INTERACTION BETWEEN ROOT LESION NEMATODE, *PRATYLENCHUS NEGLECTUS,* AND ROOT-ROTTING FUNGI OF WHEAT

by

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TABLE OF CONTENTS

Summary

It is now recognised that the root lesion nematode, *Pratylenchus neglectus*, is an important pest of crops in the wheat growing districts of South Australia. Since a number of root-rot fungi are also associated with wheat roots, investigating the interaction between *P. neglectus* and these fungi was considered important in understanding the severity, aetiology and control of root disease.

A field survey of soil-borne fungi associated with the roots of South Australian wheat crops was conducted during the 1992 growing season (Chapter 3). The fungi most frequently isolated from lesioned and non-lesioned segments of wheat roots collected from two fields infested with *P. neglectus* were *Fusarium equiseti*, *F. acuminatum*, *F. oxysporum*, *Microdochium bolleyi*, *Gaeumannomyces graminis* var. *tritici*, *Bipolaris sorokiniana*, *Pythium irregulare*, *Pyrenochaeta terrestris*, *Phoma* sp. and *Ulocladium atrum*. Root samples collected also contained a high number of nematodes. The nematodes invaded both seminal and crown roots, but were more concentrated in the seminal roots.

Although considered to be a major root pathogen of wheat, *Rhizoctonia solani* (AG-8) was not isolated from field samples in 1992, but was subsequently included in interaction tests with *P. neglectus* as the fungus is an important root pathogen occurring in South Australia. Other major root pathogens, such as *G. graminis* or *P. irregulare,* were isolated frequently, particularly from lesioned segments of root. The fungi most frequently isolated from damaged roots were those considered to be minor pathogens of wheat, such as *M. bolleyi* and *Fusarium* spp. Numerous other fungi were identified, but these occurred sporadically and at low frequencies.

Association of *P. neglectus* with species of fungi detected in field samples was examined under controlled glasshouse conditions. Field experiments were supplemented with interaction tests in the glasshouse and laboratory.

Preliminary experiments (Chapter 4) indicated a positive interaction between *P. neglectus* and some root-rot fungi tested. The number of nematodes/plant and nematodes/g dry root and severity of root lesioning increased in the presence of some fungi. When combined with the nematode, *B. sorokiniana, M. bolleyi, P. irregulare, P. terrestris* or *R. solani* resulted in significantly higher nematode numbers in the roots. *P. terrestris, P. irregulare, F. oxysporum* or *G. graminis* in combination with *P. neglectus* significantly increased root lesion rating.

Association of root lesion nematode with major fungal pathogens, *G. graminis, R. solani* (Chapter 5) or *P. irregulare* (Chapters 8 and 9), was tested. The hypothesis that some minor pathogens (such as *M. bolleyi*, *Fusarium* spp. or *P. terrestris*) interact with *P. neglectus* was also examined in detail.

The hypothesis that *M. bolleyi* influences disease caused by *G. graminis* was tested (Chapter 5). *M. bolleyi* reduced the level of *G. graminis* infection in experiments carried out in the glasshouse, consequently decreasing the severity of damage caused by *G. graminis*. A second hypothesis, that *G. graminis* and *R. solani* may have an antagonistic interaction which may then affect their interaction with *P. neglectus,* was tested both in the glasshouse and in the field, using different densities of either fungus and *P. neglectus*. The two fungi reacted antagonistically, which resulted in less root damage and increased yield.

While many fungi positively interacted with *P. neglectus*, *G. graminis* showed a negative interaction with the nematode. Plants inoculated with nematodes two weeks prior to *G. graminis* inoculum suffered less damage than plants inoculated with *G. graminis* alone.

In 1993, a field trial was conducted at Minnipa Research Centre using *G. graminis*, *R. solani* or *G. graminis* plus *R. solani* (Chapter 5). Nematicide (Temik®) reduced the number of nematodes in wheat roots by 98%. *G. graminis* alone reduced yield by 48%, whereas *R. solani* did not affect yield where nematodes were controlled. With the combination of *G. graminis* and *R. solani,* grain yield was not affected in either soil

treatment (±Temik®). However, *G. graminis* reduced grain yield by only 14% in the presence of *P. neglectus*. The nematode alone reduced yield by up to 20%.

The interaction between *F. acuminatum*, *M. bolleyi* or *P. terrestris* and *P. neglectus* was tested aseptically under growth chamber conditions using a sterilised sandy soil (Chapter 6). The hypothesis that feeding by *P. neglectus* may cause physiological changes in the host was tested using mechanical root wounding. The effect of timing of inoculation was also tested in this experiment. The results indicated that *F. acuminatum* could not be considered a major root pathogen, as the fungus alone did not cause severe damage to the root system, while both *M. bolleyi* and *P. terrestris* alone caused considerable root lesioning. Combination of *P. neglectus* with *M. bolleyi* or *P. terrestris* further increased disease rating. Mechanical lesioning on the surface of roots did not augment disease rating and was not attractive for fungi. Different inoculation times for fungus and nematode had a significant effect on the nematode-fungus interaction.

Interaction between *P. thornei* and some soil-borne fungi was also examined, and compared to the *P. neglectus*-fungus interaction results (Chapter 7). *M. bolleyi* or *F. acuminatum* in combination with *P. thornei* increased root lesion rating significantly compared to either pathogen alone. Both nematode species at higher inoculum density decreased root dry weight and caused severe root damage.

The effect of soil temperature on the nematode-fungus interaction was tested using *F. acuminatum*, *M. bolleyi* or *Pythium irregulare* and *P. neglectus* at several inoculum levels (Chapter 8). At lower soil temperature (15°C) neither fungal or nematode inoculum level caused severe damage to the root system. Nematode multiplication rate in most treatments was below 1.0. However, at higher soil temperature, the activity and pathogenicity of both nematodes and fungi increased. The highest multiplication rate of *P. neglectus* was recorded at 25°C, at which temperature a synergistic interaction between the nematode and all fungi tested occurred. In most experiments, fungi alone caused early stimulation of plant growth and increased plant tillering. However, lower initial densities of *P. neglectus* also stimulated plant growth in glasshouse experiments.

The effect of soil temperature on the *M. bolleyi-P. neglectus* interaction was tested using several wheat cultivars ranging from moderately resistant to susceptible to *P. neglectus* and the resistant triticale cultivar Abacus (Chapter 8). All wheat cultivars tested were similarly infested by both fungus and nematode. Combination of *M. bolleyi* and *P. neglectus* increased root lesion rating but had no significant effect on number of nematodes extracted from the roots. The triticale cultivar Abacus contained the lowest number of nematodes and very little root damage was observed.

The effect of the nematode-fungus interaction was tested under natural conditions in the field using microplots and a field trial (Chapter 9). In 1993 and 1994, microplot experiments were conducted at Roseworthy Campus. *M. bolleyi, F. acuminatum, Pyrenochaeta terrestris* or *Pythium irregulare* in combination with *P. neglectus* led to a higher root lesion rating and increased nematode numbers in roots.

The mechanism of the interaction between nematode and fungus was investigated in agar under laboratory conditions (Chapter 10). The hypothesis that *P. neglectus* may feed on fungal mycelium was tested. Nematodes died in all agar plates with fungi (*M. bolleyi, F. acuminatum* or *G. graminis*), indicating they were unable to feed. The attractiveness of *P. neglectus* to roots that had been infected with these fungi was also investigated. *M. bolleyi* and *G. graminis* infected roots were more attractive to the nematode than those infected with *F. acuminatum*.

From the results of this study, it was concluded that in soils in South Australia where the fungi and *P. neglectus* exist together root disease of wheat is caused by the combined effects of *P. neglectus* and some root-rotting fungi. Evidence suggests that *P. neglectus* not only contributes to this interaction through mechanical wounding of roots, but also causes biochemical and physiological changes in plants making them more prone to fungal infection.

STATEMENT OF ORIGINALITY AND CONSENT TO PHOTOCOPY OR LOAN

I hereby declare that the research work presented in this thesis is original and has not been previously submitted in full or in part to any other university or tertiary institution for any degree. This thesis contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis being available for photocopying and loan by any interested person.

ABDOLHOSSEIN TAHERI

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Publications arising from this thesis

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Nicol, J. M., A. Taheri, K. A. Davies, J. M. Fisher and T. Hancock (1995) Interaction of *Pratylenchus thonei* or *P. neglectus* with root-rotting fungi, *M. bolleyi* and *F. acuminatum* on wheat. Abstracts, 10th Biennial Conference of the Australasian Plant Pathology Society, Lincoln University, New Zealand, August 28-30.

Taheri, A., G. J. Hollamby, V. A. Vanstone and S. M. Neate (1993) Interaction between root lesion nematode (*Pratylenchus neglectus*) and root rotting fungi of wheat. Abstracts, 9th Biennial Conference of the Australasian Plant Pathology Society, Hobart, Tasmania, July 5-8.

Taheri, A., G. J. Hollamby, V. A. Vanstone and S. M. Neate (1994) Interaction between root lesion nematode (*Pratylenchus neglectus*) and *Rhizoctonia solani* or *Gaeumannomyces graminis* and relative yield losses of wheat in South Australia. Workshop on Tillage Systems, Rotations, Nutrition and Associated Cereal Diseases, Primary Industries South Australia, Charles Hawker Conference Centre, Waite Campus, March 24-25.

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Taheri, A., G. J. Hollamby, V. A. Vanstone and S. M. Neate (1994) Studies of the interaction between root lesion nematode (*Pratylenchus neglectus*) and *Rhizoctonia solani* or *Gaeumannomyces graminis*. 22nd International Nematology Symposium, University of Gent, Belgium, August 7-12. Abstract, *Nematologica* **41:** 346-347.

Taheri, A., J. M. Nicol, K. A. Davies and J. M. Fisher (1994) Description of male and female *Pratylenchus thornei* (Sher and Allen, 1953) and *Pratylenchus neglectus* (Rensch, 1924) from Australia. 22nd International Nematology Symposium, University of Gent, Belgium, August 7-12. Abstract, *Nematologica* **41:** 347.

Taheri, A., J. M. Nicol, J. Lloyd and K. A. Davies (1996) Morphometrics of South Australian population of *Pratylenchus thornei* and *P. neglectus* males and females. *Australasian Plant Pathology* (in press).

Taheri, A., V. A. Vanstone, G. J. Hollamby and S. M. Neate (1995) Interrelationship between root lesion nematode (*Pratylenchus neglectus*) and take-all (*Gaeumannomyces graminis* var. *tritici*) on wheat. Newsletter of the Crop Science Society of South Australia, November, No. 142.

Taylor, S., M. Evans, V. Vanstone, J. Nicol, R. Eastwood, A. Smith, M. Farsi and A. Taheri (1994) Root lesion nematodes (*Pratylenchus neglectus* and *Pratylenchus thornei*). Agronomy Technical Conference, Primary Industries South Australia, Charles Hawker Conference Centre, Waite Campus, March 22-23.

Taylor, S., M. Evans, V. Vanstone, J. Nicol, R. Eastwood, A. Smith, M. Farsi and A. Taheri (1994) Root lesion nematodes (*Pratylenchus neglectus* and *Pratylenchus thornei*). Newsletter of the Crop Science Society of South Australia, June No. 127.

Chapter 1

General introduction and review of literature

1.1 Introduction

With 15,000 described species, nematodes are among the most numerous multicellular animals on the Earth. It has been estimated that there are at least 500,000 species of nematodes (Bogoyavlenskii *et al.*, 1974). Plant-parasitic nematodes are found in all agricultural regions of the world. The genus *Pratylenchus* (Tylenchida: Pratylenchidae), with the common name of "root lesion nematode", currently contains about 70 valid species (Frederick and Tarjan, 1989; Loof, 1991) which are of economic importance to crops of agricultural interest.

The relationship of nematodes to other soil-borne organisms in most cases remains unclear. Interactions between nematodes and other pathogens often have important economic effects on the growth and yield of plants in agricultural ecosystems, and diseases induced by interaction between pathogens are well documented on various crops (Powell, 1971; Sikora and Carter, 1987; Evans, 1987; Storey and Evans, 1987; Evans and Haydock, 1993), including wheat (Benedict and Mountain, 1956).

Pratylenchus spp. are distributed in all agricultural soils worldwide and have a wide range of host plant species. Wheat (*Triticum aestivum* L.), a susceptible host to several root and shoot diseases caused by fungi and bacteria (Butler, 1961), is also subject to attack by various species of nematode, in particular *Pratylenchus* spp. (Goodey *et al.*, 1965).

In Australia, two species of *Pratylenchus*, *P. neglectus* (Rensch 1924) Filipjev and Schuurmans Stekhoven 1941 and *P. thornei* (Sher and Allen 1953), are the most important migratory endoparasitic nematodes causing considerable damage to root

systems of wheat and other cereals. In South Australia, *P. neglectus* is widespread and infects the roots of many crops including wheat and pasture species (Vanstone, 1991).

1.2 *Pratylenchus* spp.

Nematodes of the genus *Pratylenchus* Fillipjev 1936 (Nematoda: Tylenchida: Pratylenchidae) are migratory endoparasites, moving within the root cortex and between the root and soil (Dropkin, 1980). They are obligate parasites that feed on a wide range of cultivated and wild hosts worldwide. Their feeding and migration within the root causes considerable damage to the cortical cells. Migratory nematodes can create potential infection sites for soil fungal and bacterial plant pathogens, including some that normally are not pathogens or are only weak pathogens.

1.2.1 Symptoms

Infested wheat plants show light-brown discolouration to extended dark-brown lesions on the root cortex (Plate 1.1), rotting and degradation of root hairs, and may be stunted with fewer tillers and yellowed lower leaves. Both *P. neglectus* and *P. thornei* appear to occur in greater numbers in seminal than in crown roots (Kimpinski *et al.*, 1976; J. P. Thompson, personal communication), so that lesions are more frequent on seminal roots than on crown roots. Crown roots do not develop until about six weeks after sowing, so the nematodes first infect seminal roots. There are also more seminal than crown roots and they may be more physiologically active, and may be the preferred sites for nematode invasion (Kimpinski *et al.*, 1976). However, crown roots are produced throughout the life of the plant (under suitable growing conditions) and may offer a continuous food supply. Also, when seminals are severely damaged or over-crowded with nematodes, the nematodes may move to crown roots. Furthermore, by the time crown roots are formed, one generation of nematodes would have already developed in seminals, and rapid multiplication and competition for the food source may force nematodes to migrate to crown roots (V.A. Vanstone, personal communication).

Plate 1.1 Seminal and crown root systems of wheat cultivar Machete.

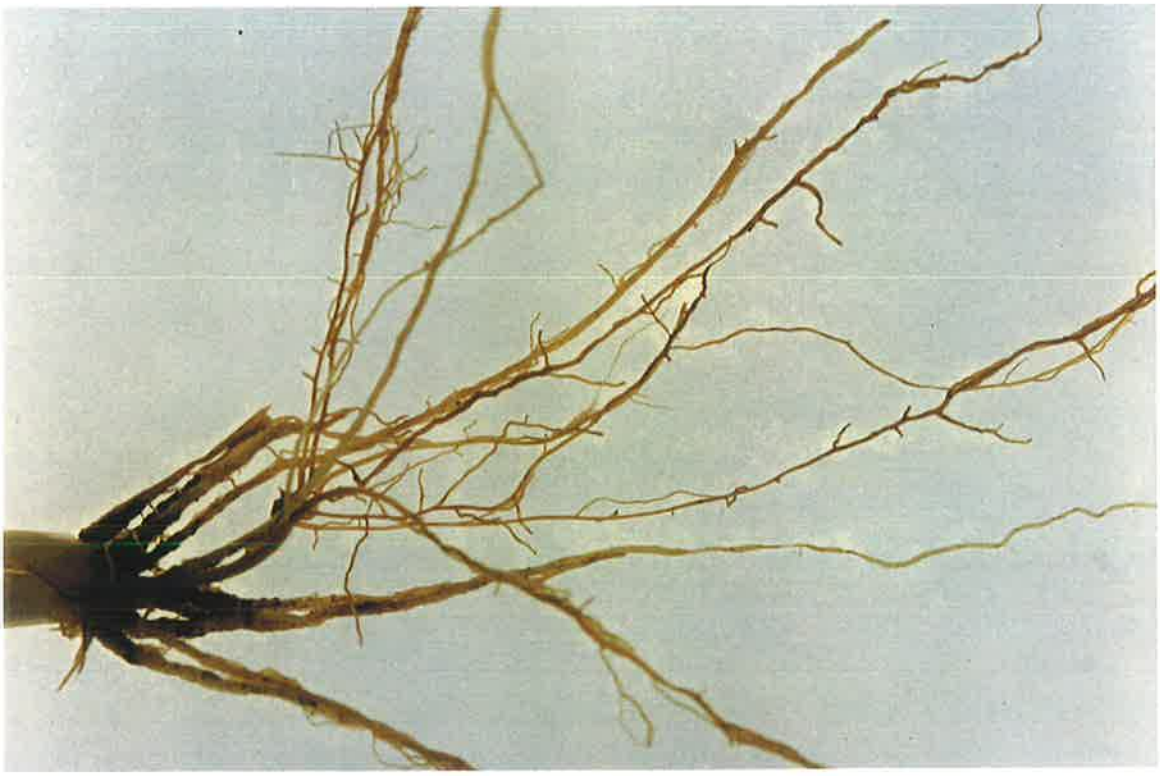
A. Healthy plant with numerous lateral root branches collected from a field plot fumigated with methyl bromide.

B. Plant collected from the field showing symptoms of *Pratylenchus neglectus* infection.

(photographs courtesy of V. A. Vanstone)

A

B



1.2.2 Economic importance

Nematodes are both major pathogens in their own right and through their interactions with other plant pathogens (Sidhu and Webster, 1977). The nematode genus *Pratylenchus* contains a number of parasitic species that infect a wide range of the most important crop species worldwide. *P. thornei* and *P. neglectus* are the most important migratory nematodes that parasitise the roots of wheat crops in Australia. *P. thornei* is predominant in heavy-textured soils whereas *P. neglectus* tends to occur in light-textured soils, although mixed populations of both nematode species can be found in both soil types (Nicol, 1996).

P. thornei decreased yield of susceptible wheat cultivars by up to 85% on the Darling Downs of Queensland (Thompson *et al.*, 1980, 1981). In northern New South Wales, severe yield losses of wheat have been reported with high populations of *P. thornei* (Doyle *et al.*, 1987). Other workers from Mexico, Canada, Israel and the USA have reported yield loss on different crops, in particular wheat and potatoes, where either *P. thornei* or *P. neglectus* occur in high populations (Benedict and Mountain, 1956; Thorne, 1961; Van Gundy *et al.*, 1974; Amir *et al.*, 1991; Orion and Shlevin 1989; Olthof, 1990).

P. neglectus has been found to be damaging to cereals (Griffin, 1992; Mojtahedi *et al.*, 1992; Umesh and Ferris, 1994). However, experiments to measure crop loss due to nematode attack are difficult to design because of the many overlapping interactions (biotic and abiotic) involved (Dowler and Van Gundy, 1984).

1.3 *Pratylenchus neglectus*

1.3.1 Description

P. neglectus (Rensch 1924) Filipjev and Schuurmans Stekhoven 1941 (Syn. *P. minyus* Sher and Allen 1953) is a vermiform, migratory endoparasite.

Female: (Townshend and Anderson, 1976) "body 0.312-0.588mm long; stylet 15-19µm and stylet knobs 4-6µm across, typically indented on anterior surfaces; head with two annules of about equal size; tail usually curving ventrally, with rounded smooth tip".

Male: (Taheri *et al.*, 1996 in press): "body large (428-432µm), assuming a straight to very open "C" shape when killed. Lateral field with four lines. Head with two annules about equal size, the apical one comprising the lips. Stylet knob 3-5µm across, typically indented on anterior surfaces. Dorsal gland orifice 3-5µm posterior to stylet. Excretory pore 66-70µm from head end". Male and female *P. neglectus* are described fully in Appendix A.

1.3.2 Life cycle

P. neglectus is an obligate parasite which reproduces parthenogenetically. Because males are very rare, females do not have functional spermatheca. Reproduction is by mitotic parthenogenesis (Townshend and Anderson, 1976). From egg to adult there are four moulting stages, the first within the egg and the others either within root cortical cells or in the soil (Wiese, 1987). All active juveniles and adults can infect roots of host plants. Females are attracted to host roots, penetrate and migrate through the root cortex, laying eggs as they feed. The nematode can penetrate anywhere along the root, particularly at the tip (Townshend and Anderson, 1976). Nematodes move from cell to cell and desposit eggs within the root cortex, or in the soil when females are outside the roots.

The life cycle of *P. neglectus* may be completed in about 28 days under optimal conditions (Mountain, 1954), depending upon host species and soil temperature. For example, the life cycle of *P. neglectus* on tobacco is completed in about 28 days at 38°C. This comprises eight to ten hours for the female to completely enter a root (eggs are laid almost immediately after root penetration) and seven to nine days for the egg to hatch into a second stage juvenile (the first stage moult occurs within the egg). After

penetration, the nematode becomes stationary in the cortex and feeds for four to six days, then migrates through the cortex to feed on other cells, resulting in breakdown and necrosis of the tissues (Townshend and Anderson, 1976). Mountain (1954) observed that cortical tissues of tobacco roots turn brown and die four to six days after penetration but the epidermis remains intact although necrotic.

Of abiotic factors influencing nematode biology (rate of development and reproduction), temperature is particularly important (Wallace, 1973). The optimum temperature for development and reproduction of *P. neglectus* on alfalfa and wheatgrass is 30°C (Griffin and Gray, 1990; Griffin, 1991, 1992). Under optimal conditions (temperature and availability of favourable hosts), the reproduction rate of *P. neglectus* is very high and nematode numbers in the root system increase exponentially. Similarly, Baxter and Blake (1968) observed that the number of *P. thornei* per root system of wheat increased exponentially from an initial 30 to 450 after 40 days. Unlike cereal cyst nematode (*Heterodera avenae*), which completes only one life cycle in the life of a wheat crop, *Pratylenchus* spp. can complete several life cycles within the life of a single wheat crop (Van der Plank, 1968).

During dry conditions, and in the absence of a host, eggs and other stages of root lesion nematodes can enter an anhydrobiotic state, similar to other nematode species (Townshend and Anderson, 1976). *P. neglectus* has a considerable capacity to survive in the soil. Meagher (1970) found that about 50% of the initial population of *P. neglectus* survived for fifteen months in a dry sandy topsoil (1.5% moisture) of a solonised brown soil from a wheat field. The nematode survives best at 2°C (Townshend, 1963), but does not survive sub-zero temperatures (Townshend and Anderson, 1976). Nematodes survive in soil or within dead roots until the next season. With increases in soil moisture early in the season and availability of host plants, the nematodes start to move, soon penetrate roots and begin feeding.

1.3.3 Penetration sites

Lownsbery (1956) noted that the favoured sites for *Pratylenchus* spp. penetration and feeding are the root hairs. Similarly, Zunke (1990) observed that all stages of *P. penetrans* are able to probe and feed on root hairs. In general, *Pratylenchus* spp. prefer seminal roots rather than crown roots for penetration and feeding. Kimpinski *et al.* (1976) reported that penetration of *P. neglectus* into wheat roots is not limited to a certain part of the root, but seminal roots are invaded about ten times more often than crown roots.

Invasion seems to occur at random along the root. However, it has been observed that once a root segment is invaded, many other nematodes are attracted to the invasion site and also enter the root. Root exudates leaking from damaged roots may attract other nematodes. This results in many areas with few or no nematodes and some areas with many nematodes (V.A. Vanstone, personal communication). However, these lesion nematodes do not penetrate or damage vascular tissues of host plants (Krusberg, 1963).

1.3.4 Distribution

Root lesion nematodes have a wide geographical distribution, ranging from the temperate zones to the tropics (Townshend, 1963). *P. neglectus* occurs in temperate regions worldwide, and has been reported from Europe (Townshend and Anderson, 1976), Canada (Olthof and Hopper, 1973), the USA (Cotten, 1970), North Western India (Sethi and Swarup, 1971) and Australia (de Beer, 1965; Thompson *et al.*, 1981; Vanstone, 1991; Nicol, 1996). Mojtahedi *et al.* (1992) noted that high numbers of *P. neglectus* were extracted from roots of stunted dryland winter wheat in Washington State, USA.

In Australia, *P. neglectus* occurs widely in the wheat belts of Victoria, northern New South Wales and some areas of Queensland, infecting cereals and other crops grown in rotation with cereals (de Beer, 1965; Meagher, 1970; Thompson, unpublished data). It

is also widespread in cereal-growing areas of South Australia (de Beer, 1965; Kimpinski, 1972; Stynes, 1975; Kimpinski *et al.*, 1976; Patel, 1983; Vanstone, 1991) infecting a wide range of cereals, pasture legumes, grain legumes, oilseeds and weeds.

1.3.5 Host range

Pratylenchus species have been associated with a wide range of plant species. In particular, *P. neglectus* is a parasite of several species of cereal, legume, potato and a wide range of other crop plants (Siddiqui *et al.*, 1973), including *Citrus* spp., *Prunus* spp., *Lolium* spp., vines and subterranean clover (Khair, 1987). Mountain (1954) reported that this nematode is quite destructive to tobacco and it is also present on red clover, soybean and peppermint (Faulkner and Skotland, 1965; Faulkner and Bolander, 1969). *P. neglectus* has also been reported on grasses, legumes, crucifer, sunflower, strawberry, carnation, fruit trees (Goodey *et al.*, 1965; Tobar-Jimenez, 1971), vetch, chickpea (Guevara-Benitez *et al.*, 1970), alfalfa (Griffin and Gray, 1990) and white clover (Townshend and Potter, 1976). Vanstone *et al.* (1993) found that all crop species tested (cereals, grain legumes, pasture species, oilseeds) were infested by *P. neglectus,* although the multiplication rate of the nematode differed both between and within crop species. At 2000 nematodes per plant, *P. neglectus* reduced shoot and root growth of five cultivars of wheatgrass tested in a glasshouse experiment (Griffin, 1992).

1.4 Root-rotting fungi

There are several species of fungi involved in root-rotting of plants in disease complexes (Gorter, 1943; Jooste, 1965; Maas and Kotze, 1981). *Rhizoctonia solani*, *Gaeumannomyces graminis* var. *tritici*, *Pythium* spp., *Phytophthora* spp., *Fusarium* spp., *Bipolaris sorokiniana* and *Microdochium bolleyi* are commonly isolated from roots of wheat, but have differing degrees of pathogenicity. *R. solani* and *G. graminis* are widely spread in agricultural soils worldwide and are known to be pathogens of wheat. Some species of *Fusarium* and *Pythium, B. sorokiniana* and *M. bolleyi* are also known to be pathogenic to wheat.

Fedel-Moen and Harris (1987) isolated a wide range of soil-borne fungi from roots of wheat and barley in South Australia, including *B. sorokiniana, F. equiseti, F. acuminatum* and *F. oxysporum*. Other fungi such as *M. bolleyi, Curvularia* spp., *Phoma* spp., *Embellisia* sp., *Athelia* sp., *Cylindrocarpon* sp., *Alternaria alternata, Ulocladium atrum* and *Periconia macrospinosa* have been also found associated with cereal roots in South Australia (Moen and Harris, 1985; Harris, 1986, 1987). Moen and Harris (1985) concluded that although many of these fungi are classified as minor pathogens (Colhoun, 1979), they should not be overlooked as a primary source of damage to roots. Vanstone (1991) also listed a similar range of species isolated from wheat roots in the field. Association of *P. macrospinosa* in "crater disease" of wheat in South Africa was reported by Scott *et al.* (1979).

1.4.1 *Gaeumannomyces graminis* var. *tritici*

G. graminis (Sacc) Von Arx and Olivier (1952) var. *tritici* (Walker) is an ascomycete which produces ascospores (Asher, 1981). The fungus is known as a cause of the root rot disease "take-all", which is found worldwide and can cause great damage to most cereal species (Garrett, 1942).

G. graminis has a cosmopolitan distribution in temperate climates (Garrett, 1981). In South Australia, the disease has been known since the middle of the last century (Anon, 1868). The fungus is the most serious cause of root disease in wheat and barley occuring in the cereal growing areas of South Australia (Murray and Brown, 1987).

Severity of take-all disease is dependent upon host and environmental conditions, such as soil moisture and soil pH (Reis *et al.*, 1982). Under conditions favouring pathogenicity of *G. graminis*, the fungus is able to grow and spreads into the root system (seminal roots, crown roots and up the culm base), resulting in production of darkly pigmented mycelial growth along the root surface or within the root cortex (Cook, 1981). Cook *et al.* (1972) found the rate of hyphal ectotrophic growth was directly proportional to matric water potential: growth was minimal in dry conditions (-

50 bars) and reached a maximum in moist conditions (-1.5 bars). The severity of take-all was greatest on areas where drainage was very poor (Yarham, 1981). In severe infections of seedlings or young plants, stunted and unthrifty plants occur within a crop in irregular patches (Butler, 1961; Rovira and Venn, 1985). Apparently healthy plants approaching maturity may senesce prematurely and heads are either completely empty of grain or the grain is severely shrivelled. The disease caused by *G. graminis* is known in Australia as "take-all" and "white-heads" or "hay-die", which are different phases of the same disease (McAlpine, 1904). Hay-die is the severe phase mentioned above. The incidence of hay-die is greatest when there is a dry finish to the season (MacNish, 1980). In contrast, take-all is always most severe in years with a wet winter and/or spring (MacNish, 1980).

It is also possible for this fungus to interact with other soil organisms, including nematodes, to either increase or decrease damage to the host. Cook (1975) demonstrated that *G. graminis* reduces formation of *H. avenae* (cereal cyst nematode) cysts by rotting roots and competing for root sites, thus reducing the nematode population. A mutual antagonism between *R. solani* and *G. graminis* was described by Patel (1983).

MacNish (1980) estimated a $20 million annual average loss in cereals due to take-all in Western Australia. Rovira (1976) found a yield decrease of 47% in a trial in South Australia, and this loss was ascribed to severe take-all alone. Yield losses attributed to take-all disease in the cereal belt of South Australia average 5-10% (Rovira, 1980; Murray and Brown, 1987).

1.4.2 *Rhizoctonia solani*

The genus *Rhizoctonia* contains heterogeneous species comprising morphologically similar basidiomycetous imperfect fungi of diverse relationship. *R. solani* Kühn 1858 (teleomorph: *Thanatephorous cucumeris* (Frank) Donk 1956) is recognised as a destructive pathogen causing root rot in a wide range of plant species, including wheat,

worldwide (Samuel, 1923; Hynes, 1933). No source of host resistance in wheat to *R. solani* has been found, but Neate (1984) suggested that cultivation reduced the incidence of disease.

Interaction between *R. solani* and various plant parasitic nematodes has been noted for several crops (de Beer, 1965; Meagher and Chambers, 1971; Patel, 1983). The work of Mountain (1956) and Benedict and Mountain (1956) showed a positive interaction between *R. solani* and *P. neglectus*. Meagher and Chambers (1971) noted that the combination of *R. solani* and cereal cyst nematode (*H. avenae*) had a significantly greater effect on wheat growth than did either pathogen alone.

1.4.3 *Bipolaris sorokiniana*

B. sorokiniana (Sacc.) Shoem. 1959 (Syn. *Helminthosporium sativum* Pamm. *et al.* 1910; teleomorph: *Cochliobolus sativus* (Ito and Kurib.) Drechsler ex Dastur 1942) is known as a cause of "common root rot" disease. The fungus is a major cause of root rot worldwide, infecting several plant species including cereals and other grasses, and is a major pathogen of wheat (Simmonds and Ledingham, 1937; Sprague, 1950; Harper and Piening, 1974; Verma *et al.*, 1974; Diehl, 1979; Hill *et al.*, 1983; Fernandez *et al.*, 1985).

In Australia, wheat is a major host of *B. sorokiniana* and the disease has been reported from every state (Samuel, 1924; Butler, 1961; Price, 1970; Harris and Moen, 1981; Mayfield, 1981; Tinline, 1984; Wildermuth, 1986; Whittle, 1992). The fungus is commonly associated with a disease complex (Jooste, 1965; Skou, 1967; Statler and Darlington, 1972; Maas and Kotze, 1981). *B. sorokiniana* is a relatively minor pathogen of several plant species worldwide (Russell, 1931; Salt, 1977) and can infect plants throughout the growing season (Tinline, 1977). However, a loss of 28% in wheat with severe lesioning of the subcrown internode was estimated by Ledingham *et al.* (1973).

1.4.4 *Microdochium bolleyi*

M. bolleyi (Sprague) de Hoog and Hermanides-Nijhof 1977 (Syn. *Gloeosporium bolleyi* Sprague 1948) is a weak pathogen and has been isolated from several graminaceous and non-graminaceous species (Sprague, 1948; Domsch *et al.*, 1980; Kirk and Deacon, 1987b). *M. bolleyi* is probably the most widely distributed soil-borne fungus associated with wheat and barley and has been found worldwide (Sprague, 1948; Salt, 1977; Domsch *et al.*, 1980; Maas and Kotze, 1981; Murray and Gadd, 1981; Jonsson, 1987; Kane *et al.*, 1987).

In Australia, *M. bolleyi* is associated with root-rotting of cereals in the field (Moen and Harris, 1985; Harris, 1986). The fungus has been isolated from wheat roots infected with *P. thornei* in northern New South Wales (Taheri, 1992). Vanstone (1991) has also reported *M. bolleyi* from South Australia, where the majority of plant samples from the field were infected with this fungus. Light to dark brown or black lesions on roots infected with *M. bolleyi* have been noted by Broom (1972).

The fungus produces small conidia (10-12µm) in a conidiogenecell and black chlamydospores which are obvious in root cortical cells (Murray and Gadd, 1981). The fungus can enter plant tissues by direct penetration or through stomatal openings, and produces groups of dark celled chlamydospores both in the outer and inner cortex (Kirk and Deacon, 1987b). *M. bolleyi* is a late coloniser of roots in field and glasshouse studies (Murray and Gadd, 1981; Kirk and Deacon, 1987b; Liljeroth and Baath, 1989; Vanstone, 1991). The species has been recently renamed *Idriella bolleyi*. However, in this thesis I will continue to use the more familiar name, *Microdochium*.

1.4.5 *Pyrenochaeta terrestris*

P. terrestris (Hansen) Gorenz, Walker and Larson (syn. *Phoma terrestris* Hansen) causes pink root rot of onion and some other crops. It was originally identified as the causal agent of onion (*Allium cepa*) pink root by Hansen in 1926. In Australia, *Phoma* spp. are widely distributed, but have received very little attention (Harris, 1986).

P. terrestris has been reported from all over the world (Punithalingam and Holliday, 1973). The fungus has been recognised as a weak pathogen causing root rot of wheat in Australia (Butler, 1961). It has also been reported on other cereals including maize and rice (Jayaweera *et al.*, 1988; Sumner *et al.*, 1990; Campbell *et al.*, 1991). Hyphae enter the young roots, grow through the cortical tissue and form pycnidial primordia in the epidermal and cortical cells (Kreutzen, 1941). Optimal temperature for growth and development of the fungus is 28°C (Gornez *et al.*, 1949).

P. terrestris produces the phytotoxin pyrenocine A which possesses general antibiotic activity against plants, fungi and bacteria (Sparace *et al.*, 1987). *P. terrestris* has been associated with populations of *H. glycines* (soybean cyst nematode) on soybean.

1.4.6 *Pythium* spp.

The genus *Pythium* Pringsheim 1858 contains numerous described species distributed worldwide (Robertson, 1980). *Pythium* spp. are cosmopolitan in soil, and capable of parasitising seeds, roots or aerial parts of a wide range of plants (Robertson, 1980). There are numerous species of *Pythium* that can infect wheat and other cereals (Sprague, 1950; Tesoriero and Wong, 1988).

In Australia, serious losses due to poor emergence of wheat and barley caused by *Pythium* spp. have been reported by several researchers (Bratoloveanu and Wallace, 1985; Blowes, 1988). The most commonly pathogenic species of *Pythium* isolated from rotted roots of wheat or barley in South Australia are *P. irregulare*, *P. graminicola*, *P. volutum* and *P. troulosum* (Bratoloveanu and Wallace, 1985). Moisture content and temperature are known to influence the abundance of *Pythium* spp. and the severity of infection. Among the fungi, *Pythium* spp. have one of the highest soil water requirements for growth. Like *Rhizoctonia* spp., *Pythium* spp. are most damaging at lower soil temperatures. For instance, infection of barley roots with *P. irregulare* was greatest at 13°C (Bratoloveanu, 1985).

1.4.7 *Fusarium* spp.

The genus *Fusarium* contains many pathogenic species which cause a wide range of plant diseases (Nelson *et al.*, 1985). Species of *Fusarium* have a worldwide distribution and are known to be pathogens of many plant species, and many are saprophytic species and common in soil (Nelson *et al.*, 1981). *Fusarium* survives in soil as chlamydospores or as hyphae in plant residues and organic matter (Burgess, 1981). The genus includes some species, for example *F. oxysporum,* which are quite variable in their pathogenicity depending upon host plant and environmental conditions.

There are over 1000 published names of *Fusarium* species due to very similar features (spores, mycelium, colony size and pigmentation in agar) and occurrence of *formae speciales* within individual *Fusarium* spp. (Booth, 1971). Some species which are parasitic in one host may be a secondary coloniser in another host. The genus contains many soil-borne populations which are isolated from diseased root tissues but appear to be secondary colonisers. In general, many *Fusarium* spp. are involved in a root disease complex rather than being pathogenic in their own right (Russell, 1931; Gorter, 1943; Jooste, 1965; Diehl, 1979; Scardaci and Webster, 1982; Hill *et al.*, 1983; Sturz and Bernier, 1987a,b). *F. acuminatum* is considered a weak pathogen, inflicting the most damage when adverse environmental conditions, such as drought stress, persist (Sprague, 1950; Hill and Blut, 1994).

There are numerous reports of *Fusarium*-nematode interactions in several plant species which are of economic importance and, in some instances, this complex disease causes considerable yield reduction to crops (Powell, 1971; Powell *et al.*, 1971). *Fusarium* spp. have been isolated from plants infected with nematodes, and association of *Fusarium* spp. with the lesions initially produced by *Pratylenchus* spp. is well documented (Powell, 1971).

1.5 Nematode-fungus interactions

> "Nature does not work with pure culture. I suspect that many plant diseases are influenced by associated organisms to a much more profound degree than we have yet realised, not only as to inhibition but also as to acceleration of the process. It may be that a number of diseases require an association of organisms for their occurrence and cannot be produced by infection of one organism alone" (Fawcett, 1931).

The soil ecosystem is a complex of various biotic and abiotic factors. Roots grow in soil containing a great number of microorganisms including fungi, bacteria, viruses, insects and nematodes. Thus, under natural conditions, plants are potential hosts to many microorganisms which can influence each other by occupying and/or modifying the same habitat. Synergism has been demonstrated in several plant disease complexes involving interaction of nematodes and fungi (Benedict and Mountain, 1956; McKeen and Mountain, 1960; Mountain and McKeen, 1960). Taylor (1990) suggested that infection by one pathogen may alter the host response to subsequent infection by another soil microorganism. Powell (1971) also noted that, in nature, plants are rarely if ever subject to infection by only one potential pathogen, especially soil-borne pathogens.

The first report of a nematode-fungus interaction was made by Atkinson in 1892. He found that *Fusarium* wilt of cotton was more severe in the presence of the root-knot nematode (*Meloidogyne* spp.) than in its absence. Since then, a number of interactions between plant parasitic nematodes and fungal plant pathogens have been reported worldwide. Although a fungus is an essential component of the interacting system of a nematode-fungus disease complex, the role of these organisms in their interactions with the nematodes has not been defined (Hasan, 1993). However, the roles of nematodes in such interactions have been thoroughly studied and are well documented (Pitcher, 1965; Powell, 1979).

Nematode-fungus disease complexes have been recognised and investigated for many years. Association of nematodes with certain fungi in which nematodes become a part of an aetiological complex is an important aspect of the role of parasitic nematodes as plant pathogens. It has been suggested that nematodes, during penetration and feeding, create potential sites of infection for fungal hyphae (Faulkner *et al.*, 1970). In the soil microbial community, many fungal pathogens interact with the nematodes and in particular the endoparasitic nematodes.

Under natural conditions in the field, a host plant is likely to be infected by more than one pathogen. Thus, the activities and effects of one are likely to be influenced by the activities and effects of the other. Nematode infections too, may modify host physiology in some way which may benefit the fungal or bacterial pathogens. The mechanism of modification of the host plant may be mechanical or physiological or a combination of both.

Interactions of plant parasitic fungi and *Pratylenchus* spp. have been reported on several plant species (Faulkner and Bolander, 1969; Faulkner *et al.*, 1970; Kotcon *et al.*, 1985; Kurppa and Vrain, 1989; LaMondia and Martin, 1989; Jin *et al.*, 1991) including wheat (Mountain, 1956; Benedict and Mountain, 1956; Mojtahedi *et al.*, 1992; Taheri, 1992; Taheri *et al.*, 1994). Of several nematodes of economic importance, root-knot nematodes (*Meloidogyne* spp.) have been most thoroughly studied in their interaction with wilt-causing or root-rotting fungi.

Considering the damage suffered by plants under combined attack by nematodes and fungi, Wallace (1983) divided the interactions between them into synergistic or positive interaction, in which the combined effect is greater than the sum of the individual effects of nematode and fungus, and antagonistic or negative interaction in which the combined effect is less than the sum of the individual effects. When the amount of damage is simply additive, it is considered that there is no interaction.

1.5.1 Synergistic interaction

Synergistic interactions occur due to the role of nematodes in favouring fungal infection, even though in some cases nematode development and reproduction are suppressed by fungi. It is not always easy to identify synergistic interactions, however they have been reported more often than other types of interaction (Evans and Haydock, 1993). Synergistic interactions between *Verticillium* spp. and several species of plant parasitic nematodes have been reported. The majority of these interactions occur with the root lesion nematode, *Pratylenchus* spp. Synergistic interactions between *P. penetrans* and *Verticillium* sp. have been reported on peppermint (Bergeson, 1963), on tomato (Mountain and McKeen, 1962; Conroy *et al.*, 1972), sugar beet (Dwinell and Sinclair, 1967), eggplant (Mountain and McKeen, 1962) and cotton (Riedel and Rowe, 1985; MacGudwin and Rouse, 1990).

1.5.2 Antagonistic interaction

There is some evidence that the interactions between plant parasitic nematodes and soil-borne fungi result in less plant damage, compared to the sum of the individual damage. El-Sherif and ElWakil (1991) reported the antagonistic effect of *F. oxysporum* f. sp. *lycopersici* on *Meloidogyne javanica* when both pathogens were individually applied to split root systems of tomato. The fungus inhibited development and reproduction of *M. javanica* and decreased number of galls on the roots. Similarly, Qadri and Saleh (1990) found that *F. oxysporum* and *F. solani* reduced tomato galling index as well as parasiting *M. javanica* and *H. schachtii* eggs. Jorgenson (1970) found an antagonistic interaction between *F. oxysporum* and *H. schachtii* on sugar beet, where combined effects of the nematode and fungus resulted in less plant damage and an increase in fresh plant weight compared to the effect of the nematode alone.

In some studies, combination of nematodes and fungi did not affect the development of symptoms or yield reduction compared to the individual pathogen, or the effect of the combinations were as great as the sum of individual effects (Abawi and Barker, 1984; Riedel and Rowe, 1985; Wheeler and Riedel, 1994).

Plant pathologists and, in particular, plant nematologists, generally agree that parasitic nematodes can predispose plants to certain fungal pathogens or to a complex disease. These are tripartite interactions, of which soil-borne fungal pathogens are the most important component of interaction. The time at which a plant is inoculated with either the fungus or nematode seems to play an important role in nematode-fungus interaction. In fact, other than a few seed-borne or seedling diseases such as *R. solani*, it is most unlikely that plant species under field conditions will be infected with fungi prior to nematode invasion and, in most cases, nematodes will be the first pathogens infecting plant roots. For instance, wheat seedlings are infected by *P. neglectus* during very early stages of growth.

1.5.3 Nematode/root-rotting fungi interaction

In recent years, there has been an increase in the number of reported interactions between nematodes and fungi other than the *Fusarium* wilt fungi. There have been over 45 reports of nematode-fungus interactions (Evans and Haydock, 1993). Evans and Haydock (1993) in their comprehensive review mentioned a list of interactions between nematode and root-rot fungi together with the genera of nematodes and fungi involved. Plant parasitic nematodes increase the severity of several fungal diseases. Benedict and Mountain (1956) stated that there was a relationship between *R. solani* and *P. neglectus*, causing root-rot disease of wheat in Canada. They have found a consistent association between *R. solani* and *P. neglectus* under field conditions (Benedict and Mountain, 1956).

In a glasshouse experiment carried out by Benedict and Mountain (1956), field soil infested with both *R. solani* and *P. neglectus* was treated with either ethylene dibromide (nematicide), Malachite green (fungicide) or methyl bromide (fumigant) to control both pathogens. Reduction of either pathogen in soil resulted in an increase in plant growth. However, when both pathogens were controlled with application of methyl bromide, growth response of wheat was twice that achieved by either of the other soil treatments. Thus, the effect of both pathogens was necessary to produce full disease expression. A

similar result from a field study confirmed this glasshouse finding but, in an aseptic test using agar medium, no interaction between nematode and fungus was obtained.

