11	190.13	0.32	17.11
53	Warigal	5	4.52	Warigal	1.73	165.93	31.94	608.66	0.19	19.05
54	Fallow	5		Warigal	1.67	264.83	443.06	3503.79	1.67	7.91
55	Yallaroi	5	3.86	GS50A	1.5	175.82	72.22	1425.96	0.41	19.74
56	Echidna	5	4.24	Warigal	1.78	459.34	1381.96	10347.2	3.01	7.49
57	Molineux	5	3.87	Warigal	1.54	253.85	6337.55	16810.3	24.97	2.65
58	Grimmett	5	3.56	Warigal	2.18	85.71	733.34	3530.96	8.56	4.81
59	Spear	5	5.15	Warigal	2.31	159.34	2413.91	3639.6	15.15	1.51
60	Canola	5	0.43	AUS4930	*	98.9	*	592.61	*	*
61	Currency	5	3.88	GS50A	1.68	31.87	158.33	734.59	4.97	4.64
62	Machete	5	3.71	Warigal	2.2	169.23	384.73	1316.08	2.27	3.42
63	Tahara	5	4.35	AUS4930	1.43	39.56	126.39	734.59	3.19	5.81
64	Linseed	5	0.53	Warigal	2.24	70.33	187.5	759.28	2.67	4.05
65	GS50A	8	2.77	GS50A	*	49.45	734.73	1760.54	14.86	2.4
66	Warigal	6	4.14	AUS4930	1.48	16.48	108.33	867.92	6.57	8.01

67	GS50A	6	2.7	Warigal	1.96	152.75	288.89	3079.09	1.89	10.66
68	Tahara	6	3.76	AUS4930	1.38	558.24	234.72	522.24	0.42	2.22
69	Molineux	6	4.44	AUS4930	1.37	746.15	766.67	1392.63	1.03	1.82
70	Currency	6	3.89	Warigal	1.78	178.02	401.39	7554.52	2.25	18.82
71	Fallow	6		Warigal	1.48	49.45	56.94	277.79	1.15	4.88
72	Spear	6	5.05	Warigal	1.45	32.97	30.56	101.24	0.93	3.31
73	Linseed	6	0.79	Warigal	1.63	68.13	151.39	354.33	2.22	2.34
74	Grimmett	6	*	Warigal	2.05	46.15	152.78	859.28	3.31	5.62
75	Yallaroi	6	3.88	Warigal	1.93	263.74	52.78	3150.7	0.2	59.7
76	Machete	6	3.43	Warigal	1.88	252.75	3350.03	6056.95	13.25	1.81
77	Canola	6	*	Warigal	1.32	241.76	863.9	8308.86	3.57	9.62
78	Echidna	6	4.14	GS50A	1.51	1012.09	2625.02	2637.11	2.59	1
79	Canola	7	0.29	Warigal	1.31	1335.16	1533.35	6876.72	1.15	4.48
80	Spear	2	4.39	GS50A	1.79	121.98	1743.07	2375.37	14.29	1.36
81	Warigal	7	4.47	Warigal	2.07	618.68	6600.05	10933.6	10.67	1.66
82	Yallaroi	7	4	AUS4930	1.42	84.62	436.11	1906.22	5.15	4.37
83	Tahara	7	4.21	AUS4930	1.47	18.68	65.28	325.93	3.49	4.99
84	Linseed	7	0.72	AUS4930	1.13	140.66	219.45	544.46	1.56	2.48
85	Grimmett	7	3.53	GS50A	1.72	136.26	108.33	2328.46	0.8	21.49
86	Fallow	7		Warigal	1.82	30.77	120.83	337.05	3.93	2.79
87	GS50A	7	2.65	GS50A	1.81	90.11	822.23	4000.1	9.12	4.86
88	Echidna	7	4.06	AUS4930	1.31	200	238.89	1113.61	1.19	4.66
89	Machete	7	3.38	AUS4930	1.16	429.67	37.5	2266.73	0.09	60.45
90	Currency	7	3.47	GS50A	1.68	587.91	745.84	888.91	1.27	1.19
91	Molineux	7	4.26	Warigal	2.08	129.67	72.22	2118.57	0.56	29.33
92	Machete	7	*	AUS4930	1.4	27.47	613.89	1196.33	22.35	1.95
93	Echidna	8	4.29	AUS4930	1.51	18.68	0	139.51	0	*
94	Tahara	8	4.29	Warigal	1.94	26.37	16.67	380.26	0.63	22.82
95	Fallow	8		Warigal	1.49	0	19.44	345.69	*	17.78
96	Canola	8	0.54	AUS4930	1.21	12.09	188.89	822.24	15.63	4.35
97	Grimmett	8	3.5	Warigal	1.8	9.89	63.89	1618.56	6.46	25.33
98	Warigal	8	3.67	Warigal	1.72	79.12	5125.04	6412.51	64.77	1.25
99	Molineux	8	2.77	Warigal	1.22	857.14	12561.2	11167	14.65	0.89
100	Spear	8	3.11	GS50A	1.61	1654.94	13077.9	4566.79	7.9	0.35
101	GS50A	8	3.12	Warigal	1.56	1873.62	879.17	7747.12	0.47	8.81
102	Linseed	8	0.92	AUS4930	1.37	1169.23	2531.96	1392.63	2.17	0.55
103	Yallaroi	8	4.18	AUS4930	*	3228.57	2972.25	2584.02	0.92	0.87
104	Currency	8	3.5	AUS4930	1.25	196.7	326.39	1930.91	1.66	5.92
105	Molineux	9	3.91	GS50A	1.71	12.09	208.34	3200.08	17.24	15.36
106	Grimmett	9	3.6	Warigal	2.42	21.98	184.72	302.48	8.4	1.64
107	Warigal	9	4.7	AUS4930	1.22	91.21	187.5	3059.34	2.06	16.32
108	Fallow	9		Warigal	1.93	687.91	354.17	696.31	0.51	1.97
109	Machete	9	3.67	GS50A	1.96	46.15	200	886.44	4.33	4.43
110	GS50A	9	2.83	AUS4930	1.52	200	361.11	1659.3	1.81	4.59
111	Echidna	9	4.01	Warigal	1.84	906.59	1375.01	7271.79	1.52	5.29
112	Yallaroi	9	3.91	AUS4930	1.62	258.24	325	1106.2	1.26	3.4
113	Tahara	8	4.35	GS50A	1.74	209.89	361.11	859.28	1.72	2.38
114	Currency	9	4	Warigal	2.02	13.19	65.28	1944.5	4.95	29.79
115	Linseed	9	0.86	GS50A	1.34	568.13	211.11	944.47	0.37	4.47
116	Canola	9	0.46	GS50A	1.57	5.49	213.89	1000.03	38.93	4.68
117	Spear	9	4.35	Warigal	2.11	159.34	619.45	4363.08	3.89	7.04
118	Warigal	10	3.51	GS50A	1.67	24.18	90.28	2400.06	3.73	26.59
119	Spoar	10	3.85	AUS4930	1.35	147.25	93.06	1361.76	0.63	14.63
120	Echidna	10	4.11	Warigal	1.75	682.42	1129.18	22799.4	1.65	20.19
121	Grimmett	10	3.29	AUS4930	0.95	2184.61	3402.81	*	1.56	*
122	Yallaroi	10	4.16	Warigal	1.81	760.44	2058.35	14059.6	2.71	6.83
123	Currency	10	3.66	Warigal	2.13	246.15	604.17	12475.6	2.45	20.65
124	Machete	10	2.69	Warigal	1.26	4230.77	4170.87	17301.7	0.99	4.15
125	Fallow	10		Warigal	1.56	0	600	3322.31	*	5.54
126	Molineux	10	4.29	Warigal	2.31	241.76	797.23	1303.74	3.3	1.64
127	Tahara	10	4.43	Warigal	2.21	39.56	111.11	462.98	2.81	4.17
128	Linseed	10	0.78	AUS4930	1.26	61.54	156.95	859.28	2.55	5.48
129	Canola	10	0.18	GS50A	1.83	29.67	333.34	692.61	11.23	2.08
130	GS50A	10	2.93	Warigal	2.32	18.68	338.89	1422.26	18.14	4.2

Appendix C : Preliminary Investigations into the Molecular Distinction of *P . thornei* and *P. neglectus*.

Appendix C
Preliminary Investigations into the Molecular Distinction of *P . thornei* and *P. neglectus.*

C1.0 General Introduction

The similar morphology of nematodes means that it is often difficult to identify species apart using morphological characters (Curran and Robinson, 1993). To overcome this problem, alternative diagnostic characters have been sought for identifying species and interspecific groupings within economically important nematode genera. Precise, reliable and rapid identification of economically important plant parasitic nematodes (eg *Meloidogyne*, *Globodera*, *Heterodera* and *Ditylenchus*) is an increasingly important component of plant protection (Curran and Webster, 1987).