Later, LaMondia (1992) investigated the effects of inoculation timing on interaction between *F. oxysporum* and *Globodera tabacum* or *M. hapla* on broadleaf tobacco. Wilt incidence and severity was greater for plants inoculated with nematodes one to three weeks prior to addition of *F. oxysporum* than for plants inoculated with nematodes and fungi simultaneously, or with *F. oxysporum* alone.

Root-rot fungi, in general, seem to increase the number of nematodes, particularly migratory endoparasitic nematodes such as *Pratylenchus* spp. A significant increase in the population of *P. penetrans* occured with high inoculum density of *F. avenaceum* on red clover, suggesting that nematode reproduction was stimulated by the fungus (Jin *et al.*, 1991). In another instance, Carter (1975) noted that interaction between *R. solani* and *M. incognita* on cotton resulted in an increase in nematode numbers. Similar stimulation of *P. neglectus* reproduction was observed in peppermint plants infected by *V. dahliae* f. sp. *menthae* (Faulkner and Skotland, 1965; Faulkner *et al.*, 1970). Presence of the nematode increased both incidence and severity of the disease, while presence of the fungus increased the reproductive rate of the nematode in a synergistic interaction (Faulkner and Skotland, 1965).

The relation of soil temperature to nematode biology and to the combined effect of nematode and fungus is likely to be important. Benedict and Mountain (1956) showed that the optimum soil temperature for reproduction of *P. neglectus* on winter wheat was approximately 32°C. Umesh and Ferris (1992) reported that the optimum temperature for development of *P. neglectus* on barley plants was about 25°C, and on soybean and alfalfa optimum temperature for reproduction of the nematode was 30°C.

Faulkner and Bolander (1969) investigated the effects of soil temperature on *P. neglectus* and *V. dahliae* f. sp. *menthae* interaction on peppermint. They used a range of soil temperatures (18, 21, 24, 27 and 30°C). The incidence and severity of

Verticillium wilt were increased at all temperatures by the presence of the nematode. Nematode reproduction in the absence of fungus was greatest at 30°C but, in combination with the fungus, nematode reproduction increased with each increase in temperature up to 24°C where the fungus causes severe symptoms on roots of peppermint. However, 27°C was optimal for disease development when both pathogens were present. Plant growth at all soil temperatures was retarded more when both pathogens were present than with either pathogen alone. *P. neglectus* alone retarded growth of peppermint most severely at 24°C.

1.5.4 Nematode/nematode interactions

Interspecific competition between plant parasitic nematodes is common and considered to be of major ecological importance in structuring natural communities (Schoener, 1982, 1983; Eisenback, 1985; Rhode, 1991). Nematode species may inhibit other nematode species through competition for feeding sites (Duncan and Ferris, 1982), or through changes in host physiology that may render the host unsuitable for other species (Estores and Chen, 1972; Kraus-Schmidth and Lewis, 1981). Feeding by one species of nematode may alter attraction of the roots to other species, or change availablity of penetration sites. It has been suggested by Eisenback (1985) that plant damage induced by a single nematode species may be increased or decreased by the presence of another species, depending on the nematode species and host plant.

Pratylenchus spp. often occur as mixed populations and are probably very competitive with each other (Eisenback, 1993). Antagonistic competition between *P. alleni* and *P. penetrans* on soybean has been reported by Ferris *et al.* (1967). However, due to mixed populations of *Pratylenchus* spp. in the field, and the difficulty of identifying individuals to species level, studies of interactions of species are difficult.

In experiments on competition between *P. neglectus* and *M. chitwoodi* on barley plants (Umesh and Ferris, 1994), the species that parasitised the root first inhibited

penetration by the later species. In the presence of *P. neglectus*, the reproductive index of *M. chitwoodi* decreased but *P. neglectus* numbers were not affected.

1.5.5 Nematode interactions with non-pathogenic fungi

Most of the literature is related to interactions between nematodes and fungi, bacteria or viruses already known as plant pathogens in their own right. There is little on interactions with non-pathogenic or weakly pathogenic fungi. Fungi non-pathogenic to a host plant may become pathogenic in the presence of nematodes, and weak pathogens may become more damaging. There is a growing body of evidence that nematodes may interact with organisms not generally recognised as plant parasites (Khan, 1993). Weakly parasitic fungi and bacteria can cause considerable damage once they gain entry into plant roots in the presence of feeding nematodes.

Mechanical wounding of roots by nematodes generally assists bacterial and fungal plant pathogens, and other soil organisms which are not normally considered as plant pathogens, to enter the plant. Saprophytic fungi such as *Curvularia trifolii*, *Botrytis cinerea*, *Aspergillus ochraceous, Penicillium martencii* and *Trichoderma harzianum* in combination with the root-knot nematode (*M. incognita*) caused extensive decay to plant roots (Powell *et al.*, 1971). These fungi were added to roots that had been exposed to *M. incognita* for three to four weeks, suggesting that physiological changes in the host plant may have favoured fungal attack.

Migratory nematodes also predispose some plant species to infection by certain fungal and bacterial plant pathogens. *Gnomonia comari*, considered a weak parasite, was extremely pathogenic to strawberry in combination with *P. penetrans* (Kurppa and Vrain, 1989). In sterilised sand, the fungus alone colonised young strawberry roots but did not develop perithecia and was not pathogenic.

1.6 Mechanisms of fungus-nematode interactions

Mechanisms responsible for the interactions between plant parasitic nematodes and soil-borne fungi are not entirely understood (Mani and Sethi, 1987). Nematodes and fungi have different roles in promoting the interaction, and the host plant is the third component in a nematode-fungus interaction.

1.6.1 The role of nematodes in the interaction

Riedel (1988) divided host predisposition into mechanical and physiological predisposition. Mechanical predisposition involving nematode-fungus interaction is mostly due to wounding of the host by nematodes. Plant parasitic nematodes frequently destroy apical meristems during feeding and this often stimulates the development of lateral roots. Natural wounding where lateral roots emerge, or injury because of nematode feeding, especially for endoparasitic nematodes such as *Pratylenchus*, *Meloidogyne* spp. (Porter and Powell, 1967) or *Heterodera* sp. (Polychronophoulos *et al.*, 1969), facilitate fungal infection of plants. Polychronophoulos *et al.* (1969) reported that *R. solani* grew along *H. schachtii* juvenile invasion tracks in sugar beet.

Based on their histopathological study of the interaction between *Globodera pallida* and *V. dahliae* on three potato cultivars, Storey and Evans (1987) reported that *G. pallida* juveniles were able to break down the intermediate resistance of potato cultivars by assisting *V. dahliae* to evade the natural defences of the root. The second stage juvenile was able to bypass the cortical cell lignotuber, and hence it provided an invasion channel for the fungus to colonise the tissue.

Evidence of mechanical predisposition was also obtained by Inagaki and Powell (1969), who imitated nematode damage by wounding roots mechanically. Results showed that in the wounding treatment, black shank disease caused by *Phytophthora parasitica* var. *nicotianae* developed faster than in the treatment with the fungus alone.

However, the role of nematodes in predisposing hosts is not just in providing access for fungi. Much research suggests that alteration of host physiology by nematodes is

the more important mechanism of predisposition (Riedel, 1988). This is supported by evidence that the incidence and severity of fungal diseases increased significantly when the nematode was inoculated onto plants prior to the fungus (Bowman and Bloom, 1966; Porter and Powell, 1967; Mani and Sethi, 1987).

The predisposition of plant tissues to fungal infection caused by nematodes is not limited to the tissues they invade, but increased susceptiblity can also be in tissues away from the infection site (Hillocks, 1986). Using split-root experiments, some researchers have found that the resistance of some tomato cultivars was broken down by *M. incognita*, even though each pathogen was inoculated on separate halves of the root system (Bowman and Bloom, 1966; El-Sherif and ElWakil, 1991). A similar result was obtained by Faulkner *et al.* (1970), who observed the interaction between *P. neglectus* and *V. dahliae* on peppermint.

1.6.1.1 Nematodes as rhizosphere modifiers

Microbial activities in the rhizosphere and rhizoplane are greatly influenced by the quantity and quality of root exudates. Many plant pathogens generally exist in soil in a resting form. However, a fungus may be in a resting stage for reasons other than soil fungistasis and root exudates can stimulate pathogen spores to germinate (Russell, 1984).

Plant parasitic nematodes appear to affect root exudates both directly and indirectly. Quantitative and qualitative changes in root exudates could occur directly as a result of parasitism or indirectly as a result of stresses imposed on the host, accompanied by a reduction of root function, photosynthesis and nutrient level (Mai and Abawi, 1987). Indeed, Powell (1971) suggested that direct quantitative change in exudates could occur by rupture of root cell membranes during feeding, penetration and migration within the root.

Bergeson *et al.* (1970) found a consistent increase in *F. oxysporum* f. sp. *lycopersici* in the rhizosphere of tomato infected with *M. javanica*. They also found a significant

decrease in the population density of actinomycetes which could be antagonistic to the fungal pathogen. Therefore, they concluded that the nematode infection may have a dual effect, by stimulating plant pathogens while at the same time inhibiting their antagonist. Subsequently, it has been reported that roots parasitised by *Meloidogyne* spp. exude higher concentrations of several elements including Ca, Mg, Na, K, Fe, and Cu (Van Gundy *et al.*, 1977; Melakebrhan *et al.* 1985), carbohydrate and amino acids (Wang and Bergeson, 1974), which are necessary to stimulate fungal germination.

Van Gundy *et al.* (1977) demonstrated that exudates from *M. incognita*-infected tomato roots attracted hyphae of *R. solani* to the galls and enhanced sclerotial formation as well as severity of root decay. However, the decay did not develop when root exudates were continuously removed by leaching. In contrast, when leachates were collected from *M. incognita*-infected roots and applied to *R. solani*-infected roots, the rot became more severe. Indeed, observing the aetiological sequence of the disease complex, they reported that during the first fourteen days after nematode infection, when carbohydrates were abundant and C/N ratio was high in *M. incognita*-infected root exudate, *R. solani* was stimulated in the rhizosphere and attracted to the root. Between 14 and 28 days after nematode infection, the C/N ratio decreased. This indicated that the concentration of N was high and therefore favourable for parasitic development of *R. solani*.

1.6.2 The role of fungi in the interaction

In fungus-nematode interactions, fungi have different effects on the nematode. In some cases they can break down plant resistance to nematodes (Hasan, 1985; Hasan and Khan, 1985), increase nematode penetration (Edmunds and Mai, 1966) and in many cases influence nematode development and reproduction (Powell, 1971; Qodri and Saleh, 1990; El-Sherif and ElWakil, 1991). However, research regarding the mechanisms whereby fungi break down resistance and increase populations of nematodes is very rare.

Fungi are likely to differ in their effects on nematode penetration and reproduction. In many cases, populations of migratory nematodes such as *Pratylenchus* spp. appear to increase, as a result of interactions with fungi, whereas populations of sedentary nematodes such as *Meloidogyne* spp. and *Heterodera* spp. are generally suppressed (Powell, 1971).

A few studies have attempted to determine the possible mechanisms whereby fungi increase nematode infection. Klingler (1965) suggested that increased nematode infection may be associated with the increase in CO_2 concentration, acting as an attractant for nematodes to roots infected by fungi. Mountain and McKeen (1962) pointed out the possibility of fungi aiding nematode entry into root tissues. A major barrier to penetration of epidermal cells by nematodes are cell walls which contain cellulose and pectin. The degradation of these cell components by fungi might facilitate penetration of root cells by nematodes (Edmunds and Mai, 1966). This is supported by the evidence of Morsink (1963, in Edmunds and Mai, 1966) that *P. penetrans* which failed to penetrate roots of potato seedlings were able to invade when roots were already infected by fungi.

Regarding the mechanism enabling fungi to increase nematode reproduction, Dwinell and Sinclair (1967) suggested that the effect of *V. dahliae* on *P. penetrans* reproduction was not direct, but determined by host reaction to fungal infection. The fungus appeared to modify the host to create a better substrate for nematode reproduction.

1.7 Conclusion

In general, the level of interactions between plant parasitic nematodes and soil-borne fungal pathogens can not be validated using appropriate statistical tests (Wallace, 1983; Sikora and Carter, 1987). There are only few studies using multi-factorial analyses to determine the significance of the interactions (Sikora and Carter, 1987). Synergistic or antagonistic interactions have been considered by comparing the combined effect of

nematode-fungus interactions to the effect of either nematode or fungus alone, rather than the sum of the individual effects.

Abawi and Barker (1984) demonstrated the effect of nematode population density on nematode-fungus interactions. In their experiments, increasing the initial level of *M. incognita* led to an increase in *Fusarium* spp. and *F. oxysporum* f. sp. *lycopersici* infections as well as wilt development in some tomato cultivars.

The timing of inoculation of the pathogens also influences fungus-nematode interactions. Sequential or simultaneous inoculations of the pathogens affects the type of interaction, determining whether it is synergistic or antagonistic. Different results obtained in experiments studying the interaction between *M. hapla* and *Fusarium* spp. on chrysanthemum seem to be due to the difference in the time of nematode inoculation. Inoculation of nematodes a week prior to the fungal inoculation resulted in a synergistic interaction (Littrell and Heald, 1967). However, when the two pathogens were inoculated simultaneously, no interaction occurred (Johnson and Littrell, 1969). A contrasting result, because of the different time of inoculation, was found by Husain *et al.* (1985). In their study, a sequential inoculation in which *R. solani* was inoculated ten days prior to nematode inoculation resulted in the inhibition of nematode multiplication and root-knot development. On the other hand, a simultaneous inoculation led to a significant increase in nematode multiplication and root galling on pea roots.

Because the nematode-fungus interactions can be altered by many factors, the phenomenon of interactions is not well established. Therefore, the results of many studies on interactions are often contradictory. Sikora and Carter (1987) even questioned the existence of such interactions in nature. According to them, most experiments regarding nematode-fungus interactions were conducted in the greenhouse where environmental conditions do not approach those of nature. Therefore, experiments could produce forced interactions. Furthermore, they also pointed out that the densities of both organisms used in experiments were often high or even higher than those under natural conditions, so that interactions may be favoured.

To obtain reasonable results, however, field observations and the nature of interactions should be taken into consideration. Experimental conditions regarding environmental factors, timing of inoculation and the population density of pathogens should simulate those in the field as closely as possible. More research dedicated to the study of the mechnism of fungi in suppressing nematode development and reproduction is also required.

1.8 Project aims

The literature indicated that attention has been paid to the effect of nematode-fungus interaction on many crops. However, the interaction between nematodes and fungi normally not considered as plant pathogens has not been studied on many crops. In South Australia, particularly, the effect of interaction between root lesion nematode, *P. neglectus,* and root-rotting fungi of wheat has not been investigated in detail. The aims of this project were:

1. To isolate and identify fungi infecting wheat roots in fields naturally infested with *P. neglectus.*

2. To determine the pathogenicity of the nematode and associated fungi and their relative contributions to disease of wheat roots.

3. To conduct glasshouse pathogenicity tests using fungi found to be associated with damaged wheat roots infected with *P. neglectus.*

4. To use aseptic pathogenicity tests to determine the effect of fungi on nematode penetration into the root and to investigate nematode-fungus interactions.

5. To study the influence of factors such as soil type, timing of inoculation, the population density of both fungus and nematode and soil temperature on the nematode-fungus interaction. Different wheat cultivars were also examined in relation to the nematode-fungus interaction.

6. To conduct field experiments using fungi positively associated with *P. neglectus* in glasshouse studies.

7. To investigate the mechanisms of the interaction between nematode, fungus and/or host.

Chapter 2

General methods

2.1 Field samples

To investigate nematode-fungus interactions, two sites were selected on the basis of preliminary observations of *P. neglectus* in the soil. Wheat roots were sampled six, ten, fourteen and eighteen weeks after sowing.

2.1.1 Sites

The first site was at Stow, approximately 125km north of Adelaide. Wheat cultivars sampled at this site were Spear and Condor. The previous crop rotation was wheat, medic pasture and wheat in 1990, 1991 and 1992 respectively. The second site was at Palmer, approximately 80km east of Adelaide (Figure 2.1). Wheat cultivars sampled at this site were Spear and Molineux. Means of rainfall for the 1992 growing season at both Stow and Palmer are listed in Table 2.1. Soil type at both sites was a sandy loam.

2.1.2 Sampling

At all sample times, five plants were sampled randomly from each of four replicate plots ($5.2m^2$) in wheat variety evaluation trials conducted by the Roseworthy wheat breeding group and by Dr. V.A. Vanstone. Plants were carefully dug to include most of the root system and the surrounding soil to a depth of 15cm (approximately 2.0kg soil). Plants and soil were placed in plastic bags, transported to the laboratory and stored at 4°C until processing on the same day or within two days. Roots were then carefully washed under running tapwater to free them of soil.

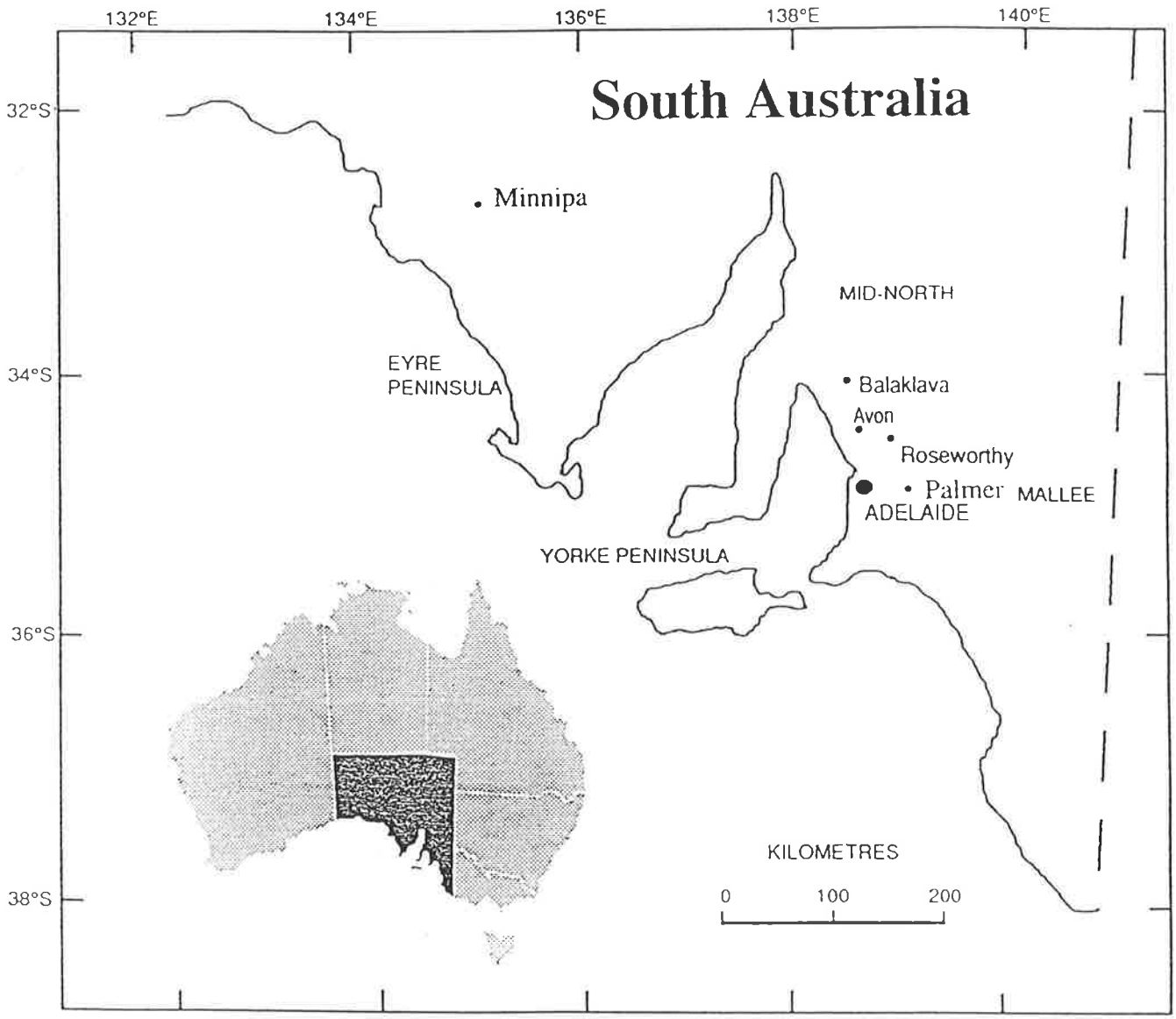


Figure 2.1 Map showing sites where field experiments were conducted.

Table 2.1 Monthly and total rainfall (mm) recorded at Balaklava, Palmer, Roseworthy and Minnipa. Source: Bureau of Meteorology, Kent Town, South Australia and Minnipa Research Centre.

	Balaklava				Palmer				Roseworthy				Minnipa			
	1991	1992	1993	1994	1991	1992	1993	1994	1991	1992	1993	1994	1991	1992	1993	1994
January	10.2	0.0	74.6	1.8	13.8	4.2	37.2	3.4	23.2	0.0	52.8	24.2	N/A	0.4	77.2	N/A
February	0.0	6.6	7.0	4.6	0.0	34.4	5.0	10.0	0.0	9.8	10.4	12.0	N/A	18.6	5.6	N/A
March	6.8	69.4	6.8	0.0	0.6	54.4	1.0	0.0	5.8	57.2	11.4	0.0	N/A	41.1	3.2	N/A
April	36.2	47.4	1.8	2.0	30.2	52.0	0.0	4.8	39.8	29.0	3.6	2.6	N/A	40.2	0.4	N/A
May	1.0	79.6	16.4	18.6	8.8	38.4	15.8	32.0	10.6	55.6	24.4	16.8	N/A	38.8	21.9	N/A
June	109.4	38.2	31.8	96.8	75.2	20.4	25.2	106.8	118.2	56.0	44.2	77.8	N/A	26.8	30.8	N/A
July	35.0	23.2	44.0	23.6	60.2	38.4	N/A	15.8	60.2	23.8	36.8	27.4	N/A	22.4	27.6	N/A
August	42.8	45.8	19.0	7.4	75.4	118.4	N/A	11.6	68.4	182.1	35.2	17.6	N/A	73.0	31.5	N/A
September	33.2	99.5	50.8	7.8	48.0	101.8	N/A	34.0	59.6	142.4	77.4	15.2	N/A	88.4	33.8	N/A
October	14.8	55.4	43.4	11.0	1.6	71.2	N/A	27.0	4.0	81.2	93.0	27.8	N/A	70.0	72.6	N/A
November	33.8	43.8	12.2	21.6	18.4	77.0	N/A	15.4	35.0	72.2	21.4	44.6	N/A	48.0	15.4	N/A
December	1.2	38.4	N/A	7.0	4.0	208.8	N/A	7.4	0.8	75.8	N/A	8.4	N/A	99.0	31.2	N/A
Total	**324**	**547**	N/A	**202**	**336**	**819**	N/A	**268**	**426**	**705**	N/A	**274**	N/A	**567**	**346**	N/A

N/A= data not available.

2.1.3 Disease rating

Lesions on root samples caused by nematodes, fungi or both were assessed for severity according to a scale of 0-5 as follows:

0= Healthy root system, free of lesioning

1= Up to 10% of root system lesioned

2= Between 10 and 25% of root system lesioned

3= Between 25 and 50% of root system lesioned

4= Between 50 and 75% of root system lesioned

5= Between 75 and 100% of root system lesioned.

Two root segments, each 1cm long, were removed randomly from lesioned parts of both the seminal and crown roots of each plant. Similarly, another two root segments from non-lesioned parts were collected (that is, 8.0cm total from each plant). The remaining roots were placed in a misting chamber to extract nematodes (Section 2.1.8.2). Lesioned and non-lesioned roots were not separated for nematode extraction.

Shoots from each sample were oven dried at 80°C for 48 hours and weighed. After four days in the mister for nematode extraction, roots were also dried and weighed.

2.1.4 Isolation of fungi

In a laminar flow cabinet, representative root segments were surface-sterilised prior to plating on agar. Half were surface sterilised by immersion in 2.5% sodium hypochlorite for 60 seconds, followed by washing in three changes of sterile distilled water.

The other half were washed in three changes of sterile distilled water only, because some soil-borne fungi (such as *R. solani* and *Pythium* spp.) are sensitive to sodium hypochlorite. All root segments were blotted dry on sterile tissue before plating on the isolation medium.

2.1.5 Isolation media

Each lesioned or non-lesioned root segment from both seminal and crown roots was cut into two parts and plated onto two different isolation media.

1) Half strength Potato Dextrose Agar (PDA) plus antibiotics (RA medium) (Harris and Moen, 1985a). This isolation medium can be used as a general medium for a large number of soil-borne fungi. The medium consisted of half strength PDA (19.5g/l PDA), which had been autoclaved at 121°C for 20 minutes then cooled to approximately 55°C. The following concentrations of antibiotics were added to each litre of medium prior to pouring into 9cm diameter plastic Petri dishes:

Streptomycin Sulphate	50ppm
Neomycin Sulphate	50ppm
Chloramphenicol	250ppm.

2) Modified VP3 medium (Pankhurst and McDonald, 1988). This medium is selective for the isolation and identification of *Pythium* spp. and *Phytophthora* spp. from roots. The medium consisted of:

Sucrose	20.0g
$CaCl_2$	10.0mg
$MgSO_4.7H_2O$	10.0mg
$ZnCl_2$	1.0mg
$CuSO_4.5H_2O$	0.02mg
MoO_3	0.02mg
$MnCl_2$	0.02mg
$FeSO_4.7H_2O$	0.02mg
Thiamine HCl	100mg
Difco® Cornmeal Agar	17.0g
Distilled Water made up to	1000ml.

The medium was autoclaved at 121°C for 20 minutes, cooled to approximately 55°C, and the following antibiotics were added to each litre before pouring:

Pimaricin	5.0 mg
Vancomycin HCl	75.0 mg
Penicillin	50.0 mg
[benzylpenicillium sodium BP]	
PCNB	100.0 mg
[pentachloronitrobenzene]	
Rifampicin	10.0 mg
[3-(4-methylpiperazinyl-iminomethyl) rifamycin SV].	

Each 1cm lesioned or non-lesioned segment of seminal or crown root was divided into two parts and plated onto each of the above media. Five segments of approximately 0.5cm length were plated per Petri dish of each medium.

2.1.6 Incubation conditions

All cultures on RA or modified VP3 media were incubated in the dark at 25°C for three to four days. After the second day, cultures were checked and all growing fungi were sub-cultured onto PDA and placed under a light bank with a 12 hour photoperiod for three to four days (incubation period) to induce sporulation of isolates. The light bank consisted of four florescent lights and one black light.

2.1.7 Identification of fungi

Two media were used for fungal identification:

1) Carnation Leaf Piece Agar (CLA) (Burgess and Liddell, 1988). This medium contained four to five (0.5cm length) γ-irradiated sterile carnation leaf pieces on each Petri dish of 2% water agar (WA). Leaves were placed on agar while it was still molten so they became partially embedded in the agar. The medium was used for identification of several species of soil-borne fungi, particularly *Fusarium* spp. as they readily sporulate on carnation leaves.

2) Potato Dextrose Agar (PDA) (Toussoum and Nelson, 1976). All fungi growing from the original root samples on RA medium or on modified VP3 medium were subcultured onto PDA and incubated under a light bank for up to one week to encourage sporulation and colony growth. Fungi were identified by microscopic examination of spores and other structures as well as colony form and colour on PDA medium.

Fusarium spp. were subcultured onto CLA medium for sporulation, and the single spore technique of Burgess and Liddell (1988) was used to identify species of *Fusarium*. A single spore germinated on WA was transferred onto PDA and stored in the dark in both 25°C and 30°C incubators for 72 hours, after which time colony diameter and colour were recorded for each temperature.

2.1.8 Nematode extraction

2.1.8.1 Soil

Ten random soil samples were collected from 10-15cm depth of top soil in the field using 10cm diameter plastic cores of 15cm length. Root lesion nematodes were extracted from 200g sub-samples using the Whitehead Tray method (Whitehead and Hemming, 1963) (Plate 2.1A). Each sample was spread on a perforated plastic basket that had been covered with three large facial tissues. Baskets containing soil were then placed within a plastic tray and water added until the soil was saturated. These Whitehead Trays were maintained at room temperature for three days. Over the extraction period, nematodes migrated into the water below the basket. This water was then passed through a 20µm sieve three times, and nematodes washed off the sieve each time until 20ml of nematode suspension in water was obtained.

2.1.8.2 Roots

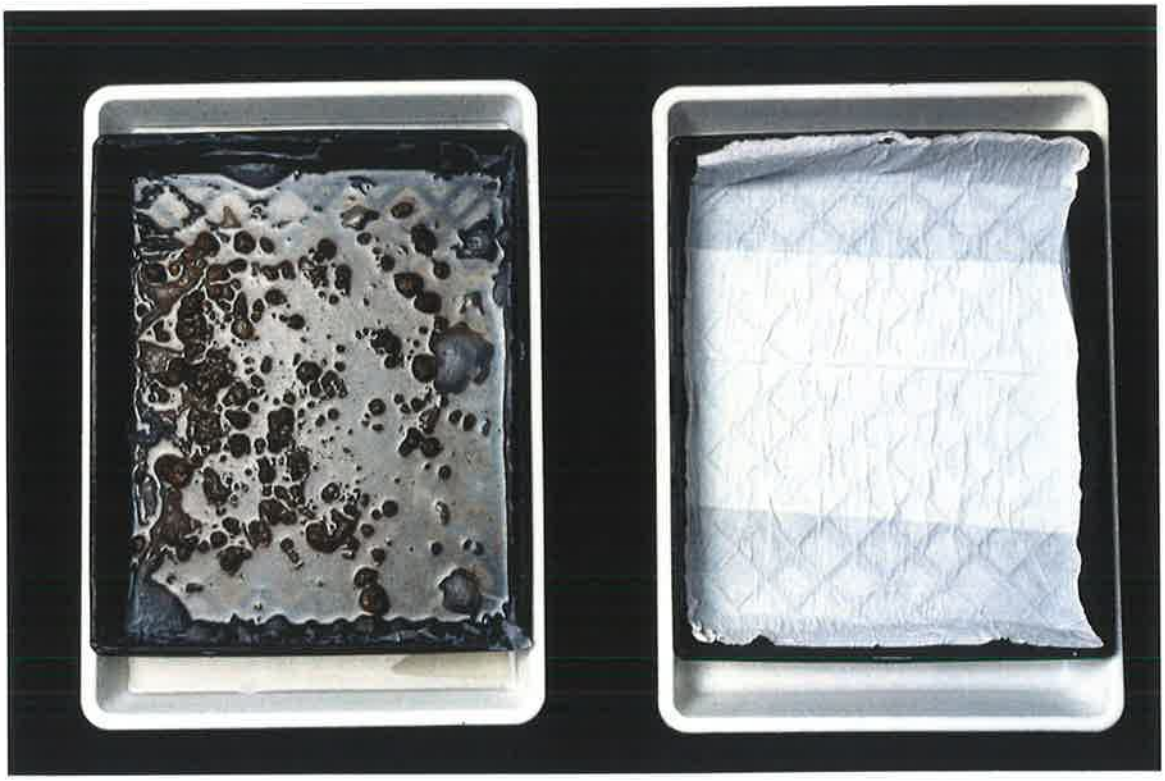
Nematodes were extracted from the roots of plants in a misting chamber (Southey, 1986) (Plate 2.2) over a four day period. Roots were cut into segments no longer than 1cm and spread on a 9cm diameter mesh disk that had been covered in a double layer of facial tissue and placed within a 10cm diameter plastic funnel. Samples were then

Plate 2.1 Nematode extraction technique and equipment required for counting.

A. Whitehead Trays used to extract nematodes from soil.

B. Nematode suspension in test tubes obtained from misting roots, and equipment required for counting (including the modified Doncaster counting dish).

A



B

Plate 2.2 Extraction of nematodes from roots using the mist chamber.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sprayed for ten seconds with a fine mist of water (25°C) at ten minute intervals. Water containing nematodes was collected in a 100ml test tube below each funnel. For counting, the nematode suspension was adjusted to a known volume.

2.1.9 Nematode counting

A 1.0ml aliquot of nematode suspension for each sample was counted microscopically at 25 or 40 times magnification using a modified Doncaster dish (40mm in diameter with four concentric rings) (Doncaster, 1962) (Plate 2.1B).

2.1.10 Staining nematodes, fungi or both in roots

2.1.10.1 Nematodes

Root tissues were stained with acid fuchsin (Byrd *et al.*, 1983) in lactoglycerol to detect larvae, adults and eggs of root lesion nematodes.

Root segments were immersed in 20ml of 4% NaOCl for approximately ten minutes, rinsed for 30 seconds and allowed to soak for ten to fifteen minutes in distilled water. They were then drained and transferred to 30ml (0.40%) of acid fuchsin and heated to boiling over a low flame for 30 seconds, and allowed to cool at room temperature. To destain, the roots were then placed in 20ml of glycerin containing ten drops of 5M HCl, and this was then heated to boiling. Finally, pure glycerin and roots were transferred into a Petri dish to observe nematodes microscopically (Plate 2.3A).

2.1.10.2 Fungi

Simultaneously, some root segments of field samples were stained to detect fungal mycelia in or around lesions, using trypan blue in lactoglycerol (Phillips and Hyman, 1970) (Plate 2.3B).

2.1.10.3 Nematodes and fungi

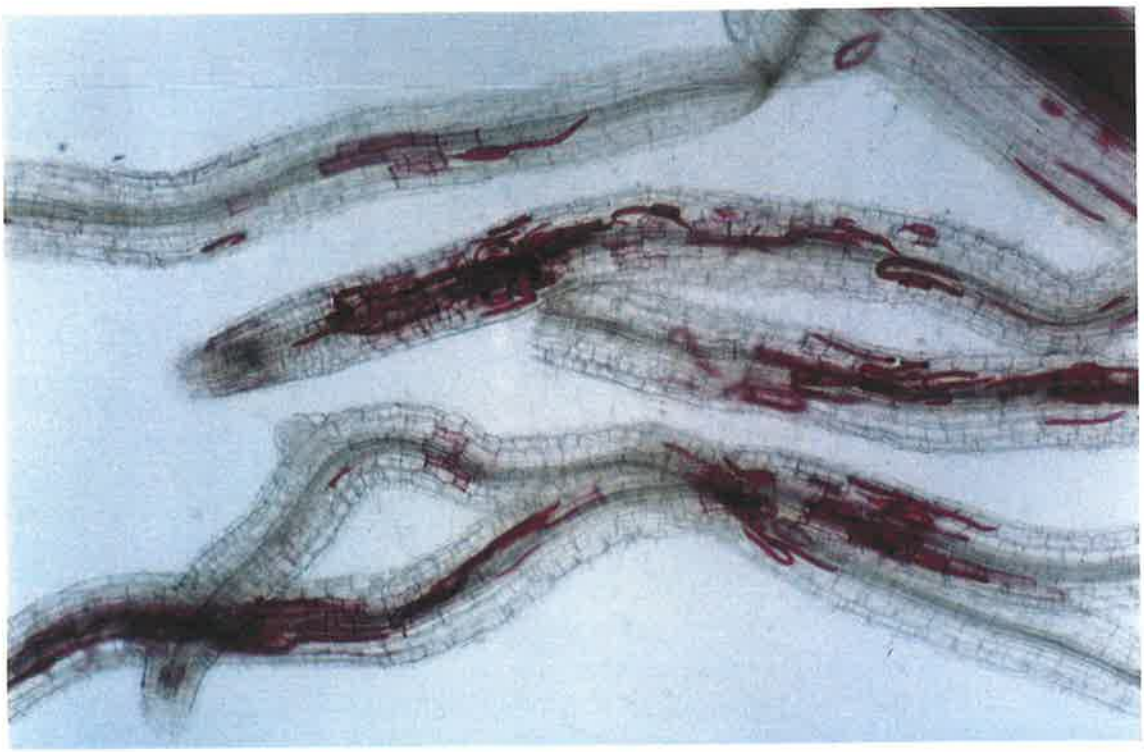
Staining roots for either nematode or fungus resulted in losing the other organism within root tissues. Developing a staining technique to detect both fungus and nematode

Plate 2.3 Staining root tissue of wheat cultivar Machete to detect nematode or fungus.

A. *Pratylenchus neglectus* within roots of Machete wheat using acid fuchsin stain to detect nematodes.

B. *Fusarium acuminatum* in the roots of Machete wheat using Trypan blue stain to detect fungus.

A



B

simultaneously was needed. Different recipes for staining nematodes and fungi within root tissues were tested using varying percentages of potassium hydroxide or sodium hydroxide (1%, 3,% 5% or 10%) and different percentages of trypan blue in lactoglycerol (0.1%, 0.05% or 0.01%). The time and temperature at which roots should be left in the staining solution were also examined. Roots were kept in KOH or NaOH for one, two, six, twelve or 24 hours as well as in trypan blue for the same period. Finally, the following procedure was developed to detect fungi and nematodes within roots in one staining procedure.

1) Fix root segments in 10:1:1 FAA (formalin:alcohol:acetic acid) or, for better results, in 4:1 FA (formalin:acetic acid) fixative overnight. Composition of 10:1:1 FAA was: 20ml distilled water, 6ml formalin (40%), 1ml acetic acid, 40ml alcohol (95%). Composition of 4:1 FA was: 10ml formalin (40%), 1ml acetic acid, 98ml distilled water.

2) Wash roots with several changes of distilled water.

3) Transfer whole roots into 5% KOH and store at 25°C or room temperature for 12-24 hours, depending upon sample age (longer for older root samples).

4) Rinse and transfer to 0.01% trypan blue in lactoglycerol for one to two hours at 25°C.

5) Rinse and transfer to destaining solution (ten drops of HCl in 20ml of glycerol) for microscopic examination.

Plate 2.4 shows *P. neglectus* with either *F. acuminatum* or *M. bolleyi* stained within root tissues of Machete wheat.

2.2 Pot experiments

2.2.1 Soil

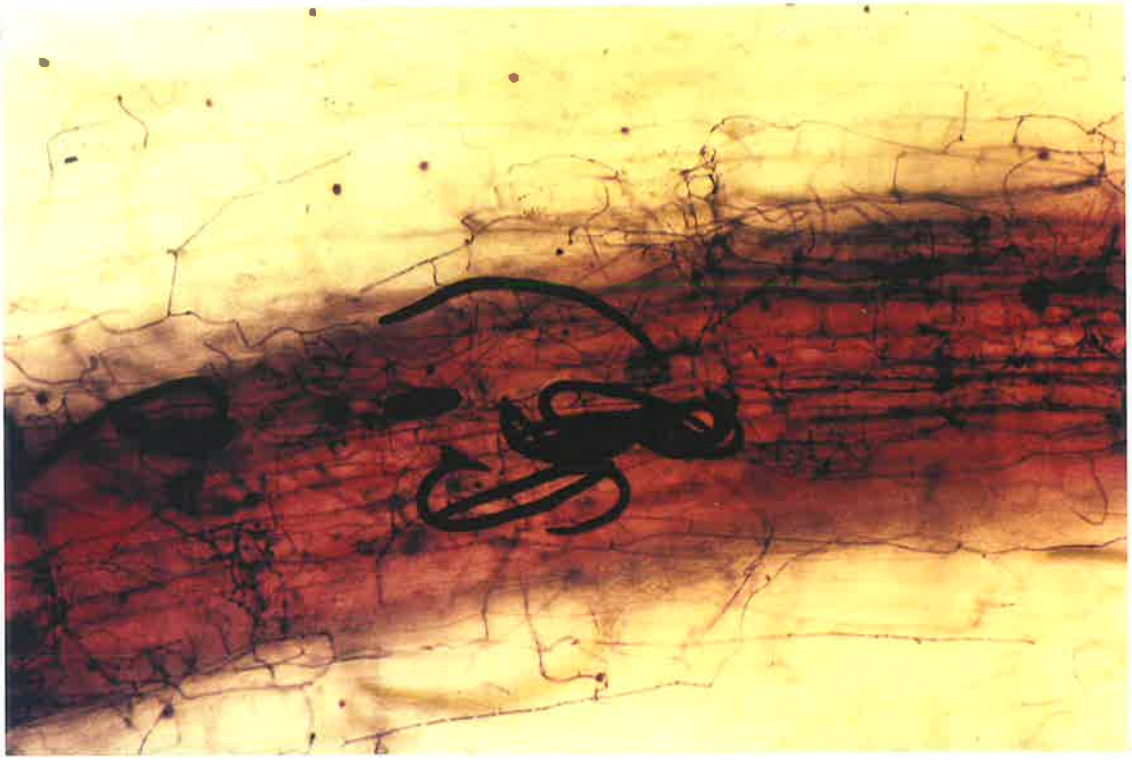
A sandy loam soil was collected from a typical wheat-growing field at Stow, adjacent to the field where the plant samples (Chapter 3) were taken. The soil was collected from

Plate 2.4 Staining root tissue of wheat cultivar Machete to detect nematode and fungus at the same time.

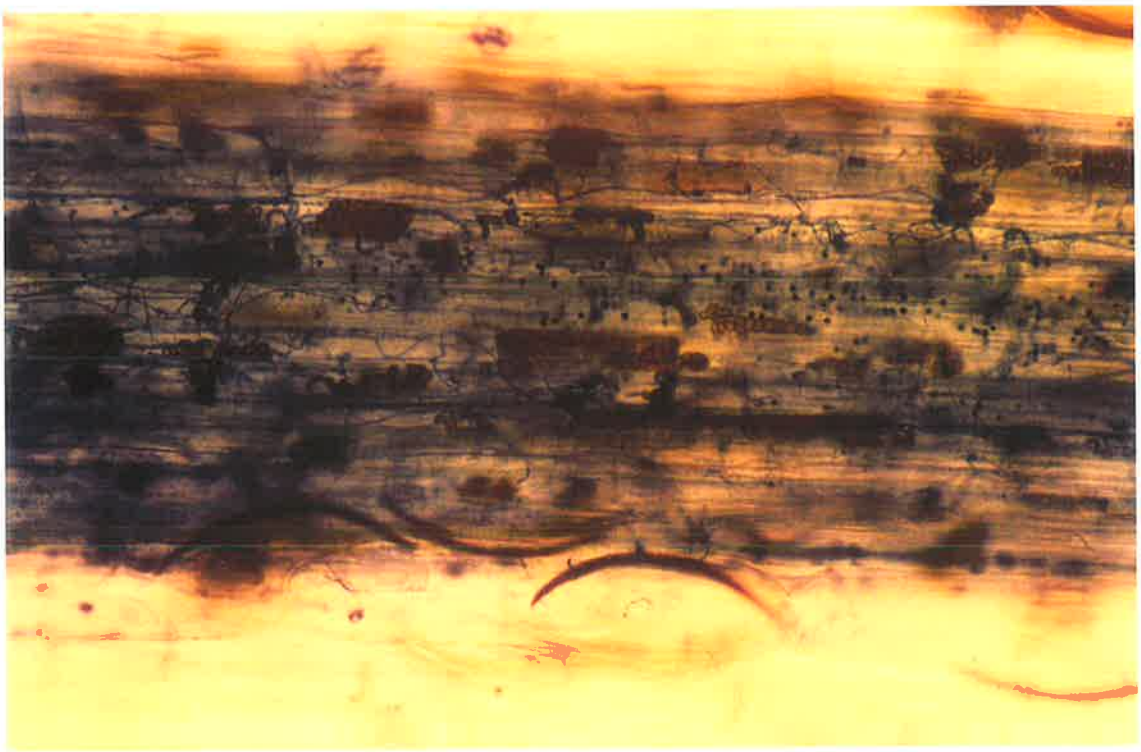
A. *Pratylenchus neglectus* and *Fusarium acuminatum* in the roots of Machete wheat.

B. *P. neglectus* and *Microdochium bolleyi* in the roots of Machete wheat. Chlamydospores of *M. bolleyi* are clumped within the cortical cells.

A



B



the top 15cm (cultivated layer) of the A horizon. The soil was pasteurised with steam at 70°C for 40 minutes, to kill most fungi, bacteria and insects as well as weed seeds (Bollen, 1985; Blom *et al.*, 1988). Soil was sieved (2mm) to remove plant debris and larger particles. This soil was used in inoculation experiments described in Chapter 5.

Soil used for other glasshouse experiments was from Avon, 100km north of Adelaide. This is a calcareous sand (Gcl. 12), also described as a solonized brown soil (Northcote *et al.*, 1975). Soil was collected from an un-cropped area adjacent to areas that were regularly cropped to cereals. Soil was sieved (2mm) to remove plant debris and larger particles.

For other glasshouse experiments, the sandy loam soil was pasteurised with steam at 70°C for 40 minutes.

2.2.2 Pots

For the interaction tests described in Chapter 5, white plastic cups (600ml) without drainage holes and with a capacity of approximately 750g of dry soil were used where two pre-germinated wheat seedlings were sown in each cup. For all other experiments, plastic cups (300ml) without drainage holes with a capacity of approximately 420g of dry soil were used.