Over the last three decades, methods of analysing protein, lipids, carbohydrates and most recently DNA are being used as technical solutions to these taxonomic problems (Curran and Robinson, 1993). Techniques to detect DNA sequence variations between organisms can be divided into at least four basic approaches : detection of restriction fragment length polymorphisms (RFLP's) between homologous DNA sequences, use of DNA probes in dot blots and DNA sequencing (Curran and Robinson, 1993), and the polymerase chain reaction (PCR).

Both root lesion nematodes, *P. thornei* and/or *P. neglectus* are found in the majority of soil samples within cropping regions of South Australia (Ch. 3.). The fact that wheat resistance differs for the two species, reinforces the importance of accurate taxonomic identification. Abad (1994) noted that in some cases the use of morphological characters is insufficient for identification because some of the definitive characteristics may overlap. As mentioned in Appendix D, the main morphological character, V% (position of the vulva), distinguishing the two *Pratylenchus* species is not always

definitive and can overlap between the two species. Field workers, who usually do not have the time or expertise to identify species beyond the dissecting microscope, are routinely confronted with such taxonomic difficulties. In order to address this problem, preliminary investigations were carried out to assess a molecular approach to identify *P. thornei* and *P. neglectus*. To do this, different methods of extracting DNA from nematodes were examined followed by an attempt to identify molecular differences between the DNA from *P. thornei* and *P. neglectus* .

C.1.1 Extraction of DNA from nematodes

C1.1.1 Introduction

Investigations of different methods were made in order to obtain the best DNA extraction procedure for both species. The first extraction method is commonly used to isolate DNA from insects (O. Schmidt, pers. comm.) and did not involve purification by CsCl gradient centrifugation. In extraction method 2, two different buffers (A and B) were used followed by either CsCl gradient centrifugation or phenol/chloroform extraction. Buffer A has been used for plant DNA extractions, while buffer B is known to be an efficient fungal DNA extraction buffer (D. Whisson, pers. comm.).

C1.1.2 Materials and Methods

P. thornei and *P. neglectus* were cultured on pure carrot using the modified method by Moody *et al.* (1973).

Preparation and purification of nematode DNA

The nematodes were removed from carrot cultures as previously described in Materials and Methods (Section 3.1). Both adults and juveniles were collected and rinsed at least three times in DDW. The two species were placed separately in 1.5ml microcentrifuge tubes to the 1ml mark which gave 300-500 thousand nematodes. The DNA was extracted from the nematodes by the following methods;

Extraction method 1 The nematodes were pelleted (320 *g*, 2 min.) and excess water removed with a pipette. The nematodes were homogenised in the tubes with 400µl SDS buffer (10mM Tris-HCl pH8, 10mM EDTA, 1% sodium dodecyl sulfate) using a microcentrifuge pestle for 2-3 minutes at room temperature. 5µl of proteinase K (20mg/ml) was added and the tubes were incubated overnight at 40°C. The following day the solution was extracted with 400µl of phenol (equilibrated with 0.1M Tris-HCl, pH8), left at room temperature for 5 minutes and centrifuged at 12 000*g* for 5 minutes at room temperature to separate the two phases. The aqueous layer was transferred to a clean microcentrifuge tube and re-extracted with phenol, followed by extraction with an equal volume of chloroform. The aqueous layer (350µl) was transferred to another 1.5ml microcentrifuge tube and the DNA was precipitated from the aqueous phase by the addition of 15µl of 5M NaCl and two volumes (730µl) of cold 100% ethanol, followed by several inversions to mix and then placed on ice. After 20 minutes, the DNA was pelleted (12 000*g*, 10min.), washed with 70% ethanol and resuspended in 50µl of TE buffer (pH 8). The DNA extracts were stored frozen at -20°C.

Extraction method 2 The second extraction method involved assessing the efficiency of two different extraction procedures and purification by CsCl gradient centrifugation as compared with method 1. *P. neglectus* was extracted with Buffer A (2 x SSC, 20mM EDTA, 2% sarcosyl, 50mM Tris, pH 8), while *P. thornei* was extracted with Buffer B (2 x SSC, 20mM EDTA, 2% sarkosyl, 150mM sodium acetate, pH 5.4).

P. thornei and *P. neglectus* (f.wt. 1.3g) were frozen in liquid nitrogen and ground to a fine powder with acid-washed sand using a mortar and pestle. The powder was then brushed into 1ml of extraction buffer A or B containing 0.2mg/ml predigested pronase and 0.05mg/ml of proteinase K and mixed gently. The contents were then placed in a 1.5ml microcentrifuge tube and made to a final volume of 1.3-1.5ml with the appropriate buffer. The solution was incubated at 37°C for 30 minutes followed by

65°C for another 30 minutes. The samples were divided into two and the following extraction procedures applied.

CsCl gradient centrifugation 50µl of DNA solution was placed into separate 1.5ml microcentrifuge tubes and buffer (20mM EDTA, 50mM Tris, pH 8) was added to make a final volume of 1.4ml. CsCl (1.4g) was added and allowed to dissolve. The solution was transferred into a 3.5ml quickseal tube and 80µl of 10mg/ml ethidium bromide was added and mixed. The tubes were heat sealed and centrifuged at 272 000*g* at 20°C for 18 hours in a Beckman TL-100 ultracentrifuge. The DNA band was removed using a syringe, placed in a 1.5ml microcentrifuge tube and made up to 250µl with TE buffer (pH 8). The ethidium bromide was removed by the addition of 250µl water saturated n-butanol followed by gentle inversion. The bottom aqueous layer was pipetted into a clean 1.5ml microcentrifuge tube and 2 volumes of TE buffer (pH 8) was added followed by 2 volumes of 100% ethanol to precipitate the DNA. The DNA was pelleted (12 000*g*, 5 min.) and washed as described previously and stored frozen at -20°C.

Phenol / Chloroform extractions The extracted DNA (750µl) from both species was placed into separate 1.5ml microcentrifuge tubes and 750µL phenol was added, mixed by gentle inversion, and centrifuged at 14 000*g* for 5 minutes. The upper aqueous phase was removed to a clean microcentrifuge tube and extracted with phenol as above. The aqueous phase was then extracted 2 times with 750µl of 24:1 chloroform:isoamylalcohol to remove residual phenol. Two volumes of cold absolute ethanol were added and the samples left overnight at -20°C to precipitate the DNA. The DNA was pelleted (12 000 *g*, 5 min.) and washed with 1ml of 70% ethanol containing 10mM magnesium acetate. The ethanol was poured off and the DNA pellet left to dry. The DNA was dissolved on 50µl of TE buffer (pH 8) and stored frozen at -20°C.

Gel Electrophoresis of undigested DNA

The amount of DNA in each sample was measured using a UV spectrophotometer. Undigested total DNA (0.2µg) from each preparation was mixed with 2µl loading buffer (0.05% bromophenol blue, 3% glycerol) and 16µl SDW to make 20µl. 10µl was removed and electrophoresed on a 1% agarose gel at 70V for 2 hours in TAE running buffer (0.04M Tris, 0.02M sodium acetate and 0.001 EDTA, pH 7.8). *Hind* III digested lambda DNA was used as a size marker. The gel was stained with ethidium bromide to visualise the nucleic acids and photographed by 354nm transmitted irradiation on a transilluminator.

C1.1.3 Results and Conclusions

Both extraction procedures yielded DNA of high molecular weight (Fig. C.1). Extraction method 1 showed a clearer DNA extraction band. Buffers A and B used in extraction method 2 in combination with the more 'rigorous' grinding procedure gave DNA that was contaminated, possibly with protein and carbohydrates that prevented the DNA from going into solution. However, the yield of DNA was higher using the CsCl gradient which is evident from the ethidium stained gel (Fig. C.1, Lanes 8-11). The CsCl extraction used for both *P. thornei* and *P. neglectus* eliminated the RNA (lower molecular weight bands on the gel, Fig. C.1).

C.1.2 RFLP Hybridisation Analysis

C1.2.1 Introduction

Digestion of genomic DNA with restriction enzymes generates a unique set of different sized DNA restriction fragments dependent upon the nucleotide sequence of the genome. Nucleotide substitutions, insertions or deletions that create or destroy restriction sites modify the restriction profile and therefore generate restriction fragment length polymorphism (RFLP) (Abad, 1994). The RFLPs in repetitive DNA and other high copy number sequences such as mitochondrial DNA and ribosomal DNA can be

detected by agarose gel electrophoresis and visualised by ethidium bromide staining of the DNA fragments under UV light (Curran and Robinson, 1993). RFLPs have been used successfully to distinguish the two closely related potato cyst nematodes, *Globodera rostochiensis* and *G. pallida* as well as other plant parasitic nematodes including *Meloidogyne* and *Heterodera glycines* (Abad, 1994).

To determine the degree of relatedness of particular fragments the homology between the bands should be established (Curran and Robinson, 1993). This can be done by hybridising labelled DNA fragments to size fractionated DNA transferred to a solid support such as a nitrocellulose filter, allowing for the visualisation of low copy number sequences (Curran and Robinson, 1993). In such cases, hybridisation of plant parasitic nematode DNA will identify the organism for which the probe is specific and as a result are quite important tools that can be used in positive or negative assay for nematode races, species or pathotypes (Abad, 1994).

The initial RFLP analyses of *P. thornei* and *P. neglectus* were done using restriction enzyme digested of total DNA of *P. thornei* and *P. neglectus* probed with labelled *P. thornei* digested of total DNA. This was done in order to determine the degree of DNA homology between the two nematode species. The rationale for this experiment was that the repetitive DNA present in the probe would be in higher copy number and should therefore be detected on Southern blots when hybridising to DNA fragments representing repeated sequences.

C.1.2.2 Materials and Methods

Alu I Digest Approximately 0.2µg of *P. thornei* and *P. neglectus* DNA was digested in a total volume of 60µl with 10 units of *Alu* I (Boehringer Mannheim ®) according to the manufacturers directions.

Twelve and 48µl aliquots were removed from the *Alu* I digests and made up to 51.5µl with 2µl loading buffer (0.05% Bromophenol Blue, 30% Glycerol) and sterile DDW. 15µl was removed from each and loaded on a 1% agarose gel containing ethidium bromide (1µg/ml). The gel was run for 1 hour at 70V in TBE (0.09M boric acid, 0.009M Tris base, 0.01M EDTA pH 8). 0.5µg of Boehringer Mannheim ® no 4 (lambda DNA with pSPTBM20 DNA digested with *Sty* I and *Sau* I) was used as a marker. The gel was photographed at 354nm transmitted irradiation.

Multi-endonuclease Digest Five µg of both *P. thornei* and *P. neglectus* total DNA extracted from the CsCl gradient were digested in a 50µl volume with 15 units of the following Boehringer Mannheim® restriction enzymes: *Pst* I, *Hind* III, *Hae* II, *Cla* I, *Bam* HI, *Xba* I and *Eco* RI according to the manufacturer's directions. The following day another 5 units of each enzyme was added and digestion was continued at 37°C for another hour.

Restriction endonuclease digested DNA samples (50µl) were mixed with 10µl volume loading buffer (0.05% Bromophenol Blue, 30% Glycerol) and 15µL was placed in slots of a 1% agarose gel containing ethidium bromide (1µg/ml) along with 0.5µg of Boehringer Mannheim ® no 4 marker. The gel was electrophoresed at 50V overnight in TAE running buffer (pH 7.8) and photographed at 354nm transmitted irradiation.

Southern Hydridization Both the *Alu I* digest and the multi-endonuclease digests (C1.2.2) were transferred from the 1% agarose gel to nylon filters by Southern blotting

as described by Sambrook *et al.* (1989). The filter containing the multi-endonucleased digests of *P. thornei* and *P. neglectus* was hybridised with [^{32}P]-dCTP labelled *Alu* I digested *P. thornei* DNA as the probe. The labelled probe was generated using the Megaprime DNA labelling system RPN 1606 (Amersham®) according to the manufacturer's protocol. The filter was prehybridized for 2 hours at 65°C followed by hybridisation at 65°C overnight (Sambrook *et al.*, 1989) in a rolling bottle hybridisation oven (Hybaid). The unbound probe was poured off and the filter washed twice in 2x SSC + 0.1% SDS for 20 minutes at 65°C. This was followed by two more 20 minutes washes in 0.2x SSC + 0.1% SDS also at 65°C. The filter was then exposed to X-ray film for 16 hours at 70°C and developed.

C1.2.3 Results

RFLP

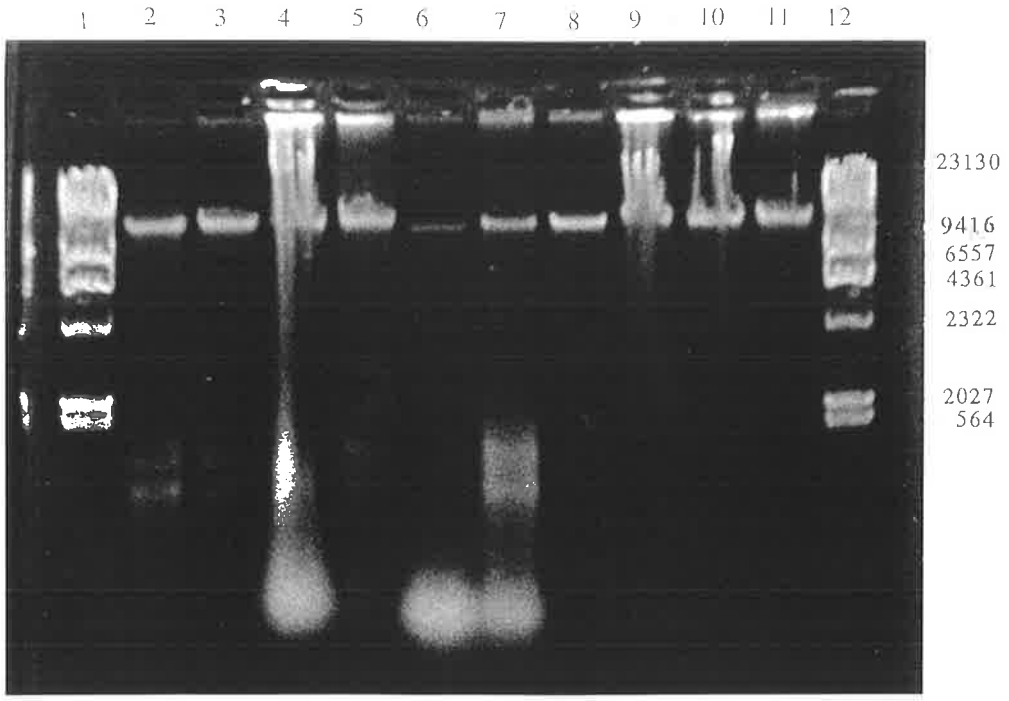
Some band differences were evident for *P. neglectus* and *P. thornei* for some of the restriction endonucleases investigated (Fig. C.2), in particular *Xba* I and *Eco* RI (Lanes 16-19). This suggests that highly repetitive DNA sequences differ between the two species. However, the bands were not clearly distinguishable between species within the range of endonucleases examined. To further verify the possible banding distinctions between species Southern blot hybridisations were performed using the radioactive labelled *Alu* I digested *P. thornei* DNA as a probe. This digest was used as a probe because small DNA fragments are necessary for efficient labelling.

Hybridisation

The radiolabelled *P. thornei* DNA probe hybridized to both *P. thornei* and *P. neglectus* digests. Some restriction enzymes, in particular *Xba* I (Lanes 16 and 17, Fig. C.3.) identified repetitive DNA bands in *P. thornei,* which were absent in *P. neglectus* . However the DNA was not sufficently digested (Fig. C.2), hence the experiment would need to be reconfirmed with completely digested DNA.

Fig C.1 Agarose gel separation of *P. thornei* and *P. neglectus* total undigested DNA. Ethidium bromide stained gel viewed under 354nm transmitted UV irradiation: Lane 2, Extraction 1 *P. thornei*; Lane 3, Extraction 1 *P. neglectus*; Lane 4, *P. neglectus* Extraction 2 (buffer A, phenol); Lane 5, *P. thornei* Extraction 2 (buffer B, phenol); Lane 6 and 7 repeat of Lane 4 and 5; Lane 8, *P. neglectus* Extraction 2 (buffer A, CsCl); Lane 9, *P. thornei* Extraction 2 (buffer B, CsCl); Lane 10 and 11 repeat of Lanes 8 and 9; Lanes 1 and 12, Hind III digested lambda DNA (in single basepairs indicated at right).