2.2.3 Fungal inoculum

Fungal inoculum for experiments described in Chapter 5 and for all experiments with *G. graminis* var. *tritici* was prepared using ryegrass seed as the culture medium. Fungus inoculum for other experiments was prepared on millet seed. Seeds were soaked in water overnight at 5°C, drained thoroughly, then transferred to plastic oven bags closed with a large cotton-wool plug and autoclaved at 121°C for one hour on each of three successive days. This dead, sterile seed was then inoculated with fungus inoculum grown on PDA medium which had been cut into 1.0cm^2 segments and mixed through the bag by shaking. The bags were incubated at 25°C in an incubator with a 12 hour light:12 hour dark cycle. After one week, bags were shaken to encourage colonisation by the fungus.

When the medium had been thoroughly colonised, after about four weeks, it was air dried in a laminar flow cabinet for one week either for immediate use or storage.

The following fungi were used in all interaction experiments: *Microdochium bolleyi* isolate #9251, *Fusarium acuminatum* isolate #9211, *F. equiseti* isolate #9221, *F. oxysporum* isolate #9231 and *Bipolaris sorokiniana* isolate #9241. *Pythium irregulare* isolate #9261, *Pyrenochaeta terrestris* isolate #9281, *Gaeumannomyces graminis* var. *tritici* isolate #9271, *Rhizoctonia solani* Anastomosis Group-8 (AG-8) isolate #Rs21 were also used in the experiments described in Chapter 5.

The above fungi, except *R. solani,* were originally isolated in the 1992 growing season from lesioned wheat roots collected from Stow. *R. solani* isolate #Rs21 was originally isolated from wheat roots by H. McDonald, CSIRO Division of Soils, Adelaide, South Australia. The isolate of *G. graminis* used in all glasshouse and field experiments, except experiments described in Chapter 5, was also isolated by H. McDonald.

P. terrestris isolate #9110, used for the experiments described in other chapters, was originally isolated from lesioned wheat roots at Blue Hills and Deni, Narrabri, NSW, in August 1991 (Taheri, 1992). A culture of *P. irregulare* isolate #P300= DAR 63863, originally isolated from wheat roots by Dr Len Tesoriero (NSW Department of Agriculture), was used for the temperature test and the microplot experiment described in Chapters 8 and 9. Cultures used for inoculum were always those that had been recently sub-cultured from the original inoculum on grain seed.

2.2.4 Inoculation of soil with fungi

M. bolleyi, F. acuminatum, F. equiseti, F. oxysporum, B. sorokiniana or *P. terrestris* were added to the soil in pots at 1% w/w, *R. solani* (AG-8) was added at 0.02% w/w, *G. graminis* var. *tritici* at 0.05% w/w and *P. irregulare* at 0.1% w/w. The inoculum was thoroughly mixed with the soil in experiments described in Chapter 5 and was added at two levels into pots for other experiments.

2.2.5 Seed sterilisation and germination

Cereal cultivars used for both field and glasshouse studies are listed in Table 2.2. Wheat seed used to produce the host plants was surface-disinfested in 2.5% sodium hypochlorite for ten minutes, then thoroughly washed with three changes of sterile distilled water. Seed was pre-germinated on sterile, moist filter paper in Petri dishes in the dark at 5°C for two days to allow uniform moisture imbibition, and then kept for one day at 25°C to germinate. Only healthy seedlings with three roots at least 1cm long were transplanted.

Table 2.2 Wheat and triticale cultivars used in experiments, Australian Winter Cereals Collection (AUS) accession numbers and origins.

Cultivar	AUS	Origin
Abacus	99164	Australia
Excalibur	25292	Australia
Machete	23038	Australia
Molineux	24457	Australia
Spear	22254	Australia
Tatiara	99144	Australia
Xiaoyang huomai	13963	China

2.2.6 Nematode inoculum

Inoculum of *P. neglectus* in aseptic carrot culture was obtained from Dr V.A. Vanstone (Nicol and Vanstone, 1993, using the modified technique of Moody *et al.*, 1983). *P. neglectus* used to establish cultures were originally obtained from Machete wheat roots grown in pots of naturally infested field soil from the Palmer site (Figure 2.1). After inoculation, cultures in plastic containers were kept at 20°C for three to five months, after which time they contained 300,000-500,000 nematodes (Plate 2.5). In a laminar flow cabinet, carrots were chopped into small pieces and immersed in distilled

Plate 2.5 *Pratylenchus neglectus* growing in aseptic carrot culture, two months after inoculation.

(photographs courtesy of V. A. Vanstone)

water for about two hours. Carrots were then washed, retaining the rinse water, and the liquid volume reduced through a sintered glass funnel (No. 4) over a Buchner flask attached to a Venturi pump. A known number of nematodes were pipetted in 1.0ml of distilled water onto the soil surface around each plant.

2.2.7 Growing conditions

The experiments were conducted in a controlled temperature Wisconsin tank in a glasshouse with an air temperature of 25±3°C. Plants were watered with distilled water as required.

2.2.8 Harvesting and measurements

Harvest time varied according to the aims of each experiment, but the harvest and measurement techniques were always the same. Soil was washed from roots under running tapwater. Fungi were re-isolated from representative root segments from each treatment. Roots and shoots from each pot were oven dried at 80°C for 48 hours and weighed.

2.3 Field experiments

Field sites were chosen where pure populations of *P. neglectus* were found from preliminary observations.

2.3.1 Nematode population in soil

Ten soil samples of 200g were taken at random from the experimental sites. Nematodes were extracted using the Whitehead Tray method, and numbers/g of dry soil were calculated (Plate 2.1).

2.3.2 Sampling

Plots were sampled at eight and twelve weeks after sowing. At each sample date, five plants were carefully dug from each plot in order to remove as much as possible of the root system. Plants were placed in plastic bags, and kept cool until processing. Plant

samples were processed as described in Section 2.1, and fungi re-isolated from roots. Nematodes were extracted from roots and number of nematodes per plant and per gram dry root were calculated.

Plots were harvested in all field experiments and grain yields were recorded.

2.4 Experimental design

For field experiments, a split plot design was used to reduce risk of contamination of plots with different fungi or with fumigant.

2.5 Data analyses

All data were subjected to analyses of variance using the Super Anova program (The Accessible General Linear Modeling Package published by Abacus Concepts, Inc., 1984). All data are presented as mean values of the number of replicates. Analyses of variance were performed, and the least significant difference (LSD) calculated at the $P<0.05$, $P<0.01$ and $P<0.001$ significance levels.

Percentage data (i.e. fungal isolation frequencies) were transformed using either $\log_e (x+1)$ or square root transformation prior to analysis of variance where required (Zar, 1984).

On all graphs, error bars represent the standard error of the mean at $P= 0.05$.

Chapter 3

Field survey of fungi infecting roots of wheat in soil naturally infested with *Pratylenchus neglectus*

3.1 Introduction

Wheat is subject to attack from numerous root-rotting fungi. *Rhizoctonia solani*, *Gaeumannomyces graminis* var. *tritici*, *Pythium* spp. (particularly *P. irregulare*) are considered to be major causes of cereal root disease in southern Australia (Patel, 1983; Mayfield, 1984; Bratoloveanu and Wallace, 1985; Rovira, 1987). *Bipolaris sorokiniana*, *Fusarium* spp. *Microdochium bolleyi*, *Pyrenochaeta terrestris* and *Phoma* spp. are considered to be minor pathogens of wheat (Moen and Harris, 1980; Harris, 1987; Rovira, 1987). Numerous saprophytic fungi have also been isolated and identified from wheat roots infested with major pathogens (Fedel-Moen and Harris, 1987).

Root lesion nematode, *Pratylenchus neglectus,* occurs throughout the state of South Australia infecting cereals and other crops grown in rotation with cereals (Vanstone, 1991). Wheat roots suffer considerable damage from *P. neglectus*.

The major aim of this study was to identify fungi from wheat roots naturally infected with *P. neglectus* in the field. The species of fungi most frequently associated with wheat roots infected with *P. neglectus* were determined. Complex effects of these fungi together with root lesion nematodes have generally been over-looked as a cause of cereal root disease in South Australia (Stynes, 1975).

3.2 Methods

3.2.1 Site selection

In the 1992 growing season, two field sites were chosen on the basis of observation of *P. neglectus* in the soil (V. A. Vanstone, personal communication). One was at Palmer, approximately 80km east of Adelaide, and the other was at Stow, approximately 125km north of Adelaide (Figure 2.1). The population of *P. neglectus* at Palmer was 300-400 nematodes/200g of soil and at Stow 800-1000 nematodes/200g of soil. The survey sites consisted of a sandy loam soil.

3.2.2 Wheat cultivars

Wheat cultivars Spear and Condor were sampled at Stow, and Spear and Molineux at Palmer. Spear is tolerant of a range of root and shoot diseases and is also tolerant of high soil boron levels. The opposite is true of Condor and Molineux.

3.2.3 Sampling

Root samples were taken from both sites at six, ten, fourteen and eighteen weeks after sowing. At all sample dates, five plants were sampled randomly from each of four replicate plots. Plants were carefully dug to include most of the roots and the surrounding soil. Root samples were placed in plastic bags for transport to the laboratory and stored in a 4°C cold room until soil was washed from the roots under running tapwater. A total of twenty plants was collected from each site at each sample date. The first sampling at Stow was on June 16, 1992, and at Palmer on June 30, 1992.

Seminal and crown roots were removed from each set of five plants. In a laminar flow cabinet, ten 1cm long segments from lesioned areas of both seminal and crown roots were removed randomly. A similar number of root segments was also selected from non-lesioned (clean) parts of the same root system. Both lesioned and non-lesioned root segments were surface sterilised as described in the General Methods, dried on sterilised tissues and plated on RA and VP3 media (General Methods).

Fungi grown on each medium were sub-cultured onto PDA medium and incubated under a light bank for up to one week to encourage sporulation and colony growth. Fungi were identified by microscopic examination as well as by colony form and colour on PDA medium. *Fusarium* spp. were sub-cultured onto CLA medium to induce sporulation, and the single spore technique of Burgess and Lidell (1988) was used to identify species of *Fusarium*. A single spore germinated on WA medium was transferred onto PDA and stored in the dark in 25°C and 30°C incubators for 72 hours, after which time colony diameter and colour were recorded.

Frequencies of fungal isolation from both lesioned and non-lesioned segments of either seminal or crown roots were determined and recorded separately.

Nematodes were extracted from the remaining seminal and crown roots separately in a mist chamber over four days (extraction period) and counted. Roots for misting were not separated into lesioned and non-lesioned sections. After extracting nematodes, roots were oven dried at 80°C for 48 hours and weighed.

3.3 Results

Symptoms on the roots ranged from light brown cortical lesions to large dark brown or black stelar lesions. Distinct dark black lesions on both seminal and crown roots were caused by *G. graminis*. The majority of samples collected from both sites showed such symptoms. Number of nematodes extracted from roots increased as the frequency of fungi increased late in the season (Figure 3.1).

Seminal roots contained significantly more nematodes than crown roots at all sample dates (Figure 3.2a). Greater populations of fungi were isolated from lesioned parts of roots than from non-lesioned (clean) parts (Figure 3.2b). However, root lesion rating increased with increase in nematode number or fungal population in seminal roots more than in crown roots (Figure 3.2c).

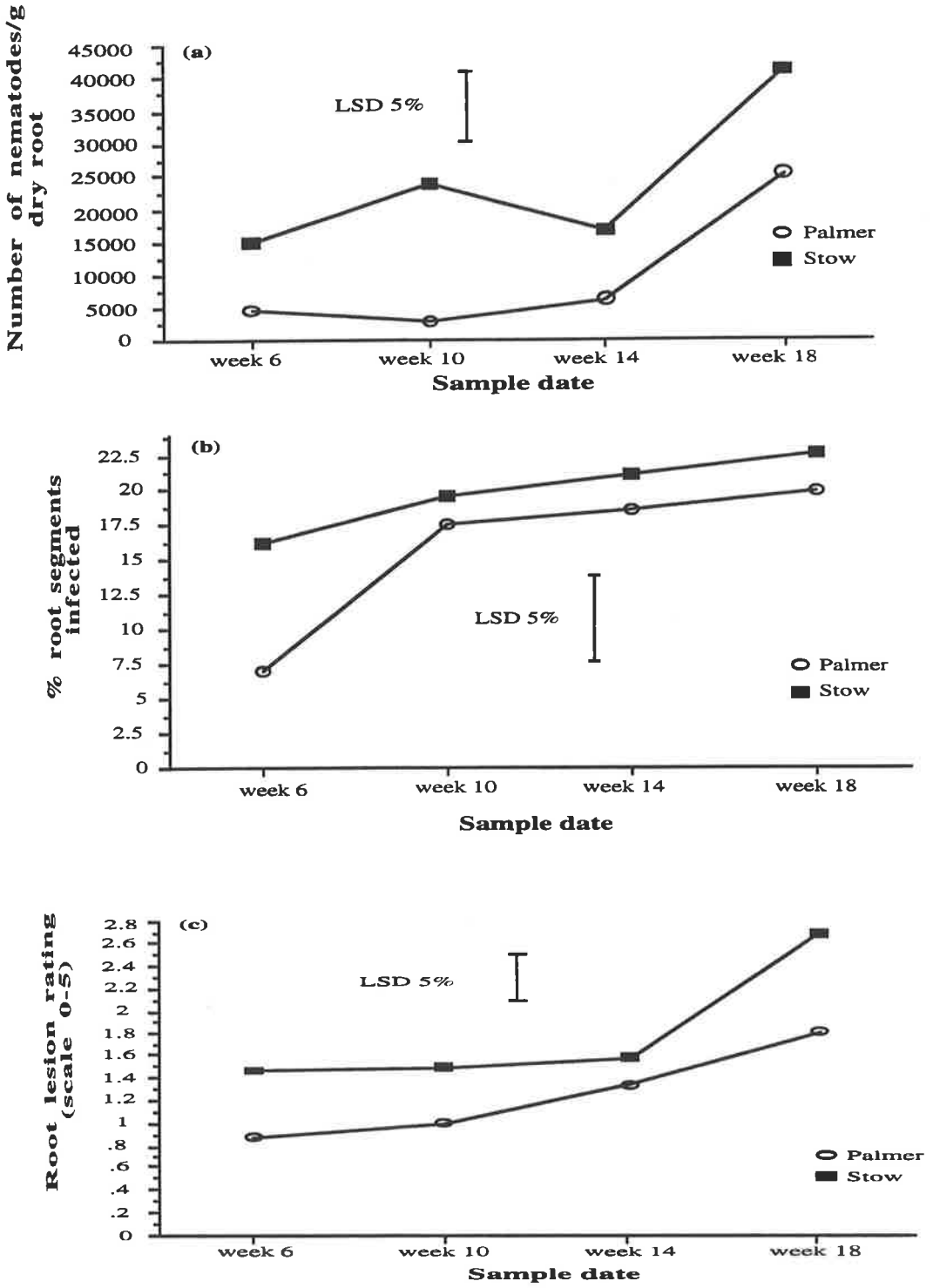


Figure 3.1 Changes with time at two sites (Palmer or Stow) in **(a)** number of nematodes extracted from roots of wheat, **(b)** percentage root segments infected with fungi, and **(c)** root lesion rating over four sample dates (6, 10, 14 or 18 weeks after sowing). Data are means of two wheat cultivars at two sites (Spear and Condor from Stow; Spear and Molineux from Palmer).

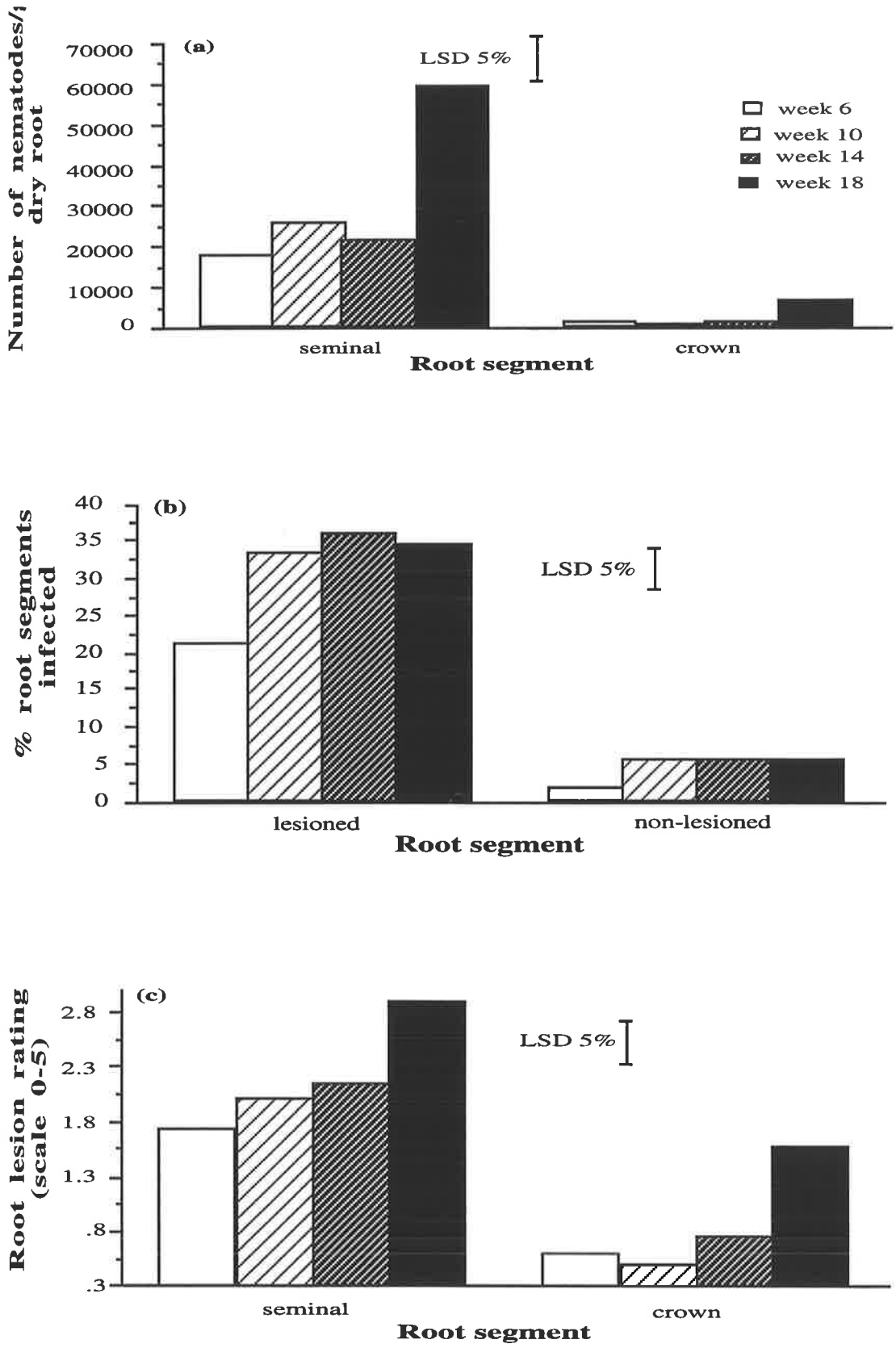


Figure 3.2 **(a)** Number of *P. neglectus* extracted from seminal or crown roots of wheat, **(b)** percentage root segments infected with fungi, and **(c)** root lesion rating of seminal and crown roots over four sample dates. (Data are means of two wheat cultivars at two sites).

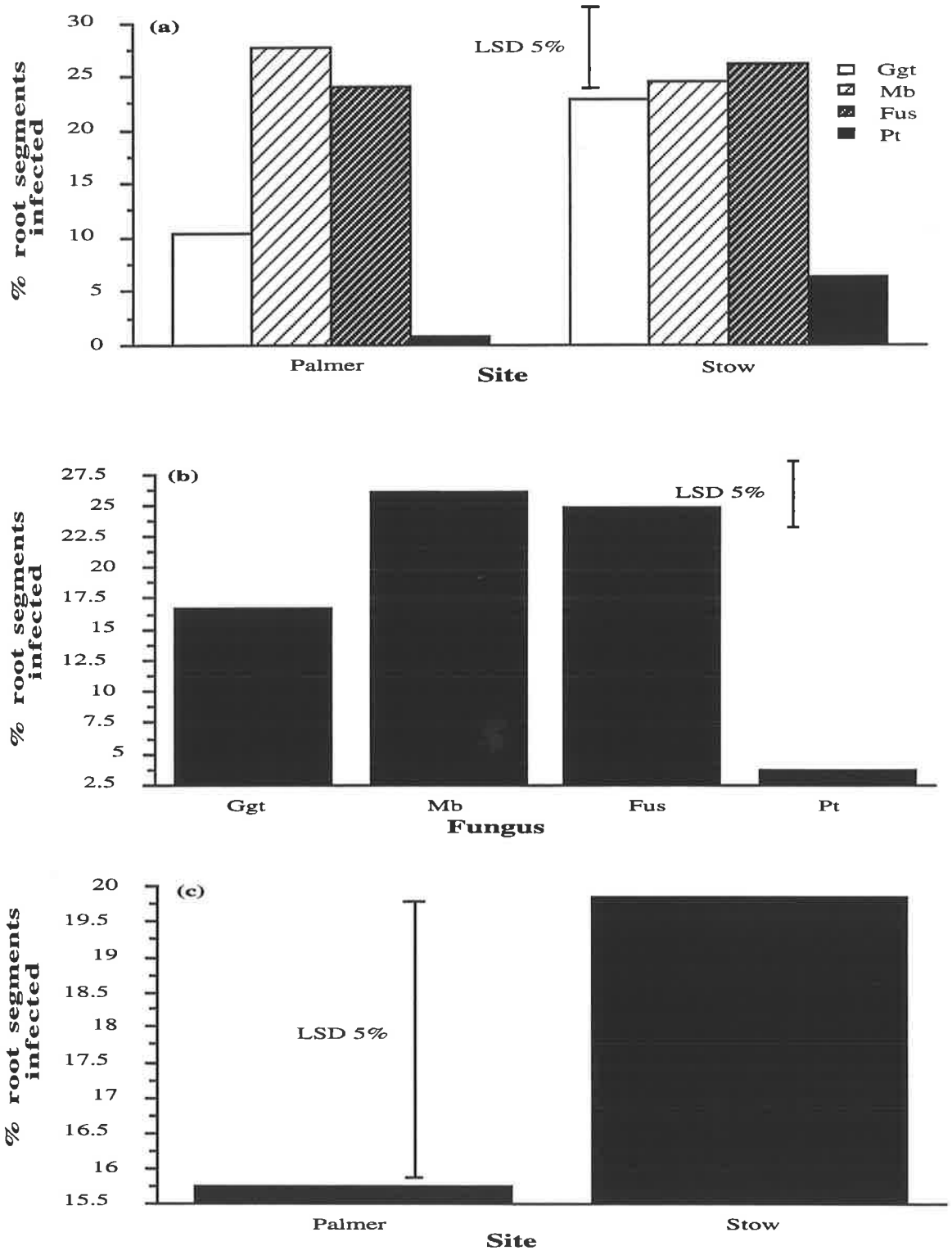


Figure 3.3 Percentage root segments infected with fungi in a soil naturally infested with *P. neglectus* at Palmer or Stow over four sample dates (6, 10, 14 and 18 weeks after sowing) in the 1992 growing season. Ggt= *Gaeumannomyces graminis* var. *tritici*, Mb= *Microdochium bolleyi*, Fus= *Fusarium* spp. and Pt= *Pyrenochaeta terrestris*.

Of the most commonly isolated fungi, *M. bolleyi* or *Fusarium* spp. were higher at both sites (Figure 3.3a), whereas *G. graminis* was more common at Stow than at Palmer (Figure 3.3a). However, *M. bolleyi* or *Fusarium* spp. were generally the most commonly isolated fungi from field samples during the 1992 field survey (Figure 3.3b). Samples from Stow contained more fungi than those from Palmer (Figure 3.3c).

3.3.1 Isolation of fungi

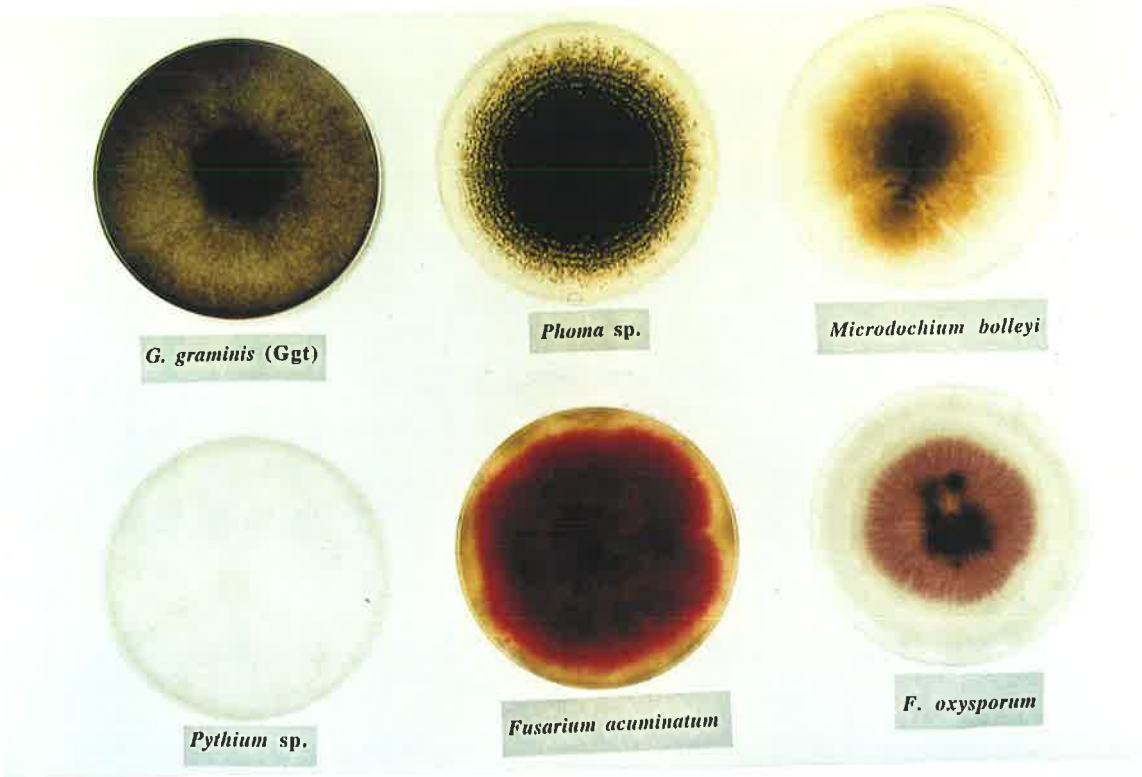
A number of fungi were isolated from both lesioned and non-lesioned sections of seminal and crown roots. Apart from pathogenic fungi, such as *G. graminis* and some *Pythium* spp., many minor pathogens were also isolated. *G. graminis* was common at both sites and on all wheat cultivars tested throughout the growing season, becoming more frequent late in the season. Infection was much greater at Stow than at Palmer. *G. graminis* was isolated from both seminal and crown roots in the same proportions.

Species of *Pythium* were commonly isolated from samples collected from Stow (Tables 3.3 and 3.4), but none of the samples from Palmer contained these species (Tables 3.1 and 3.2).

Fungi were isolated more frequently from lesioned areas of both seminal and crown roots than from non-lesioned parts (Tables 3.1, 3.2, 3.3 and 3.4). At six weeks after sowing, there were fewer fungi present on seminal and crown roots except for cultivar Condor which showed higher fungus populations in early growth stages than in later growth stages (Table 3.4).

The most frequently isolated fungi from lesioned and non-lesioned segments of wheat roots were *Gaeumannomyces graminis*, *Microdochium bolleyi*, *Fusarium* spp., *Bipolaris sorokiniana*, *Pythium irregulare*, *Pyrenochaeta terrestris*, *Phoma* sp. and *Ulocladium atrum* (Tables 3.1, 3.2, 3.3 and 3.4) (Plate 3.1). Species of *Fusarium* commonly isolated were *F. equiseti*, *F. oxysporum*, *F. acuminatum*, *F. solani* and *F. subglutina*. *F. graminearum* Group 1 was also isolated infrequently from crown roots. The majority of

Plate 3.1 Some species of fungi isolated from wheat roots infested with *Pratylenchus neglectus* in the field.


G. graminis (Ggt)
Phoma sp.
Microdochium bolleyi
Pythium sp.
Fusarium acuminatum
F. oxysporum

isolates of fusaria recorded were either *F. equiseti* or *F. acuminatum*. Fusaria were more common on crown roots than on seminal roots.

Highest fungal populations were isolated from wheat roots at ten weeks after sowing and there was little change up to the last sampling at 18 weeks. The majority of isolates recorded were *M. bolleyi*, *G. graminis* or *Fusarium* spp. *M. bolleyi*, at all sample dates, was more common than any other species of fungus.

Table 3.1 Mean isolation freqencies (%) of fungi from lesioned and non-lesioned segments of seminal and crown roots of wheat cultivar Molineux from Palmer.

Fungi		Lesioned (%)		Non-lesioned (%)		
	Sampling*	Seminal	Crown	Seminal	Crown	Total
Gaeumannomyces graminis	1	6	0	0	0	6
	M	12	17	0	2.5	31.5
Microdochium bolleyi	1	20	15	0	0	35
	M	36	30	5	15	86
Pyrenochaeta terrestris	1	3	0	0	0	3
	M	0	0	0	0	0
Fusarium equiseti	1	6	10	0	0	16
	M	12.5	25	0	2.5	35
F. oxysporum	1	0	0	0	0	0
	M	0	2.5	0	0	2.5
F. acuminatum	1	3	0	0	0	3
	M	5	5	0	2.5	12.5
F. solani	1	0	0	0	0	3
	M	3	0	0	0	3
F. subglutina	1	0	0	0	0	0
	M	0	5	0	0	5
Bipolaris sorokiniana	1	0	0	0	0	0
	M	1.5	7.5	0	0	9
Cylindrocarpon sp.	1	15	0	0	0	15
	M	0	0	0	0	0
Ulocladium atrum	1	0	0	0	0	0
	M	1.5	5	0	0	6.5
U. botrytis	1	0	0	0	0	0
	M	3	2.5	0	0	5.5
Phoma sp.	1	0	5	0	0	5
	M	3	0	0	0	3
Embellisia chlamydospora	1	0	0	0	0	0
	M	1.5	0	0	0	1.5
Alternaria sp.	1	0	0	0	0	0
	M	1.5	0	0	0	1.5
Roselina sp.	1	6	0	0	0	6
	M	0	0	0	0	0
Cylindrocarpon sp.	1	15	0	0	0	15
	M	0	0	0	0	0
Trichoderma sp.	1	5	0	0	0	5
	M	1.5	0	0	0	1.5

* 1 = Six weeks after sowing.
M = Mean isolation frequencies of fungi over three sample dates (10, 14 and 18 weeks after sowing).

Table 3.2 Mean isolation freqencies (%) of fungi from lesioned and non-lesioned segments of seminal and crown roots of wheat cultivar Spear from Palmer.

Fungi		Lesioned		Non-lesioned		
	Sampling*	Seminal	Crown	Seminal	Crown	Total
Gaeumannomyces graminis	1	3	5	0	0	8
	M	13	7.5	0	0	20.5
Microdochium bolleyi	1	28.5	0	0	0	28.5
	M	26	27	0	2.5	65.5
Pyrenochaeta terrestris	1	3	0	0	0	3
	M	0	2.5	0	0	2.5
Fusarium equiseti	1	6	0	0	0	6
	M	7	37.5	0	0	43.5
F. oxysporum	1	0	5	0	0	5
	M	1.5	0	0	0	1.5
F. acuminatum	1	0	0	0	0	0
	M	8	2.5	0	0	10.5
F. subglutina	1	0	0	0	0	0
	M	0	5	1.5	0	6.5
Bipolaris sorokiniana	1	0	0	0	0	0
	M	1.5	5	0	0	6.5
Periconia sp.	1	0	0	0	0	0
	M	1.5	0	0	0	1.5
Cylindrocarpon sp.	1	0	0	0	0	0
	M	3	2.5	0	2.5	8
Aspergilum sp.	1	0	2.5	0	0	2.5
	M	1.5	0	0	0	1.5
Colletotrichum sp.	1	0	0	0	0	0
	M	0	0	0	2.5	2.5
Phoma sp.	1	0	5	0	0	5
	M	3	2.5	0	0	5.5
Embellisia chlamydospora	1	0	0	0	0	0
	M	0	5	0	0	5
Trichoderma sp.	1	0	0	0	0	0
	M	3	0	0	0	3

* 1 = Six weeks after sowing.
M = Mean isolation frequencies of fungi over three sample dates (10, 14 and 18 weeks after sowing).

Table 3.3 Mean isolation freqencies (%) of fungi from lesioned and non-lesioned segments of seminal and crown roots of wheat cultivar Spear from Stow.

Fungi		Lesioned (%)		Non-lesioned (%)		
	Sampling*	Seminal	Crown	Seminal	Crown	Total
Gaeumannomyces graminis	1	0	5	0	0	5
	M	16	32.5	4	2.5	55
Microdochium bolleyi	1	25	10	8.5	5	48.5
	M	14	18	1.5	15	48.5
Pyrenochaeta terrestris	1	0	0	0	0	0
	M	5	2.5	5	5	17.5
Fusarium equiseti	1	11	25	3	5	44
	M	17	25	0	0	42
F. oxysporum	1	3	0	0	0	3
	M	1.5	0	0	0	1.5
F. acuminatum	1	0	10	0	10	20
	M	0	2.5	0	0	2.5
F. solani	1	0	0	0	0	0
	M	1.5	2.5	0	0	4
F. subglutina	1	3	20	0	0	23
	M	0	0	0	0	0
Bipolaris sorokiniana	1	0	0	3	0	3
	M	0	0	0	0	0
Periconia sp.	1	3	0	0	0	3
	M	0	0	0	2.5	2.5
Macrophomina sp.	1	3	0	0	0	3
	M	2.5	0	0	0	2.5
Pythium irregulare	1	0	30	0	0	30
	M	1.5	0	0	0	1.5
P. graminicola	1	6	15	3	0	24
	M	0	0	0	0	0
Trichoderma sp.	1	0	0	0	0	0
	M	0	2.5	0	0	2.5

* 1 = Six weeks after sowing.
M = Mean isolation frequencies of fungi over three sample dates (10, 14 and 18 weeks after sowing).

Table 3.4 Mean isolation freqencies (%) of fungi from lesioned and non-lesioned segments of seminal and crown roots of wheat cultivar Condor from Stow.

Fungi		Lesioned (%)		Non-lesioned (%)		
	Sampling*	Seminal	Crown	Seminal	Crown	Total
Gaeumannomyces graminis	1	34	15	0	0	49
	M	23	25	3	2.5	53.5
Microdochium bolleyi	1	23	15	0	0	38
	M	16	22.5	6	12.5	51
Pyrenochaeta terrestris	1	0	10	0	0	10
	M	7.5	2.5	1.5	2.5	14
Fusarium equiseti	1	8.5	10	0	0	18.5
	M	8.5	20	0	0	28.5
F. oxysporum	1	0	0	0	0	0
	M	4	0	1.5	0	5.5
F. acuminatum	1	0	0	0	0	0
	M	3	0	0	0	3
F. solani	1	0	0	0	0	0
	M	7.5	2.5	1.5	2.5	14
F. graminearum (group1)	1	0	0	0	0	0
	M	0	2.5	0	2.5	5
Periconia sp.	1	0	0	0	0	0
	M	1.5	0	3	2.5	7
Cylindrocarpon sp.	1	0	0	0	0	0
	M	0	0	0	2.5	2.5
Colletotrichum sp.	1	3	0	0	0	3
	M	0	0	0	0	0
Pythium irregulare	1	6	0	0	0	6
	M	1.5	2.5	0	2.5	6.5
Phoma sp.	1	0	0	0	0	0
	M	3.5	0	0	0	3.5
Trichoderma sp.	1	3	0	0	0	3
	M	0	2.5	0	0	2.5
Bipolaris sorokiniana	1	0	0	0	0	0
	M	0	2.5	0	0	2.5

* 1 = Six weeks after sowing.
M = Mean isolation frequencies of fungi over three sample dates (10, 14 and 18 weeks after sowing).

In late July at Palmer, where nematode numbers at the beginning of the season were much less than at Stow (Figure 3.1a), *M. bolleyi* was isolated more often on seminal roots than *G. graminis* or *Fusarium* spp. However, *G. graminis* or *Fusarium* spp. were isolated more often on crown roots than on seminal roots (Table 3.1). *M. bolleyi* was also isolated more often from non-lesioned parts of seminal and crown roots late in the season than other species of fungi (Table 3.1). With less *G. graminis* var. *tritici* at Stow early in the season, the frequency of *M. bolleyi* was greater (Table 3.3). However, later in the season, when *G. graminis* became the dominant species, the amount of *M. bolleyi* was lower (Table 3.3). The reverse was true at Palmer: when *M. bolleyi* was dominant, the frequency of *G. graminis* was lower (Tables 3.1 and 3.2).

3.3.2 Other species of fungi isolated

Many species of fungi, besides those mentioned above, were isolated from both seminal and crown roots, particularly from lesioned sections, but at low frequencies. Some were pathogens of cereals and many were saprophytes. *Alternaria alternata*, *Colletotrichum* sp., *Cylindrocarpon* sp., *Embellisia chlamydospora*, *Macrophomina* sp., *Periconia macrospinosa*, *Phoma* sp., *Roselina* sp., *Ulocladium atrum* and *U. botrytis* were isolated from root samples. Isolates of *Aspergillus, Rhizopus* and *Trichoderma* were also recorded, but at very low frequencies.

The observed levels of root damage increased at the later sample dates (Figures 3.1c and 3.2c). Also, the root systems of Condor at Stow and Molineux at Palmer suffered greater damage than did those of Spear. Overall, samples of all wheat varieties from Stow had more damage to roots than did those from Palmer (Figure 3.1c).

3.3.3 Nematode number in roots

Among wheat cultivars, Condor had the highest nematode numbers associated with its roots. At six weeks after sowing, Condor at Stow had very high numbers of nematodes in the root system, and by ten weeks both Condor and Spear showed a further increase in nematode numbers. However, by fourteen weeks, the number of nematodes in the root

system decreased, insignificantly, but had increased again by eighteen weeks until the highest number of nematodes was reached (Figure 3.1a).

There was a significant difference between seminal and crown roots in number of nematodes extracted. Seminal roots supported almost eight times the number of *P. neglectus* (Figure 3.2a). Cultivars of wheat showed no significant difference in ability to support nematodes at either site. However, samples from Stow contained three times more nematodes in both seminal and crown roots compared to samples collected from Palmer (Figure 3.1a). In late July, at the beginning of the season, nematode numbers were much higher at Stow that at Palmer.

In general, root samples from Stow were more damaged than those from Palmer (Figure 3.1c). Within wheat cultivars, although Spear had greater fungal populations, Condor and Molineux had more damage to their root systems, perhaps due to greater nematode and fungal infections. The dominant fungal species at both sites were *G. graminis*, *Fusarium equiseti*, *F. acuminatum* and *Microdochium bolleyi* (Figures 3.3a and 3.3b). The number of *P. neglectus* extracted from roots and soil was greater at Stow than at Palmer.

3.4 Discussion

Overall, this survey agreed with those of Fedel-Moen and Harris (1987) and Vanstone (1991), but the difference was that samples were taken from soil known to be infected with *P. neglectus,* and this was confirmed by extracting the nematode from all samples throughout the survey. Presumably, as *Pratylenchus* spp. are now known to occur in almost all crops (Vanstone *et al.*, 1993), Fedel-Moen and Harris (1987) and Vanstone (1991) also took their samples from areas that were infested with *Pratylenchus* spp. Isolation techniques and media used in the survey were adequate to detect fungi present, and the chosen sections of lesioned and non-lesioned roots were statistically different and clearly indicated that fungi are present more commonly on lesioned areas of roots than on non-lesioned (clean) sections.

At the first sample dates at the end of June, both seminal and crown roots were present. Lesions were more obvious on seminal roots, and crown roots seemed to be very clean. Therefore, the majority of fungi isolated were from seminal roots. There were no differences in fungal species between the first and last sampling, but the proportion of different fungi varied between sites and sample dates. Although at the second, third and fourth sampling times, in July and August, both seminal and crown roots were infested by fungi and the nematode, the lesion severity on crown roots was more than on seminal roots.

R. solani is a cereal pathogen in Australia (Neate, 1984) and is the cause of bare patches. In South Australia the fungus is of major concern in wheat (de Beer, 1965). *R. solani* is often found in association with other pathogens. Its association with *P. neglectus* in patches of unthrifty plants in South Australia was reported by de Beer (1965). In glasshouse conditions, association of *R. solani* and *H. avenae* caused greater reduction in tillering, plant height and fresh weight of wheat than when acting alone (Meagher and Chambers, 1971). The fungus, however, was not isolated from any plants sampled from either site on different dates during the 1992 growing season.

G. graminis is a major root pathogen of wheat in southern Australia and is a dominant pathogen of cereals at tillering (Rovira, 1980, 1987). Vanstone (1991) claimed that *G. graminis* did not have a major impact on the level of root disease at the sites she sampled during the 1987 growing season. However, this fungus was isolated from 31-55% of roots collected from either Palmer or Stow in the 1992 growing season, showing a high incidence at both sites. This is probably due to the high rainfall in 1992 favouring the fungus (Table 2.1).

Take-all has been recorded in South Australia since 1852 (Anon, 1868) and McAlpine (1904) concluded that *G. graminis* is the primary cause of the disease, and reported that severity on cereals and particularly on wheat varies in different years. The highest infection of wheat crops with *G. graminis* was recorded by Mayfield (1984), who

claimed that 72-98% of South Australian wheat crops were infected with this fungus in 1980 and 1981.

G. graminis occurred at low frequency at Stow early in the season, while the population of *M. bolleyi* was greater (Table 3.3). However, later in the season, when *G. graminis* became the dominant species, the population of *M. bolleyi* was lower (Tables 3.3 and 3.4). The reverse was true at Palmer, where *M. bolleyi* was dominant over *G. graminis* (Tables 3.1 and 3.2).

The extent of lesions on seminal roots was greater than on crown roots. At the first sampling, with the exception of one wheat cultivar, there were almost no *G. graminis* lesions on crown roots and very few on seminal roots. On the other hand, at the same time, amount of *M. bolleyi* on seminal roots particularly was high, suggesting a possible negative relationship between these two fungi. At the second sampling time, 30% of root samples from Palmer and 50% from Stow had *G. graminis* lesions on both seminal and crown roots, with a higher proportion on seminal roots where *M. bolleyi* was isolated less frequenly. At the late sampling in August, there was almost a ten-fold increase in *G. graminis* infection, with most plants having at least a few lesions caused by *G. graminis*. This could be due to environmental conditions such as soil temperature favouring the fungus.

Seminal and crown roots were also colonised by *M. bolleyi*, *F. equiseti*, *F. acuminatum*, *Pyrenochaeta terrestris* and *Pythium* spp. as well as many other saprophytic or pathogenic fungi which were isolated infrequently. At both sites, *M. bolleyi* was frequent at the second sampling in early August, but *F. acuminatum* was the dominant species at the early sampling in mid-June. *M. bolleyi* is common in the South Australian wheat belt (Fedel-Moen and Harris, 1987; Vanstone, 1991). The fungus has been regarded as a minor pathogen (Kirk and Deacon, 1987a, 1987b), but Harris and Moen (1985a, 1985b; Harris, 1986) suggested that it had the potential to cause cereal root disease in South Australia. Species of *Fusarium* were frequent at later sampling times when soil temperature would have been above 20°C.

Fungi were isolated more often from seminal roots than from crown roots, suggesting that greater availability and activity of seminal roots caused them to be more attractive to invasion by fungi and nematodes, or the fact that crown roots are produced later than seminal roots and are therefore exposed less to infection. The higher frequency of fungi on lesioned parts of roots than on clean areas suggests that when roots of wheat are infected by either nematodes or fungi, they become more favourable for a number of fungi including saprophytic soil fungi.

Nematodes were much more concentrated in seminal roots than in crown roots. Kimpinski *et al.* (1976) also found many more *P. neglectus* in seminal roots of wheat than in crown roots. *P. neglectus* initially attacks seminal roots as early as one week after sowing (Benedict and Mountain, 1956). Similarly, Corbett (1972) found that *P. vulnus* had penetrated the roots of barley and wheat within one week. However, crown roots are produced six to seven weeks after sowing, by which time at least one generation of *P. neglectus* will have developed, and the number of nematodes will be increasing exponentially. Kimpinski *et al.* (1976) suggested that seminal roots were the preferred site for nematode invasion and reproduction due to their greater physiological activity.

Increase in number of *P. neglectus* in roots of wheat at the late sampling (eighteen weeks) suggests that number of nematodes is associated with an increase in soil temperature by that time. The optimum temperature for the development and multiplication of *P. neglectus* is 25˚C depending upon the host plant (Vanstone and Nicol, 1993). The results of the fungal survey and related nematode numbers together suggest that these organisms are closely related to each other in producing and expanding root lesions. It is particularly important late in the season when plants require more nutrients and higher water uptake which are affected by nematode damage.