Fig C.2 Agarose gel separation of *P. thornei* and *P. neglectus* of DNA digested with restriction endonucleases. Ethidium bromide stained gel viewed under 354nm transmitted UV irradiation showing the fragment size distribution of the 7 restriction endonucleases: Lane 2, *Pst* I *P. neglectus*; Lane 3, *Pst* I *P. thornei*; Lane 4, *Hind* III *P. neglectus*; Lane 5, *Hind* III *P. thornei*; Lane 6, *Hae* II *P. neglectus*; Lane 7, *Hae* II *P. thornei*; Lane 8, *Cla* I *P. neglectus*; Lane 9, *Cla* I *P. thornei*; Lane 11 *Bam* HI, *P. neglectus*; Lane 12, *Bam* HI *P. thornei*; Lane 13, *Cla* I *P. neglectus*; Lane 14, *Cla I P. thornei*; Lane 15, *Cla* I *P. thornei*; Lane 16, *Xba* I *P.neglectus*; Lane 17, *Xba* I *P. thornei*; Lane 18, *Eco* RI *P. neglectus*; Lane 19, *Eco* RI *P. thornei.* Lane 1 and 10, DNA marker, Boehringer Mannhein ® no. 4 (size in basepairs indicated on right).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23130
9416
6557
4361
2322
2027
564

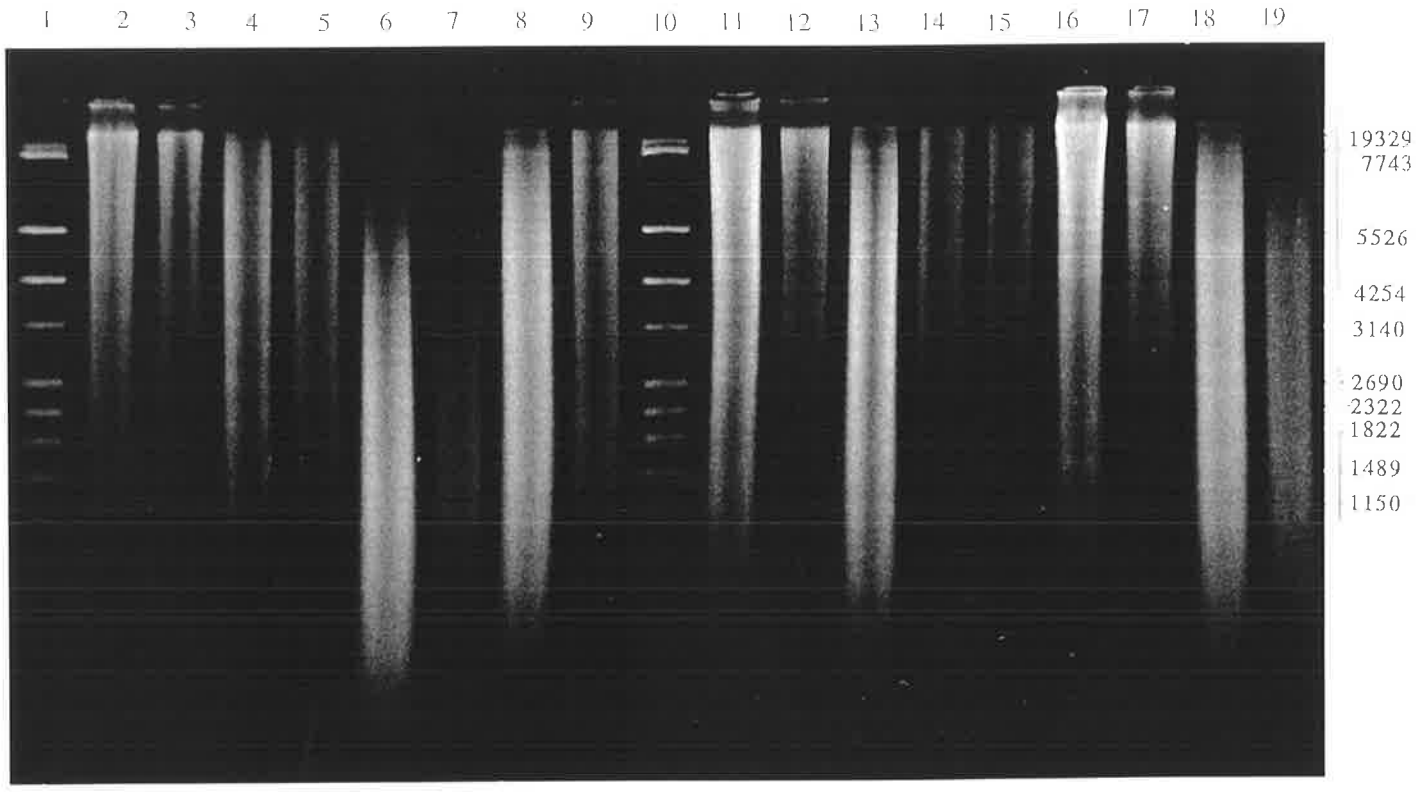
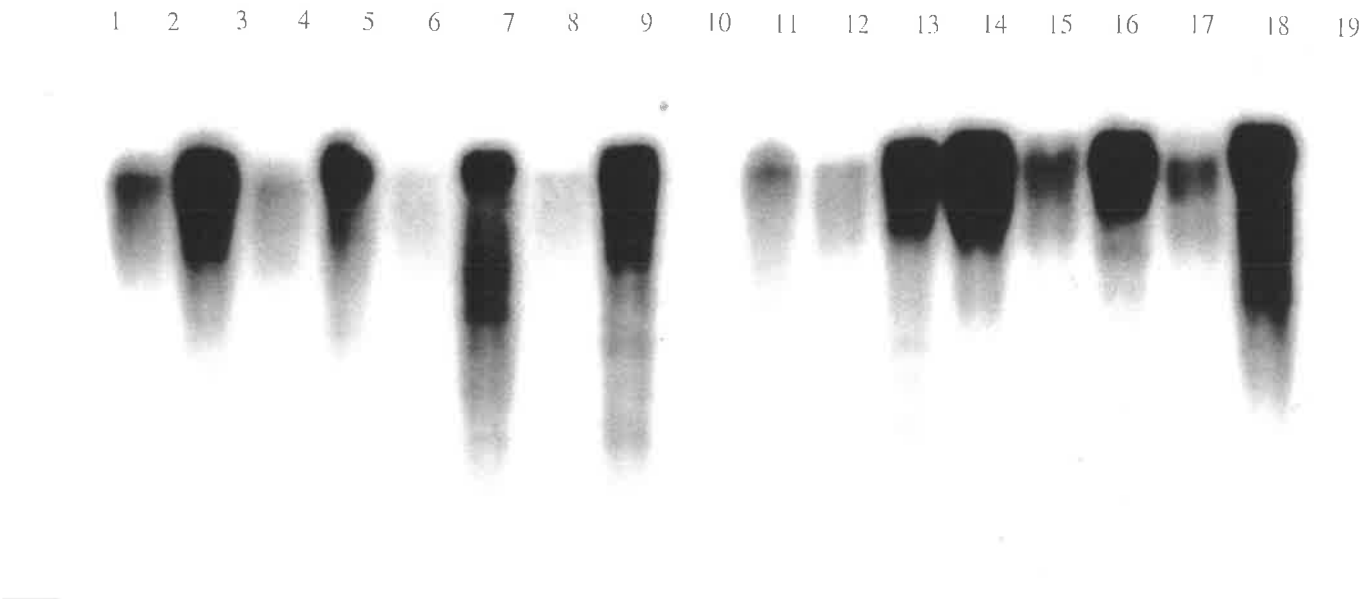

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
19329
7743
5526
4254
3140
2690
2322
1822
1489
1150

Fig. C.3 : Autoradiograph of Southern blot showing hybridization of *P. thornei* [^{32}P]-dCTP labelled *Alu* I digested *P. thornei* DNA to the multi-endonuclease digest of both nematode species (see Fig. C.2).


1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

C.1.2.4 General Conclusion

Although RFLP data is valuable in preliminary studies, usually relatively large quantities of DNA are required (0.1-1µg per sample) and the time consuming gel electrophoresis, transfer and hybridisation steps preclude this approach when processing large numbers of samples in a routine diagnostic assessments (Curran and Robinson, 1993). Preliminary evidence is presented here to indicate that DNA polymorphisms are evident between *P. thornei* and *P. neglectus*, although the band differences were not resolved sufficiently due to the poor quality of the CsCl prepared DNA. However, hybridisation differences between nematode species have been detected, suggest the possibility of using species-specific probes.

To obtain specific probes several possibilities exist. A phage library may be constructed with genomic DNA of *P. thornei*. This could then be hybridised against the Alu digest of both species and screened for clones with strong signals. These clones would then need to be verified as species-specific by hybridisation studies using genomic DNA of both species. If species specificity exists it may be possible to sequence them and develop PCR primers. An alternative method is to use a genomic library to subclone some of the gel eluted fragments. The fragments would need to be cut out of the gel, ligated into an appropriate vector and hybridised with genomic DNA from both species to verify species-specific fragments. It is once again possible to sequence the DNA and develop PCR primers.

Such a PCR based technique has been developed to identify individual nematodes in the genus *Pratylenchus* (Samas and Linden, 1994). This technique is simple and rapid, producing differential reproducible banding patterns between *P. penetrans*, *P. scribneri*, *P. hexincisus* and *P. agilis*. Curran *et al.* (1993) are developing species specific probes for many species of *Pratylenchus* with the possibility of extracting 'PCR' grade DNA

from soil and plant tissues in addition to the quantitative assessment of these nematodes.

The success of the development of such molecular techniques for diagnostic identification, detection (pre-planting) and quantification of nematode populations will be influenced by the economic viability of the assay. This will be determined by commercial considerations such as market size and regulatory requirements (Curran and Robinson 1993). However, it is likely that the importance of biotechnology-based methods for diagnosis will increase as management systems are developed to fully utilise the management practices, such as the use of resistant and tolerant cultivars in nematode problems (Curran and Robinson, 1993).

Appendix D: Morphometrics of South Australian population of *P. thornei* and *P. neglectus* males and females

Please note the first two authors are the primary contributors.

This Appendix is currently In Press.

Description of the Male and Redescription of the Female *Pratylenchus thornei* and *Pratylenchus neglectus* from Australia.

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D1.0 Abstract

The first descriptions of male and female *Pratylenchus thornei* and *Pratylenchus neglectus* from Australia are presented, based on scanning electron microscopy (SEM) and light microscopy. Nematodes from populations of *P. thornei* and *P. neglectus* found in South Australia are similar to those previously described from Europe, Africa, North America and the United Kingdom. As reported by other workers, there is considerable variation and overlap of measurements, making it difficult to determine suitable taxonomic characters to distinguish the two species. Body length, vulval percentage and number of lip annules are considered the most important characters by which to distinguish the two species.

D1.1 Introduction

Members of the genus *Pratylenchus* (Nematoda: Pratylenchidae) parasitise a wide variety of plants (Loof, 1991). *Pratylenchus thornei* (Sher and Allen, 1953) and the

related species *P. neglectus* (Rensch, 1924) have a cosmopolitan distribution (Loof, 1991) and are also widespread in South Australia (Nicol, unpublished data), often having overlapping distributions. They cause yield losses of wheat in glasshouse tests and in the field (Thompson et al., 1981; Doyle et al., 1987; Nicol, 1991; Taheri et al., 1994). The difficulty of identifying *Pratylenchus* species in Australia is a major impediment to sound ecological studies (Stirling and Stanton, 1993). *P. thornei* and *P. neglectus* are parthenogenetic and males although found are rare. Several were collected from cultures which allowed a more detailed description of males than has previously been published. Descriptions and morphometric measurements of *P. thornei* and *P. neglectus* from Australia were made to compare Australian populations with those from other countries, and to determine which are the most useful characters for Australian field workers to use to distinguish the two species.

D1.2 Materials and Methods

Laboratory cultures of *P. thornei* and *P. neglectus* were reared on aseptic carrots by a method modified from Moody *et al.*(1973). Field populations of both species were derived from cereal and legume fields in South Australia, and extracted from soil using a modified Baermann technique. Nematodes were killed and fixed in hot (100°C) formalin/acetic acid 4:1. Specimens for light microscopy were processed by slow evaporation through ethanol - glycerol at 40°C over 2 weeks, mounted in glycerol on permanent slides, and examined using a Nomarski microscope. Nematodes for SEM were dehydrated in an alcohol series, critical point dried, coated with 30nm of gold and examined under 20kV using a Cambridge S250 microscope. All measurements have been rounded to the nearest whole number.

D1.3 Descriptions

Pratylenchus thornei

(Figs. 1, 3 & 4)

Measurements: Table 1.

Females. As per decription by Fortuner (1977).

Males. Body forming a very open "C" shape when killed. Cuticle with fine inconspicuous transverse striae, appears smooth in some specimens in light microscope. Body annules 1.9µm wide (1.5-2.3µm). Lateral field with four incisures. Three lip annules, continuous with body outline. SEM of the lip region showed oral disc fused to sub-median segments which broadened towards outer edge. Amphid openings rounded, on the inner edges of the lateral segments. Outer margin of sclerotized labial framework extends about two annules into body and one annule into lip region. Stylet guiding apparatus extends posteriorly from basal plate for about four annules. Stylet medium size (13-19µm long) with broadly rounded to almost anteriorly flattened basal knobs. Orifice of the dorsal oesophageal gland about 4µm behind stylet base. Nerve ring directly behind oesophageal bulb. Excretory pore opening 59 to 84µm behind head. Hemizonid about two annules long, one annule anterior to excretory pore. Oesophageal glands in one lobe, 29 to 47µm long, extending longitudinally and ventrolaterally over intestine. Outstretched testis with spermatocytes in a single row, followed by a region of multiple rows. Phasmids slightly posterior to mid-tail, not extending to edge of bursa. Bursa sometimes shorter in region of phasmid, edges smooth; peloderan. Spicules 18 to 21µm; arcuate, hafted. Gubernaculum trough-shaped. Tail dorsally convex-conoid, terminus bluntly rounded to truncate, unstriated.

Collection sites. Field specimens *Triticum aestivum*, Tarlee, South Australia. Nematodes in carrot cultures were originally collected from Waite Agricultural Research Institute soils, Urrbrae, South Australia.

Voucher specimens. Specimens deposited in the Waite Institute Nematode Collection (WINC), Adelaide, South Australia. Field specimens are numbered 661A and 661B, and specimens from cultures are 816B.

Morphometrics of the male and female P. thornei and P. neglectus collected from laboratory and field populations in South Australia.

	Females								Males							
	P. thornei				*P. neglectus*				*P. thornei*				*P. neglectus*			
Measurement (µm)	Field			Culture	Field			Culture	Field			Culture	Field			Culture
	n		n		n		n		n		n		n		n	
Body Length	7	522	21	691	14	483	20	475	3	515	9	566	1	431	3	431
		471-609		610-774		421-524		425-503		488-557		411-618		-		428-432
Anterior to Excretory Pore	7	78	15	86	14	90	18	75	3	73	8	-	-	-	3	68
		74-84		80-91		82-95		71-79		59-84		-		-		66-70
Stylet Length	7	17	18	17	14	18	20	17	3	14	7	17	1	16	3	16
		16-18		16-18		17-19		16-19		13-15		16-19		-		15-18
Width of Stylet Knobs	7	4	18	5	14	5	20	5	3	4	9	4	1	5	3	3
		3-5		4-5		5-6		4-5		4-4		3-5		-		3-4
Greatest Body Width	7	18	21	24	14	19	20	20	3	16	8	18	1	20	3	16
		16-19		18-28		17-21		16-24		16-18		16-19		-		-
Width of Median Bulb	7	9	21	10	14	10	20	10	3	9	8	9	1	9	3	8
		7-9		9-12		9-12		8-11		8-11		8-10		-		7-9
Total Gonad Length	not measured				not measured				3	227	7	317	-	-	3	208
										203-255		264-364		-		203-213
Width at Cloaca	N/A				N/A				3	14	8	15	1	12	3	13
										12-15		13-17		-		12-13
Width at Vulva	7	18	21	24	14	19	17	19	N/A				N/A			
		15-21		21-26		16-21		17-23								
Lips to Vulva	7	408	21	514	13	402	17	359	N/A				N/A			
		360-500		403-586		361-446		346-411								
a	7	29	21	29	14	26	20	24	3	31	9	32	1	23	3	27
		27-33		25-38		24-28		20-28		30-31		22-39		-		27-27
c	7	19	17	21	14	23	17	23	3	38	8	20	1	16	3	22
		9-23		18-25		20-25		20-26		35-41		16-24		-		21-24
c'	7	29	21	29	14	26	17	24	3	38	8	39	1	38	3	34
		26-34		25-33		24-28		21-28		35-41		28-47		-		34-35
V%	7	78	21	74	13	82	17	82	N/A				N/A			
		76-82		66-79		80-86		75-84								
T%	N/A				N/A				3	44	8	57	-	-	3	48
										40-51		45-89		-		47-49
Lip Annules	7	3	21	3	14	2	20	2	3	3	9	3	1	2	3	2
Height Lips	7	4	21	3	14	3	20	3	3	2	9	2	1	2	3	3
		3-5		2-4		2-4		2-4		2-3		2-3		-		3-3
Length Post Vulva Sac	6	19	17	25	14	16	17	17	N/A				N/A			
		15-22		14-43		11-23		9-24								
Spicule Length	N/A				N/A				-	-	9	20	1	15	3	17
										-		18-23		-		16-18
Tail Length	7	27	21	33	14	21	17	21	3	27	9	28	1	18	3	19
		22-39		25-39		18-23		18-23		24-30		24-30		-		18-21

TABLE II

Published morphometrics of male and female P. thornei and P. neglectus worldwide.