Generally, this study confirmed that *P. neglectus* is widely distributed in soil, infecting wheat crops. Numerous species of fungi are associated with the damage to roots caused by the nematodes. The fungi most frequently isolated from roots infested with *P. neglectus* were *G. graminis* var. *tritici*, *M. bolleyi*, *Fusarium* spp., *B. sorokiniana*,

Phoma spp. and *Pythium irregulare*. These fungi in fact may interact with *P. neglectus* and cause extensive damage on the roots of wheat crops under South Australian conditions. Therefore, it is appropriate to study the relationship between root-rotting fungi of wheat and *P. neglectus*.

Although most of the literature related to interactions between nematodes and fungi concerns fungi already known as plant pathogens in their own right, the population of fungi normally considered to be minor pathogens is high and these fungi may become pathogenic in the presence of nematodes. Thus, it is also appropriate to investigate the relationship between weakly pathogenic fungi and the root lesion nematode.

Chapter 4

Glasshouse pathogenicity tests: Effect of root-rotting fungi on wheat in combination with *Pratylenchus neglectus*

4.1 Introduction

Many fungi were isolated from wheat roots naturally infected with *P. neglectus* (Chapter 3). The fungi most frequently isolated were tested singly or in selected combinations with and without *P. neglectus*. *Rhizoctonia solani* Anastomosis Group-8 was included as it is a major root pathogen of wheat in South Australia, although it was not isolated from field samples. The role of these fungi in combination with root lesion nematode has not been clearly defined.

In view of the frequent association of *G. graminis* var. *tritici*, *M. bolleyi*, *Fusarium* spp., *B. sorokiniana*, *P. terrestris* and *P. irregulare* with the diseased roots of wheat in the field, and their possible interaction with *P. neglectus*, inoculation experiments were conducted to investigate the effect of these fungi alone and in selected combinations with and without the nematode on the growth and disease of wheat.

Three experiments were conducted in the glasshouse: the first and second experiments were designed to investigate the effects of a wide range of fungi alone and some selected combinations with and without the nematode. The third experiment was based on the results of experiments one and two using some fungi which positively interacted with *P. neglectus*.

Soil type is an important abiotic factor which can influence nematode penetration and pathogenicity (Wallace, 1963). The two species of *Pratylenchus* infecting cereals in South Australia usually inhabit different soil types: *P. neglectus* is found more commonly in lighter textured soils while *P. thornei* more commonly inhabits heavier,

clay based soils (Nicol, 1996). However, the optimal soil conditions for nematode movement (Wallace, 1963) suggest that both nematodes should move well in sandy soil. To investigate the effect of soil type on penetration rate of *Pratylenchus* spp. a glasshouse experiment was conducted using either *P. neglectus* or *P. thornei* and several soil types (Experiment 4).

4.2 Methods

4.2.1 Experiments 1 and 2

4.2.1.1 Soil

A sandy loam soil (pH 6.5) was collected from a typical wheat-growing field at Stow where plant samples (Chapter 3) were taken. Half of this soil was pasteurised as described in the General Methods. The other half remained untreated. The soil was sieved through a 2mm sieve. Plastic cups (600ml) with no drainage holes were filled with 750g of air dried soil which was then watered with distilled water to field capacity.

4.2.1.2 Fungal inoculum

Inoculum of fungi was produced on sterilised ryegrass seed as described in the General Methods (2.2).

Air dried fungus inoculum was added to the soil and thoroughly mixed by hand. *M. bolleyi*, *F. equiseti*, *F. acuminatum*, *F. oxysporum*, *B. sorokiniana* or *P. terrestris* were added to the soil at 1% w/w, *R. solani* was added at 0.02% w/w infested ryegrass seeds/cup, *G. graminis* at 0.05% w/w and *P. irregulare* at 0.1% w/w. Fungi were tested singly and in combinations of *G. graminis*+*R. solani*, *G. graminis*+*M. bolleyi* or *G. graminis*+*F. equiseti* with or without *P. neglectus*.

4.2.1.3 Seed germination and planting

Seeds of wheat cultivar Spear were surface disinfested in 2.5% sodium hypochlorite for ten minutes, then washed with three changes of sterile distilled water. Seed was pre-

germinated as described in the General Methods. Two healthy, uniform pre-germinated seeds were sown in each cup to a depth of 2cm.

4.2.1.4 Nematode inoculum

P. neglectus was grown aseptically in carrot culture (as described in the General Methods). Two thousand aseptically grown *P. neglectus* (mixed juvenile stages and adults) were pipetted in 1.0ml of distilled water around each plant immediately after planting (4000 nematodes/pot).

4.2.1.5 Growing conditions

The experiments were conducted in a glasshouse with an air temperature of 25±3°C. Soil temperature was maintained at 20±1°C in a water tank (Plate 4.1).

4.2.1.6 Experimental design

The experiment was a randomised complete block design with six replications. All data were subjected to analyses of variance.

4.2.1.7 Measurements and harvest

After three weeks, plant growth was recorded weekly by measuring plant height, number of tillers/plant, and number of leaves/plant and /main tiller. The experiment was harvested 42 days after sowing, at which time roots were washed free of soil and root lesioning was scored using a scale of 0-5, where 0= healthy roots and 5= complete lesioning of the whole root system (General Methods, 2.1.3). Number of tillers, plant height and fresh and dry weights of shoots and roots were also recorded. Nematodes were extracted from whole root systems in a mist chamber for five days (General Methods, 2.1) and counted.

4.2.2 Experiment 3

Results of Experiment 1 indicated that *M. bolleyi*, *F. acuminatum* and *P. terrestris* are damaging to wheat in natural soil (untreated). Combination effects of *G. graminis*+*F.*

Plate 4.1 Plants growing in a controlled temperature water tank in the glasshouse.

equiseti, *G. graminis+M. bolleyi* and *G. graminis+R. solani* were investigated in Experiments 1 and 2 in this Chapter. However, as *G. graminis*, *F.acuminatum* and *M. bolleyi* occur in complex on wheat roots, combination effects of *G. graminis+F. acuminatum* and *F. acuminatum+M. bolleyi* were also investigated.

***Soil and pots*:** A naturally infested sandy soil naturally infested with *P. neglectus* was used as for previous experiments. Plastic pots with 300ml capacity were used. One pre-germinated seed/pot was sown at a depth of 1.5cm.

***Fungus and nematode inoculum*:** Inoculum of fungi was prepared as described for Experiments 1 and 2 (although milett seed rather than ryegrass was used) and mixed thoroughly with the soil of each pot at the same rates as used for previous experiments. One, two and four thousand aseptically grown nematodes in 1ml of water were pippeted around each plant after planting.

4.2.3 Harvest and measurements

The experiment was harvested 49 days after sowing, at which time roots were washed free of soil and root lesion rating was scored using a scale of 0-5, where 0= healthy roots and 5= complete lesioning of the whole root system (General Methods, 2.1.3). Number of tillers and dry weight of shoots and roots were also recorded. Nematodes were extracted from whole root systems in a mist chamber for five days (General Methods, 2.1) and counted.

4.2.4 Experiment 4

Penetration rates of both nematode species, *P. thornei* and *P. neglectus,* into the roots of Machete wheat was considered in relation to soil type. The efficiency of the mister extraction process was also examined. This was done in collaboration with Ms. Julie Nicol, Department of Crop Protection, Waite Campus.

4.2.4.1 Soil

Four different soil types were used, two of a high clay content and two of a sandy composition: non-sieved Urrbrae loam (Ulns), sieved Urrbrae loam (Uls), sieved Palmer sand (Ps) and Roseworthy sand (Rs) also sieved through a 2mm sieve. Plants were inoculated with 2000 *P. thornei* or *P. neglectus*/plant.

4.2.4.2 Experiment design and harvest

Plants were grown in a controlled environment (Plate 4.2A) in a split plot design with six 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 harvested one week after inoculation.

The nematodes were extracted in a mist chamber for a period of four days and counted. The nematodes remaining in the root system were counted microscopically once stained with acid fuschin (Plate 4.2B).

4.3 Results

4.3.1 Experiments 1 and 2

Assessment of root dry weight, the number of nematodes/plant and nematodes/g dry root, and the severity of root lesioning showed a significant interaction between some fungi and *P. neglectus*. Symptoms on the roots ranged from light brown cortical lesions to large dark brown or black stelar lesions.

Experiment 1 (pasteurised soil): Root lesion rating, number of *P. neglectus*/plant and nematodes/g dry root and nematode multiplication rate showed a positive interaction between some fungi and the nematode (Table 4.1a).

Plate 4.2

A. Plants grown to assess the penetration of *P. neglectus* and *P. thornei* under controlled environmental conditions.

B. A representative Machete wheat root segment, stained seven days after inoculation with 2000 nematodes. Evidence of masses of nematodes (represented by the dark pink area) in cortical cells of seminal roots.

A

B

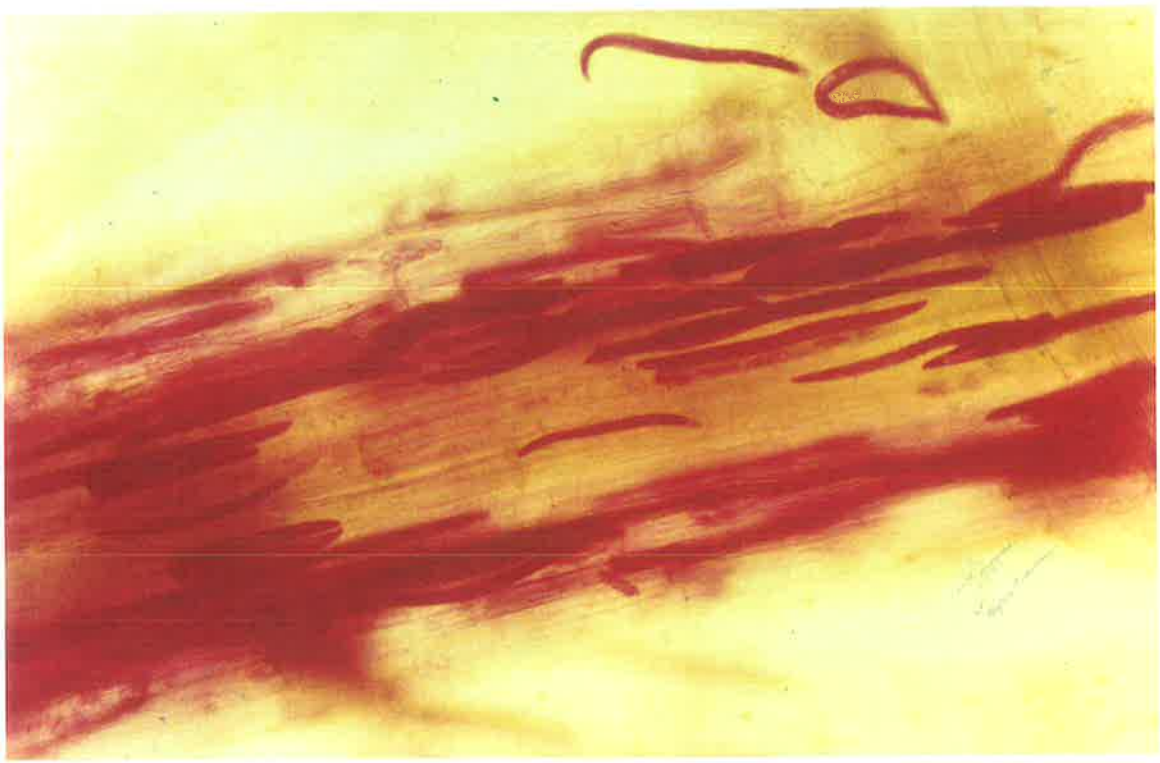


Table 4.1 Summary of analyses of variance for the effect of nematode-fungus interaction on extent of root lesioning, number of nematodes/plant and nematodes/g dry root, shoot and root dry weights, total dry weight of plants and nematode multiplication rate for wheat cultivar Machete, 49 days after sowing (Experiments 1 and 2).

(a) Experiment 1 pasteurised soil

Source	df	MS RL	P	MS N/p	P	MS N/gdr	P	MS dws/p	P	MS dwr/p	P	MS tdw/p	P	MS MR	P
Block	5														
Fungus	14	24.37	***	1.49E6	**	4.83E6	**	.715	**	.029	***	.971	***	1.07	**
Nematode	1	3.20	***	1.14E8	***	3.35E8	***	.001	ns	.324	ns	.002	ns	0.02	ns
Nematode × fungus	14	.47	**	1.49E6	**	4.83E6	**	.010	ns	.002	ns	.015	ns	0.32	ns
Residual	145	.20		5.53E5		1.88E6		.020		.003		.028		0.28	

(b) Experiment 2 unpasteurised soil

Source	df	MS RL	P	MS N/p	P	MS N/gdr	P	MS dws/p	P	MS dwr/p	P	MS tdw/p	P	MS MR	P
Block	5														
Fungus	14	8.55	***	1.81E5	ns	1.34E7	*	.193	***	.041	***	.394	***	.028	*
Nematode	1	3.06	**	1.41E7	***	6.38E8	***	.090	**	.341	ns	.103	**	.507	***
Nematode × fungus	14	.22	ns	1.52E5	ns	9.41E6	ns	.011	ns	.006	**	.016	ns	.021	ns
Residual	145	.34		1.16E5		7.56E6		.013		.003		.019		.014	

*** significant at P= 0.001 ** significant at P= 0.01 * significant at P= 0.05 ns= not significant P= Probability.
RL= root lesioning; N/p= nematodes/plant; N/gdr= nematodes/g dry root; dws/p= shoot dry weight/plant; dwr/p= root dry weight/plant; tdw/p= total dry weight/plant; MR= multiplication rate.

Tiller numbers/plant: Number of tillers/plant was not significantly affected by any treatment.

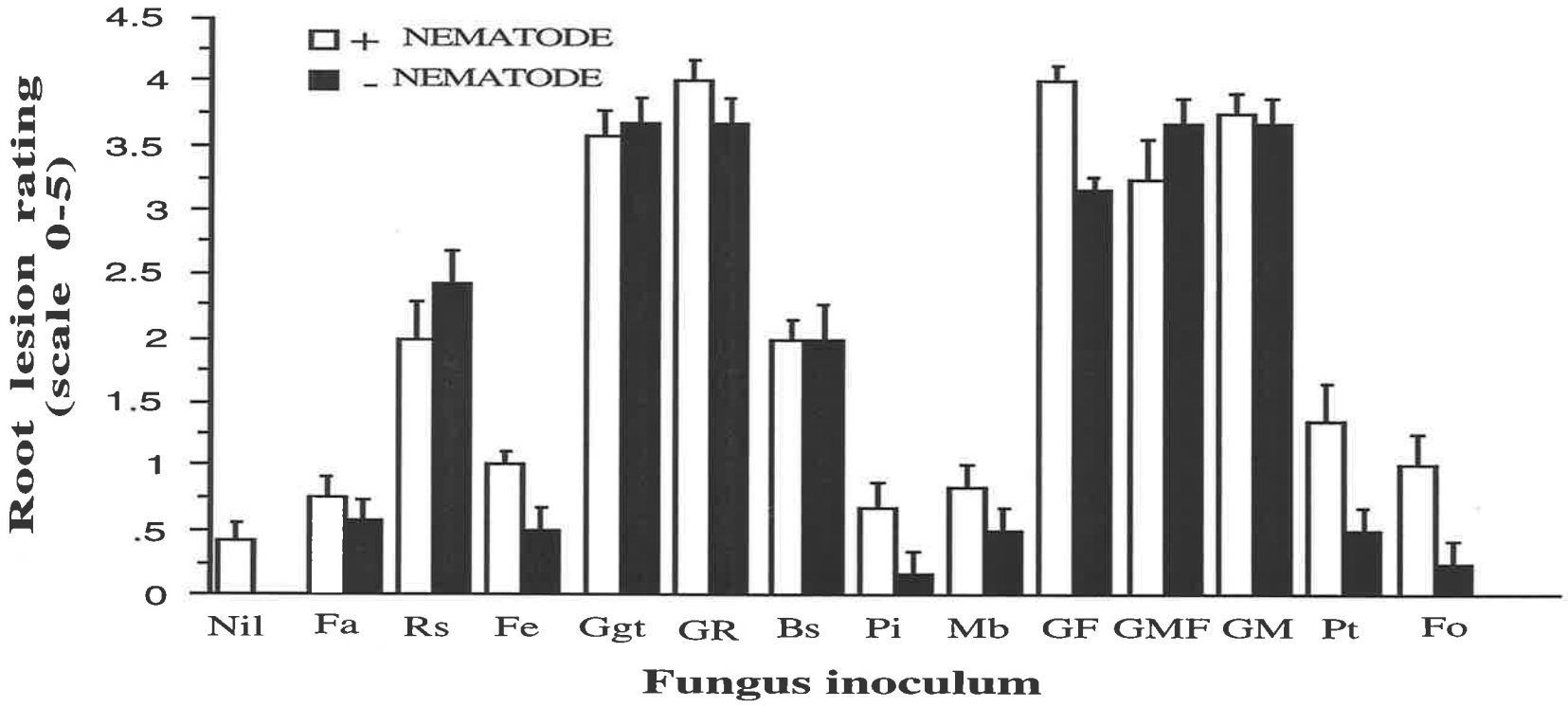
Root lesion rating: Inoculation with *F. oxysporum*, *P. irregulare*, *P. terrestris* or *G. graminis*+*F. equiseti* in combination with the nematode significantly increased severity of root lesion rating compared to that without the nematode (Figure 4.1). With or without the nematode, there was more lesioning on wheat roots with *G. graminis*, *G. graminis*+*M. bolleyi*, *G. graminis*+*F. equiseti* or with *R. solani* than with other fungi. However, inoculation with other fungi in combination with the nematode had no significant effect on severity of root-rotting (Figure 4.1).

Nematode numbers: Inoculation with *R. solani*, *B. sorokiniana*, *M. bolleyi*, *P. irregulare* or *G. graminis*+*F. equiseti* resulted in a significantly higher nematode population/plant compared to the uninoculated treatment (Figure 4.2a). However, with *G. graminis*+*R. solani*, nematode numbers were significantly reduced compared to *G. graminis* or *R. solani* alone (Figure 4.2a). In the presence of other fungi, number of *P. neglectus* did not change significantly, although there was some reduction with *F. oxysporum* and some increase in the presence of *P. terrestris*, *F. equiseti*, *F. acuminatum* or *G. graminis* (Figure 4.2a).

Selected combinations of fungi showed no increase or a decrease in number of nematodes extracted from wheat roots compared to the control (no fungal inoculum added). The combination of *G. graminis*+*F. equiseti* showed an increase in nematode number, and with *G. graminis*+*R. solani* there was a reduction in the number of *P. neglectus,* but only when compared with either fungus alone. With *G. graminis*+*M. bolleyi*, the nematode number was significantly reduced when compared with *M. bolleyi* alone. Number of *P. neglectus*/gram dry root showed a similar result to nematode number/plant, except for *G. graminis* and *F. equiseti* where nematode number/g dry root was significantly increased (Figure 4.2b).

Figure 4.1 Effect of nematode-fungus interaction on root lesion rating of wheat cultivar Machete, 49 days after sowing.

Nil= ± nematode, Fa= *Fusarium acuminatum*, Rs= *Rhizoctonia solani*, Fe= *F. equiseti,* Ggt= *Gaeumannomyces graminis* var. *tritici*, GR= Ggt+Rs, Bs= *Bipolaris sorokiniana*, Pi= *Pythium irregulare*, Mb= *Microdochum bolleyi*, GF= Ggt+Fa, GMF= Ggt+Mb+Fa, GM= Ggt+Mb, Pt= *Pyrenochaeta terrestris* and Fo= *F. oxysporum.*



Combinations of the nematode and some fungi significantly increased multiplication rate of *P. neglectus* (Figure 4.2c). Reproduction of *P. neglectus* in conjunction with the fungi *R. solani*, *B. sorokiniana*, *P. irregulare*, *M. bolleyi* or *G. graminis+F. equiseti* was increased significantly (P=0.05) compared to the nematode alone. However, with *G. graminis+R. solani* and with *F. oxysporum*, reproduction of the nematode was reduced but not by a significant level (Figure 4.2c).

Plant dry matter: Nematodes alone or in combination with fungi had no significant effect on root and shoot dry weight, plant height or on number of leaves and tillers/plant (Table 4.1a). Plant height, number of leaves/plant or leaves/main tiller and number of tillers/plant measured at three, four and five weeks after sowing were not significantly different in either treatment so the data are not presented.

Experiment 2 (unpasteurised soil): A similar experiment to Experiment 1 was conducted under the same growing conditions, but with a natural soil collected from a field infested with 400 nematodes/200g of soil. The soil remained untreated (not steam pasteurised). The summary of analyses of variance is presented in Table 4.1b.

Tiller numbers/plant: Number of tillers/plant was not significantly affected by any treatment.

Root lesion rating: Root lesion rating was significantly affected by either fungus or nematode alone (Figure 4.3). Plants grown in natural soil inoculated with 2000 nematodes showed a 17% increase in root lesion rating compared to the control (no nematode or fungus added). Root lesion rating in plants inoculated with *R. solani*, *F. equiseti*, *G. graminis*, *G. graminis+F. equiseti*, *G. graminis+M. bolleyi+F. equiseti* or *G. graminis+M. bolleyi* increased by 49%, 71%, 71%, 68%, 62%, or 66%, respectively, compared to the control (no fungus inoculum added) (Figure 4.3).

Nematode numbers: The effect of nematode-fungus interaction on number of nematodes/plant and nematodes/g dry root together with nematode multiplication rate showed a similar pattern to the previous experiment using pasteurised soil (Figure 4.4).

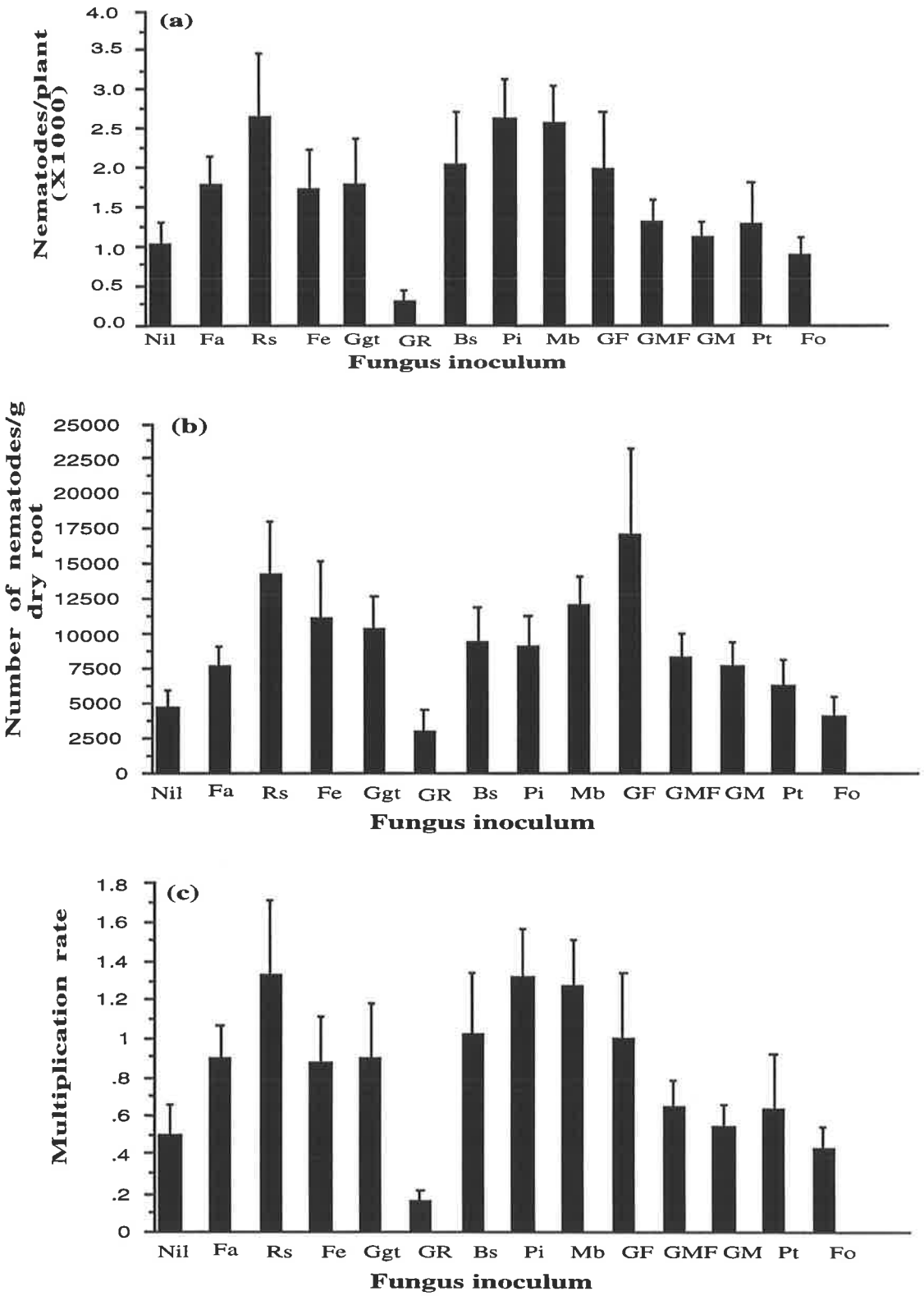
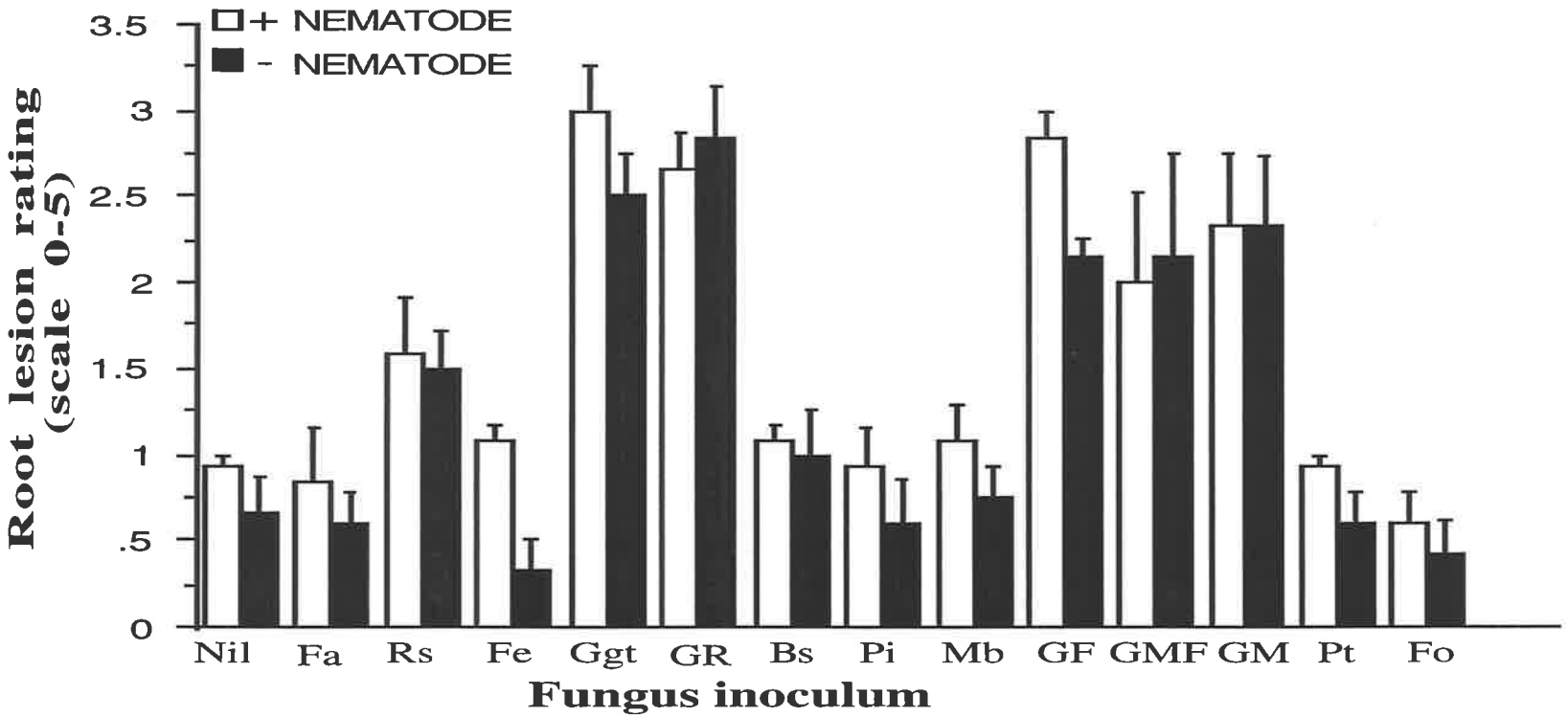


Figure 4.2 Effect of nematode-fungus interaction on **(a)** number of nematodes/plant, **(b)** number of nematodes/g dry root and **(c)** nematode multiplication rate on wheat cultivar Machete 49 days after sowing.

Nil= ± nematode, Fa= *Fusarium acuminatum*, Rs= *Rhizoctonia solani*, Fe= *F. equiseti*, Ggt= *Gaeumannomyces graminis* var. *tritici*, GR= Ggt+Rs, Bs= *Bipolaris sorokiniana*, Pi= *Pythium irregulare*, Mb= *Microdochum bolleyi*, GF= Ggt+Fa, GMF= Ggt+Mb+Fa, GM= Ggt+Mb, Pt= *Pyrenochaeta terrestris* and Fo= *F. oxysporum*.

Figure 4.3 Effect of nematode or fungus inoculum on root lesion rating of wheat cultivar Machete 42 days after inoculation.

Nil= ± nematode, Fa= *Fusarium acuminatum*, Rs= *Rhizoctonia solani*, Fe= *F. equiseti,* Ggt= *Gaeumannomyces graminis* var. *tritici*, GR= Ggt+Rs, Bs= *Bipolaris sorokiniana*, Pi= *Pythium irregulare*, Mb= *Microdochum bolleyi*, GF= Ggt+Fa, GMF= Ggt+Mb+Fa, GM= Ggt+Mb, Pt= *Pyrenochaeta terrestris* and Fo= *F. oxysporum.*



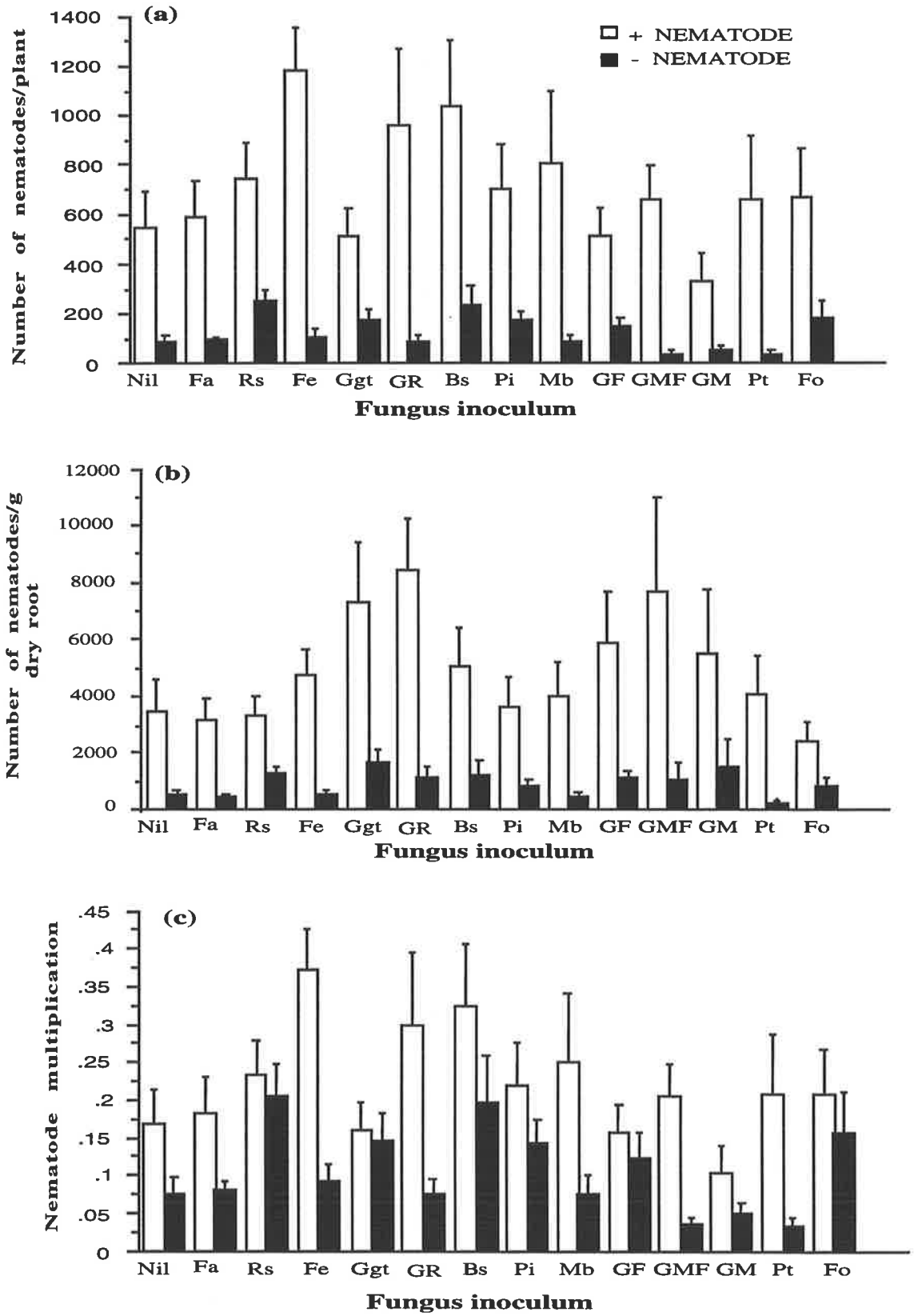


Figure 4.4 Effect of nematode-fungus interaction on (**a**) number of *P. neglectus*/plant, (**b**) nematodes/g dry root and (**c**) multiplication rate of nematodes 42 days after inoculation.

Nil= ± nematode, Fa= *Fusarium acuminatum*, Rs= *Rhizoctonia solani*, Fe= *F. equiseti*, Ggt= *Gaeumannomyces graminis* var. *tritici*, GR= Ggt+Rs, Bs= *Bipolaris sorokiniana*, Pi= *Pythium irregulare*, Mb= *Microdochum bolleyi*, GF= Ggt+Fa, GMF= Ggt+Mb+Fa, GM= Ggt+Mb, Pt= *Pyrenochaeta terrestris* and Fo= *F. oxysporum*.

Plant dry matter: Combination of the nematode with the fungi *G. graminis*, *G. graminis+F. equiseti*, *M. bolleyi*, *P. terrestris*, *B. sorokiniana* or *F. acuminatum* significantly (P=0.05) reduced root dry weight of wheat seedlings compared with the fungus alone (Figure 4.5). *R. solani*, *F. equiseti*, *F. oxysporum* or *G. graminis+M. bolleyi+F. equiseti* in combination with the nematode significantly increased root dry weight. However, with *G. graminis+R. solani*, *P. irregulare* or *G. graminis+M. bolleyi* in conjunction with *P. neglectus* there was no effect on root dry weight (Figure 4.5).

4.3.2 Experiment 3

Number of nematodes/plant and nematodes/g dry root, root, shoot and total dry weight of plants, root lesion rating and number of tillers/plant were affected by nematode, fungus and the combination of nematode and fungus (Table 4.2).

Tiller numbers/plant: Number of tillers/plant was affected by fungus inoculum but not by nematodes. However, there was a significant interaction (P=0.05) between nematode and fungus for number of tillers/plant (Figure 4.6). All fungi increased number of tillers compared to the control (no fungus added). Wheat cultivars differed significantly in tiller production. Machete produced 32% more tillers than Spear.

Root lesion rating: Both wheat cultivars (Spear and Machete) had a similar disease rating when inoculated with fungi and the nematode, so data for the two cultivars was combined (Table 4.2). Root lesion rating was increased by 41%, 41%, 39% or 38%, respectively, on plants inoculated with *M. bolleyi*, *M. bolleyi+F. acuminatum*, *G. graminis+F. acuminatum*, *G. graminis* or *F. acuminatum*, when compared to the control (no fungus inoculum added). These increases were statistically significant (P=0.01) (Figure 4.7).

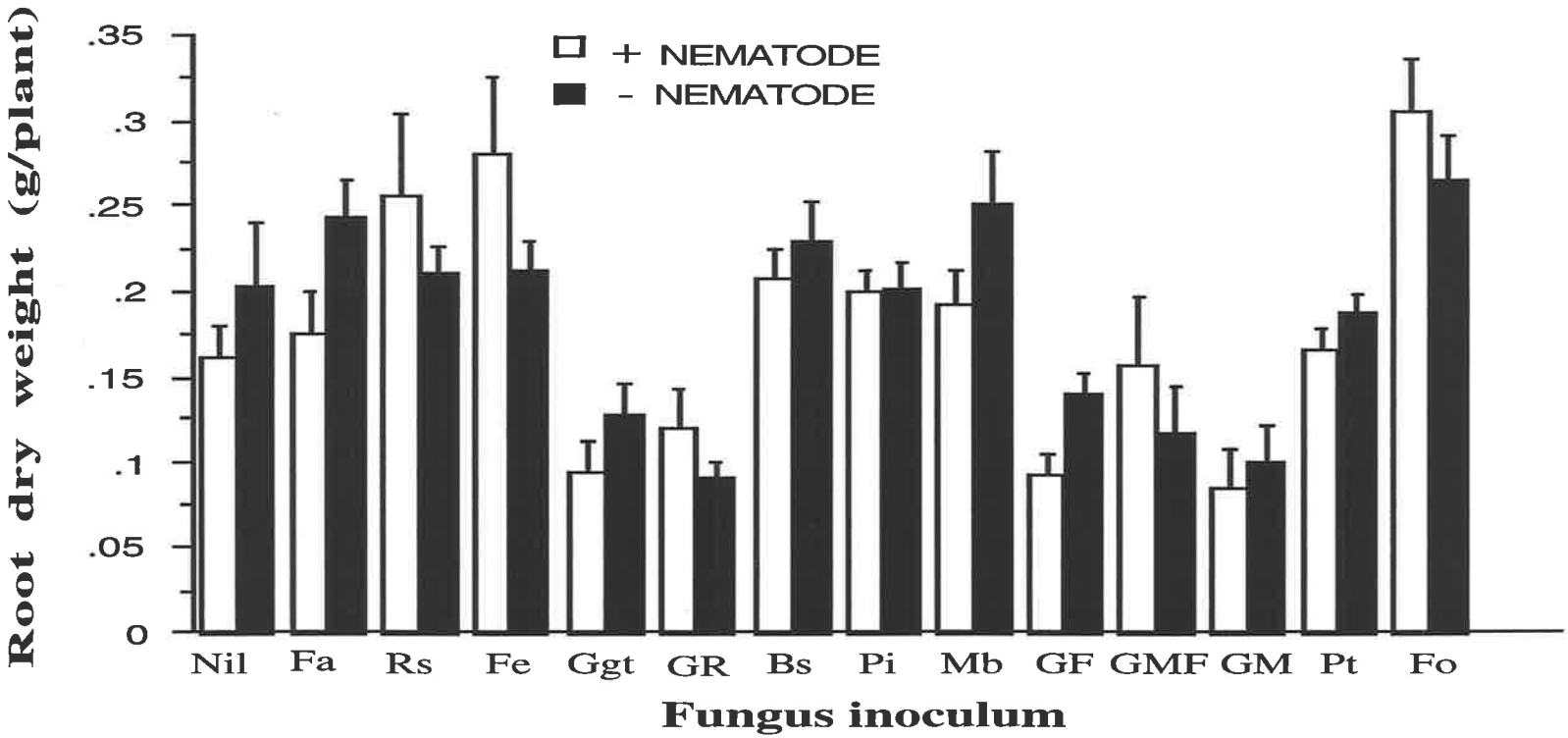
Table 4.2 Summary of analyses of variance for the effect of nematode-fungus interaction on extent of root lesioning, number of nematodes/plant and nematodes/g dry root, shoot and root dry weights, total dry weight of plants, nematode multiplication rate and number of tillers for wheat cultivars Machete and Spear, 49 days after sowing (Experiment 3).

Source	df	MS RL	P	MS N/p	P	MS N/gdr	P	MS dws/p	P	MS dwr/p	P	MS tdw/p	P	MS MR	P	MS tillers	P
Block	3																
Cultivar	1	.03	ns	1.26E3	ns	4.91E4	ns	.03	*	.007	*	.04	ns	.11	ns	9.21	***
Fungus (fun)	5	2.68	***	1.58E5	***	7.03E6	***	.09	***	.01	***	.15	***	1.10	**	3.35	***
Nematode (nem)	3	6.27	***	1.33E6	***	8.10E7	***	.05	***	.02	***	.13	***	1.14	*	.33	ns
Cultivar × fungus	5	.33	ns	7.14E3	ns	5.44E5	ns	.o1	ns	.004	ns	.02	ns	.12	ns	.23	ns
Cultivar × nematode	3	.06	ns	1.20E4	ns	4.91E5	ns	.01	ns	.003	ns	.01	ns	.21	ns	.94	*
Nematode × fungus	15	1.23	**	4.95E4	**	2.96E6	***	.01	ns	.01	ns	.04	ns	.66	**	.63	*
Nem × fun × cultivar	15	.81	*	1.01E4	ns	1.37E6	ns	.01	ns	.002	ns	.02	ns	.34	ns	.33	ns
Residual	141	.45		1.92E4		9.55E5		.02		.002		.01		.36		.38	

*** significant at P= 0.001 ** significant at P= 0.01 * significant at P= 0.05 ns= not significant P= Probability.
RL= root lesioning; N/p= nematodes/plant; N/gdr= nematodes/g dry root; dws/p= shoot dry weight/plant; dwr/p= root dry weight/plant; tdw/p= total dry weight/plant; MR= nematode multiplication rate; tillers= tillers/plant.

Figure 4.5 Effect of nematode-fungus interaction on the root dry weight of wheat cultivar Machete 42 days after inoculation.

Nil= ± nematode, Fa= *Fusarium acuminatum*, Rs= *Rhizoctonia solani*, Fe= *F. equiseti*, Ggt= *Gaeumannomyces graminis* var. *tritici*, GR= Ggt+Rs, Bs= *Bipolaris sorokiniana*, Pi= *Pythium irregulare*, Mb= *Microdochum bolleyi*, GF= Ggt+Fa, GMF= Ggt+Mb+Fa, GM= Ggt+Mb, Pt= *Pyrenochaeta terrestris* and Fo= *F. oxysporum*.



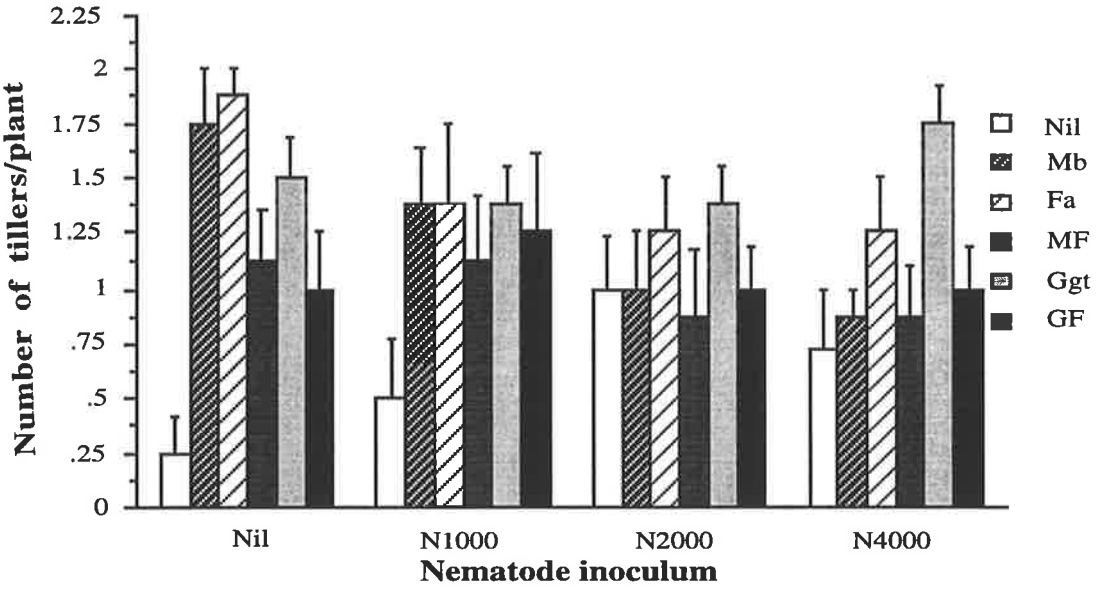


Figure 4.6 Effect of nematode-fungus interaction on number of tillers/plant 49 days after sowing. Nil= no fungus inoculum added, Mb= *Microdochium bolleyi*, Fa= *Fusarium acuminatum*, Ggt= *G. graminis* var. *tritici*, MF= Mb+Fa and GF= Ggt+Fa. Nil= no nematodes added, N1000= 1000 nematodes/plant, N2000= 2000 nematodes/plant, N4000= 4000 nematodes/plant.