	Females										Males				
	P. thornei						*P. neglectus*				*P. thornei*			*P. neglectus*	
Authors	Frederick & Tarjan (1989)	Handoo & Golden (1989)	D'Errico (1970)	Loof (1960)	Corbett (1970)	Nicol (1991)	Frederick & Tarjan (1989)	Handoo & Golden (1989)	Sher & Allen (1953)	Corbett (1970)	Sher & Allen (1953)	Fortuner (1977)	Loof (1960)	Sher & Allen (1953)	Loof (1960)
Measurement (µm)															
Body Length	540	-	-	-	-	709	490	461	-	-	480	551	492	340	472
	460-610	450-770	454-614	408-708	420-690	620-825	410-530	312-588	310-550	370-450	-	-	-	-	420-524
Stylet Length	17	-	15	-	-	14	17	-	-	-	16	16	16	14	16
	15-18	15-19	-	15-19	14-17	12-18	16-18	15-19	16-18	16-17	-	-	-	-	15-17
Greatest Body	-	-	-	-	25	-	not measured				not measured			not measured	
Width	-	-	-	-	20-33	-									
Width of Median	-	-	-	-	-	23	not measured				not measured			not measured	
Bulb	-	-	-	-	-	8-30									
a	33	-	-	-	-	29	27	-	-	-	32	39	29	22	27
	26-34	26-36	28-32	25-36	27-37	25-31	23-31	16-32	18-25	24-30	-	-	-	-	25-29
b	-	-	-	-	-	-	-	-	-	-	6	6	6	5	6
	-	6-8	5-8	5-8	5-8	-	-	5-8	4-6	5-6	-	-	-	-	-
c	20	-	-	-	-	13	21	20	-	-	20	19	20	20	19
	18-24	19-25	19-28	17-25	18-27	10-17	17-23	14-27	16-18	17-21	-	-	-	-	17-22
c'	not measured						-	-	-	-	not measured			not measured	
							-	1.5-2.5	-	-					
T%	N/A						N/A				30	-	-	-	49
											-	-	-	-	42-56
V%	76	-	-	-	-	75	82	82	-	-	N/A			N/A	
	75-79	74-79	76-79	74-79	76-79	-	80-84	75-87	80-88	78-83					
Lateral Incisures	-	4	-	-	-	-	-	4	-	-	not measured			not measured	
Spicule Length	N/A						N/A				21	-	-	not measured	
Lip Annules	-	3	-	-	3	-	-	2	-	2	not measured			not measured	
Tail Annules	-	-	-	-	-	-	-	-	-	-	not measured			not measured	
	20-29	-	-	-	-	-	16-21	-	-	-					

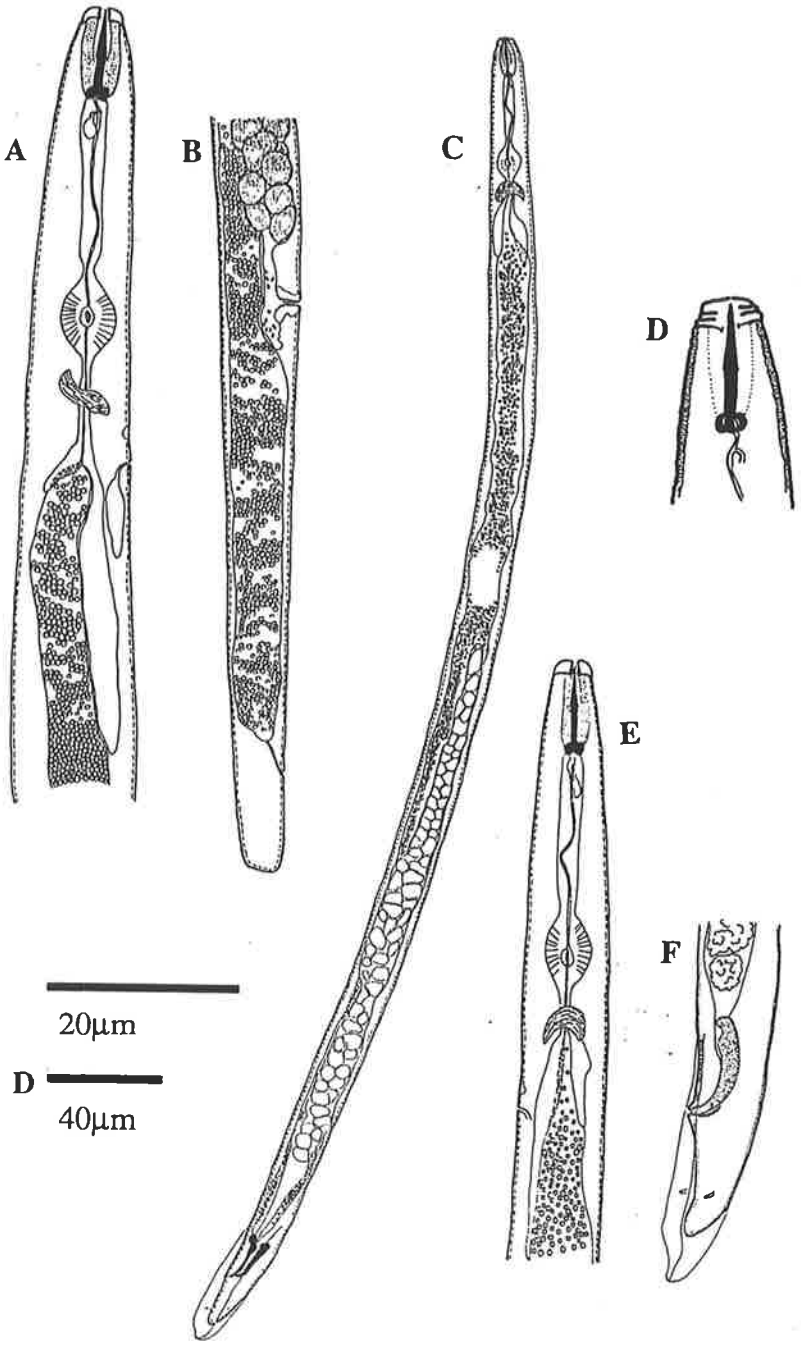

Fig. 1. *Pratylenchus thornei*. A, oesophageal region female; B, tail region female; C, entire male; D, head end female; E, oesophageal region male; F, tail region male.

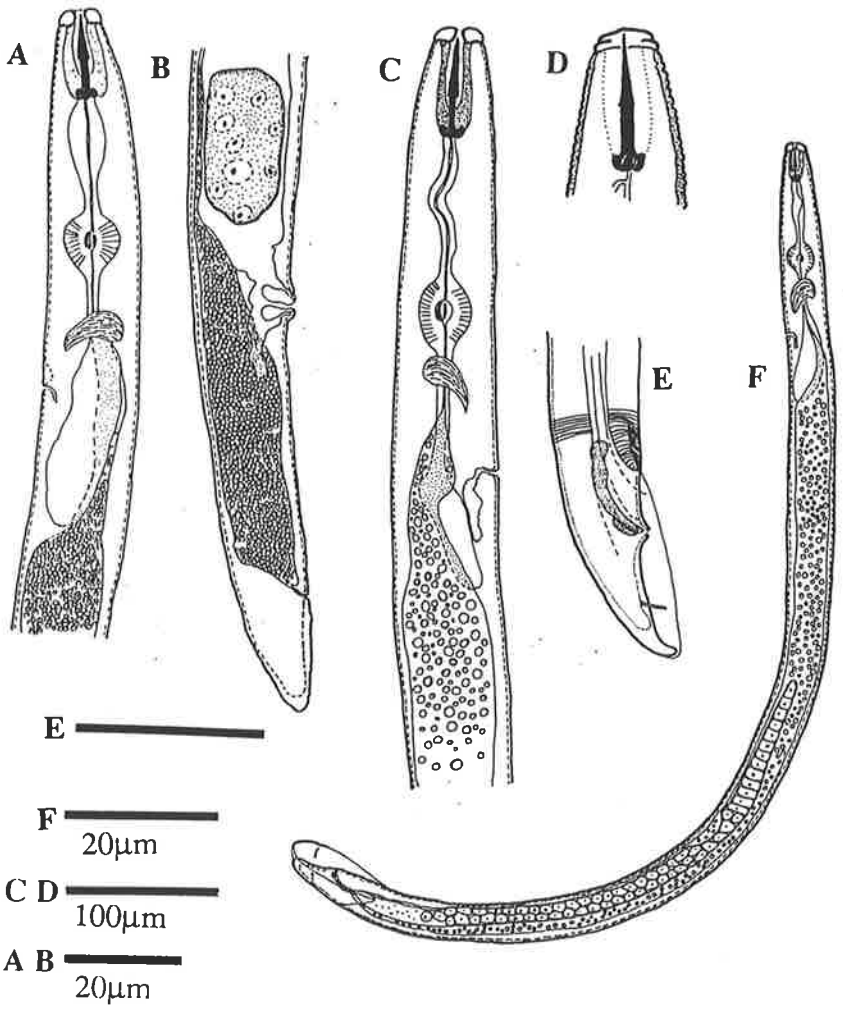

Fig. 2. *Pratylenchus neglectus*. A, oesophageal region female; B, tail region female; C, oesophageal region male; D, head end female; E, tail region male; F, entire male.