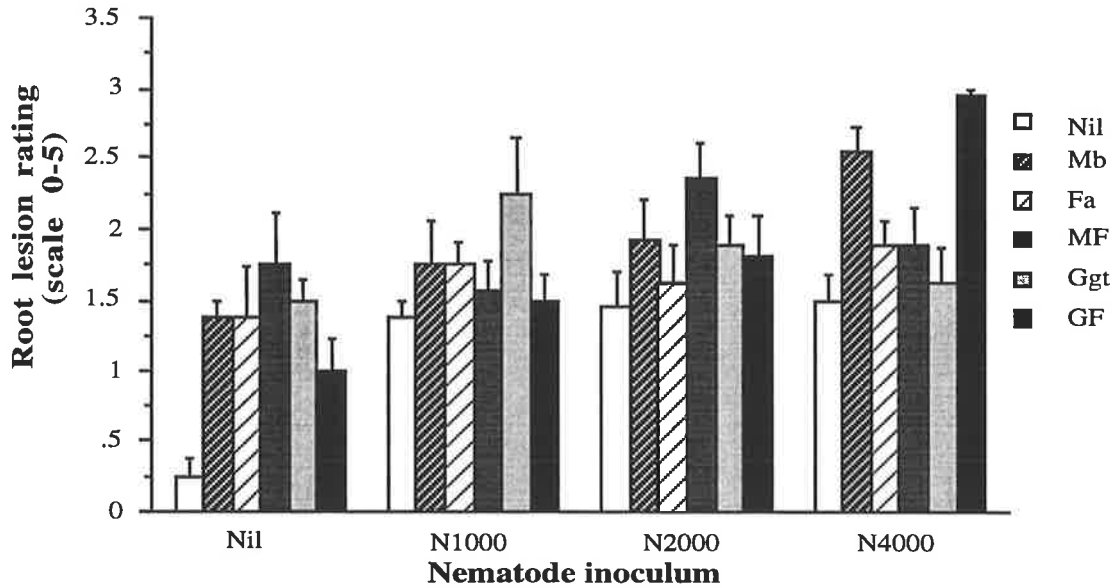


Figure 4.7 Effect of nematode-fungus interaction on root lesion rating of wheat plants grown in 300ml pots for 49 days. Nil= no fungus inoculum added, Mb= *Microdochium bolleyi*, Fa= *Fusarium acuminatum*, Ggt= *G. graminis* var. *tritici*, MF= Mb+Fa and GF= Ggt+Fa. Nil= no nematodes added, N1000= 1000 nematodes/plant, N2000= 2000 nematodes/plant, N4000= 4000 nematodes/plant.

With all nematode densities (1000, 2000 or 4000 nematodes/plant), root lesion rating was increased significantly compared to the control (no nematodes added). With 1000 or 2000 nematodes/plant, lesions were increased by 29% and 34%, respectively, when compared to the control (no nematodes added) (Figure 4.7). While there was no significant difference between root lesion rating caused by 1000 or 2000 nematodes/plant and 2000 or 4000 nematodes/plant, there was a significant increase in root lesion rating with 4000 nematodes/plant when compared to the control (no nematodes added) or 1000 nematodes/plant.

The effects of fungi and *P. neglectus* in combination were also statistically significant for root lesion rating. *M. bolleyi* in combination with 1000, 2000 or 4000 nematodes/plant increased root lesion rating by 22%, 29% or 46%, respectively, compared to the effect of fungus alone (Figure 4.7). Plants inoculated with 1000, 2000 or 4000 nematodes had 33%, 20% or 8%, respectively, higher lesion rating than those inoculated with the fungus alone (Figure 4.7).

While *F. acuminatum* or *G. graminis* in combination with 4000 nematodes/plant increased root lesion rating by 26% or 8%, respectively, with the combination of *G. graminis*+*F. acuminatum* and 4000 nematodes/plant root lesion rating increased by 45% or 38%, respectively, when compared to either fungus alone or 4000 nematodes. In comparison, the combination of *M. bolleyi* and *F. acuminatum* with any level of nematode inoculum did not significantly increase disease.

***Nematode number*:** Both wheat cultivars (Spear and Machete) had similar nematode numbers in their root system, so the data were combined. Inoculation with *M. bolleyi*, *F. acuminatum* or *G. graminis* significantly (P=0.05) increased number of nematodes/plant or nematodes/g dry root (Figures 4.8 and 4.9). Number of nematodes/plant increased by 35%, 37% or 39% when plants were inoculated with 4000 nematodes and *M. bolleyi*, *F. acuminatum* or *G. graminis*, respectively, compared to the control (no fungus added) (Figure 4.8).

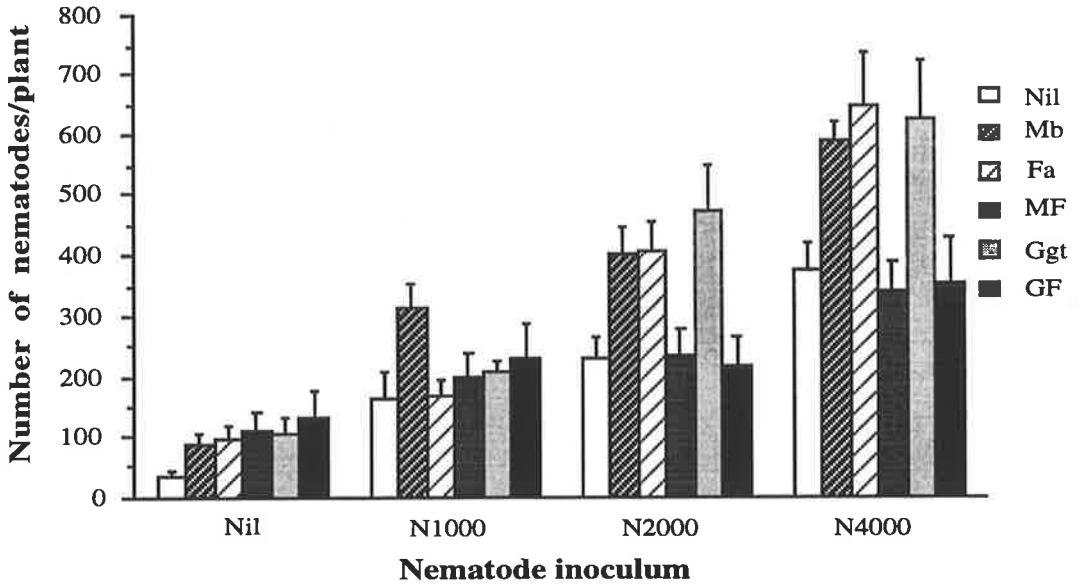


Figure 4.8 Effect of nematode-fungus interaction on number of nematodes extracted from roots of wheat plants 49 days after sowing. Nil= no fungus inoculum added, Mb= *Microdochium bolleyi*, Fa= *Fusarium acuminatum*, Ggt= *G. graminis* var. *tritici*, MF= Mb+Fa and GF= Ggt+Fa. Nil= no nematodes added, N1000= 1000 nematodes/plant, N2000= 2000 nematodes/plant, N4000= 4000 nematodes/plant.

With *M. bolleyi*, *F. acuminatum* or *G. graminis* and 4000 nematodes, number of nematodes/g dry root increased by 71%, 47% or 32%, respectively, compared to nematodes alone at the same density (Figure 4.9). However, with *M. bolleyi+F. acuminatum* or *G. graminis+F. acuminatum* nematode numbers/plant at either level remained unchanged compared to when nematodes were added alone (Figure 4.8). Nematode numbers/g dry root were significantly affected by *M. bolleyi+F. acuminatum* or *G. graminis+F. acuminatum* (Figure 4.9).

Nematode multiplication rate, however, was significantly affected by both nematode and fungus. A 2-way interaction between nematode and fungus was also significant at P=0.05, and is illustrated in Figure 4.10.

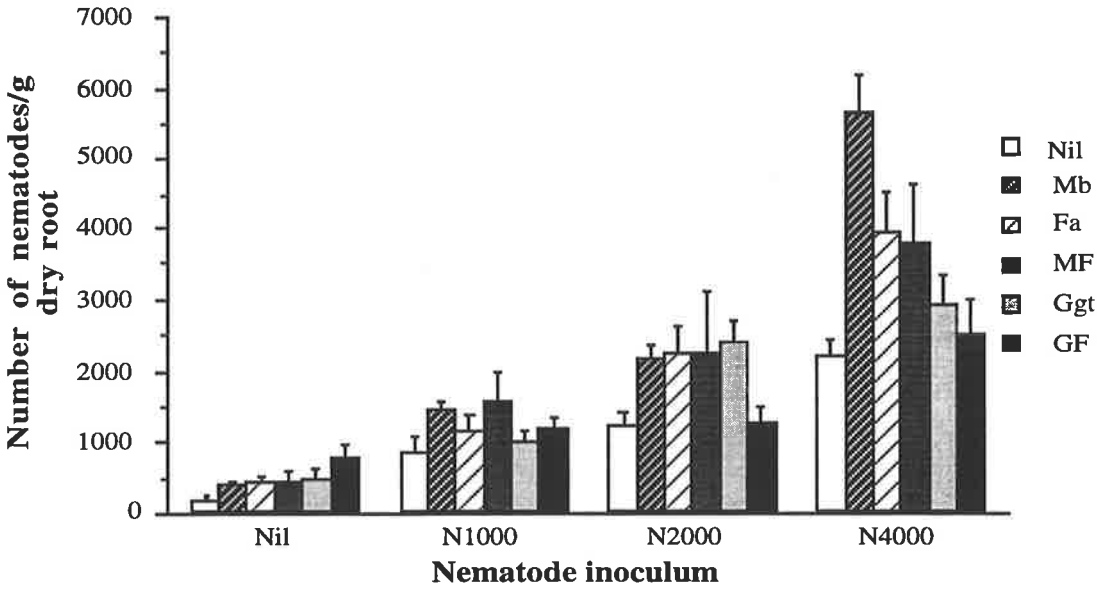


Figure 4.9 Effect of nematode-fungus interaction on number of nematodes/g dry root extracted from roots of wheat plants 49 days after sowing. Nil= no fungus inoculum added, Mb= *Microdochium bolleyi*, Fa= *Fusarium acuminatum*, Ggt= *G. graminis* var. *tritici*, MF= Mb+Fa and GF= Ggt+Fa. Nil= no nematodes added, N1000= 1000 nematodes/plant, N2000= 2000 nematodes/plant, N4000= 4000 nematodes/plant.

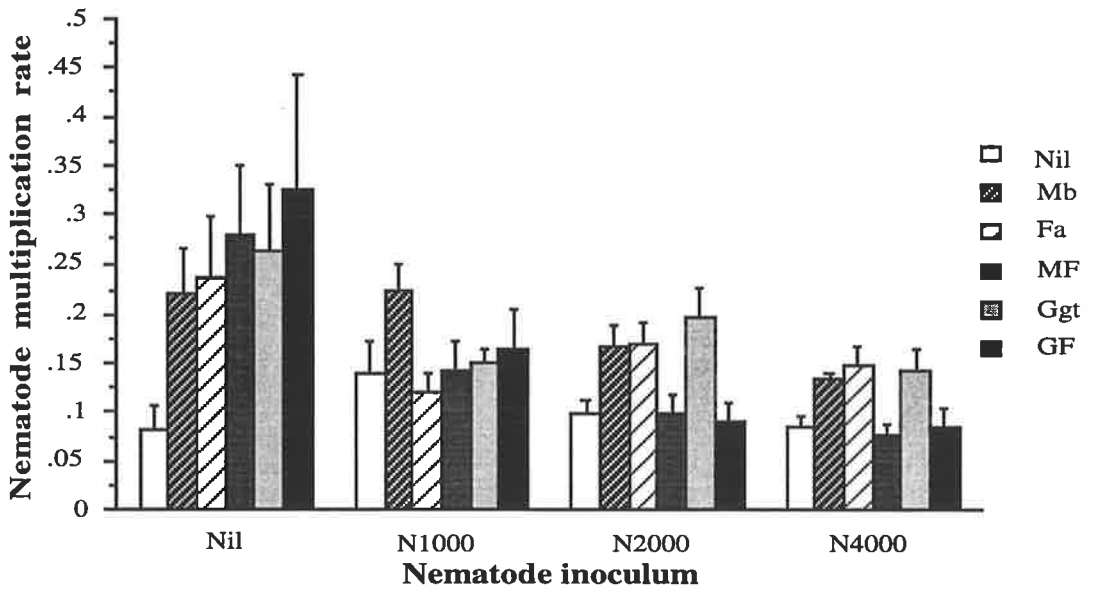


Figure 4.10 Effect of nematode-fungus interaction on *P. neglectus* multiplication rate 49 days after sowing. Nil= no fungus inoculum added, Mb= *Microdochium bolleyi*, Fa= *Fusarium acuminatum*, Ggt= *G. graminis* var. *tritici*, MF= Mb+Fa and GF= Ggt+Fa. Nil= no nematodes added, N1000= 1000 nematodes/plant, N2000= 2000 nematodes/plant, N4000= 4000 nematodes/plant.

***Plant dry matter*:** Shoot, root and total dry weight of plants were affected by fungus or nematode inoculum. Shoot and root dry weights of plants inoculated with *G. graminis* increased by 13% or 30%, respectively, when compared to the control (no fungus added) (Figures 4.11 and 4.12). *G. graminis,* however, increased total dry weight by 25% (P=0.05) compared to the control (no fungus inoculum added) (Figure 4.13). Plants inoculated with *F. acuminatum*, *M. bolleyi*, *M. bolleyi+F. acuminatum* or *G. graminis+F. acuminatum* showed no significant effect on root dry weight compared to the control (no fungus added) (Figure 4.12). Both shoot and root dry weights of plants inoculated with 4000 nematodes/plant decreased by 19% or 23%, respectively, compared to the control (no nematode inoculum added).

Root and total dry weight of plants were significantly affected by nematode-fungus interactions (P=0.05). Inoculation of plants with *F. acuminatum*, *M. bolleyi* or *M. bolleyi+F. acuminatum* and the nematode resulted in a significant reduction in root dry weight compared to the effect of fungus alone (Figure 4.12). The combination of nematode and *F. acuminatum*, *M. bolleyi* or *M. bolleyi+F. acuminatum* decreased root dry weights by 24%, 56% or 52%, respectively, when compared to either fungus alone.

Total dry weight of plants inoculated with *M. bolleyi* and 4000 nematodes/plant decreased by 39% compared to fungus alone or by 22% compared to the effect of nematodes alone at the same density (Figure 4.13). This decrease was statistically significant. Plants inoculated with *F. acuminatum* and 4000 nematodes also showed a significant (P=0.05) reduction in dry matter (Figure 4.13). With *M. bolleyi+F. acuminatum* and 4000 nematodes/plant, total dry weight decreased by 44% compared to fungus alone. However, with or without nematodes, *G. graminis* or *G. graminis+F. acuminatum* had no significant effect on total dry weight of plants (Figure 4.13).

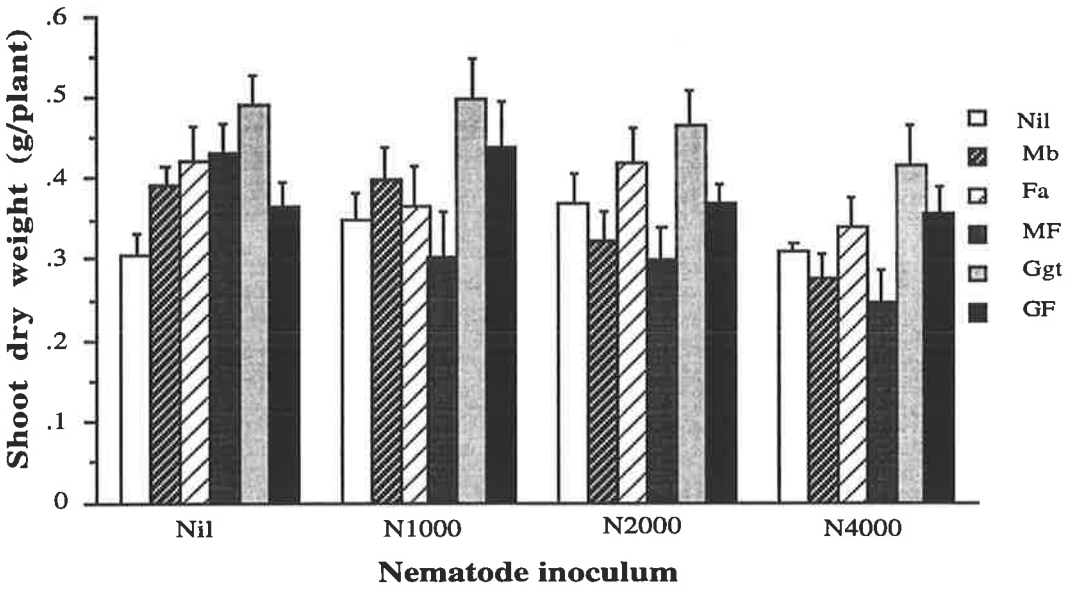


Figure 4.11 Effect of nematode-fungus interaction on shoot dry weight of wheat plants 49 days after sowing. Nil= no fungus inoculum added, Mb= *Microdochium bolleyi*, Fa= *Fusarium acuminatum*, Ggt= *G. graminis* var. *tritici*, MF= Mb+Fa and GF= Ggt+Fa. Nil= no nematodes added, N1000= 1000 nematodes/plant, N2000= 2000 nematodes/plant, N4000= 4000 nematodes/plant.

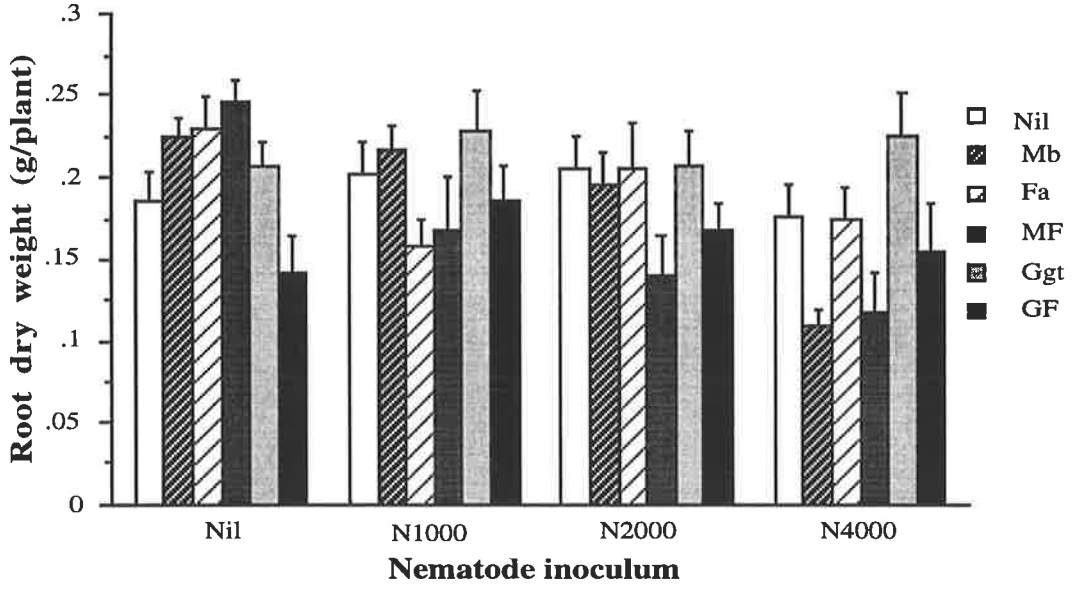


Figure 4.12 Effect of nematode-fungus interaction on root dry weight of wheat plants 49 days after sowing. Nil= no fungus inoculum added, Mb= *Microdochium bolleyi*, Fa= *Fusarium acuminatum*, Ggt= *G. graminis* var. *tritici*, MF= Mb+Fa and GF= Ggt+Fa. Nil= no nematodes added, N1000= 1000 nematodes/plant, N2000= 2000 nematodes/plant, N4000= 4000 nematodes/plant.

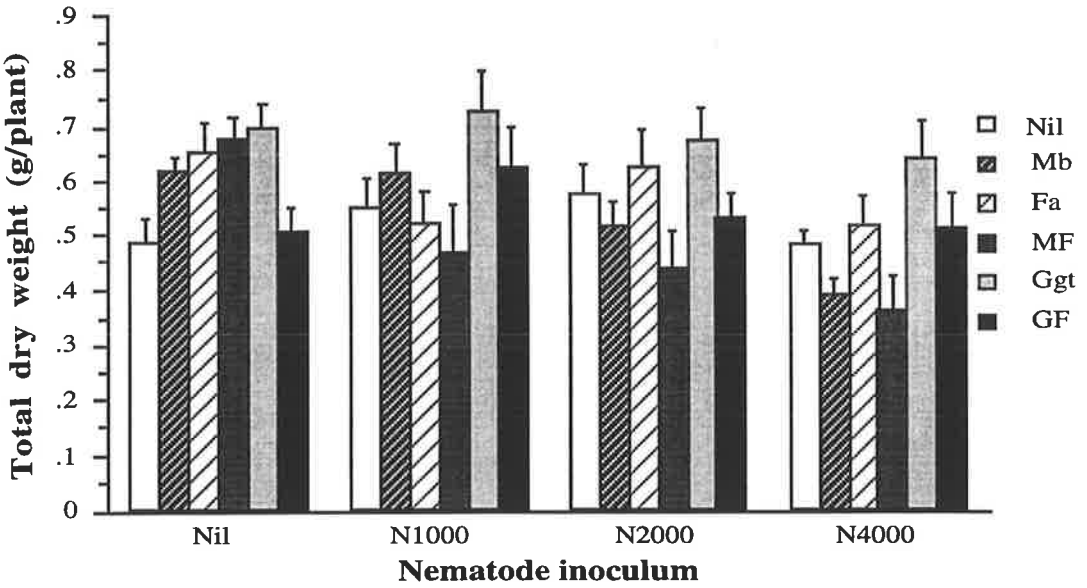


Figure 4.13 Effect of nematode-fungus interaction on total dry weight of wheat plants 49 days after sowing. Nil= no fungus inoculum added, Mb= *Microdochium bolleyi*, Fa= *Fusarium acuminatum*, Ggt= *G. graminis* var. *tritici*, MF= Mb+Fa and GF= Ggt+Fa. Nil= no nematodes added, N1000= 1000 nematodes/plant, N2000= 2000 nematodes/plant, N4000= 4000 nematodes/plant.

4.3.3 Experiment 4

Analyses of variance showed that number of nematodes/plant extracted from roots, number of nematodes/plant remaining in the roots after the extraction period and total number of nematodes/plant penetrating the roots of Machete wheat were significantly affected by different soil types (Table 4.3). However, there was no difference between the two nematode species in total number of nematodes/plant (Table 4.3).

The two species, *P. neglectus* and *P. thornei,* behaved similarly regardless of soil type. Figure 4.14 illustrates the significant interaction between soil type and number of nematodes penetrating roots. Sandy soil was by far the best medium (P=0.05) for maximum penetration of roots (up to 40%). However, the clay soil was very inefficient, particularly if unsieved, with fewer nematodes (only 5%) able to penetrate the roots of Machete.

Further analysis (Table 4.3) of the total number of nematodes/plant (mister extracted and stained nematodes within the roots) revealed there was a significant nematode

species effect, both with the mister extraction and the numbers of stained nematodes remaining in the root system (Figure 4.15). From the mister extraction, 42% of *P. neglectus* migrated from the root system over four days while for *P. thornei* the number leaving the roots was significantly less, almost three times fewer (only 16%). However, when *P. thornei* were counted inside the root there was a significantly greater number left (84%), compared with *P. neglectus* (58%).

During investigation of stained roots to determine efficiency of mister extraction, hypersensitive reaction of root cells was observed (Plate 4.3).

Table 4.3 Summary of analyses of variance for the effect of soil type and extraction technique on number of nematodes extracted from roots over four days (extraction period), number of nematodes remaining within root tissues and total number of nematodes penetrating roots of Machete wheat seven days after inoculation.

Source	df	MS M. E/p	P	MS S. N/p	P	MS T/p	P
Block	5	10647		26095		62727	
Nematode	1	140400	*	150521	*	176	ns
Block × nematode	5	11646		22292		51304	
Soil type	3	105525	**	846109	**	1495092	**
Nematode × soil type	3	31694	ns	93902	ns	36646	ns
Residual	29	11941		93902		70092	

** significant at P= 0.01, * significant at 0.05, ns= not significant, P= probability.
M. E/p= Mister extraction of nematodes/plant, S. N/p= Remaining stained nematodes within root tissues/plant, T/p= Total number of *Pratylenchus* spp. that had penetrated roots.

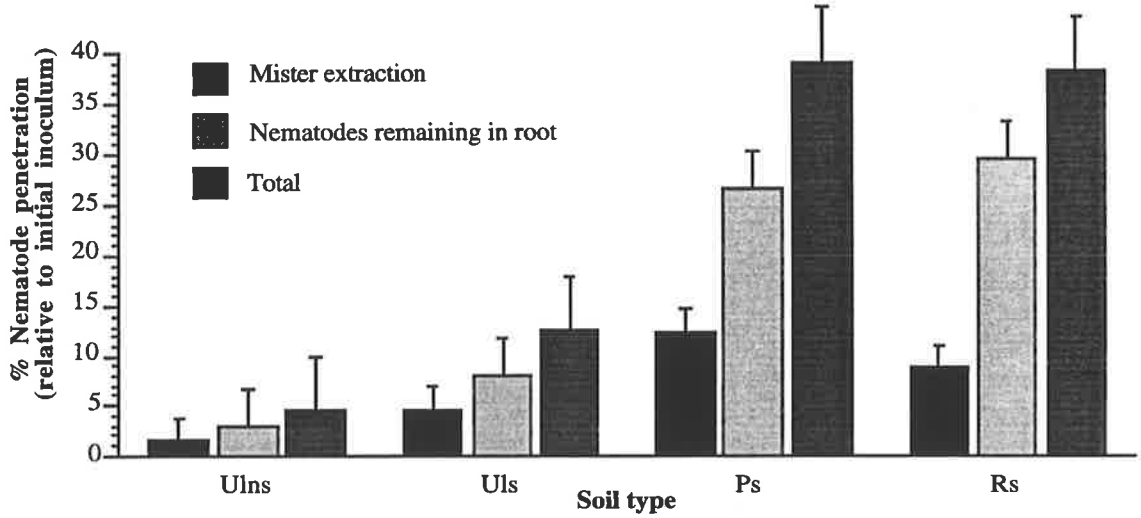


Figure 4.14 The effect of soil type and nematode extraction technique on the numbers of *Pratylenchus* spp. in Machete roots. Ulns= non-sieved Urrbrae loam, Uls= sieved Urrbrae loam, Ps= sieved Palmer sand and Rs= Roseworthy sand.

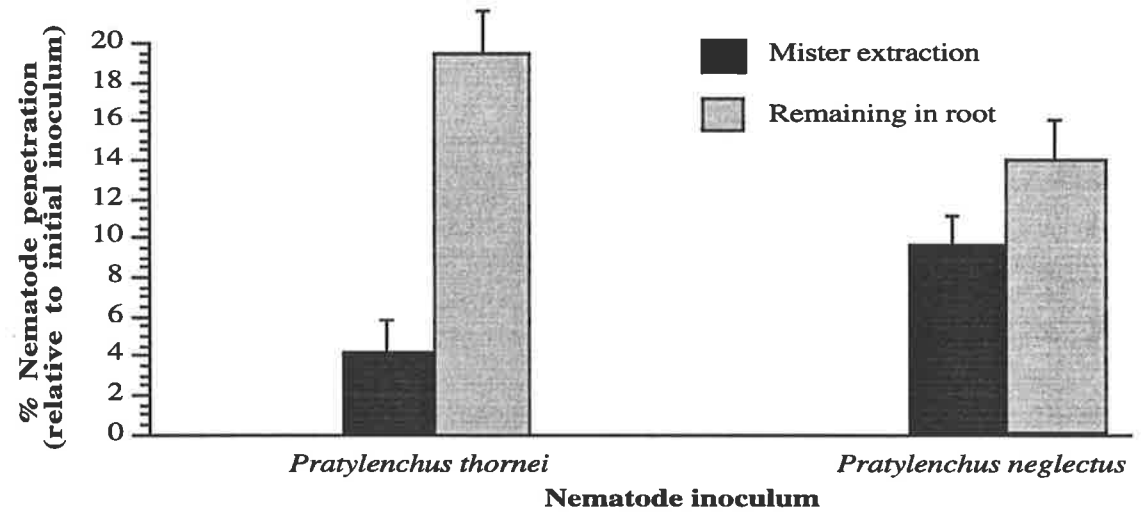
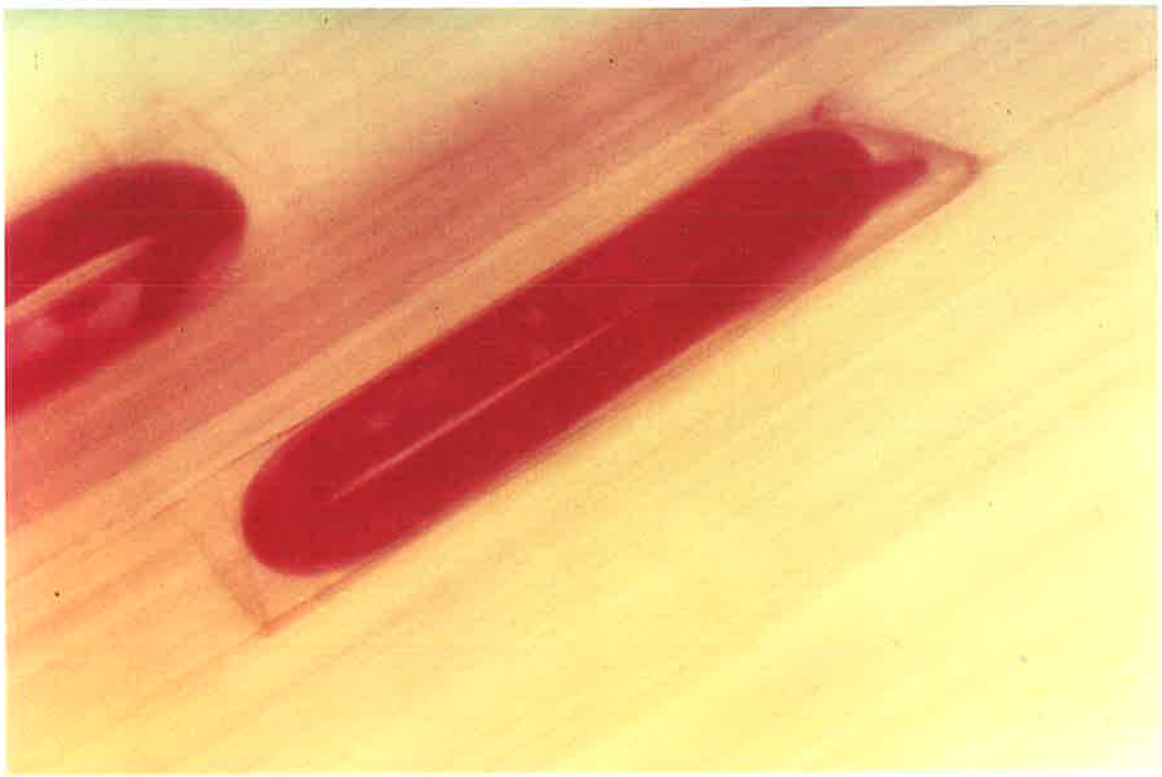
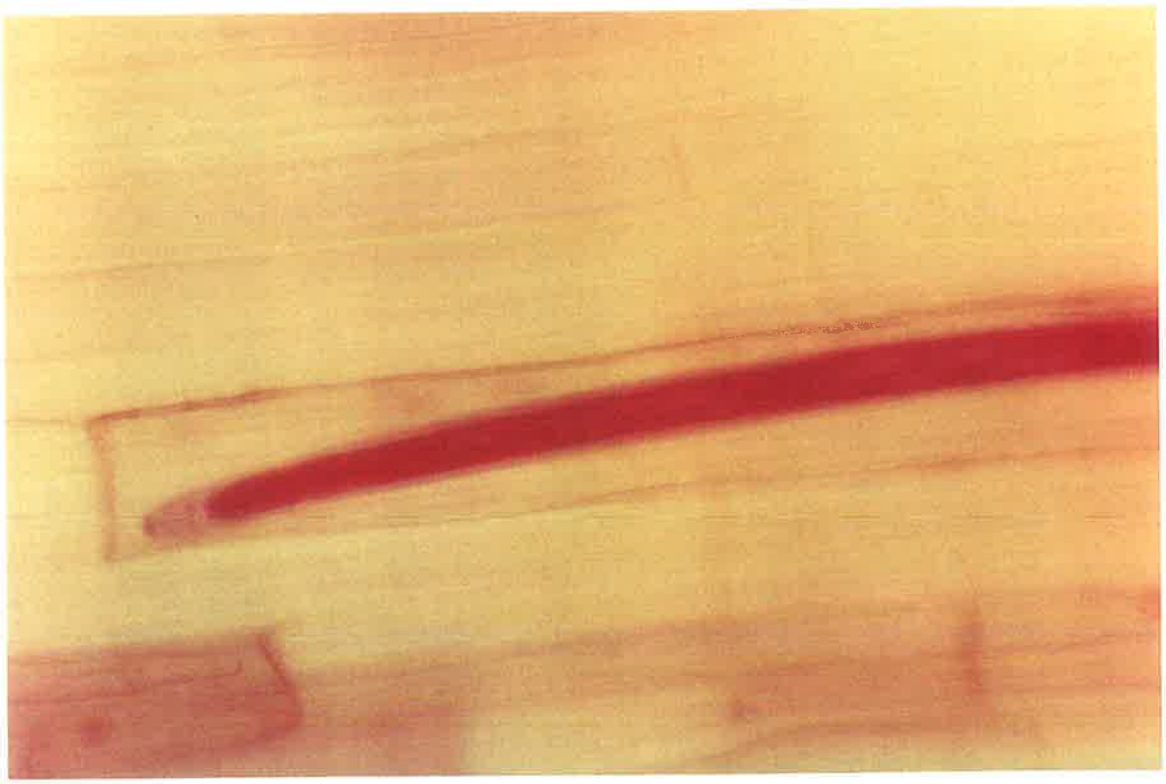


Fig 4.15 Number of *Pratylenchus* extracted from roots of Machete wheat by misting, and number remaining in roots after four days of mister extraction.

Plate 4.3 Evidence of plant hypersensitive reaction to nematode attack. Nematodes are represented by dark pink colour. Necrotic cell walls are present around the nematode body.





4.4 Discussion

Increases in the numbers of *P. neglectus* in roots and an increase in the amount of rotting on the root systems of wheat when both fungus and the nematodes are combined together compared to either pathogen alone indicate a positive interaction between the root lesion nematode and some root infecting fungi of wheat. In pasteurised soil with a very low level of only saprophytic fungi as a microbial buffer, but in the absence of all pathogenic fungi, bacteria and nematodes, the nematode population increased when plants were also infected with some root-rotting fungi. Number of *P. neglectus* in the root system was higher when the fungi *R. solani*, *B. sorokiniana*, *P. irregulare*, *M. bolleyi* or the combination of *G. graminis* var. *tritici* and *F. equiseti* were present. Thus, it is suggested that some fungi may render the roots more suitable for nematode reproduction. The number of root lesion nematodes generally increases in combination with root-rotting fungi (Mountain and McKeen, 1962; Vrain, 1987; Hasan, 1988).

There are several species of fungi involved in root-rotting of wheat in a disease complex (Jooste, 1965; Maas and Kotze, 1981; Vanstone, 1991). *M. bolleyi*, *Fusarium* spp., *Pythium* spp. and *B. sorokiniana* occur in the South Australian wheat belt as well as *R. solani*, *G. graminis* var. *tritici* and several non or weakly pathogenic fungi (Rovira, 1980; Fedel-Moen and Harris, 1987; Vanstone, 1991). Field survey results (Chapter 3), which agree with those of previous investigators, showed that *M. bolleyi* and *Fusarium* spp. were at high levels in late July and August, when roots of wheat plants are likely to be infected by *P. neglectus*.

M. bolleyi, *F. acuminatum* and *P. terrestris* are known to be common soil-borne fungi but are considered non or weak pathogens of cereals (Sprague, 1950; Butler, 1961; Fernandez *et al.*, 1985; Hill and Blunt, 1994). These fungi increased *P. neglectus* numbers within roots under controlled conditions in pasteurised soil. However, in unpasteurised soil (natural field soil), the above fungi in combination with the nematode decreased root dry weight, but no significant increase in *P. neglectus* numbers in the roots occurred.

It seems that more root damage and lower production of roots may have resulted in lower nematode numbers in roots. Another possibility could be the influence of other soil micro-organisms which were expected to be present in a natural soil. Physiological changes occurring in plants infected with nematodes can enhance susceptibility to attack by fungi, whether pathogenic, non or weakly pathogenic (Powell, 1979). Fernandez *et al.* (1985) indicated that non or weakly pathogenic fungi, under certain conditions, may become pathogenic.

The extent of rotting caused by *P. terrestris*, *F. oxysporum*, *Pythium irregulare* or *G. graminis+F. equiseti* increased in the presence of *P. neglectus* compared to when fungus was added alone. Nematode reproduction, however, was slightly decreased in the presence of *F. oxysporum* and increased in the presence of *P. irregulare* but was not affected by the presence of *P. terrestris*. A combination of *Pythium aphanidermatum* and/or *R. solani* interacted with the root lesion nematode (*Pratylenchus coffeae)* which caused rotting in chrysanthemum roots and further increased lesioning when plants were inoculated by all three organisms (Hasan, 1988).

Root damage initiated by root lesion nematodes may increase fungal infection of roots. Some fungi normally considered to be non or weak pathogens have been reported to be able to damage the roots of the host when nematodes are present (Powell, 1971). High levels of infection by *Thielaviopsis basicola,* a minor pathogen of pea, occurred when *P. crenatus* was also present (Green *et al.*, 1983). It appears that wounding of root tissue by nematodes predisposes them to fungal infection and increases disease severity caused by the nematode or fungus. It has been well documented that pre-infection of roots with nematodes enhances symptom development (Davide and Dela Rosa, 1979; Chandel and Sharma, 1989).

With or without the nematode, *G. graminis*, *G. graminis+F. equiseti*, *G. graminis+M. bolleyi* or *G. graminis+R. solani* increased lesion severity on wheat roots compared to the control (no fungus or nematode added to the soil), but no difference was observed between the above treatments. The severity of these lesions is likely to be the

result of the presence of *G. graminis*, as the isolate of fungus used in this test was highly pathogenic to wheat roots. However, in untreated soil (natural) there were no significant differences between the effect of fungus alone or in combination with the nematode. Thus, it is suggested that under natural conditions, combination of several factors is likely to influence the activity and pathogenicity of fungi that are highly virulent when tested in sterile conditions.

In unpasteurised soil, combination of the nematode with some fungi decreased root dry weight of wheat. *P. terrestris*, which was the next most commonly isolated fungus after *F. equiseti*, *M. bolleyi* or *G. graminis* (Chapter 3), was isolated with a similar frequency to *F. acuminatum* and to *F. oxysporum* and is a weak pathogen of wheat (Butler, 1961). The fungus reduced root dry weight of wheat when combined with *P. neglectus* compared to when added alone.

M. bolleyi, *B. sorokiniana*, *F. acuminatum* or *G. graminis* in combination with the nematode significantly reduced root dry weight when compared with the fungus alone. Both *M. bolleyi* and *G. graminis* were isolated from wheat roots naturally infected with *P. neglectus* in the field at very high frequencies (Chapter 3). *M. bolleyi* is known to be a weak pathogen of several crops, including wheat, when soil is infested with high levels of fungus (Domsch *et al.*, 1980; Kirk and Deacon, 1987a,b). It also appears that the fungus is present in infected roots from as early as 4-6 weeks, and reaches its highest level late in the season when soil temperature increases (Vanstone, 1991; see also Chapter 3). Harris (1986) has also isolated *M. bolleyi* from rotted roots of cereals in Australia. The fungus could be a potential agent for interaction with the root lesion nematode, as both the fungus and the nematode are widely distributed in South Australia.

Nematode infection depends on the movement of nematodes through soil and 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 penetration rate in both experiments was higher in sand (Palmer and Roseworthy) than in sieved Urrbrae loam, and higher in sieved 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 contained in the soil organic matter.

The fourth experiment confirms that sandy soil provides the best medium for maximum penetration by either nematode species, *P. thornei* or *P. neglectus*. However, this experiment has highlighted the caution required in interpretation of results when comparing two similar nematode species.

Total number of nematodes (mister extraction plus those remaining in the roots) does not vary, however the individual components vary significantly. Because of this, quantitative comparisons of the two species using mister extraction of nematodes may be confounding results. Unfortunately to stain and count nematodes in all root systems would be too time consuming. For reasons unknown, *P. neglectus* exited the roots much faster than *P. thornei*, however this is not a reflection of total nematode number. It is important to have an understanding of the nematode penetration in different soil types and extraction efficiency of the method in use. If comparative data is required a standardised method should be used.

One very important observation in roots stained for nematode detection was that cell hypersensitivity to nematode attack occured as early as seven days after inoculation (Plate 4.3). Although Machete wheat is very susceptible to root diseases, particularly root lesion nematodes, within this short period of time cell hypersensitivity occurred. Thus plant breeders should not select plants showing cell hypersensitivity to nematode attack as the sole evidence of resistance to the nematodes. Other factors such as nematode number and the extent of lesioning on the root system should also be considered.

M. bolleyi, F. acuminatum, Pyrenochaeta terrestris, Pythium irregulare, R. solani and *G. graminis* may be important in exacerbating root lesioning caused by *P. neglectus*. Further detailed studies are needed to determine the proportion of the contribution of these fungi to root damage and possible wheat yield decline in South Australia.

Chapter 5

Interaction between *Pratylenchus neglectus* and *Rhizoctonia solani* and/or *Gaeunmannomyces graminis* var. *tritici*

5.1 Introduction

Both *R. solani* and *G. graminis* var. *tritici* are major root pathogens of wheat in South Australia (Rovira, 1987). The field survey conducted in 1992 (Chapter 3) suggested that *G. graminis* is widespread in soil, infecting wheat roots and causing severe damage to root systems. Rovira (1980) also reported that *G. graminis* was the dominant pathogen of cereals at tillering and there is a strong negative correlation between the percentage of plants infected at tillering and the grain yield.

G. graminis, the causal organism of the disease take-all, is found worldwide and can cause immense damage to most cereal species (Garrett, 1942). The fungus has been known from South Australia since the middle of the last century (Anon, 1868) and is the most serious root disease of wheat and barley (Cotterill and Sivasithamparam, 1989). Under conditions favouring pathogenicity, the fungus is able to grow on root systems and spread up to 1.5m in diameter from the original inoculation point (Wehrle and Ogilvie, 1955).

R. solani too is an important root pathogen of wheat and other crops worldwide. In Australia, and particularly in southern Australia, the fungus is of economic importance and can cause severe yield reductions in wheat on highly infected areas (Samuel, 1923; de Beer, 1965; Neate, 1984). Interactions between *R. solani* and plant parasitic nematodes have been reported on a number of crops (de Beer, 1965; Meagher and Chambers, 1971). Benedict and Mountain (1956) found a consistent association between *R. solani* and *P.*

neglectus on the roots of wheat within patches of chlorotic and stunted plants. De Beer (1965) also found that *P. neglectus* was associated with *R. solani* in bare patches.

Although *R. solani* was not isolated from field samples in 1992 (Chapter 3), the isolate used in pathogenicity tests described in Chapter 4 was pathogenic to wheat, and results suggested that both *G. graminis* and *R. solani* play an important role in the rotting of wheat roots and can lead to increased nematode numbers in the roots. However, interaction between *G. graminis* and/or *R. solani* and the root lesion nematode on wheat in South Australia needed to be investigated further. The role of these important root pathogens in combination with *P. neglectus* was thus investigated under controlled environmental conditions in a glasshouse and under natural conditions in the field.

5.2 Methods

5.2.1 Glasshouse experiments

5.2.1.1 Experiment 1

A pot experiment was conducted in the glasshouse. A controlled temperature water tank was used to maintain soil temperature at 20°C for all treatments. A sandy loam soil was collected from a typical wheat growing paddock at Stow. Soil was not treated with either steam or chemical. The soil was naturally infested with *P. neglectus* at 300 nematodes/200g of soil (600 nematodes/pot).

Pots: Plastic pots with a 300ml capacity were used (General Methods).