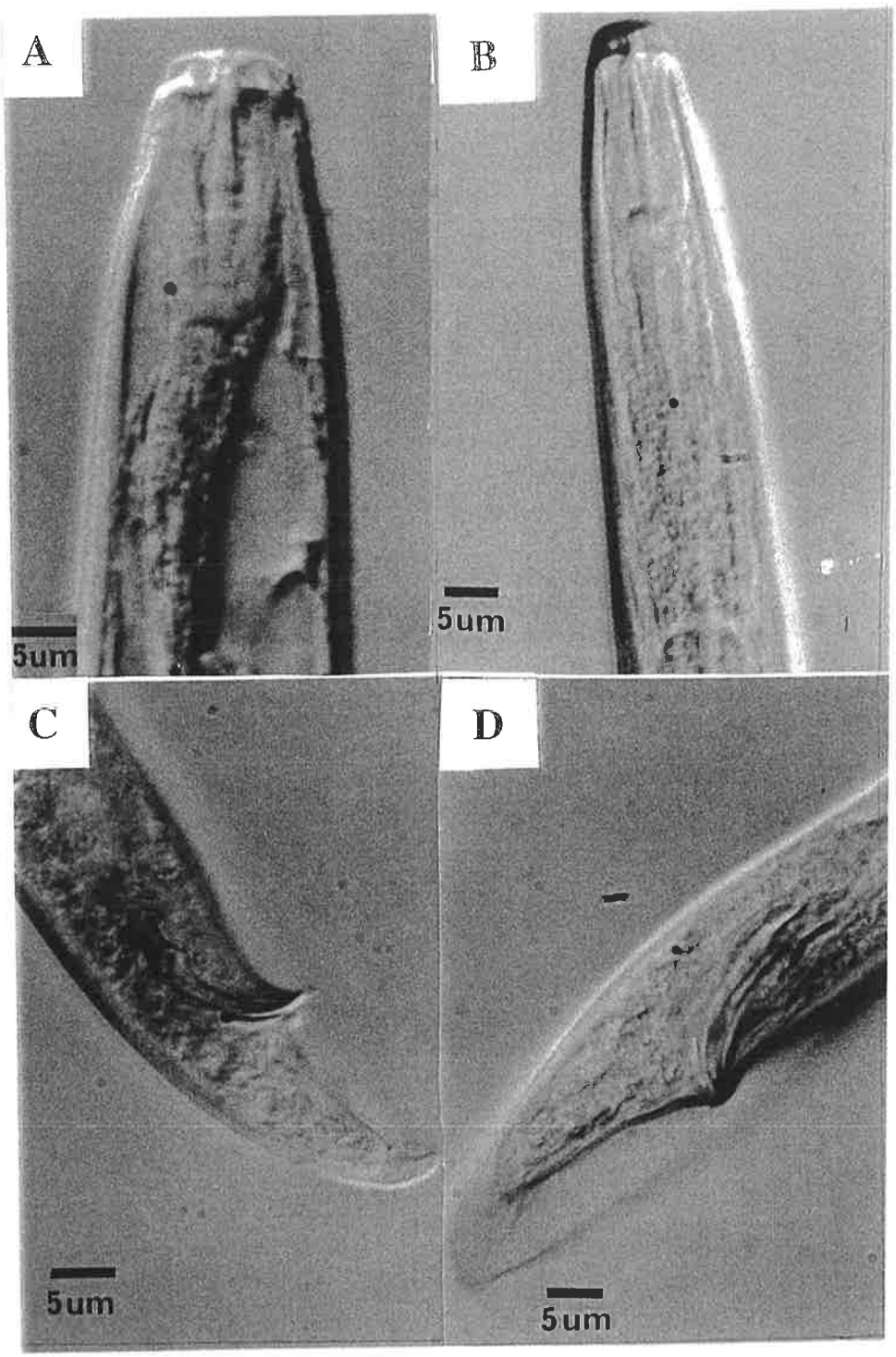

Fig. 3. Light microscopy of *Pratylenchus thornei* and *Pratylenchus neglectus*. A, head *P. neglectus* male; B, head *P. thornei* female; C, tail *P. neglectus* male; D, tail *P. thornei* male.

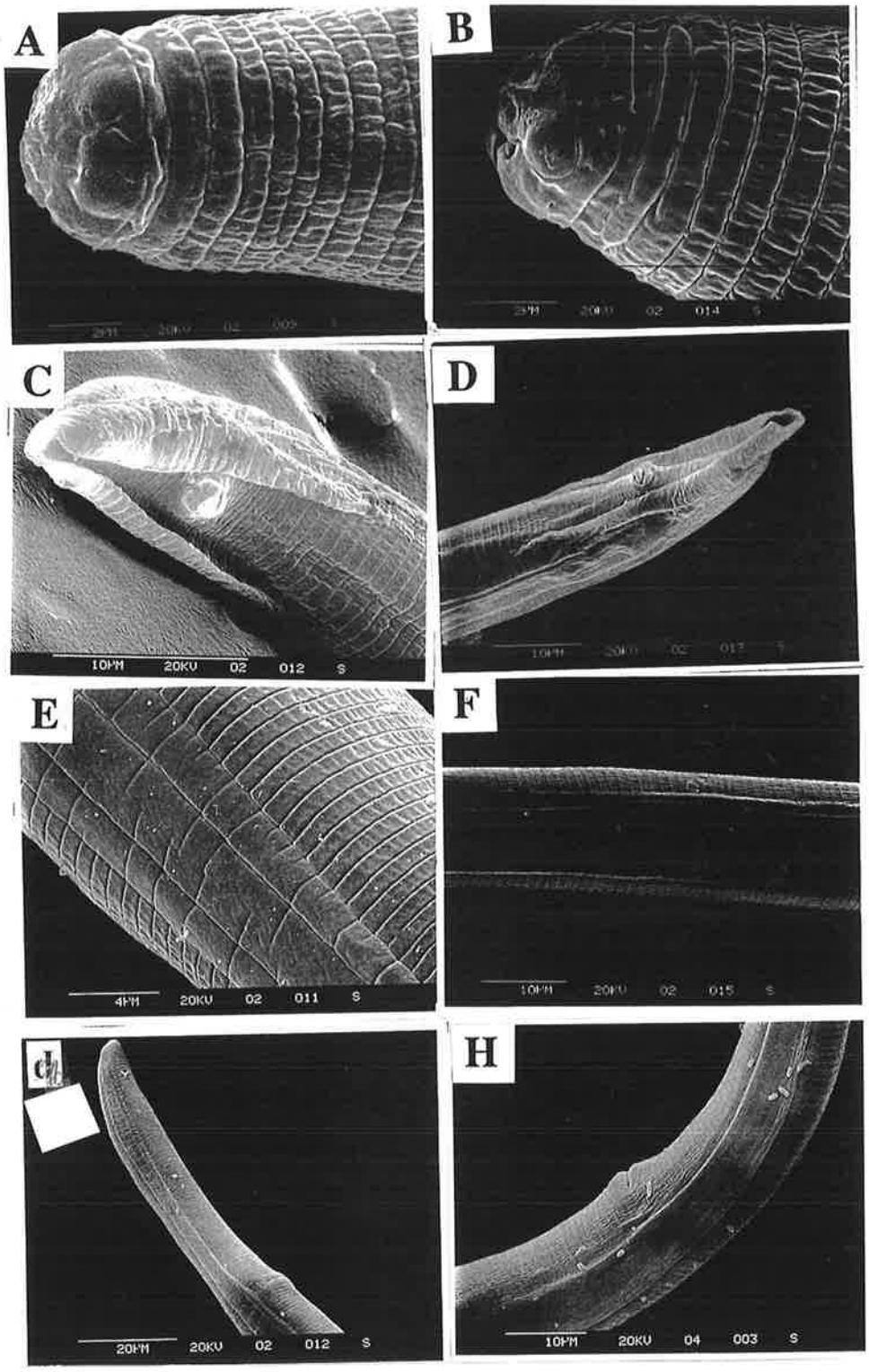

Fig. 4. SEM micrographs of *Pratylenchus thornei* and *Pratylenchus neglectus*. A, head *P. neglectus* male; B, head *P. thornei* male; C, tail *P. neglectus* male; D, tail *P. thornei* male; E, lateral field *P. neglectus* male; F, lateral field *P. thornei* female; G, vulva *P. neglectus* female; H, vulva *P. thornei* female.