Fungus and nematode inoculum: Inoculum of *G. graminis* var. *tritici* (#Ggt 9271) and *R. solani* (#Rs-21) were prepared on ryegrass seed as described in the General Methods. *G. graminis* at 0.05% w/w, *R. solani* at 10 infested seeds and the combination of *G. graminis* + *R. solani* at the same rate were mixed thoroughly with the soil of each pot. This mixing may have reduced the original nematode numbers in soil (Taylor,

1993). Wheat cultivars Spear and Machete were used in this experiment. One pre-germinated seedling was sown in each pot. Plants were inoculated with 2000 nematodes/plant which were grown aseptically on carrot cultures in addition to the natural inoculum of 600 nematodes/pot, so the total number of nematodes was between 2000 and 2600/plant. The nematode inoculum was pipetted in 1.0ml of distilled water around each plant.

Experiment layout: The experiment was set out as a completly randomised design with six replicates.

Harvest: Plants were harvested 49 days after planting, at which time roots were washed free of soil and scored for lesion severity. Fresh and dry weights of shoots were recorded, and nematodes extracted from roots as described in the General Methods in a mist chamber over a four day extraction period and counted. Roots were then oven dried and weighed. Nematode numbers/plant and nematodes/g dry root were calculated.

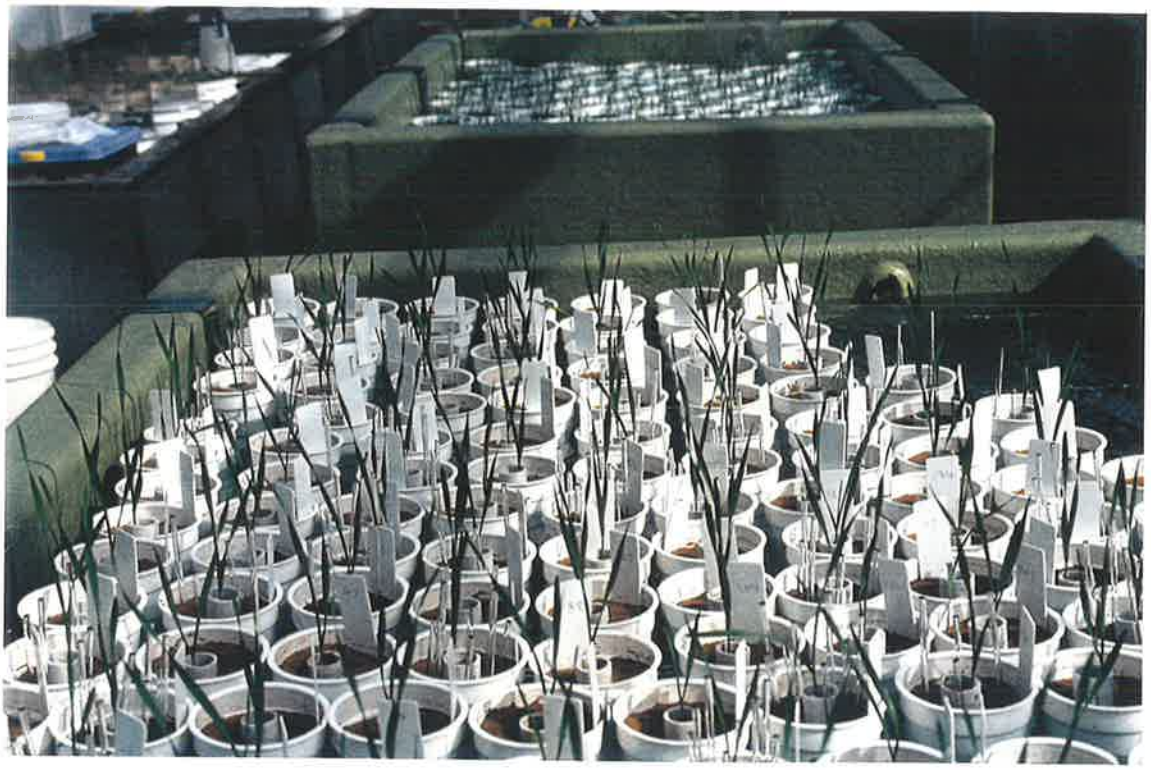
5.2.1.2 Experiments 2 and 3

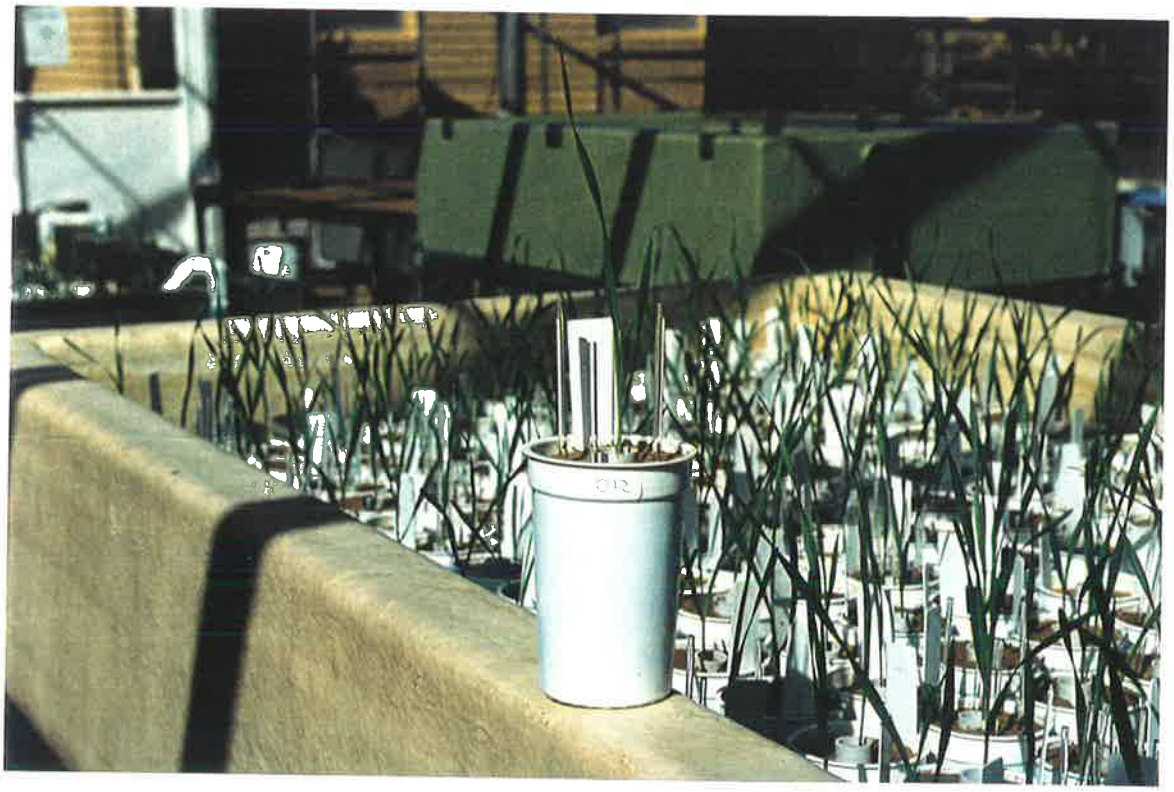
Two experiments were conducted at CSIRO Division of Soils (South Australia) in a glasshouse with an air temperature of 25±3°C. Soil temperature was maintained at 20°C in a controlled temperature tank (Plate 5.1). Various combinations of fungi and aseptically grown *P. neglectus* were added at different inoculation times.

Soil: A sandy loam soil was collected from an uncropped area at Avon, South Australia (Chapter 2). The population of known pathogenic fungi in this soil was extremely low. Therefore, the soil was not treated with steam or chemical.

Fungus inoculum: Inoculum of *G . graminis* var. *tritici* (#Ggt-500 originally isolated from wheat roots in Western Australia) on ryegrass seed was obtained from S. M. Neate. *R. solani* Anastomosis Group-8 (#Rs-21) on millet seed (Fang, 1991) was also supplied by S.M. Neate. These isolates were known to be highly pathogenic to wheat (S. M. Neate, personal communication).

Plate 5.1 Glasshouse experiment with *G. graminis* and/or *P. neglectus* using a water tank to maintain soil temperature at 20°C. Glass rods in each pot were removed to allow for second inoculation time (two weeks after sowing). Similar experiments were conducted using *R. solani* and/or *P. neglectus*.





Both fungi were added to the soil at three different rates. Ggt-500 at 0, 8 or 16 propagules (colonised seed) per pot and Rs-21 at 0, 4 or 8 propagules per pot. Fungus inoculum was added to the soil in two layers: 150g of soil was added to the pot and the first layer of fungus inoculum was added, then another 150g of soil and the second layer of inoculum added. Finally, pots were filled with the remaining soil up to 400g (Figure 5.1). Inoculum of each fungus in either layer was added in four different places as there was one further fungus inoculation time at two weeks after planting (Figure 5.1). One pre-germinated Machete wheat seed was planted in each pot.

Nematode inoculum: *P. neglectus* from carrot cultures were pipetted in 1.0ml of distilled water around each plant soon after planting at the following densities: 0, 1000, 2000 or 4000 nematodes/pot (mixed stages minus eggs). Control pots received the same amount of distilled water.

Inoculation times: Three inoculation times were used: fungus at sowing, nematode two weeks later; nematode at sowing, fungus two weeks later; both fungus and nematode at sowing. Eight 3.5mm diameter glass rods 10cm long were inserted in each pot at two separate levels corresponding to positions of fungus inoculum placement before planting (Figure 5.1). After two weeks, rods were gently removed and fungus inoculum was placed at the bottom of each hole. Holes were then filled with soil and watered.

Harvest and measurements: Plants were harvested 49 days after planting. Roots were washed free of soil and scored for the amount of lesions on root systems. A few representative (five segments/treatment) root segments from different treatments were plated on RA medium to re-isolate the fungus and were also stained to detect nematode invasion in cortical cells. Remaining roots were placed in a mist chamber to extract nematodes over a four day extraction period and nematodes were counted. Roots were then oven dried for 48 hours and weighed. Number of nematodes/plant and nematodes/g dry root were calculated.

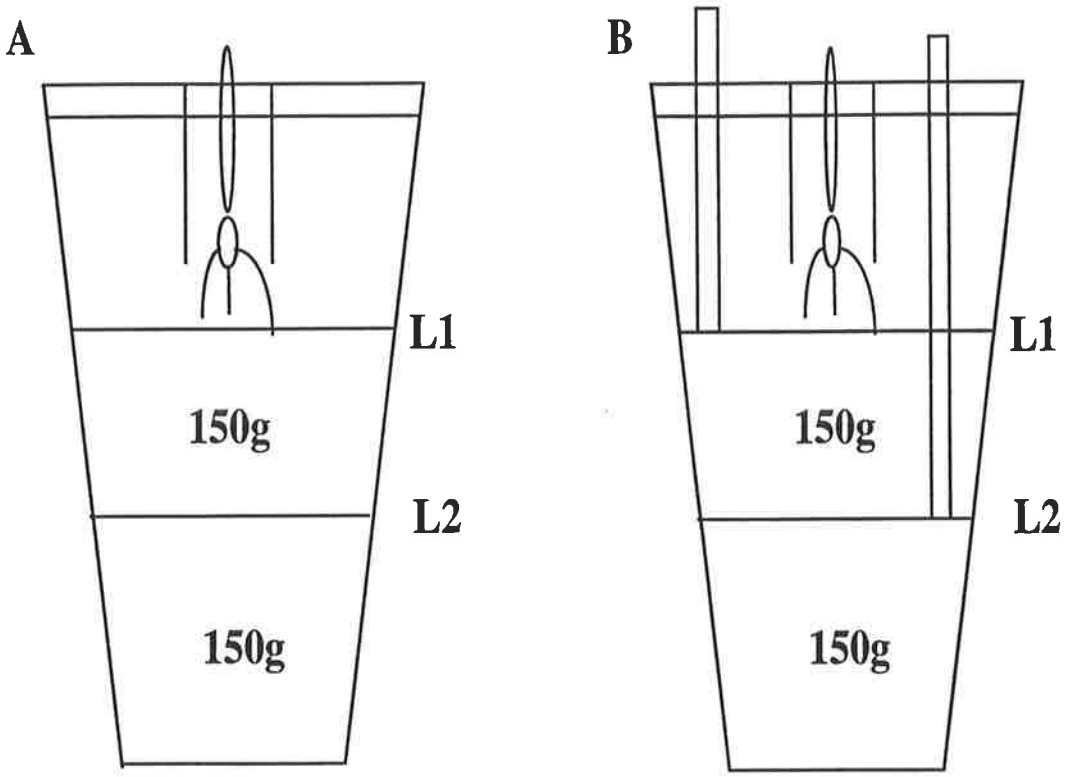


Figure 5.1 Diagram of fungal inoculation technique used for sequential inoculations.

A. Fungus inoculum added at sowing at two levels (L1 and L2).

B. Fungus inoculum added two weeks after sowing. Glass rods, placed in pots at sowing, were removed after two weeks and fungus inoculum placed in the holes at two levels (L1 and L2). Holes were then filled with fresh soil.

5.2.2 Field experiments

Experiments under controlled conditions in the glasshouse indicated that plants inoculated with *G. graminis* and *P. neglectus* suffered less damage than those inoculated with *G. graminis* alone. Conversely, plants inoculated with *R. solani* and *P. neglectus* suffered more damage than when inoculated with *R. solani* alone (Chapter 4). The aim of this experiment was to determine the effect of nematode-*R. solani* and/or *G. graminis* interaction on plant growth, root damage, nematode multiplication and grain yield of two wheat varieties (Spear and Machete) in field soil naturally infested with *P. neglectus.*

A field experiment was conducted in 1993 at the Minnipa Research Centre on the Eyre Peninsula of South Australia approximately 700 km West of Adelaide (Figure 2.1). The site was chosen on the basis of preliminary observations of root lesion nematode, *P. neglectus.*

Soil type and nematode number: The site consisted of a sandy loam soil with a pH of 8.5. The previous crop in this paddock was Mustard cv. Pusa Bold which is also a good host for *P. neglectus* (V. A. Vanstone, unpublished data), so nematode numbers in the soil were expected to be high. Ten random soil samples of 200g from 0-10cm depth were taken from a $300m^2$ area of the experimental site. Nematodes from each soil sample were extracted and counted. Mean number of nematodes was seven *P. neglectus*/g dry soil (1400 nematodes/200g soil). Average monthly rainfall is listed in Table 2.1.

Sowing: Plots were sown on June 20, 1993, as described in the General Methods. Inoculum of Ggt-500 at 2000 infected ryegrass seeds/m^2, Rs-21 at 1000 infected millet seeds/m^2, or both fungi at half the rates of either fungus (ie. 1000 infected ryegrass seeds of Ggt-500 and 500 infested millet seeds of Rs-21/ m^2). Control plots received a similar amount of autoclaved dead seeds at sowing. The nematicide Temik® (aldicarb) was applied to the soil at the rate of 5kg/ha a.i. with the seed. Fertiliser and herbicide were applied as per the usual farming practices of the region. Seeding rate and plot size are described in the General Methods.

Experiment layout: The experiment was set out as a split plot design with four blocks (Plate 5.2). Main treatments were: two wheat cultivars (Machte and Spear), two soil treatments (with Temik® or without Temik®), four fungus treatments (*G. graminis*, *R. solani*, *G. graminis*+*R. solani* and no fungus inoculum) arranged as four blocks.

Plots of Machete wheat were included as borders, and also as buffers between main plots (fungi) to eliminate the risk of contamination between the fungal treatments.

Sampling: Plots were sampled at eight and twelve weeks after sowing. At all sample dates, five plants were dug randomly from each plot in each replicate. Roots were washed free of soil, and in a laminar flow cabinet representative root segments (five/treatment) were plated from lesioned parts of root systems of either treatment to re-isolate fungi. After four days of nematode extraction, roots were oven dried and weighed. The number of nematodes/g dry root was calculated.

Harvest: Total plant tops were taken from two 50cm long rows of each plot at maturity. Number of heads, grams per head, total dry weight, total grain yield and 1000 grain weight were determined for each sample. Plots were harvested for grain yield.

5.3 Results

5.3.1 Pot experiments

5.3.1.1 Experiment 1

The analyses of variance for all measurements are shown in Table 5.1. Fungus or nematode separately showed a significant effect on plant growth, dry matter, root lesioning and nematode numbers (Table 5.1). Nematode inoculum was successful as is indicated by highly significant differences between nematode numbers in inoculated and uninoculated treatments. This difference was also expressed in the degree of lesioning and plant growth. There was no significant difference between wheat cultivars (Spear or Machete) on number of nematodes extracted from roots, but plant dry matter and root lesioning differed between the two cultivars.

Plate 5.2 The field trial at Minnipa Research Centre in 1993 using *G. graminis*, *R. solani*, both fungi together and control (no fungus added), two wheat cultivars (Spear and Machete) and plus and minus Temik® (aldicarb).

A. Photo taken nine weeks after sowing. Plots (0.75cm × 6m) of sixteen different treatments were arranged as a factorial split plot design.

B. Photo of above plots taken five months after sowing.

A

B

Few interactions were significant in this experiment (Table 5.1). There was a significant interaction between fungus and wheat cultivars on root dry weight or total dry weight of plants (Table 5.1).

Tiller numbers/plant: Number of tillers/plant decreased when plants were inoculated with *G. graminis* compared to control (no fungus added) or *R. solani*. Number of tillers was significantly different between the two wheat cultivars. Machete produced more tillers than Spear (Table 5.2). Nematodes alone had no significant effect on tiller number. However, a significant interaction between fungus and wheat cultivar was detected (Table 5.2).

Table 5.1 Summary of analyses of variance for the effect of interaction between *G. graminis* var. *tritici* and/or *R. solani* and *P. neglectus* on root lesion rating, number of nematodes/plant and nematodes/g dry root, shoot and root dry weights, total dry weight of plants, number of tillers and nematode multiplication rate for wheat cultivars Spear and Machete, 49 days after sowing (Experiment 1).

Source	df	MS RL	P	MS N/p (log)	P	MS N/gdr (log)	P	MS dws/p	P	MS dwr/p	P	MS tdw/p	P	MS tillers	P	MS MR	P
Block	5																
Nematode (nem)	1	5.5	***	8.46	***	9.29	***	0.022	**	0.02	**	0.03	**	0.02	ns	1.86	***
Fungus (fun)	3	5.7	***	0.26	**	0.32	**	0.011	**	0.01	*	0.018	*	1.07	**	0.35	**
Cultivar (cult)	1	2.3	*	0.04	ns	0.01	ns	0.009	*	0.009	*	0.0028	ns	8.37	***	0.13	ns
Nematode × fungus	3	0.5	ns	0.01	ns	0.02	ns	0.001	ns	0.7-E3	ns	0.1-E3	ns	0.32	ns	0.03	ns
Nematode × cultivar	1	0.04	ns	0.01	ns	0.01	ns	0.016	***	0.002	ns	0.3-E4	ns	0.06	ns	0.04	ns
Fungus × cultivar	3	0.1	ns	0.17	*	0.22	*	0.002	ns	0.016	ns	0.021	**	0.79	*	0.28	**
Nem × fun × cult	3	0.04	ns	0.16	*	0.20	*	0.003	ns	0.003	***	0.0074	ns	0.13	ns	0.28	**
Residual	75	0.4		0.05		0.07		0.002		0.0024		0.0055		0.28		0.06	

*** significant at P= 0.001 ** significant at P= 0.01 * significant at P= 0.05 ns= not significant P= Probability.
RL= root lesioning; N/p= nematodes/plant; N/gdr= nematodes/g dry root; dws/p= shoot dry weight/plant; dwr/p= root dry weight/plant; tdw/p= total dry weight/plant; tillers= tillers/plant; MR= nematode multiplication rate.

Table 5.2 Effect of nematode-fungus (*R. solani* or *G. graminis*) interaction on the number of tillers/plant of wheat cultivar Machete 49 days after sowing in a pasteurised soil under controlled glasshouse conditions. Values in the 3-way table are the average of six single plant blocks.

	Spear		Machete	
Nematode	N0	N2000	N0	N2000
Nil	1.40	1.17	2.00	1.83
Rs	1.33	1.20	1.83	1.50
Ggt	1.00	1.00	1.17	1.33
Rs+Ggt	0.50	1.00	1.83	1.83

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD at P= 0.05 level). LSD

Fungus	Nil	Rs	Ggt	Rs+Ggt	
	1.61	1.48	1.12	1.28	*0.30*
Nematode	N0	N2000			
	1.38	1.35			*ns*
Cultivar	Spear	Machete			
	1.06	1.67			*0.21*

Fungus	Nil	Rs	Ggt	Rs+Ggt	
Nematode					
N0	1.73	1.58	1.08	1.17	*ns*
N2000	1.50	1.36	1.17	1.38	

Fungus	Nil	Rs	Ggt	Rs+Ggt	
Cultivar					
Spear	1.27	1.27	1.00	0.77	*0.43*
Machete	1.92	1.67	1.25	1.83	

Nematode	N0	N2000			
Cultivar					
Spear	1.04	1.08			*ns*
Machete	1.71	1.62			

Rs= *R. solani*, Ggt= *G. graminis*. N0= no nematodes added, N2000= 2000 nematodes/plant.

Root lesion rating: The amount of damage to the root system was significantly (P= 0.001) affected by both fungus and nematode. Inoculation of plants with 2000 nematodes increased root lesion rating of both wheat cultivars by 27% compared to the controls (no nematodes added) (Table 5.3). Inoculation by either fungus resulted in increased root lesion rating when compared to the control (no fungus added). Among fungus treatments, there were no significant differences (Table 5.3).

***Number of nematodes*:** Number of *P. neglectus*/plant and nematodes/g dry root were significantly (P= 0.001) affected by both fungus and nematode inoculum (Table 5.1). The significant 3-way interaction between fungus, nematode and variety as well as the 2-way interaction between fungus and variety is shown in Table 5.4.

The two wheat varieties, Spear and Machete, behaved differently to the fungus and nematode interactions. Inoculation with *G. graminis* or *R. solani* increased number of nematodes/plant of Spear by 68% and 107%, respectively, compared to the control (no fungus added) (Table 5.4). However, with the combination of *G. graminis* and *R. solani,* nematode numbers did not differ from the control but were significantly (P= 0.05) less than with either fungus alone. Numbers of *P. neglectus*/plant for Machete wheat inoculated with *R. solani, G. graminis* or *R. solani*+*G. graminis* increased by 38%, 86% and 54%, respectively, compared to the control (no fungus added) (Table 5.4). Number of nematodes/g dry root for both cultivars showed similar results to nematodes/plant. A significant positive correlation was found between number of nematodes/plant and nematodes/g of dry root (Figure 5.1).

Table 5.3 Effect of nematode-fungus (*R. solani* or *G. graminis*) interaction on the root lesion rating of wheat cultivar Machete 49 days after sowing in a pasteurised sandy loam soil under controlled glasshouse conditions. Values in the 3-way table are the average of six single plant blocks.

	Spear		Machete	
Nematode	N0	N2000	N0	N2000
Nil	0.40	1.00	0.58	1.00
Rs	1.50	1.80	1.83	2.25
Ggt	1.33	1.50	1.75	1.92
Rs+Ggt	0.92	1.86	1.33	2.08

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD at P= 0.05 level). LSD

Fungus	Nil	Rs	Ggt	Rs+Ggt	
	0.76	1.85	1.62	1.56	*0.33*

Nematode	N0	N2000			
	1.22	1.68			*0.24*

Cultivar	Spear	Machete			
	1.31	1.59			*0.24*

Fungus	Nil	Rs	Ggt	Rs+Ggt	
Nematode					
N0	0.50	1.67	1.54	1.12	*ns*
N2000	1.00	2.05	1.71	1.96	

Fungus	Nil	Rs	Ggt	Rs+Ggt	
Cultivar					
Spear	0.73	1.64	1.42	1.42	*ns*
Machete	0.79	2.04	1.83	1.71	

Nematode	N0	N2000			
Cultivar					
Spear	1.07	1.54			*ns*
Machete	1.38	1.81			

Rs= *R. solani*, Ggt= *G. graminis*. N0= no nematodes added, N2000= 2000 nematodes/plant.

Table 5.4 Effect of nematode-fungus (*R. solani* or *G. graminis*) interaction on the number of nematodes/plant 49 days after sowing in a pasteurised soil under controlled glasshouse conditions. Values in the 3-way table are the average of six single plant blocks.

	Spear		Machete	
Nematode	N0	N2000	N0	N2000
Nil	124	526	163	483
Rs	227	1092	210	667
Ggt	350	883	136	897
Rs+Ggt	130	588	190	747
(3-way interaction is significant)				LSD^b 5%= *0.26*

1 and 2-way treatment means (with appropriate LSD at P= 0.05 level). LSD^b

Fungus	Nil	Rs	Ggt	Rs+Ggt	
	333	525	566	421	*0.13*

Nematode	N0	N2000	
	192	725	*0.09*

Cultivar	Spear	Machete	
	487	437	*ns*

Fungus	Nil	Rs	Ggt	Rs+Ggt	
Nematode					
N0	145	218	243	160	*ns*
N2000	505	860	890	661	

Fungus	Nil	Rs	Ggt	Rs+Ggt	
Cultivar					
Spear	343	620	617	377	*0.18*
Machete	323	438	517	468	

Nematode	N0	N2000	
Cultivar			
Spear	211	752	*ns*
Machete	175	698	

$LSD^b = \log_e$

Rs= *R. solani*, Ggt= *G. graminis*. N0= no nematodes added, N2000= 2000 nematodes/plant.

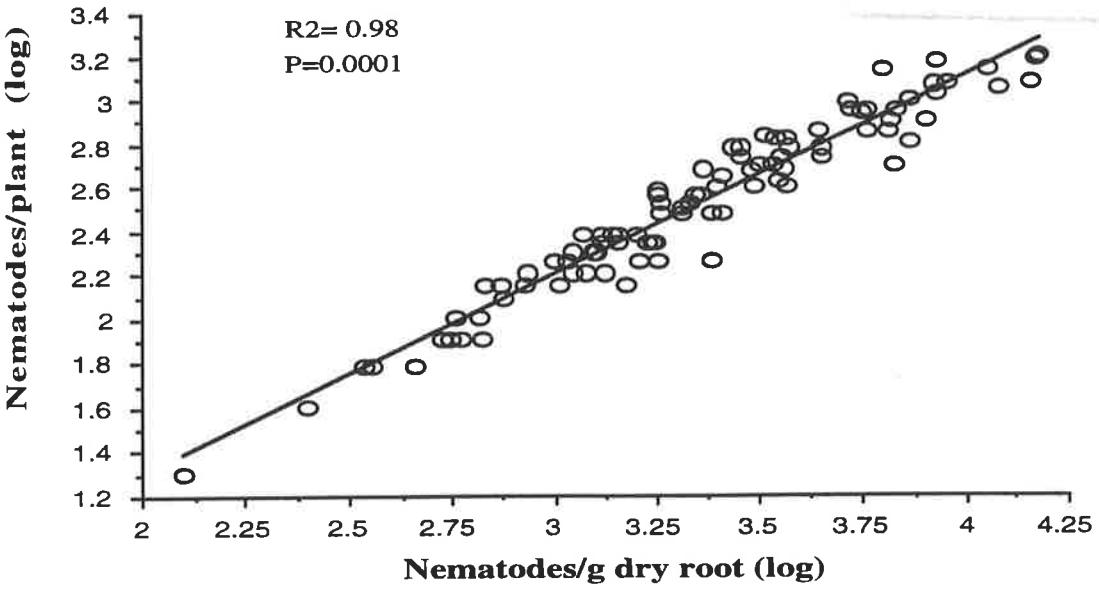


Figure 5.2 The relationship between nematodes/plant and number of nematodes/g dry root. Values in the correlation figure are the average of twelve single plant blocks (Spear and Machete).

Nematode multiplication (final number of nematodes/initial number) was another factor which was affected by fungus and nematode inoculum. The 3-way (nematode × fungus × cultivar) and 2-way (fungus × cultivar) interactions were also significant at P= 0.05 for nematode multiplication rate (Figure 5.3).

***Dry matter*:** Shoot dry weights were significantly (P= 0.05) affected by both nematode and fungus inoculum. Shoot dry weight decreased by 8% in plants inoculated with 2000 nematodes/pot when compared to the control (no nematodes added) (Table 5.5).

The effect of nematode inoculation on shoot dry weight was different for Spear and Machete, as the highly significant nematode × cultivar interaction term shows (Table 5.5), but there were no over-riding effects across both cultivars.

Root dry weight decreased where nematodes were added, but this reduction was not significant. Significant interactions between cultivar and fungus treatment also occurred for root dry weight. Similarly, total dry weight of plants was affected by inoculation with

nematodes. With increased nematode numbers in the root system, root and total dry weight decreased (Figure 5.4).

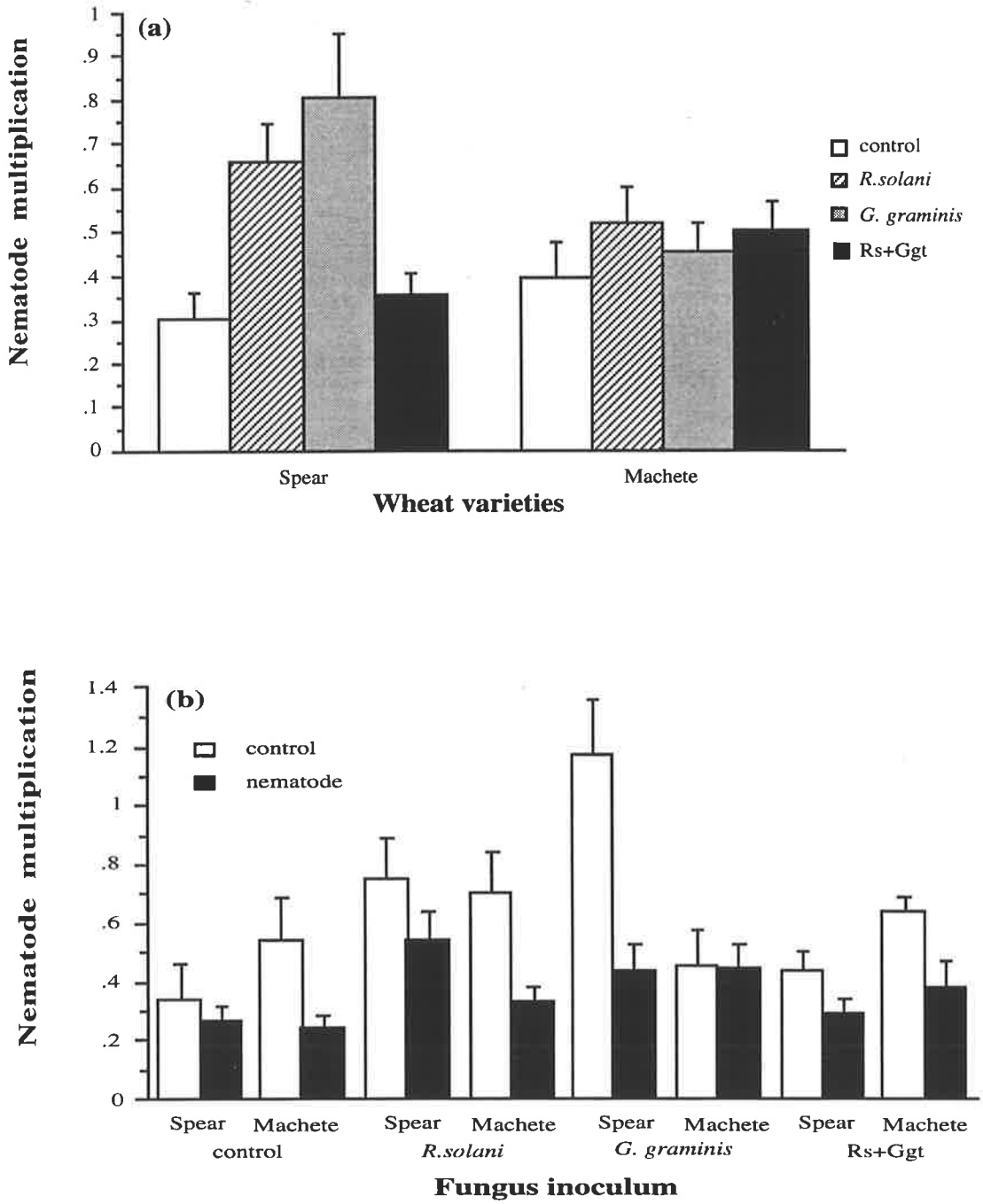


Figure 5.3 The effect of **(a)** 2-way interaction (nematode × cultivar) and **(b)** 3-way interaction (nematode × fungus × cultivar) on the multiplication rate of *Pratylenchus neglectus* 49 days after inoculation in a natural (untreated) field soil under controlled glasshouse conditions. Values in the 2-way interaction figure are the average of twelve single plant blocks and in the 3-way figure are the average of six single plant blocks.

Table 5.5 Effect of nematode-fungus (*R. solani* or *G. graminis*) interaction on the shoot dry weight (g/plant) of wheat cultivar Machete 49 days after sowing in a pasteurised soil under controlled glasshouse conditions. Values in the 3-way table are the average of six single plant blocks.

	Spear		Machete	
Nematode	N0	N2000	N0	N2000
Nil	0.32	0.30	0.43	0.37
Rs	0.40	0.36	0.37	0.37
Ggt	0.41	0.39	0.39	0.37
Rs+Ggt	0.40	0.39	0.44	0.37

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD at P= 0.05 level).

					LSD
Fungus	Nil	Rs	Ggt	Rs+Ggt	
	0.35	0.37	0.39	0.40	*0.02*
Nematode	N0	N2000			
	0.40	0.36			*0.02*
Cultivar	Spear	Machete			
	0.37	0.39			*ns*
Fungus	Nil	Rs	Ggt	Rs+Ggt	
Nematode					
N0	0.38	0.38	0.40	0.42	*ns*
N2000	0.33	0.36	0.38	0.38	
Fungus	Nil	Rs	Ggt	Rs+Ggt	
Cultivar					
Spear	0.31	0.38	0.40	0.39	*0.03*
Machete	0.40	0.37	0.38	0.41	
Nematode	N0	N2000			
Cultivar					
Spear	0.38	0.41			*ns*
Machete	0.36	0.37			

Rs= *R. solani*, Ggt= *G. graminis*. N0= no nematodes added, N2000= 2000 nematodes/plant.

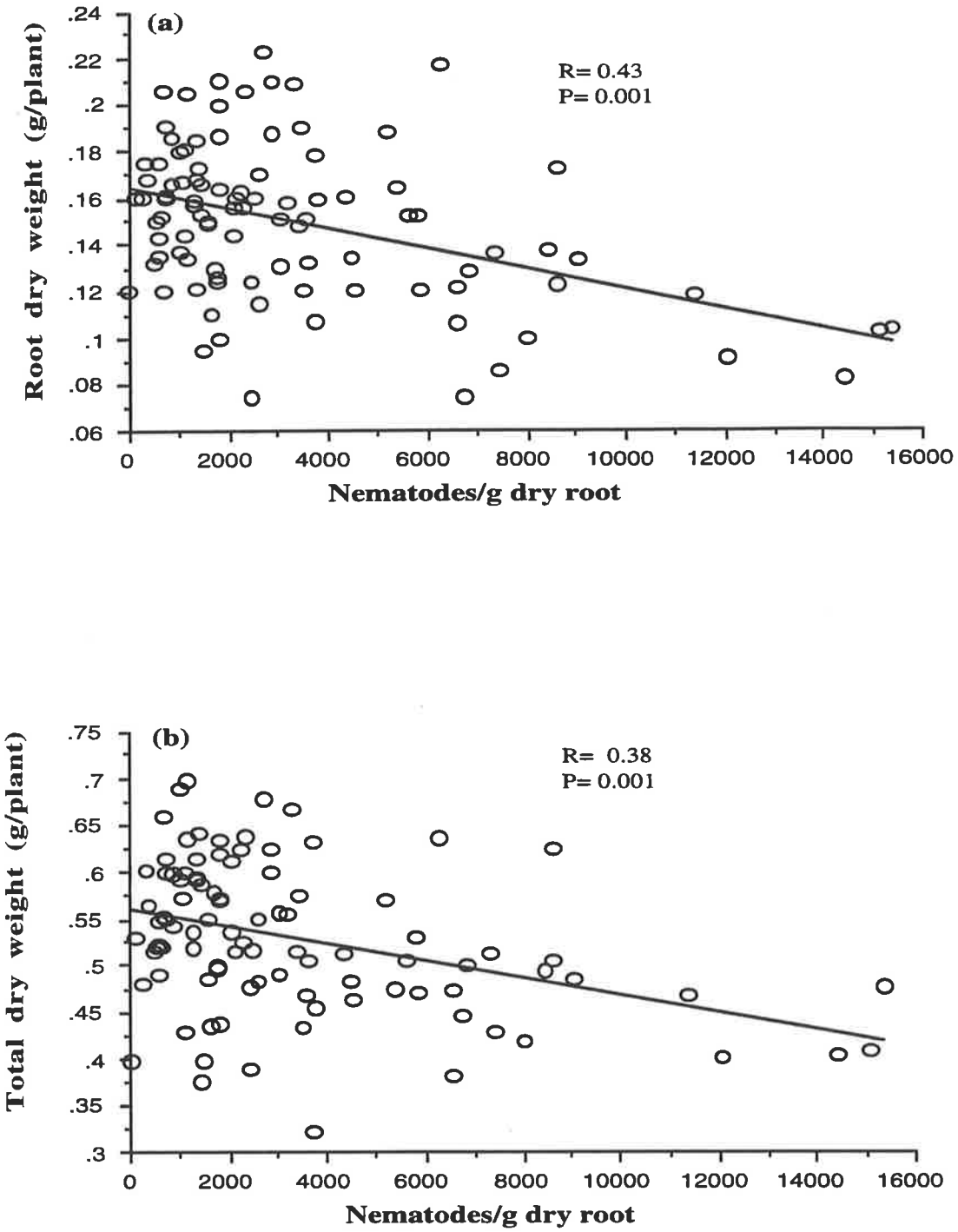


Figure 5.4 The relationship between **(a)** number of nematodes/g dry root and root dry weight and **(b)** total dry weight of wheat cultivars Spear and Machete 49 days after sowing in a natural (untreated) field soil under controlled glasshouse conditions.

5.3.1.2 Experiment 2

The analyses of variance for all measurements are shown in Table 5.6. Fungus inoculum significantly affected all variates measured (nematodes/plant or nematodes/g dry root, shoot and root dry weights and root lesion rating). Inoculation time also had a significant affect on all variates measured except for nematodes/g dry root. Nematode inoculum however, only affected nematodes/plant and nematodes/g dry root and root lesion rating but had no affect on plant dry matter.

Tiller numbers/plant: Number of tillers/plant was not significantly affected by any treatment.

Root lesion rating: At 49 days after planting in soil inoculated with sixteen propagules of *G. graminis* before or at the same time as the nematodes, take-all lesions were present on almost all roots. Root lesioning, however, was affected by nematode, fungus and inoculation time and there was a significant interaction between fungus and inoculation time.

With 2000 or 4000 nematodes/plant, root lesion rating increased by 14% and 18%, respectively, compared to the control (no nematodes added) (Table 5.7). At 1000 nematodes per plant, there was no significant effect on root lesion rating (Table 5.7).

Inoculation of plants with fungus and nematode at planting or pre-inoculation with fungus increased root lesion rating by 18% and 21%, respectively, compared to when nematodes were added prior to fungus inoculum (Table 5.7). Interaction between fungus inoculum and inoculation time, however, resulted in significant affects on root lesioning (Table 5.7).

Table 5.6 Summary of analyses of variance for the effect of interaction between *G. graminis* var. *tritici* and *P. neglectus* on extent of root lesioning, number of nematodes/plant and nematodes/g dry root, root and shoot dry weights and total dry weight of plants for wheat cultivar Machete, 49 days after sowing (Experiment 2).

Source	df	MS		MS		MS		MS		MS		MS	
		RL	P	N/p	P	N/gdr	P	dwr/p	P	dws/p	P	tdw/p	P
Block	4												
Fungus (fun)	2	148.4	***	2760E4	***	2685E4	*	0.436	***	0.308	***	1.477	***
Nematode (nem)	3	2.94	***	7106E4	***	1916E4	*	0.005	ns	0.0004	ns	0.023	ns
Time	2	7.23	**	4947E4	***	4181E3	ns	0.044	**	0.037	**	0.155	***
Fungus × nematode	6	0.57	ns	9117E3	**	6848E3	ns	0.003	ns	0.002	ns	0.007	ns
Fungus × time	4	4.04	**	1649E4	***	9245E3	ns	0.053	***	0.02	***	0.134	***
Nematode × time	6	0.55	ns	1191E4	**	1682E4	*	0.008	*	0.0002	ns	0.009	ns
Nem × fun × time	12	0.48	ns	4757E3	ns	1294E4	*	0.002	ns	0.003	**	0.008	ns
Residual	140	0.33		2993E3		7957E3		0.004		0.001		0.007	

*** significant at P= 0.001 ** significant at P= 0.01 * significant at P= 0.05 ns= not significant P= Probability.
RL= root lesioning; N/p= nematodes/plant; N/gdr= nematodes/g dry root; dwr/p= root dry weight/plant; dws/p= shoot dry weight/plant; tdw/p= total dry weight/plant.

***Nematode numbers*:** Number of nematodes extracted from the root system was significantly affected by fungus, nematode, inoculation time and all possible combinations of these treatments (nematode × fungus, nematode × inoculation time, fungus × inoculation time and the three way interaction of nematode × fungus × inoculation time) (Table 5.8). As the density of nematode inoculum increased, number of *P. neglectus* extracted from roots increased significantly (Table 5.8).

High fungus inoculum resulted in a lower number of nematodes within the root system (Table 5.8). However, there was no significant difference between either fungus inoculum level and the control (no fungus added) (Table 5.8). Number of *P. neglectus* was higher at the higher nematode inoculum densities, but was reduced up to 60% at all levels on *G. graminis* infected plants compared to the control (no fungus inoculum added) (Table 5.8).

Nematode multiplication rate, measured as nematodes recovered/plant and as nematodes/g dry root, was reduced by take-all infection on plants inoculated with 1000, 2000 or 4000 nematodes, but there was a significant reduction with 2000 and 4000 nematodes/plant (5 and 10 nematodes/g soil) (Figure 5.5). At the low level of take-all (8 propagules/pot), nematode multiplication was the same as for uninoculated roots.

***Dry matter*:** Shoot and root dry weights were significantly (P= 0.001) reduced with either 8 (53%, 70%, respectively) or 16 (54%, 72%, respectively) propagules of *G. graminis* compared to the control (no fungus added) (Tables 5.9 and 5.10). Nematodes alone had no effect on plant growth or dry matter production. With or without the nematode, root dry weight was significantly reduced by take-all infection (Table 5.10).

Table 5.7 Effect of nematode-fungus (*G. graminis*) interaction on the root lesion rating (0-5) of wheat cultivar Machete 49 days after sowing in a pasteurised soil under controlled glasshouse conditions. Values in the 3-way table are the average of five single plant blocks.

	Nil			Ggt (low)			Ggt (high)		
Nematode	0	1	2	0	1	2	0	1	2
N0	0.20	0.60	0.40	4.10	3.30	3.00	4.20	3.40	3.60
N1000	0.70	1.20	1.00	4.20	3.70	2.20	4.10	3.90	2.90
N2000	0.60	1.30	1.70	4.30	3.80	2.80	4.20	4.50	3.30
N4000	1.40	1.60	1.30	3.90	4.10	3.16	4.7	4.10	3.62

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD at P= 0.05 level)

Fungus	Nil	Ggt (low)	Ggt (high)		LSD
	1.00	3.54	3.88		*0.22*
Nematode	N0	N1000	N2000	N4000	
	2.53	2.66	2.94	3.09	*0.25*
Inoculation time	0	1	2		
	3.05	2.96	2.41		*0.22*

Nematode	N0	N1000	N2000	N4000	
Fungus					
Nil	0.40	0.96	1.20	1.43	
Ggt (low)	3.46	3.36	3.63	3.68	*ns*
Ggt (high)	3.73	3.63	4.00	4.18	

Inoculation time	0	1	2		
Fungus					
Nil	0.92	1.17	1.10		
Ggt (low)	4.12	3.72	2.81		*0.38*
Ggt (high)	4.30	3.97	3.34		

Nematode	N0	N1000	N2000	N4000	
Inoculation time					
0	2.83	3.00	3.03	3.33	
1	2.43	2.93	3.20	3.27	*ns*
2	2.33	2.03	2.60	2.67	

Ggt= *G. graminis*. Inoculation time: 0= fungus at sowing, nematodes two weeks later; 1=fungus and nematodes at sowing; 2= nematodes at sowing, fungus two wees later. N0= no nematodes added, N1000= 1000 nematodes/plant, N2000= 2000 nematodes/plant, N4000= 4000 nematodes/plant.

Table 5.8 Effect of nematode-fungus (*G. graminis*) interaction on the number of *P. neglectus* extracted from roots of wheat cultivar Machete 49 days after sowing in a pasteurised soil under controlled glasshouse conditions. Values in the 3-way table are the average of five single plant blocks.

	Nil			Ggt (low)			Ggt (high)		
Nematode	0	1	2	0	1	2	0	1	2
N0	416	432	272	112	424	120	8	248	100
N1000	712	2448	1644	1392	292	2456	276	308	2380
N2000	1098	3632	2664	2276	996	2067	100	24	4176
N4000	1896	7628	4532	804	4096	7172	260	280	3405

(3-way interaction is significant) LSD 5%= *0.039*

1 and 2-way treatment means (with appropriate LSD at P= 0.05 level). LSD

Fungus	Nil	Ggt (low)	Ggt (high)		
	2281	1850	908		*648*
Nematode	N0	N1000	N2000	N4000	
	236	1299	1892	3411	*739*
Inoculation time	0	1	2		
	787	1734	2571		*643*

Nematode	N0	N1000	N2000	N4000	
Fungus					
Nil	373	1601	2464	4685	
Ggt (low)	218	1380	1779	4024	*1269*
Ggt (high)	118	888	1433	1235	

Inoculation time	0	1	2		
Fungus					
Nil	1030	3535	2278		
Ggt (low)	1146	1452	2953		*1099*
Ggt (high)	155	215	2473		

Nematode	N0	N1000	N2000	N4000	
Inoculation time					
0	178	793	1158	1038	
1	368	1016	1550	4001	*1269*
2	164	2144	2969	5152	

Ggt= *G. graminis*. Inoculation time: 0= fungus at sowing, nematodes two weeks later; 1=fungus and nematodes at sowing; 2= nematodes at sowing, fungus two wees later. N0= no nematodes added, N1000= 1000 nematodes/plant, N2000= 2000 nematodes/plant, N4000= 4000 nematodes/plant.

Table 5.9 Effect of nematode-fungus (*G. graminis*) interaction on the shoot dry weight (g/plant) of wheat cultivar Machete 49 days after sowing in a pasteurised soil under controlled glasshouse conditions. Values in the 3-way table are the average of five single plant blocks.

	Nil			Ggt (low)			Ggt (high)		
Nematode	0	1	2	0	1	2	0	1	2
N0	0.202	0.163	0.198	0.066	0.065	0.162	0.030	0.071	0.108
N1000	0.195	0.193	0.172	0.041	0.061	0.171	0.043	0.042	0.088
N2000	0.234	0.213	0.187	0.037	0.059	0.135	0.040	0.041	0.109
N4000	0.193	0.208	0.177	0.069	0.069	0.132	0.035	0.025	0.135

(3-way interaction is significant) LSD 5%= *0.039*

1 and 2-way treatment means (with appropriate LSD at P= 0.05 level).

Fungus	Nil	Ggt (low)	Ggt (high)		LSD
	0.194	0.090	0.063		*0.011*
Nematode	N0	N1000	N2000	N4000	
	0.118	0.112	0.117	0.116	*ns*
Inoculation time	0	1	2		
	0.099	0.101	0.148		*0.011*
Nematode	N0	N1000	N2000	N4000	
Fungus					
Nil	0.188	0.187	0.211	0.192	
Ggt (low)	0.098	0.091	0.077	0.093	*ns*
Ggt (high)	0.070	0.058	0.063	0.060	
Inoculation time	0	1	2		
Fungus					
Nil	0.206	0.196	0.184		
Ggt (low)	0.053	0.064	0.148		*0.019*
Ggt (high)	0.037	0.045	0.109		
Nematode	N0	N1000	N2000	N4000	
Inoculation time					
0	0.099	0.093	0.104	0.099	
1	0.100	0.099	0.104	0.101	*ns*
2	0.156	0.144	0.144	0.148	

Ggt= *G. graminis*. Inoculation time: 0= fungus at sowing, nematodes two weeks later; 1=fungus and nematodes at sowing; 2= nematodes at sowing, fungus two wees later. N0= no nematodes added, N1000= 1000 nematodes/plant, N2000= 2000 nematodes/plant, N4000= 4000 nematodes/plant.

Table 5.10 Effect of nematode-fungus (*G. graminis*) interaction on the root dry weight (g/plant) of wheat cultivar Machete 49 days after sowing in a pasteurised soil under controlled glasshouse conditions. Values in the 3-way table are the average of five single plant blocks.

	Nil			Ggt (low)			Ggt (high)		
Nematode	0	1	2	0	1	2	0	1	2
N0	0.214	0.246	0.221	0.044	0.021	0.195	0.018	0.032	0.109
N1000	0.217	0.257	0.192	0.023	0.060	0.183	0.025	0.021	0.102
N2000	0.334	0.226	0.197	0.025	0.046	0.142	0.032	0.020	0.092
N4000	0.252	0.216	0.170	0.071	0.038	0.151	0.035	0.019	0.128

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD at P= 0.05 level).

Fungus	Nil	Ggt (low)	Ggt (high)		LSD
	0.228	0.084	0.051		*0.002*
Nematode	N0	N1000	N2000	N4000	
	0.122	0.120	0.124	0.120	*ns*
Inoculation time	0	1	2		
	0.107	0.100	0.157		*0.002*

Nematode	N0	N1000	N2000	N4000	
Fungus					
Nil	0.227	0.222	0.252	0.212	
Ggt (low)	0.087	0.089	0.071	0.091	*ns*
Ggt (high)	0.053	0.049	0.048	0.056	

Inoculation time	0	1	2		
Fungus					
Nil	0.254	0.236	0.195		
Ggt (low)	0.041	0.041	0.167		*0.03*
Ggt (high)	0.027	0.023	0.107		

Nematode	N0	N1000	N2000	N4000	
Inoculation time					
0	0.092	0.088	0.131	0.119	
1	0.100	0.113	0.097	0.091	*ns*
2	0.175	0.159	0.144	0.151	

Ggt= *G. graminis*. Inoculation time: 0= fungus at sowing, nematodes two weeks later; 1=fungus and nematodes at sowing; 2= nematodes at sowing, fungus two wees later. N0= no nematodes added, N1000= 1000 nematodes/plant, N2000= 2000 nematodes/plant, N4000= 4000 nematodes/plant.

Sequential or simultaneous inoculation of fungus and nematode significantly affected root and shoot dry weight as well as nematode number extracted from roots. Plants inoculated with the fungus two weeks prior to the nematode showed a significant reduction in both root and shoot dry weight (Tables 5.9 and 5.10). Shoot and root dry weight decreased by 32% and 30% respectively where fungus was added before the nematode and by 31% and 38% respectively when added at the same time as nematodes, compared to when fungus was added two weeks after nematode inoculum (Tables 5.9 and 5.10).

However, shoot and root weight differed, with healthy roots significantly heavier than fungus infected ones, irrespective of nematode inoculum (Tables 5.9 and 5.10). Nematode density did not significantly effect shoot dry weight (P= 0.05), although there was a small reduction with 1000 nematodes/plant and an increase with 2000 or 4000 nematodes/plant (Table 5.9). Also, root dry weight was not affected by the nematode when compared to the control, but roots inoculated with 2000 nematodes had a significantly higher weight compared to those inoculated with 1000 nematodes (Table 5.10).

Prior inoculation of roots with the nematode reduced the infection of roots by the fungus *G. graminis*. On the other hand, invasion of wheat roots by take-all increased when fungus was added prior to nematodes or at the same time as nematodes (at planting) which resulted in decreased root and shoot dry weights. When nematode infection was established on roots, invasion of take-all was reduced. This effect was not due to reduction in root size (as determined by root dry weight) since the population of nematodes also increased by 69% compared to those inoculated with the fungus at the time of planting and by 33% compared to those inoculated with both fungus and nematode at the time of planting (Table 5.8).

5.3.1.3 Experiment 3

The analyses of variance for all measurements are shown in Table 5.11.

Tiller numbers/plant: Number of tillers/plant was not significantly affected by any of the treatments.

Root lesion rating: The effects of both nematode and fungus alone on root lesion rating were significantly greater when compared to the control (no nematodes or fungus added). At 2000 or 4000 nematodes/plant, root lesion rating increased by 78% and 75%, respectively, compared to the control (no nematode or fungus added) (Table 5.12). Root damage increased with the increase in fungus inoculum from four to eight propagules/pot by 87% and 89%, respectively, compared to the control (no nematodes or fungus added) (Table 5.12). However, with the combination of nematode and fungus, root lesion rating was further increased.

With 2000 or 4000 nematodes/plant and the higher level of fungus inoculum (8 propagules per pot), root lesion rating was increased by 56% and 62%, respectively, compared to inoculation with nematodes alone at either level, and by 11% and 13%, compared to the effect of the fungus alone (Table 5.12).

Although there was a significant difference between different levels of both nematode and fungus, combinations of both had no significant effect on root lesion rating when compared to the effects of either alone. However, root lesion rating was not significantly affected by different nematode-fungus inoculation times, although there was a 13% reduction in root lesion rating when fungus was added later compared to other inoculation times.

Table 5.11 Summary of analyses of variance for the effect of interaction between *R. solani* and *P. neglectus* on extent of root lesioning, number of nematodes/plant and nematodes/g dry root, shoot and root dry weights and total dry weight of plants for wheat cultivar Machete, 49 days after sowing (Experiment 3).

Source	df	MS RL	P	MS N/p (log)	P	MS N/gdr (log)	P	MS dws/p	P	MS dwr/p	P	MS tdw/p	P
Block	4												
Fungus (fun)	2	65.08	ns	0.12	ns	0.25	*	1.210	***	0.136	***	2.4	***
Nematode (nem)	3	2.5	***	29.67	***	32.13	***	0.244	***	0.017	**	0.396	***
Time	2	1.025	*	8.67	***	10.53	***	1.463	***	0.042	***	2.07	***
Fungus × nematode	6	0.55	ns	0.06	ns	0.10	ns	0.134	***	0.010	**	0.226	***
Fungus × time	4	0.24	ns	0.02	ns	0.05	ns	0.089	***	0.008	*	0.12	**
Nematode × time	6	0.09	ns	0.63	***	0.67	***	0.85	**	0.007	*	0.129	**
Nem × fun × time	12	0.536	ns	0.06	ns	0.07	ns	0.085	***	0.003	ns	0.132	***
Residual	140	0.409		0.07		0.08		0.021		0.004		0.033	

*** significant at P= 0.001 ** significant at P= 0.01 * significant at P= 0.05 ns= not significant P= Probability.
RL= root lesioning; N/p= nematodes/plant; N/gdr= nematodes/g dry root; dws/p= shoot dry weight/plant; dwr/p= root dry weight/plant; tdw/p= total dry weight/plant.

Table 5.12 Effect of nematode-*R. solani* (Rs) interaction on the root lesion rating (0-5) of wheat cultivar Machete 49 days after sowing in a pasteurised sandy soil under controlled glasshouse conditions. Values in the 3-way table are the average of five single plant blocks.

	Nil			Rs (low)			Rs (high)		
Nematode	0	1	2	0	1	2	0	1	2
N0	0.30	0.50	0.00	2.20	2.00	2.40	2.90	2.40	2.20
N1000	0.80	0.60	0.20	2.05	2.70	2.10	2.75	2.80	2.50
N2000	1.45	1.90	0.70	2.55	2.75	2.19	2.70	3.00	2.75
N4000	0.60	1.25	1.40	2.60	2.20	2.25	3.00	3.00	2.60

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD at P= 0.05 level).

Fungus	Nil	Rs (low)	Rs (high)		LSD
	0.80	2.33	2.72		*0.23*
Nematode	N0	N1000	N2000	N4000	
	1.65	1.83	2.22	2.10	*0.27*
Inoculation time	0	1	2		
	1.99	2.09	1.77		*0.23*
Nematode	N0	N1000	N2000	N4000	
Fungus					
Nil	0.26	0.53	1.35	1.08	
Rs (low)	2.20	2.28	2.50	2.35	*ns*
Rs (high)	2.50	2.68	2.82	2.87	
Inoculation time	0	1	2		
Fungus					
Nil	0.78	1.06	0.57		
Rs (low)	2.35	2.41	2.23		*ns*
Rs (high)	2.84	2.80	2.51		
Nematode	N0	N1000	N2000	N4000	
Inoculation time					
0	1.80	1.86	2.23	2.06	
1	1.63	2.03	2.55	2.15	*ns*
2	1.53	1.60	1.88	2.08	

Ggt= *G. graminis*. Inoculation time: 0= fungus at sowing, nematodes two weeks later; 1=fungus and nematodes at sowing; 2= nematodes at sowing, fungus two wees later. N0= no nematodes added, N1000= 1000 nematodes/plant, N2000= 2000 nematodes/plant, N4000= 4000 nematodes/plant.

***Nematode number*:** Number of nematodes/plant and nematodes/g dry root was increased by the addition of nematodes and by inoculation time. As the nematode inoculum rate increased, number of nematodes/plant also increased at all three densities (Tables 5.13). Number of nematodes/g dry root showed a similar pattern as for nematodes/plant.

The two way interaction between nematode and fungus and nematode × inoculation time on number of nematodes extracted from roots was significant (P= 0.05). At 1000 nematodes/plant and low fungus inoculum, number of nematodes extracted from the root systems decreased. However, there was no difference between plants inoculated with fungus and uninoculated plants (Table 5.13).

Different nematode and fungus inoculation times were significantly (P=0.001) different. Pre-inoculation of pots with fungus and two weeks later with nematodes resulted in a significant reduction (79%) in number of nematodes extracted from roots compared to when nematodes were added before fungus inoculum (Table 5.13). A 68% reduction in nematode number also resulted when plants were inoculated with nematode and fungus at the same time (Table 5.13). However, a 34% increase in nematode number resulted where nematode and fungus were added to the pots at planting compared to those inoculated with nematodes at planting and fungus inoculum added two weeks later (Table 5.13).

There was a significant increase in nematode numbers/plant and nematodes/g dry root with increase in initial density. At 4000 nematodes/plant, there was an increase of 80% and 70%, respectively, in final number of nematodes compared to 1000 or 2000 nematodes/plant, respectively. However, there was no difference between the 1000 and 2000 nematode densities.

Table 5.13 Effect of nematode-*R. solani* (Rs) interaction on the number of nematodes/plant (log) 49 days after sowing in a pasteurised soil under controlled glasshouse conditions. Values in the 3-way table are the average of five single plant blocks. Data are natural log transformed.

	Nil			Rs (low)			Rs (high)		
Nematode	0	1	2	0	1	2	0	1	2
N0	1.52	1.67	1.81	1.56	1.77	1.74	1.56	1.69	1.88
N1000	2.53	3.08	3.21	2.60	2.89	3.27	2.71	3.21	3.38
N2000	2.44	2.99	3.58	2.42	3.35	3.52	2.63	3.17	3.38
N4000	2.84	3.92	3.80	3.03	4.03	4.05	3.03	3.84	3.95

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD at P= 0.05 level).

Fungus	Nil	Rs (low)	Rs (high)		LSD
	2.78	2.85	2.87		*ns*
Nematode	N0	N1000	N2000	N4000	
	1.69	2.99	3.05	3.61	*0.11*
Inoculation time	0	1	2		
	2.41	2.97	3.14		*0.09*
Nematode	N0	N1000	N2000	N4000	
Fungus					
Nil	1.67	2.94	3.00	3.52	
Rs (low)	1.96	2.92	3.10	3.70	*ns*
Rs (high)	1.71	3.11	3.06	3.61	
Inoculation time	0	1	2		
Fungus					
Nil	2.33	2.91	3.10		
Rs (low)	2.40	3.01	3.15		*ns*
Rs (high)	2.48	2.98	3.15		
Nematode	N0	N1000	N2000	N4000	
Inoculation time					
0	1.55	2.62	2.50	2.96	
1	1.71	3.06	3.17	3.93	*0.19*
2	1.81	3.29	3.49	3.94	

Ggt= *G. graminis*. Inoculation time: 0= fungus at sowing, nematodes two weeks later; 1=fungus and nematodes at sowing; 2= nematodes at sowing, fungus two wees later. N0= no nematodes added, N1000= 1000 nematodes/plant, N2000= 2000 nematodes/plant, N4000= 4000 nematodes/plant.

Interaction between nematode and inoculation time was also significant (P= 0.001). Pre-inoculation or inoculation of plants with nematodes at planting resulted an increase of final nematode number at all nematode densities (Table 5.13). At 1000 or 2000 nematodes/plant, final number increased when nematodes were added before fungus inoculum compared to those inoculated with nematode and fungus at planting or pre-inoculation with fungus (Table 5.13). A significant, positive, linear regression was found between nematode numbers/plant and nematodes/g dry root (Figure 5.5).

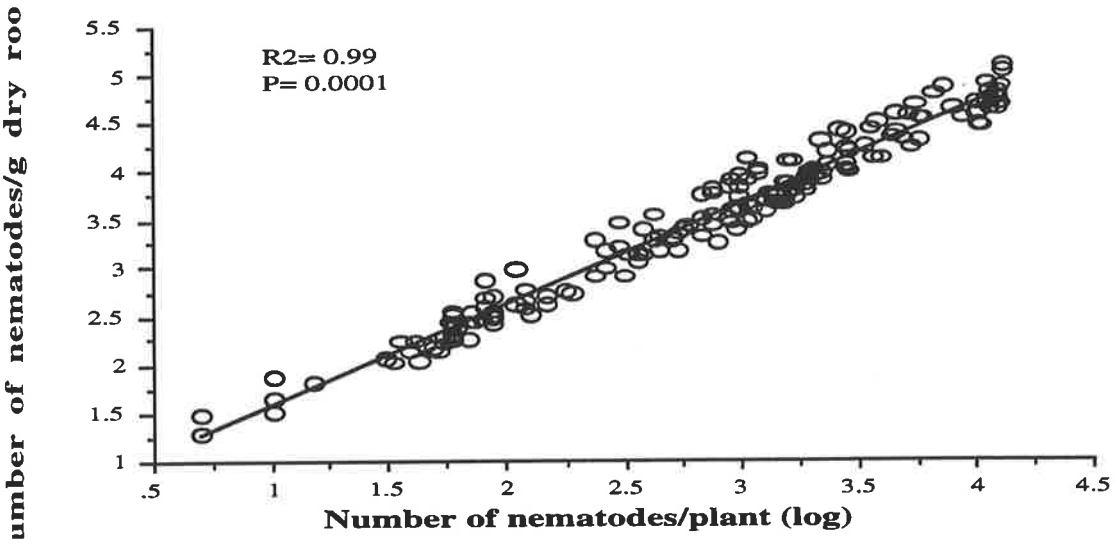


Figure 5.5 The relationship between number of nematodes/plant and number of nematodes/g dry root 49 days after inoculation.

***Dry matter*:** Shoot dry weight was significantly affected by fungus, nematode, and inoculation time (Table 5.14). Root dry weight was also significantly affected by the 2-way interaction between nematode and fungus and by inoculation time and fungus (Table 5.15).

In the presence of *R. solani*, shoot and root dry weights were increased significantly (P= 0.01). With low (4 propagules/pot) fungus inoculum, both shoot and root dry weight increased by 44% and 39%, respectively, and by 33% and 2%, respectively, with high inoculum (8 propagules/pot) compared to the control (no fungus added) (Tables 5.14 and 5.15). Nematodes, however, reduced both root and shoot dry weight of plants

significantly (Tables 5.14 and 5.15). With 4000 nematodes/plant, root and shoot dry weights were reduced by 15% and 30%, respectively, compared to the control (no nematodes added) (Tables 5.14 and 5.15).

The two way interaction between nematode and fungus on shoot and root dry weight was also significant (P= 0.01). While there was no interaction between nematode and fungus on either root or shoot dry weight, there was a significant increase in dry matter where fungus was applied at either density in combination with the nematode compared to the effects of the nematode alone. Nematodes alone at 1000 or 4000 nematodes/plant reduced both root and shoot dry weight by 47% and 77%, or 35% and 41%, respectively, compared to the control (no nematode or fungus added) (Tables 5.14 and 5.15). However, with 2000 nematodes/plant, there was no significant reduction of root and shoot dry weight compared to the control.

The effect of different inoculation times on the fungus-nematode interaction was significant (P= 0.001) (Table 5.11). Shoot dry weight was reduced by 46% when fungus inoculum was added to the pots two weeks after nematodes compared to when fungus was added before or at the same time as nematodes (Table 5.14). A 20% reduction in shoot and root dry weight also occurred when fungus was added two weeks after nematode inoculum (Tables 5.14 and 5.15).

Total dry weight of plants, however, was reduced by 38% when fungus was applied after nematode inoculum. Total dry weight of plants was significantly affected by different nematode and fungus inoculation times. Inoculation of pots with fungus two weeks after planting reduced plant dry matter by 22% compared to when the fungus was added at the time of planting irrespective of nematode inoculum. However, with 1000, 2000 or 4000

Table 5.14 Effect of nematode-*R. solani* interaction on the shoot dry weight (g/plant) of wheat cultivar Machete 49 days after sowing in a pasteurised soil under controlled glasshouse conditions. Values in the 3-way table are the average of five single plant blocks.

	Nil			Rs (low)			Rs (high)		
Nematode	0	1	2	0	1	2	0	1	2
N0	0.35	0.71	0.52	0.77	0.74	0.35	0.64	0.69	0.34
N1000	0.58	0.13	0.14	0.75	0.68	0.39	0.66	0.68	0.38
N2000	0.47	0.61	0.24	0.78	0.75	0.38	0.68	0.64	0.34
N4000	0.10	0.13	0.13	0.72	0.60	0.34	0.63	0.61	0.33

(3-way interaction is significant) LSD 5%= *0.19*

1 and 2-way treatment means (with appropriate LSD at P= 0.05 level). LSD

Fungus	Nil	Rs (low)	Rs (high)		
	0.34	0.61	0.55		*0.05*
Nematode	N0	N1000	N2000	N4000	
	0.57	0.49	0.54	0.40	*0.06*
Inoculation time	0	1	2		
	0.59	0.58	0.32		*0.05*
Nematode	N0	N1000	N2000	N4000	
Fungus					
Nil	0.53	0.28	0.44	0.12	
Rs (low)	0.62	0.61	0.64	0.55	*0.11*
Rs (high)	0.55	0.57	0.55	0.52	
Inoculation time	0	1	2		
Fungus					
Nil	0.38	0.39	0.26		
Rs (low)	0.75	0.70	0.36		*0.10*
Rs (high)	0.65	0.65	0.35		
Nematode	N0	N1000	N2000	N4000	
Inoculation time					
0	0.59	0.66	0.65	o.48	
1	0.71	0.50	0.67	0.45	*0.11*
2	0.40	0.30	0.32	0.26	

Rs= *R. solani*. Inoculation time: 0= fungus at sowing, nematodes two weeks later; 1=fungus and nematodes at sowing; 2= nematodes at sowing, fungus two wees later. N0= no nematodes added, N1000= 1000 nematodes/plant, N2000= 2000 nematodes/plant, N4000= 4000 nematodes/plant.

Table 5.15 Effect of nematode-*R. solani* (Rs) interaction on the root dry weight (g/plant) of wheat cultivar Machete 49 days after sowing in a pasteurised sandy soil under controlled glasshouse conditions. Values in the 3-way table are the average of five single plant blocks.

	Nil			Rs (low)			Rs (high)		
Nematode	0	1	2	0	1	2	0	1	2
N0	0.223	0.246	0.263	0.311	0.248	0.256	0.265	0.286	0.238
N1000	0.215	0.135	0.127	0.343	0.266	0.265	0.254	0.264	0.214
N2000	0.226	0.233	0.170	0.364	0.296	0.238	0.287	0.276	0.220
N4000	0.111	0.189	0.131	0.293	0.275	0.212	0.275	0.275	0.231

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD at P= 0.05 level).

Fungus	Nil	Rs (low)	Rs (high)		LSD
	0.189	0.281	0.255		*0.022*
Nematode	N0	N1000	N2000	N4000	
	0.259	0.231	0.257	0.219	*0.026*
Inoculation time	0	1	2		
	0.264	0.247	0.214		*0.022*
Nematode	N0	N1000	N2000	N4000	
Fungus					
Nil	0.244	0.159	0.210	0.143	
Rs (low)	0.272	0.291	0.299	0.260	*0.042*
Rs (high)	0.263	0.244	0.261	0.254	
Inoculation time	0	1	2		
Fungus					
Nil	0.194	0.200	0.173		
Rs (low)	0.328	0.271	0.243		*0.039*
Rs (high)	0.270	0.271	0.226		
Nematode	N0	N1000	N2000	N4000	
Inoculation time					
0	0.266	0.271	0.292	0.226	
1	0.260	0.221	0.268	0.241	*ns*
2	0.252	0.202	0.209	0.191	

Rs= *R. solani*. Inoculation time: 0= fungus at sowing, nematodes two weeks later; 1=fungus and nematodes at sowing; 2= nematodes at sowing, fungus two wees later. N0= no nematodes added, N1000= 1000 nematodes/plant, N2000= 2000 nematodes/plant, N4000= 4000 nematodes/plant.

nematodes/plant, total dry weights decreased by 45%, 48% and 33%, respectively, compared to treatments with nematodes added at the same time or after fungus inoculum.

Generally, as shown in Tables 5.14 and 5.15, there was a reduction in both shoot and root dry weight as the population of *P. neglectus* in the root system increased.

5.3.2 Field experiments

The analyses of variance for all measurements are shown in Table 5.16.

Minimum and maximum soil temperatures at 10cm depth on June 20, 25 and 30 and July 1, 10, 20 and 30 were 12/15°C, 7/12°C and 9/12°C and 7/14°C, 8/13°C, 9/14°C and 8/14°C, respectively. Soil remained cold throughout August but temperature increased to 15°C average in September. Minimum and maximum soil temperatures at 10cm depth on September 1, 15 and 30 and October 1, 15 and 30 were 12/15°C, 14/23°C or 20/26°C and 16/22°C, 18/26°C and 18/27°C, respectively. The warmest soil temperature (20/30°C) during this period occurred on October 25 (data recorded by Minnipa Research Centre staff).

Root lesion rating: Little difference in root-rotting or plant growth occurred among the two wheat cultivars at either sample date. Plant growth and root lesion rating also were similar for either *R. solani* or *G. graminis,* but a little higher with *G. graminis*. Both fungi were re-isolated from all root segments plated from corresponding treatments, confirming that the fungus inoculum was well established on the roots.

There was no evident *R. solani* root-rot native inocula in the un-infested control treatment, but *G. graminis* was isolated from control plots and rated at the low frequency. Both *R. solani* and *G. graminis* caused a high level of damage on seminal roots at the first sample date (eight weeks after sowing). At the second sample date (twelve weeks after sowing), both seminal and crown roots were colonised by either pathogen (*G. graminis* or *R. solani*) or both pathogens. At either sample date (eight or twelve weeks after

Table 5.16 Summary of analyses of variance for the effect of interaction between *G. graminis* var. *tritici*, *R. solani* and *P. neglectus* on number of nematodes/g dry root at eight and twelve weeks, total dry biomass of plants, total head number/sample, number of heads (minus dead heads), seed weight/head and total grain yield for wheat cultivars Machete and Spear in the field (Minnipa Research Centre) in the 1993 growing season (Field Experiment).

Source	df	MS N/gdr (8 weeks)	P	MS N/gdr (12 weeks)	P	MS bio	P	MS total heads	P	MS heads (-dead)	P	MS sw /head	P	MS Grain yield	P
Block	3														
Fungus (fun)	3	1.597E8	***	6.756E6	***	333	***	179	ns	156	ns	0.261	***	10050	*
Nematode (nem)	1	3.322E7	**	2.985E7	**	334	***	661	***	688	***	0.004	ns	35079	***
Cultivar	1	1.630E7	**	8.747E6	*	2.5	ns	0.141	ns	1	ns	0.002	ns	20235	**
Fungus × nematode	3	2.995E7	**	2.858E7	**	107	*	158	ns	184	ns	0.007	ns	5543	*
Nematode × cultivar	1	7.486E6	*	1.539E7	**	83	ns	25	ns	21.8	ns	0.021	ns	67	ns
Fungus × cultivar	3	1.845E7	*	5.810E6	ns	32	ns	166	ns	169	ns	0.013	ns	121	ns
Nem × fun × cultivar	3	2.092E7	**	5.230E6	ns	17	ns	27	ns	35.8	ns	0.008	ns	663	ns
Residual	45	6.445E7		6.952E7		43		84		82		0.011		1659	

*** significant at P= 0.001 ** significant at P= 0.01 * significant at P= 0.05 ns= not significant P= Probability.
N/gdr= nematodes/g dry root; Bio= shoot dry weight/plant at maturity; sw/head= seed weight (g/head).

sowing), roots from plots inoculated with the combination of *R. solani* and *G. graminis* showed less severe root-rot symptoms, particularly in the plots treated with Temik®.

Application of Temik® had a great effect on severity of both pathogens. In plots where nematodes were controlled by application of Temik® and inoculated with *R. solani,* there was greater plant growth and less root-rot symptoms on either wheat cultivar at either sample date. Seedlings infected with *G. graminis* were always shorter than seedlings in control plots or plots inoculated with *R. solani*. In contrast, *R. solani* never suppressed seedling height in this experiment.

Number of nematodes: Application of the nematicide Temik® significantly (P=0.001) reduced nematode number in root systems of all wheat cultivars. At the first sampling (eight weeks after sowing), Temik® had reduced nematode number by 95% compared to those with no nematicide (Figure 5.6a). At the second sampling there was a 98% reduction in nematode number where Temik® was applied (Figure 5.6b). Of the two wheat cultivars tested, Spear showed a significantly lower nematode number in its roots compared to Machete (Figure 5.6a). Wheat cultivar Machete had 45% and 51% more nematodes at the first and second sample dates, respectively, compared to Spear (Figure 5.6a).

At the first and the second sampling, *R. solani* in the control plots with no application of nematicide increased nematode numbers by 65% compared to control (no fungus added) (P= 0.05) (Figure 5.6). *G. graminis* had no effect on nematode number at the first sampling. However, *G. graminis* in combination with *R. solani* increased the nematode population by 39% compared to *G. graminis* alone or the control (no fungus added) (Figure 5.6b).

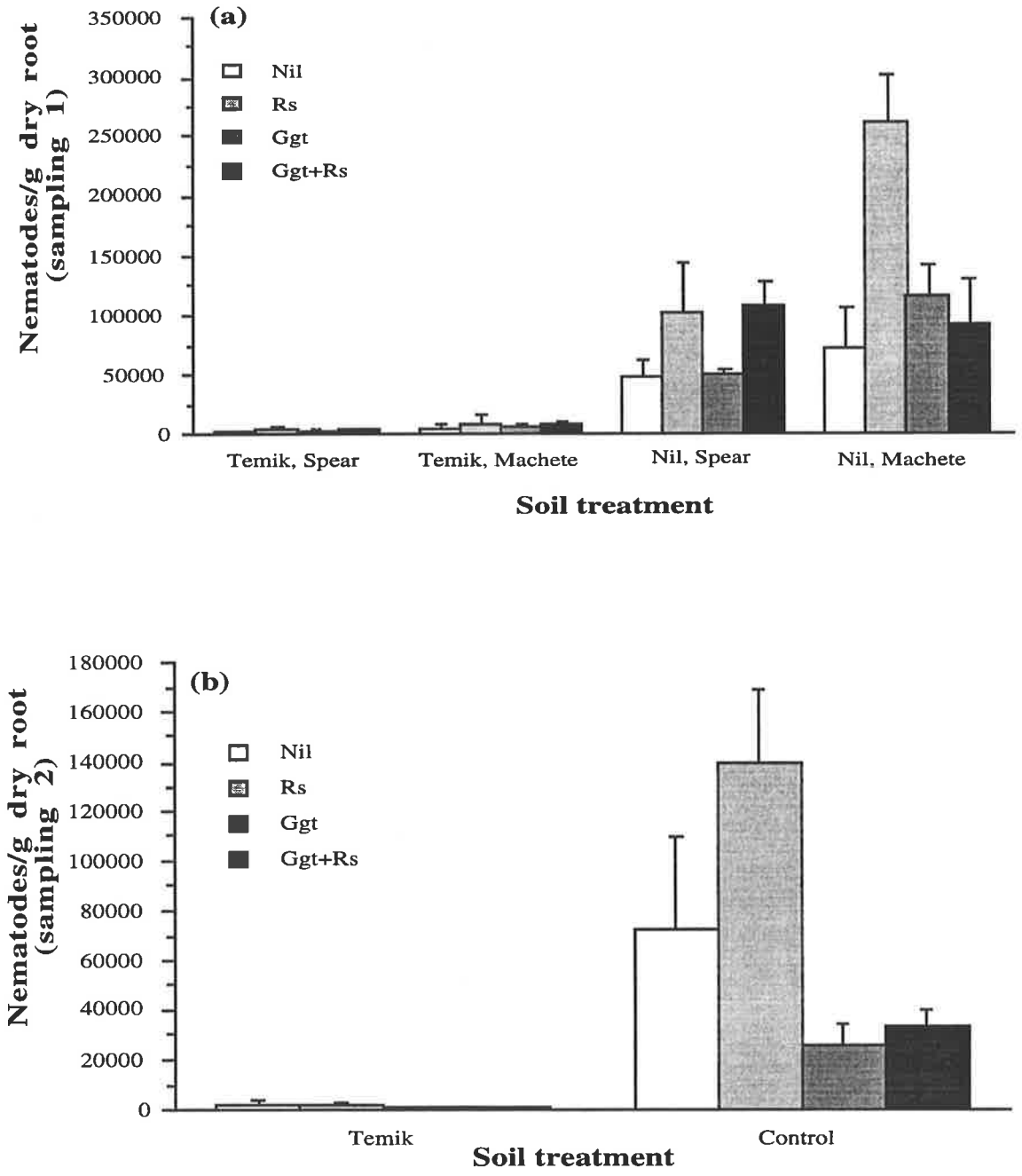


Figure 5.6 The effect of nematicide (Temik®) on number of nematodes/g dry root **(a)** eight weeks after sowing and **(b)** twelve weeks after sowing on number of nematodes extracted from roots of Spear or Machete eight weeks after sowing under natural conditions in the field (Minnipa Research Centre). Nil= no fungus added, Rs= *R. solani*, Ggt= *G. graminis*.

Interaction between nematicide and wheat varieties (Spear and Machete) was significant (P= 0.05). Both cultivars showed an extremely low number of nematodes in the roots at the first and second sample dates where the nematodes were controlled by application of nematicide. In control plots, however, wheat cultivar Spear supported fewer nematodes than Machete. In all fungus treatments too, Spear showed a lower number of nematodes than Machete, and there were no differences between the two cultivars with *G. graminis* or *G. graminis+R. solani* inoculum treatments and the control (no fungus added) (Figure 5.6).

At the second sampling, where *R. solani* inoculun was added, there was a 42% increase in number of nematodes compared to the control (no fungus added), although this was not statistically significant (Figure 5.6).

With *G. graminis* or *G. graminis+R. solani* in control plots with no application of nematicide, nematode number decreased by 59% and 55% compared to control (no fungus added) or by 77% and 75% compared to *R. solani* (P= 0.01). The influence of *R. solani* on both wheat cultivars was similar in increasing nematode populations. *G. graminis*, however, increased nematode number in Machete but not in Spear and there was no significant difference between control with no fungus inoculum and *G. graminis* or *G. graminis+R. solani* for Spear and *R. solani*, *G. graminis* or *G. graminis+R. solani* for Machete (Figure 5.6).

At harvest, number of heads per 0.16 m^2 subsample of each plot was significantly affected by fungus inoculum, nematode and the two way interaction between nematode and fungus (Table 5.16). Nematodes in plots with no application of nematicide increased total number of heads but, excluding sterile heads, there was a 10% increase in number of heads where nematodes were controlled by application of nematicide (Figure 5.7a). Temik® also increased head size (g/head) 14% compared to the untreated plots (control) (Figure 5.7b). However, grain yield increase averaged 8% where the nematode was controlled by application of nematicide.

Fungus inoculum, however, in most cases showed a significant effect on grain yield. *G. graminis* alone decreased total number of heads by 21% compared to control (no fungus added) (Figure 5.8). Grain yield was also decreased by 22% where *G. graminis* inoculum was added to the soil compared to the control (no fungus added) (Figure 5.8). Inoculum of *R. solani* or *G. graminis+R. solani*, however, had no significant effects on grain yield compared to when fungus was not added to the soil (Figure 5.8).

The two way interaction effects of nematode and fungus were also significant on most measured characters. With *G. graminis*, total number of heads decreased by 21% where the nematode was controlled using nematicide. However, excluding dead heads, there was a 13% increase in heads with *R. solani* and 14% increase with *G. graminis+R. solani* or control (no fungus added) where nematodes were controlled with application of nematicide (Figure 5.7a).

Number of seeds per head was also affected by the fungus and nematode interaction. Application of Temik® increased number of seeds/head by 13% where *R. solani* or *G. graminis* was added to the plots. In control plots with no fungus inoculum, head number increased similarly. Combined effects of *G. graminis+R. solani* and nematode decreased seed weight of each head by 22% where nematicide was not applied to control nematodes (Figure 5.7b).

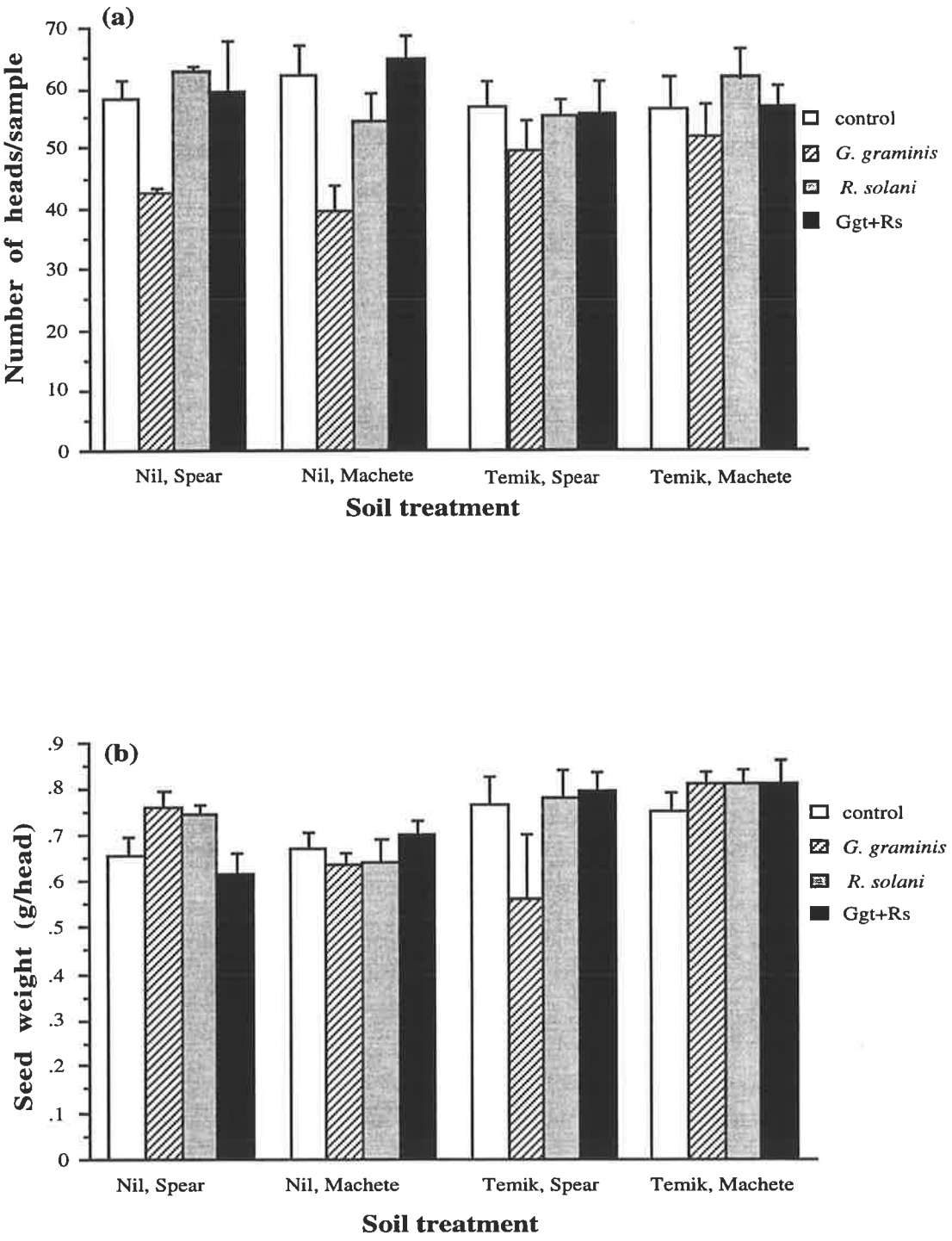


Figure 5.7 The effect of 3-way interaction on **(a)** number of heads/sample (0.16 m^2) collected at maturity and **(b)** seed weight (g/head) of both wheat cultivars (Spear and Machete).

Grain yield was significantly affected by either fungus inoculum. *R. solani* increased grain yield by 13% where the nematode was controlled. Similarly, in control plots with no fungus inoculum, grain yield increased by 13% where the nematode was controlled (Figure 5.8). Therefore, there was no difference between plots with or without *R. solani*. *G. graminis* alone and/or in combination with the nematode decreased plant growth and grain yield by 33% compared to the control (no fungus added) and by 9% compared to when nematodes were not controlled (Figure 5.8). With *G. graminis*+*R. solani* grain yield increase averaged 6% where the nematode was controlled. However, with application of Temik® in control plots (no fungal inoculation), grain yield was increased by 12.3% for Spear and by 20% for Machete compared to plots with no Temik® (Figure 5.8).

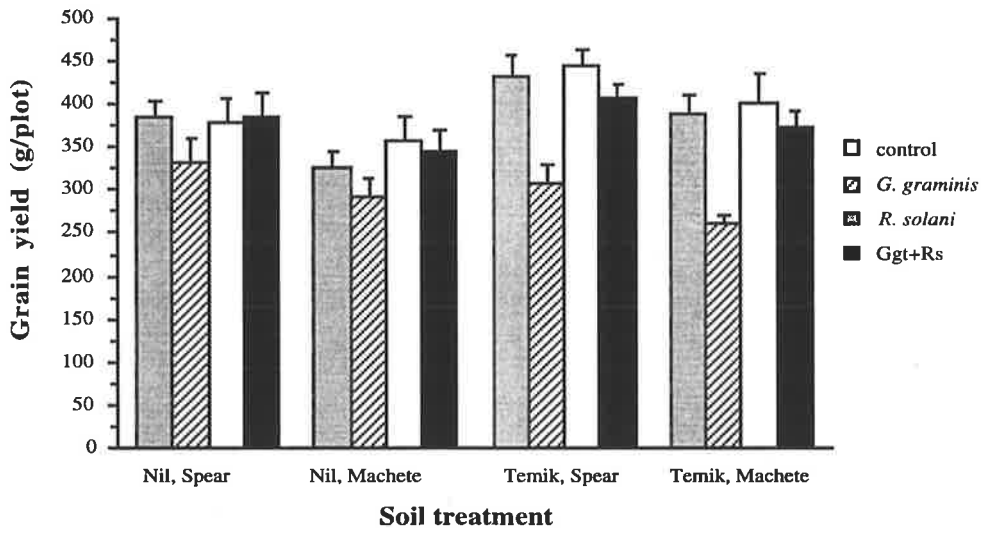


Figure 5.8 The effect of nematode-fungus interaction on grain yield of wheat cultivars Spear and Machete grown under natural field conditions over the 1993 growing season at Minnipa Research Centre.

5.4 Discussion

Antagonism between *G. graminis* and *R. solani* occurred both in the glasshouse and in the field. The term antagonism is used where interactions between plant parasitic nematodes and soil-borne fungi result in less plant damage, compared to the sum of the individual damage (Wallace, 1983). Combination of *G. graminis* and *R. solani* resulted in less root damage compared to *G. graminis* alone. *G. graminis* alone both in the glasshouse and in the field caused severe root damage particularly late in the season. *R. solani,* however, caused severe root lesioning early in the season but later plants seemed to recover from the damage caused by the fungus and gave a reasonable yield at harvest. However, the combination of these two pathogens and *P. neglectus* did not increase root lesioning and grain yield from corresponding plots and was similar compared to the control (no fungal inoculum added).

Wheat cultivar Machete suffered more damage to its root system than Spear, suggesting that this cultivar is more susceptible to fungal and nematode diseases. But, Machete produced more tillers than Spear which may have resulted in even greater yield reduction because unhealthy root systems suffer even more damage late in the season, and may be unable to support the number of tillers later in the season. The result will be many sterile tillers and finally yield losses. It is not clear what causes this stimulation of growth to occur. One possibility might be that plants are trying to recover from the extensive damage caused by these organisms. This, indeed, would result in more tillers early in the season but at maturity, due to extensive root damage, the plant would be unable to support the extra tillers, and therefore most of them would be sterile.

The number of nematodes extracted from the roots of both cultivars was affected significantly by fungus inoculation. *R. solani* increased number of *P. neglectus* on Spear, to three times more than on Machete. However, the degree of root lesioning on Machete was twice as much as Spear, and may be the reason why yield loss with Machete was greater than Spear. As mentioned earlier in this section, *Pratylenchus* spp. are

known to vacate damaged and rotted roots in order to find a new food source and penetrate new roots.