Pratylenchus neglectus

(Figs. 2, 3 & 4)

Measurements: Table 1.

Females. As per decription by Townshend and Anderson (1976)

Males. Body assuming a straight to very open "C" when killed. Cuticle has fine, inconspicuous transverse striae. Body annules 1.7µm wide (1.3-2.3). Lateral field with four lines, areolation of all bands seen with SEM. Head with two annules about equal size, the apical one comprising the lips. SEM showed oral disc fused to sub-median segments which broadened at outer edges, forming a distinct "head cap". Amphid openings small and slit-like, seen with SEM on inner edges of lateral segments. Stylet medium size (15-18µm long). Stylet knobs 3 to 5µm across, typically indented on anterior surfaces. Dorsal gland orifice 3 to 5µm posterior to stylet. Nerve ring directly behind oesophageal bulb. Oesophageal glands in one small lobe, 15 to 17µm long, extending longitudinally and ventrolaterally over intestine. Excretory pore 66 to 70µm from head end. Hemizonid immediately anterior to excretory pore, extending two or three body annules. Outstretched testis with spermatocytes in single row, followed by a region of multiple rows. Phasmids slightly posterior to mid-tail, extending to near the edge of bursa. Edges of bursa are smooth at tail tip but crenulated near the point of origin; peloderan. Spicules 15 to 18µm long, hafted; gubernaculum arcuate. Tail terminus without annulation, bluntly rounded to truncate.

Collection sites. Field specimens from *Triticum aestivum*, Minippa, South Australia. Nematodes in carrot cultures were obtained from Dr. V. A. Vanstone, University of Adelaide, who originally collected them from field soil, Palmer, South Australia.

Voucher specimens . Specimens deposited in the WINC, Adelaide, South Australia.

D1.4 Discussion

Males. In more than half the described species of *Pratylenchus* males are infrequent, rare or unknown (Sher and Allen, 1953). Until recently, only three specimens of male *P. neglectus* had been described (Sher and Allen, 1953; Loof, 1960) and similarly for *P.*

thornei (Sher and Allen, 1953; Fortuner, 1977; Loof, 1960). Vovlas and Castillo (1995) reported finding 2 males for every 1000 females of *P. thornei* grown on carrot disc cultures. Similar ratios have been observed for both *P. thornei* and *P. neglectus* grown on carrot cultures in our work. Morphometrics of the Australian isolates are comparable to those previously documented (Table 2).

The basal knobs of the stylet of Australian *P. thornei* males are broadly rounded (Fig. 1), but in *P. neglectus* they are typically indented on the anterior surfaces and less robust (Fig. 2). The spicule length is longer in Australian specimens of *P. thornei* than *P. neglectus* (Table 1, Fig. 3), as previously documented by Loof (1960) and Sher and Allen (1953). The caudal alae could be used to distinguish the two species (Fig. 4). However, while the bursa of *P. neglectus* has crenulated edges near its origin anterior to the cloaca, and that of *P. thornei* is smooth, it is difficult to see this with light microscopy. The bursa tended to roll inwards during preparation for S.E.M. and this obscured the edges. The position of the opening of the phasmids on the bursa was variable, although they opened closer to the edge of the bursa in *P. neglectus,* and in some specimens of *P. thornei* the bursa appeared shorter in the vicinity of the phasmids.

Females . The morphology and morphometrics of both *P. neglectus* and *P. thornei* (Table 1, Figs 1, 2) from Australia showed that the females are similar to isolates of each species from other countries (Table 2), except for the ratio c in *P. neglectus* females. This was larger for the Australian isolates than for isolates from the U.S.A. (Handoo and Golden, 1989) or for the averages of published data calculated by Frederick and Tarjan (1989), suggesting that the Australian isolates had shorter tails. However, Loof (1991) stated that the number of tail annules showed a wide range within species of *Pratylenchus*.

Length of the post-uterine sac of European specimens of female *P. thornei* was more than one and half times the body width at the vulva (Fortuner, 1977), but in *P. neglectus* the sac was less than or equal to the body width (Townshend and Anderson, 1976). The

measurements reported here (Table 1) show that Australian isolates of both species have similar or equal measurements for the length of the post uterine sac and body width at the vulva. Roman and Hirschmann (1969) found that the length of the post-uterine sac was very variable within species.

Characters for diagnosis of species by field workers . Body length and distance from lips to vulva of female *P. thornei* are significantly greater for specimens from cultures than from the field, and males from cultures were also larger than specimens from the field. Male and female *P. neglectus* from carrot cultures and the field are similar in size (Table 1). De Man's ratios confirmed that there was little variation in the morphometrics of these two species whether from cultures or the field (Table 1). Roman and Hirschmann (1969) found extensive morphological variation in *P. vulnus* from greenhouse cultures compared with specimens from callus cultures. This may reflect host physiology or nutritional status of the nematodes. Loof (1991) stated that, in general, *Pratylenchus* spp. extracted from roots are longer and stouter than specimens extracted from soil, and Olowe and Corbett (1984) found that body length was greater on favourable than on unfavourable hosts. The size difference of *P. thornei* from the field versus carrot cultures, which was not seen for *P. neglectus,* may suggest that carrots are a more suitable host for *thornei* than for *neglectus.* In general, Australian workers could assume that adults of *P. neglectus* from the field would be smaller than those of *P. thornei*, but body size alone would not be diagnostic.

Corbett and Clark (1983) suggested that the number of lip annules was a reliable character distinguishing the two species. This study confirmed that *P. neglectus* has two offset head annules (Handoo and Golden, 1989; Corbett, 1970), while *P. thornei* has three (Sher and Allen, 1953; Corbett, 1970) which are continuous with the body (Figs. 3, 4, Table 2). However, this character can only be checked using an oil immersion lens.

Australian specimens of both species have four incisures in the lateral field of both sexes (Fig. 4), as reported by Sher and Allen (1953) and Handoo and Golden (1989). In *Pratylenchus* spp. the lateral field starts on the seventh to ninth body annule and for the greater part of its length has four lines or three bands (Corbett and Clark, 1983). However, across or within the bands there may be further lines which are said to be characteristic for each species but are found to vary greatly, as did the distance and depth of the transverse striae. This was particularly so for *P. neglectus* where complete or partial areolation of the middle band has been found. In the Australian specimens, male *P. neglectus* showed areolation of all three bands in SEM, but the bands of *P. thornei* (in both species) were smooth and no transverse striae were seen. However, these differences in the lateral fields cannot be seen with the light microscope, and thus, the lateral fields cannot be used by field workers to separate the two species. Males of both species had body annules with similar widths. Corbett and Clark (1983) found that *P. thornei* females, with average annule width of 1.4µm, were more finely annulated than *P. neglectus*, with 1.6µm, and that the transverse striae of the latter were deeper than in *P. thornei* which sometimes looked as if the cuticle was smooth. This was also true of the striae of the Australian isolates of the two species.

Loof (1991) stated that the diagnostic value of the shape of the stylet knobs, stylet length, length of the oesophageal gland, and length of the post-uterine sac was limited due to difficulty of measurement or intraspecific variability. Number of tail annules is variable even within one species and cannot be used as a diagnostic character (Roman and Hirschman, 1969; Loof, 1991), but shape of the terminus is more reliable (Loof, 1991). The Australian specimens of *thornei* had broadly rounded to truncate tail ends, but those of *neglectus* were rounded to oblique, as described for specimens from other countries.

Current identification of both species of *Pratylenchus* using light microscopy is difficult for workers in applied field research who rely heavily on body length and vulval percentage, characters which can be checked using a dissecting microscope. Study of nine morphometric characters of six *Pratylenchus* species revealed vulval percentage to have the lowest coeffeciency of variation (Roman and Hirschmann, 1969). However, care should be taken in using this character to distinguish *P. neglectus* and *P. thornei* from Australia as an overlap (80-86% and 76-82% respectively) was observed, particularly with field populations. Hence, an increase in sample size may be necessary to distinguish the two species. Loof (1991) recommended that 25 specimens be measured for diagnosis. Use of the compound microscope can delineate species more accurately on the basis of the number of lip annules and head shape, but is time consuming and requires considerable technical expertise, and therefore may not be practical for field workers. There is an urgent need for development of a molecular technique to confirm visual identifications of *Pratylenchus* species.

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