In the second experiment, interaction between *G. graminis* and *P. neglectus* was examined using several levels of either pathogen and different inoculation times. *G. graminis* at both low (8 propagules) and high (16 propagules/plant) levels caused severe damage to the plant, increased root lesioning and decreased plant dry matter. With 16 propagules/plant, some plants died. This indicates that the isolate of fungus used (Ggt-500) was highly pathogenic to wheat, particularly the cultivar Machete.

Timing of inoculation of fungus and nematode also had a significant effect on plant growth and nematode multiplication. Pre-application of fungus or inoculation at the same time as the nematodes at sowing caused significantly more damage to the plant. Roots were severely lesioned and the plants had very poor growth. General shoot yellowing was noticeable. This suggests that the fungus grew rapidly and penetrated almost all of the root system, causing severe damage to the plant. Favourable conditions for *G. graminis* growth are moist soil, higher soil temperature and high soil pH. All these conditions were maintained in this experiment. Severe damage in the field tends to be associated with a wet, early season, and sandy loam soils of high pH.

Pre-inoculation with nematodes, on the other hand, reduced disease rating caused by *G. graminis*. This may be a simple competitive relationship between the two organisms for penetration sites. Nevertheless, involvment of physiological and biochemical changes in the host are strongly suggested. It has been reported that *P. neglectus* alters host physiology causing greater susceptibility to *Verticillium* sp., even with a split root system where nematode inoculum was added to one part and the fungus to the other side (Faulkner *et al.*, 1970).

There are a number of synergistic interactions reported in the literature involving *R. solani* and *Pratylenchus* spp. on different crops. However, on wheat, there are few reports of experimentally assessed losses in yield due to *R. solani-Pratylenchus* spp. interactions, particularly in controlled glasshouse conditions.

The glasshouse experiment was designed to investigate the interaction between *R. solani* and *P. neglectus* on wheat under controlled conditions. *R. solani* alone increased root lesioning, but in combination with the nematode, further increase occurred. The fungus also increased number of *P. neglectus* in the roots, but not significantly.

Inoculation timing of both fungus and nematode had a significant effect on plant growth, root lesioning and number of nematodes extracted from roots. The greatest amount of root lesioning was observed when plants were inoculated with both fungus and nematodes at sowing. However, number of nematodes was high when nematodes were applied prior to the fungus. This may indicate that the nematodes are able to modify root systems or the root rhizosphere in a way that somehow favours fungus infection. This situation may put nematodes under stress and stimulate them to multiply faster. The other possibility is that after a host is occupied by nematodes, *R. solani* mycelium may be used as a source of food by the nematodes.

These observations suggest that early colonisation of the roots by *P. neglectus* encourages infection by *R. solani*, while *G. graminis* has the opposite effect. A similar result was observed by Stynes and Veitch (1983) who showed that in the field, where plants contained high levels of *P. neglectus* at three weeks after sowing, they were colonised by high levels of *R. solani* later in the season.

The field experiments described in this Chapter suggest that natural levels of *P. neglectus* found in the soil of the experimental site (Minnipa Research Centre) caused significant damage to the roots of the host and decreased grain yield. This supported the results of glasshouse experiments reported earlier in this chapter and in Chapter 4. High nematode numbers caused root damage and reduced plant growth and dry matter accumulation. Treatments with aldicarb had a major effect on nematode numbers and plant growth. Grain yield significantly increased where Temik® was applied at sowing. Adverse effects of Temik® on plant growth have been reported (Fisher, 1993). Therefore, application of nematicide before sowing may give a better indication of the real effect of nematodes on plant growth. It would give slightly earlier protection to the

seminal roots, and may control fungal diseases occurring early in the season, thus reducing the damage caused by the nematode-fungus interaction throughout the season. However, root lesioning was reduced where the nematode was controlled by application of Temik®.

These results partly agree with those of Stynes and Veitch (1983) that *R. solani* increases root lesioning and number of *P. neglectus* in the roots at early stages of plant growth (up to twelve weeks), but its combined effect on grain yield did not differ from the effect of the nematode alone. This may suggest that in fields with no history of *R. solani*, artificial inoculum of the fungus is not able to cause yield loss in the first year of its establishment in the soil.

G. graminis in plots treated with Temik® reduced grain yield by 48% but corresponding plots with no Temik® resulted in only 14% reduction in yield. This further supports previous evidence from glasshouse experiments (Section 5.3.1.3) that there is a negative interaction between *P. neglectus* and the fungus *G. graminis* both under controlled conditions and in the field.

R. solani, on the other hand, did not affect plant growth and grain yield in this experiment. The experimental site had no history of *Rhizoctonia* bare patch. Thus, the fungus may need to be stabilised in the soil before it can affect the host. Then *Rhizoctonia* bare patch would actually be a complex of different micro-organisms that cause damage to the plants. It has been well known for some time that *R. solani* is a major root pathogen of many crops including wheat, but why the fungus occurs in certain areas and not in others is not understood. However, the fungus increased number of *P. neglectus* in roots significantly. High nematode numbers in the soil may be important for subsequent crops, particularly if they are intolerant of *P. neglectus*.

R. solani however, cannot be completely disregarded as a cause of root damage, as there was some indication that this species is associated with *P. neglectus* (Chapter 4). The interaction between *R. solani* and *Pratylenchus* spp. has been studied on many crops, and in most of these studies a synergistic interaction has been reported. A synergistic

interaction between *R. solani* does occur on wheat in Canada, although this is detected as patches of poor growth in the crop (Benedict and Mountain, 1956). Since *R. solani* infects root tissues early in the season (Samuel and Garrett, 1932), it may play an important role in initiating root damage, although secondary organisms occur subsequently at higher frequencies in root tissues and cause more damage than the initial *R. solani* infection (Harris and Moen, 1985a, 1985b; Moen and Harris, 1985). However, with experiments reported here (glasshouse and field) the fungus increased nematode numbers and enhanced root lesioning but had no effect on plant growth and grain yield.

There are a number of reports on the negative relationship between *G. graminis* and *R. solani* (Stynes, 1975; Patel, 1983). Glasshouse and field studies of the interactions between these two major root pathogens confirm the previous finding by other researchers. Interestingly, the combination of these two pathogens and *P. neglectus* resulted in less damage than did either fungus alone. In the field, although *G. graminis* alone caused significant reduction in yield, the combination of *G. graminis* and *R. solani* had no effect on plant growth and grain yield as compared to the corresponding plots with no fungus inoculum.

This negative interaction could be explained in two ways. Firstly, competition for infection sites may occur, and the first fungus in the root may inhibit invasion by the other. Secondly, physiological and biochemical changes in the host caused by one pathogen may not favour infection by the other fungus. Further work on the interrelationship between *R. solani* and *G. graminis* is required.

A negative interaction between *G. graminis* and *P. neglectus* was also found in the field. It was shown that there is a negative relationship between *G. graminis* and *P. neglectus*. Presence of either pathogen reduced infection by the other. *G. graminis* alone in both glasshouse and field studies significantly damaged roots of wheat. In the glasshouse test inoculation with *G. graminis* with or without *P. neglectus* at sowing

increased root lesioning and reduced plant dry matter. Early infection of plants with *P. neglectus* resulted in less infection with *G. graminis* and increased plant growth.

Again, these results may be due to competition for infection sites. Infection by one pathogen may reduce the chance for the other pathogen to infect the plant. However, it is more likely to be due to physiological and biochemical changes in the host. Plants infected by *P. neglectus* may not be attractive and favourable for *G. graminis* infection. In the field, nematodes are among root pathogens penetrating in the very early stages of plant growth. Therefore, it is important to investigate further the relationship between root lesion nematode and *G. graminis* in order to determine what density of nematode is reducing the incidence and severity of *G. graminis,* while not causing problems for the plant.

It is not clear why this situation occurred later in the season, although other workers (Stynes, 1975) have reported for some crops that when both *G. graminis* and *P. neglectus* are present, the severity of disease is disproportionately high. It is possible that when plants are infected by one pathogen they may be protected (immune) from other pathogens. However, physiological and biochemical change in the host may also be involved. Nevertheless, the results reported in this Chapter are reliable, as they follow a similar pattern to the glasshouse experiments where early infection of plants by *P. neglectus* protected plants from *G. graminis* infection.

The importance of *P. neglectus* and associated fungi relates to the fact that the growth of plants between anthesis and maturity depends on the transport of water from the roots to the plant shoots. Any damage to the root system consequently induces water stress. Under the optimum conditions for plant growth and development, presence of the nematodes may not affect plant growth until the host is placed under stress, for instance water or nutritional stress.

When soil moisture is low late in the season, nematodes seem to migrate from dry soil to the root tissues and multiply faster in order to survive. This will cause considerable damage to the roots as well as increase inoculum level of both nematodes and soil fungi

that could be of danger for following crops. In August and September, there are increases in the nematode numbers in the roots and the population of fungi isolated from roots (particularly seminal). At this time of year water stress occurs, which may be favourable for nematode multiplication and development, and also suitable for fungal infection and growth. A large number of nematode eggs were detected in the root tissues at this time of year.

Chapter 6

Aseptic fungus-nematode interaction tests

6.1 Introduction

Microdochium bolleyi, *Fusarium acuminatum*, *Pyrenochaeta terrestris* or *Pythium irregulare* were frequently isolated from field samples (Chapter 3). Combinations of the above fungi and *Pratylenchus neglectus* caused severe damage to the roots of wheat and enhanced nematode multiplication (Chapter 4). Experiments were therefore conducted to further investigate and determine whether these fungi augment root damage caused by *P. neglectus*, thus causing root damage similar to that observed in the field.

Two aseptic inoculation experiments were conducted under controlled conditions in a growth chamber to determine the effect of different fungi on nematode penetration rates and to determine whether there was a significant interaction between the above mentioned root-rotting fungi and root lesion nematodes infecting wheat plants. A preliminary experiment (Experiment 1) involving two different nematode densities was undertaken. Machete wheat was inoculated with *M. bolleyi*, *F. acuminatum* or *P. terrestris* at one density and *P. neglectus* at two densities, and harvested at seven or fourteen days after inoculation, to determine the influence of different fungi on penetration rates of the nematode.

Experiment 2 was conducted to determine the effect of nematode-fungus interactions on plant growth and nematode multiplication. *M. bolleyi*, *F. acuminatum* or *P. terrestris* with or without *P. neglectus* (and also with or without mechanical lesions on the surface of seedling roots prior to planting) were added to the soil at three different inoculation times. Mechanical lesioning and different inoculation times were used to determine the role of *P. neglectus* in the nematode-fungus interaction.

To explain the role of nematodes in a nematode-fungus interaction there are several

possibilities, namely: nematodes may merely provide infection sites for fungi; they may change plant physiology to be attractive for both fungi and other nematodes; or the role of nematodes could be both by providing mechanical wounding and causing physiological changes in the host plants.

6.2 Methods

Pre-germinated, surface-sterilised seed of Machete wheat was planted in pots of steam pasteurised soil, as described in the General Methods for pot experiments. Each pot contained one plant. Soil used for these experiments was a sandy loam collected from Avon.

For Experiments 1 and 2, 500ml plastic jars with lids were used as the growing containers. Jars were filled with 200g of soil and autoclaved for one hour at 121°C on each of two successive days. The aim was to determine the pathogenicity of either fungus and the nematode and/or their interaction under completely aseptic conditions. Soil rather than agar was used for this experiment because agar medium by itself could be a rich source of nutrients for the fungi. Hence the pathogen may not prefer to colonise the root system within the experimental duration (five weeks).

6.2.1 Fungus and nematode inocula

Inocula of fungi was prepared on millet seed as described in the General Methods. Inocula of *M. bolleyi*, *F. acuminatum* or *P. terrestris* used for Experiments 2 and 3 were prepared on PDA plates grown for ten days. Each pot was inoculated with five 5mm diameter colonised agar cores of the appropriate fungus.

Aseptically grown nematodes were pipetted in 1ml of distilled water (as described in the General Methods) around each plant. Inoculum levels differed depending upon experimental aims and are indicated in the results of each experiment. Mechanical wounding on the root surface was made using a fine sterilised needle (15 wounds/root system).

Three inoculation times were used in Experiment 2: fungus at sowing, nematode two

weeks later (T0); fungus and nematode at sowing (T1); nematode at sowing, fungus two weeks later (T2). For Experiment 1, only one inoculation time was used (fungus and nematode at sowing).

6.2.2 Experimental design

The experiments were set out in a Complete Randomised Design. Plants were grown in a growth chamber with 20°C day and 15°C night temperature and 12 hour day length and light intensity of 65μEinsteins.

6.2.3 Harvest and measurements

Experiment 1 was harvested one or two weeks after inoculation and Experiment 2 at five weeks after planting. At harvest, roots were washed, scored for root lesioning, nematodes were extracted from roots and counted as described in the General Methods. Shoot and root dry weights were also measured. Nematodes were extracted from roots in Experiment 1. After nematode extraction, roots from the mister were stained and the nematodes counted in order to determine total nematodes/root system.

6.3 Results

6.3.1 Experiment 1

Nematode penetration increased in the presence of some root-rotting fungi. Table 6.1 summarises the effect of nematode, fungus and harvest time alone and their interactions on nematode penetration.

Table 6.1 Effect of fungi on the penetration of *P. neglectus* (nematodes/plant) seven and fourteen days after inoculation with fungus in a sterilised sandy loam soil under controlled growth room conditions. Values in the 3-way table are the average of three single plant blocks. (N1500= 1500 *P. neglectus*/plant, N15000= 15000 *P. neglectus*/plant).

	Harvest 1 (7 days)		Harvest 2 (14 days)	
Fungus	N1500	N15000	N1500	N15000
Nil	194	5223	1466	13573
F. acuminatum	486	2799	1673	12567
M. bolleyi	537	6693	1627	15867
P. terrestris	543	7935	2247	15367

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD at P= 0.05).

Fungus	Nil	*F. acuminatum*	*M. bolleyi*	*P. terrestris*	LSD
	5114	4381	6181	6523	*1265*
Nematode	N1500	N15000			
	1097	10003			*895*
Harvest	7 days	14 days			
	3051	8048			*895*
Nematode	N1500	N15000			
Harvest					
7 days	440	5663			*1265*
14 days	1753	14343			

Fungus	Nil	*F. acuminatum*	*M. bolleyi*	*P. terrestris*	
Nematode					
N1500	830	1079	1082	1395	*1790*
N15000	9398	7683	11280	11651	

Fungus	Nil	*F. acuminatum*	*M. bolleyi*	*P. terrestris*	
Harvest					
7 days	2708	1642	3615	4239	*ns*
14 days	7520	7120	8747	8807	

As shown in Table 6.1, the 2-way interaction between nematode and fungus was significant. With the lower nematode density (1500 nematodes/plant), penetration of *P. neglectus* increased by 30%, 30% or 68%, respectively, when *F. acuminatum*, *M. bolleyi* or *P. terrestris* were also present, but this increase was not statistically significant. Similarly, at the higher nematode density (15000 nematodes/plant), with *M. bolleyi* or *P. terrestris,* nematode penetration increased by 20% or 24%, respectively, compared to those without fungus inoculum. At higher nematode inoculum and in the presence of *F. acuminatum,* penetration of the nematode was not significantly affected (Table 6.1).

Of the original low inoculum density at seven days, only 13% of the nematodes had penetrated roots, while at fourteen days almost 98% of them were inside the roots of plants where fungus inoculum was not added (Table 6.1). In the presence of the fungi *F. acuminatum*, *M. bolleyi* or *P. terrestris,* penetration rate of the nematode increased by 33%, 36% or 36% respectively at seven days and by 110%, 108% or 133% respectively at fourteen days compared to the initial (1500 nematodes/plant) inoculum.

At fourteen days at the higher nematode inoculum density, however, there was only a 14% increase in nematode penetration with *M. bolleyi* and 11% increase with *P. terrestris* compared to the control (no fungus inoculum added). With *F. acuminatum,* again there was a 7% decrease in penetration (Table 6.1).

6.3.2 Experiment 2

The analyses of variance for all measurements are shown in Table 6.2. Fungus, nematode or inoculation times separately show a significant effect on plant growth, dry matter, root lesion rating and nematode numbers (Table 6.2). Nematode inoculation was successful as is indicated by the very significant differences between nematode numbers in inoculated and uninoculated treatments. This difference was also expressed in the degree of lesioning and plant growth (Table 6.2). Most interactions were significant in this experiment for most of the variables measured.

Tiller numbers/plant: Number of tillers/plant was not significantly affected by any treatment.

Root lesion rating At harvest (five weeks after sowing), all main treatments (fungus, nematode or inoculation times) and their possible interactions (fungus× nematode, nematode × inoculation time, fungus × inoculation time or the three way interactions) were significant (Table 6.2). Nematodes alone at either 1500 or 15000 nematodes/plant increased root lesion rating by 62% and 77%, respectively, compared to the control (no fungus or nematode added). A significant 3-way interaction between nematode, fungus and inoculation time is shown in Figure 6.1. The amount of lesions on the root system further increased when fungi were also present.

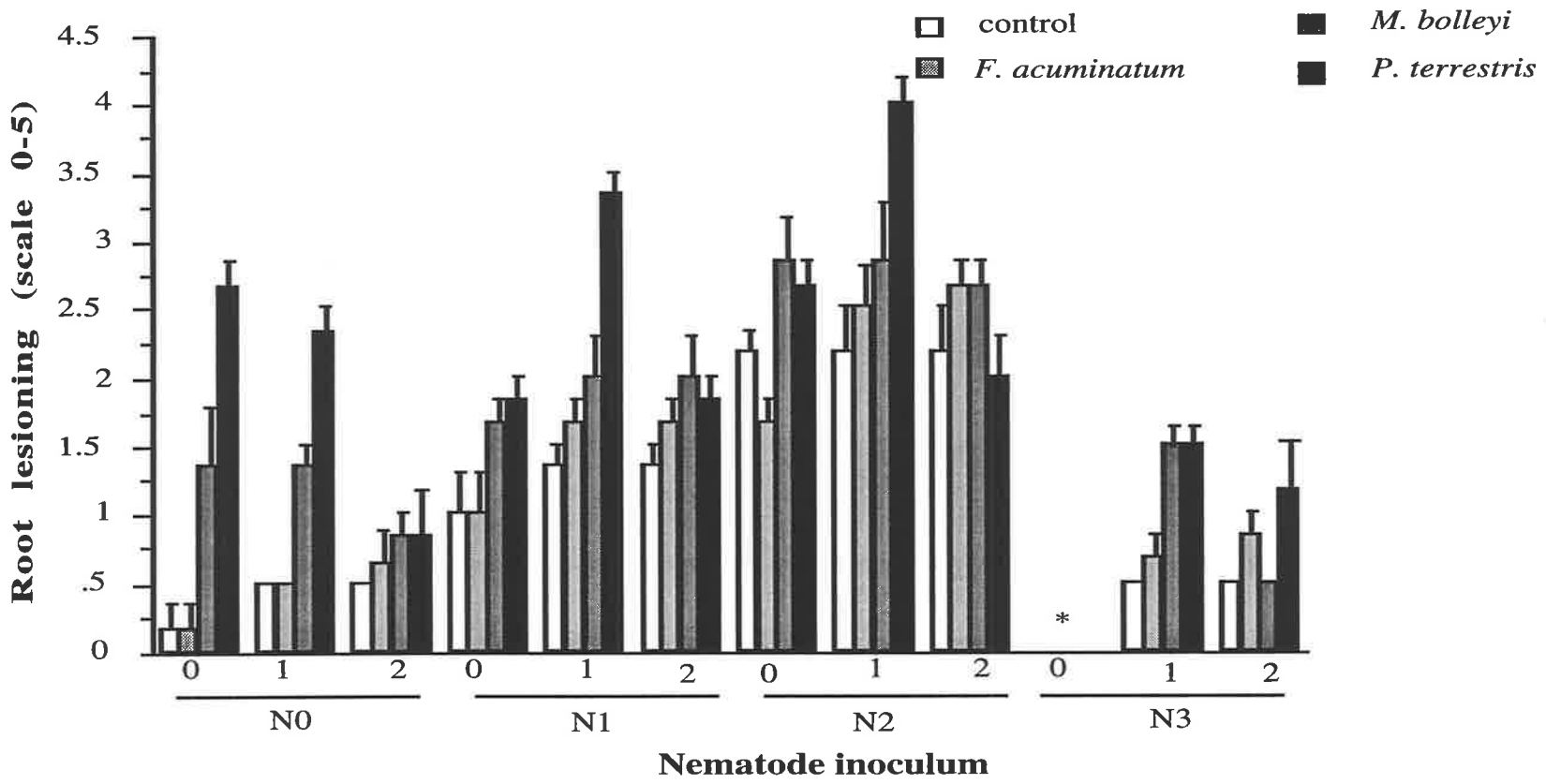
F. acuminatum, in combination with either 1500 or 15000 nematodes/plant added at the time of sowing, increased root lesion rating by 20% and 14%, respectively, compared to nematodes alone at the same densities, or by 70% and 80%, respectively, when compared to the effect of fungus alone (Figure 6.1). However, with mechanical lesions on the roots (fifteen fine lesions on each pre-germinated seedling at planting) there was no increase in root lesion rating compared to fungus alone or in combination with the nematodes. Nevertheless, pre-inoculation of the plants with the fungus had no significant effect on root lesioning, but pre-infection of plants with the nematode increased severity of lesioning by *F. acuminatum* when the fungus was applied two weeks after nematodes compared to when fungus was applied at sowing (Figure 6.1).

Table 6.2 Summary of analyses of variance for the effect of interaction between *F. acuminatum, M. bolleyi or P. terrestris* and *P. neglectus* on extent of root lesioning, number of nematodes/plant and nematodes/g dry root, root and shoot dry weights, total dry weight of plants and nematode multiplication rate for wheat cultivar Machete, 35 days after sowing.

Source	df	MS RL	P	MS N/p	P	MS N/gdr	P	MS dwr/p	P	MS dws/p	P	MS tdw/p	P	MS MR	P
Fungus	3	2.94	***	2.91E6	**	8.733E11	**	0.001	***	0.001	***	0.002	***	8.3	ns
Nematode	3	18.99	***	1.10E11	***	3.001E13	***	0.001	***	3.640E-4	**	0.002	***	594.3	***
Time	2	2.67	***	1.99E6	***	2.283E12	***	4.115E-4	**	0.002	***	0.004	***	81.4	***
Fungus × nematode	9	0.30	*	2.25E6	**	8.818E11	***	1.252E-4	*	1.439E-4	*	2.950E-4	ns	5.9	ns
Fungus × time	6	1.59	***	1.61E6	*	3.351E11	ns	7.466E-5	ns	4.070E-4	***	0.001	***	6.6	ns
Nematode × time	5	0.40	*	7.60E6	***	2.121E12	***	5.060E5	ns	9.884E-5	ns	1.582E-4	ns	28.6	***
Fungus×nematode×time	15	0.37	**	1.66E6	**	5.611E11	**	1.579E-4	**	1.472E-4	*	4.730E-4	**	8.0	*
Residual	88	0.14		6.54E5		1.941E11		6.725E-5		7.653E-5		1.725E-4		4.2	

*** significant at P= 0.001 ** significant at P= 0.01 * significant at P= 0.05 n= not significant P= Probability.
RL= root lesioning; N/p= nematodes/plant; N/gdr= nematodes/g dry root; dwr/p= root dry weight/plant; dws/p= shoot dry weight/plant; tdw/p= total dry weight/plant; MR= nematode multiplication rate.

Figure 6.1 Effect of three way interaction between nematode*fungus*inoculation time on the extent of root lesioning of wheat cultivar Machete 35 days after sowing. (**Inoculation time:** 0= fungus at sowing, nematode two weeks later; 1= fungus and nematode at sowing; 2= nematode at sowing, fungus two weeks later. **Nematode inoculum:** N0= no nematodes added; N1= 1500 nematodes/plant; N2= 15000 nematodes/plant; N3= only mechanical lesioning on root system at sowing). * Data not available.



M. bolleyi alone or in combination with *P. neglectus* at sowing increased severity of root lesioning. With 1500 nematodes/plant there was a 35% increase in root symptoms compared to nematode or fungus alone, and a 75% increase compared to the control (no fungus or nematode added) (Figure 6.1). There was no significant difference between inoculation times. With pre-infection of plants with *M. bolleyi* or *P. terrestris* alone, root lesion rating increased significantly compared to when fungi were applied to the soil two weeks after sowing (Figure 6.1). With *P. terrestris* alone or in combination with either level of nematode, root lesion rating increased significantly compared to *M. bolleyi*, *F. acuminatum* or nematode alone (Figure 6.1).

Nematode or fungus alone did not cause any significant damage to the root system, whereas the combination of both fungus and the nematode caused severe lesioning on the roots (Plate 6.1). *M. bolleyi* not only increased root lesion rating but also increased nematode numbers within the root system as well as egg production (Plate 6.2a) compared to the control (nematode only). It was also noted that calls containing nematodes appeared to contain no fungal hyphse, whereas adjacent cells with no nematodes did contain hyphae of *M. bolleyi* (Plate 6.2b).

At lower nematode inoculum density and in the presence of *P. terrestris*, root lesion rating increased by 60% compared to the nematode alone or by 85% and 56%, respectively, compared to the control (no fungus or nematode added) or mechanical lesioning of the roots (Figure 6.1). At high nematode inoculum density, *P. terrestris* increased root lesion rating by 46%, 87% or 62%, respectively, compared to nematode alone, control (no fungus or nematode added) and mechanically injured roots (Figure 6.1).

***Number of nematodes*:** Number of nematodes/plant and nematodes/g dry root increased significantly with increased inoculum density (Figure 6.2). There was also a significant interaction between nematodes and fungi on the number of nematodes/root system and nematodes/g dry root (Figures 6.2).

Plate 6.1 *Pratylenchus neglectus* in association with *P. terrestris* caused extensive damage to the roots of Machete wheat. Dark black lesion caused by the combination of nematodes and fungus. Photo taken five weeks after inoculation with both fungus and nematodes.

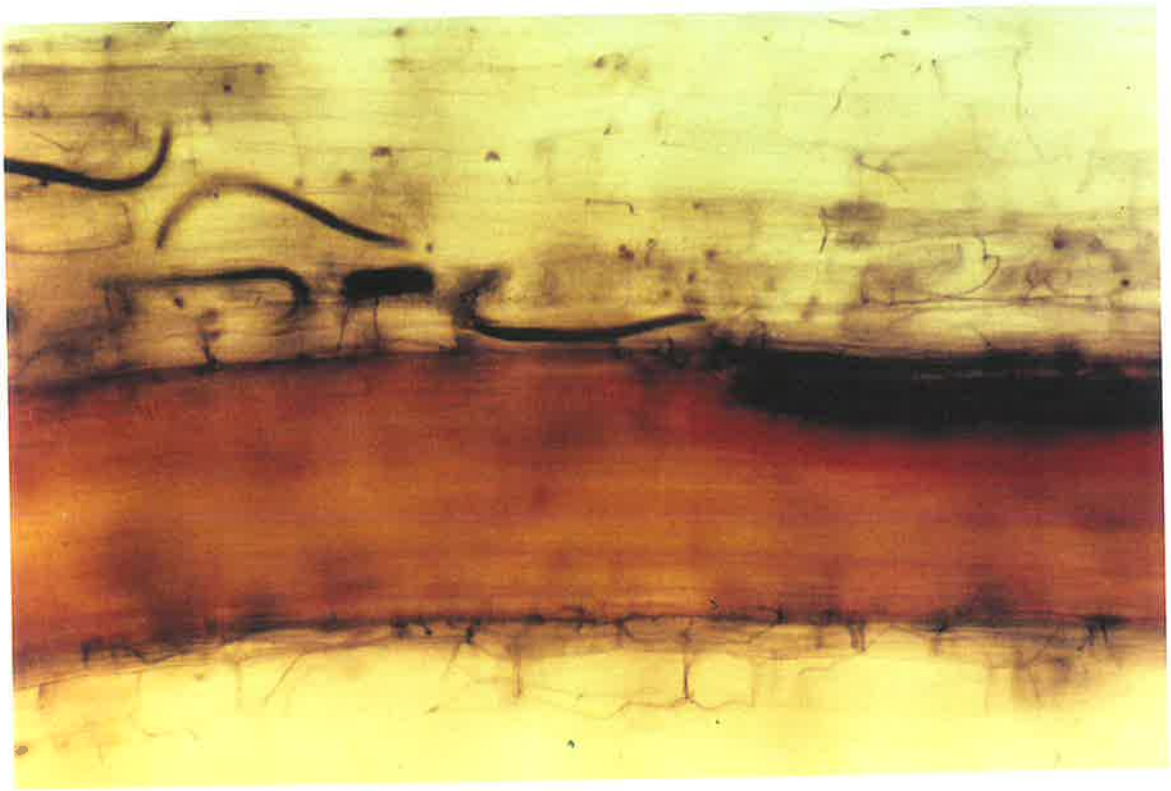


Plate 6.2 Stained roots of Machete wheat infected with *M. bolleyi* and *P. neglectus*.

A. Large number of *P. neglectus* (stained dark blue) and their eggs (oval shaped rods). Evidence of extensive cortical degradation resulting in the loss of outer cortical layers.

B. Stained roots of the same plant infected by both *P. neglectus* and *M. bolleyi*. Evidence of digestion of fungal hyphae in cells containing nematodes. No fungal hyphae are apparent in cells containing nematodes, whereas hyphae are present in adjacent cells.

A

B

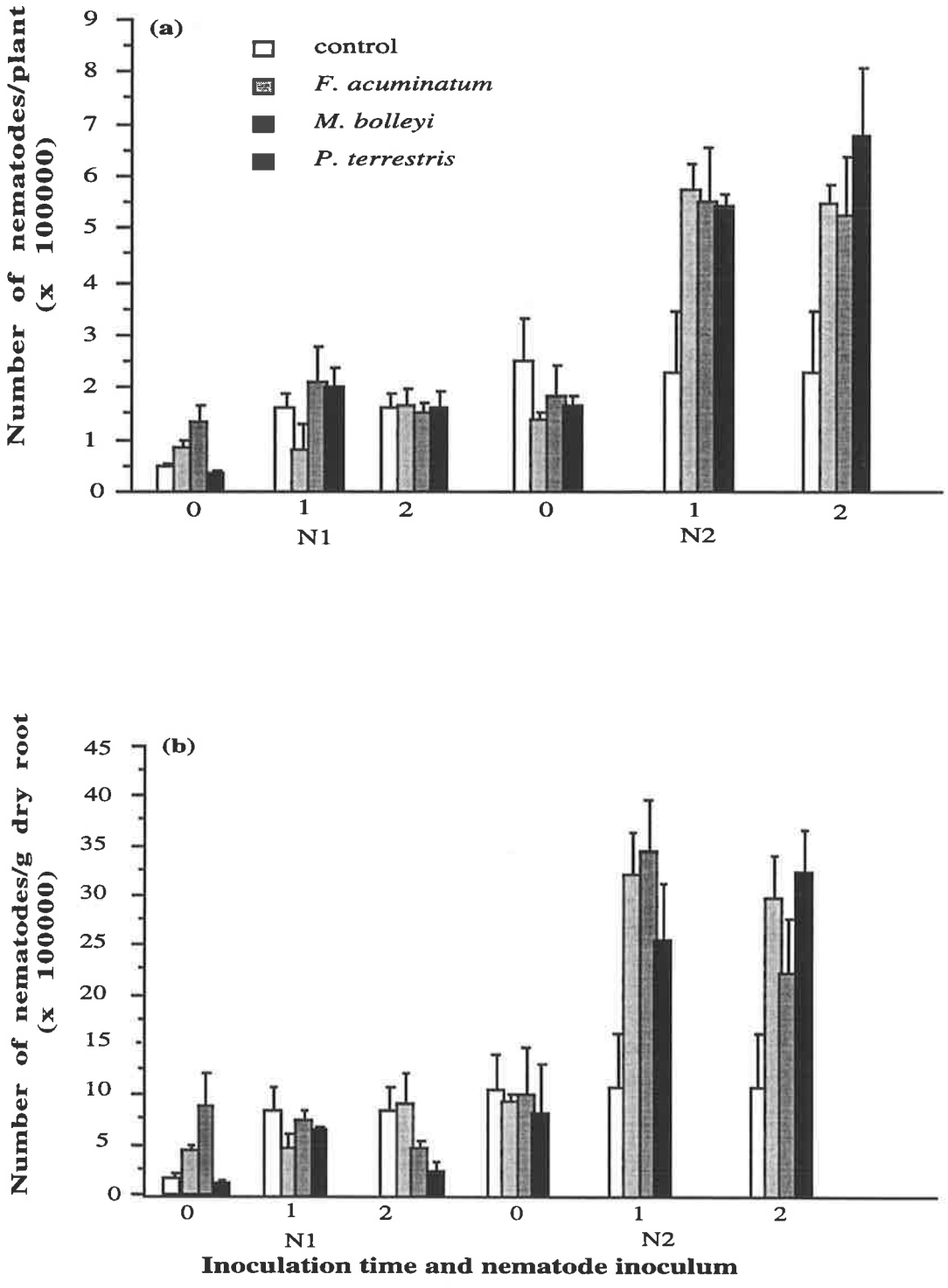


Figure 6.2 Effect of three way interaction of nematode × fungus × inoculation time on **(a)** number of *P. neglectus*/plant and **(b)** nematodes/g dry root 35 days after sowing. (**Inoculation time:** 0= fungus at sowing, nematode two weeks later; 1= fungus and nematodes at sowing; 2= nematodes at sowing, fungus two weeks later. **Nematode inoculum:** N1= 1500 nematodes/plant; N2= 15000 nematodes/plant).

At lower nematode inoculum (1500/plant) and with pre-inoculation of plants with *F. acuminatum* or *M. bolleyi,* nematode numbers/plant increased by 49% and 64%, respectively, compared to control (no fungus inoculum added) (Figure 6.2a). This increase was significant at P= 0.01. However, *P. terrestris* decreased nematode numbers significantly compared to *F. acuminatum* or *M. bolleyi* and did not differ from the control (no fungus added) (Figure 6.2a). A similar result was obtained for number of nematodes/g dry root (Figure 6.2b).

Inoculation of plants with fungus and nematode at sowing did not affect the nematode numbers in the roots. Although there was a significant reduction in number of nematodes/plant with *F. acuminatum*, this reduction was not significant on nematodes/g dry root (Figure 6.2). Pre-inoculation of plants with *P. neglectus* at sowing and two weeks later with *F. acuminatum*, *M. bolleyi* or *P. terrestris* had no effect on number of nematodes/root system. However, with *M. bolleyi* or *P. terrestris,* number of nematodes/g dry root decreased significantly compared to the control (no fungus added), but with *F. acuminatum*, nematode numbers were not changed (Figure 6.2).

With higher nematode inoculum density (15000 nematodes/plant) and in the presence of all fungi, nematode numbers/plant or nematodes/g dry root increased significantly (P=0.001). With *F. acuminatum*, *M. bolleyi* or *P. terrestris* number of nematodes/root system and nematodes/g dry root increased by 44%, 44% or 49%, respectively, and by 54%, 51% or 51%, respectively, compared to the control (no fungus added) (Figure 6.2).

Pre-inoculation of pots with the fungi and inoculation with the nematode two weeks later generally decreased nematode numbers significantly compared to the application of fungus and nematode at sowing or pre-inoculation with the nematodes (Figure 6.2). However, when *F. acuminatum*, *M. bolleyi* or *P. terrestris* and the nematode were added to the soil at sowing or nematodes at sowing and fungus two weeks later, number of nematodes/plant or nematodes/g dry root increased significantly (P=0.001) compared to the control (no fungus added) and the treatment where fungus was added before the

nematode (Figure 6.2).

Nematode multiplication rate was also affected by nematode and inoculation time and by some combinations (nematode × inoculation time or fungus × nematode × inoculation time) (Table 6.2). As shown in Figure 6.3, in plants inoculated with *F. acuminatum* or *M. bolleyi* two weeks before nematodes (1500 nematodes/plant) nematode multiplication rate increased by 48% or 65%, respectively, compared to the control at the same nematode level (no fungus added). However, with *P. terrestris* at either inoculation time, there was no change in multiplication rate of the nematode compared to the control (no fungus added) (Figure 6.3). There were also no significant differences between fungal inoculum with either pre-inoculation or with inoculation of plants with the nematode at sowing.

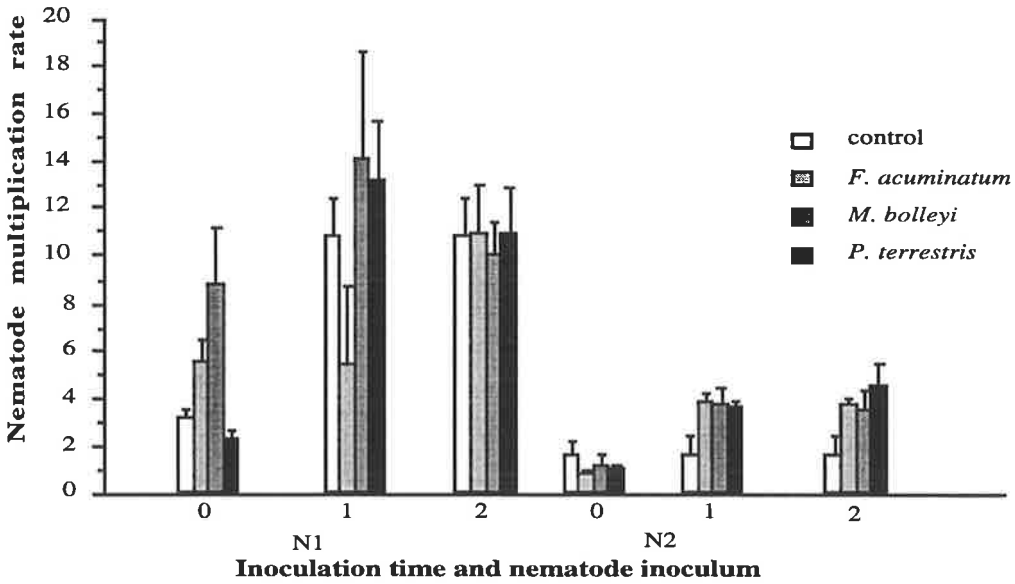


Figure 6.3 Effect of three way interaction of nematode × fungus × inoculation time on the multiplication rate of *P. neglectus* 35 days after sowing. (**Inoculation time:** 0= fungus at sowing, nematode two weeks later; 1= fungus and nematodes at sowing; 2= nematodes at sowing, fungus two weeks later. **Nematode inoculum:** N1= 1500 nematodes/plant; N2= 15000 nematodes/plant).

When fungus and nematode were added to the pots at sowing, *F. acuminatum* decreased nematode multiplication rate by 49% compared to the control (no fungus

added). This reduction was significant (P= 0.1). *M. bolleyi* or *P. terrestris* increased nematode multiplication rate by 23% or 19%, respectively, compared to those inoculated only with 1500 nematodes/plant. However, at higher nematode inoculum density (15000 nematodes/plant), nematode multiplication rate decreased significantly (P=0.001) compared to those inoculated with the lower nematode inoculum (1500 nematodes/plant) (Figure 6.3).

There was a significant increase in the multiplication rate of the nematode at the higher inoculum level when plants were inoculated with both fungus and nematode at sowing or with pre-infection of plants with the nematodes at sowing compared to controls (no fungus added) (Figure 6.3). *F. acuminatum*, *M. bolleyi* or *P. terrestris* increased multiplication rate of the nematode by 60%, 59% or 58%, respectively, compared to the control (15000 nematodes/plant).

Plant dry matter: Shoot dry weight decreased as the nematode inoculum increased. Plants inoculated with 15000 nematodes/plant at sowing produced 41% less shoot dry matter than those inoculated with 1500 nematodes/plant at sowing (Figure 6.4). With the combination of nematodes and fungi, shoot, root or total dry weights of plants generally increased. However, with no nematode application or with mechanical lesioning, there was no significant difference among fungi or between fungi and the controls (no nematode or fungi added).

Neither nematode inoculum density (1500 or 15000 nematodes/plant) alone had a significant affect on root dry weight compared to the control (no fungus or nematode added) (Figure 6.5). Root dry weight was affected significantly by *F. acuminatum* alone. Further reduction occurred when *F. acuminatum* and the nematodes were combined. With *F. acuminatum* alone, root dry weight decreased by 25%, whereas with *F. acuminatum* and the nematodes at either 1500 or 15000/plant root dry weight decreased by 36% compared to the control (no fungus or nematode added) (Figure 6.5). Other fungi alone did not cause any reduction in root dry matter.

Figure 6.4 Effect of three way interaction between nematode*fungus*inoculation time on shoot dry weight/plant of wheat cultivar Machete 35 days after sowing. (**Inoculation time:** 0= fungus at sowing, nematode two weeks later; 1= fungus and nematodes at sowing; 2= nematode at sowing, fungus two weeks later. **Nematode inoculum:** N0= no nematodes added; N1= 1500 nematodes/plant; N2= 15000 nematodes/plant; N3= only mechanical lesioning on root system at sowing). * No data available.

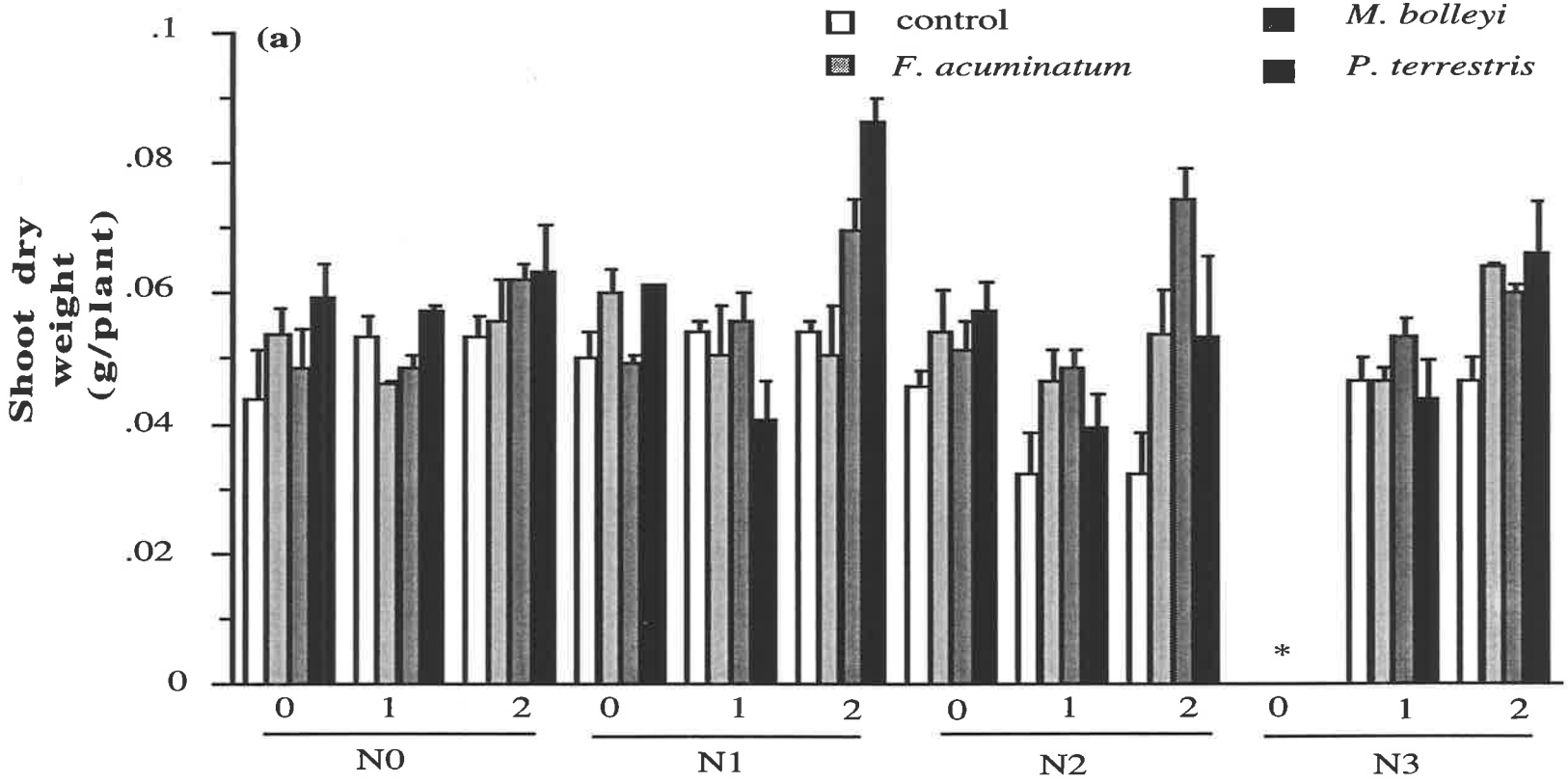


Figure 6.5 Effect of three way interaction between nematode*fungus*inoculation time on root dry weight/plant of wheat cultivar Machete 35 days after sowing. (**Inoculation time:** 0= fungus at sowing, nematode two weeks later; 1= fungus and nematodes at sowing; 2= nematode at sowing, fungus two weeks later. **Nematode inoculum:** N0= no nematodes added; N1= 1500 nematodes/plant; N2= 15000 nematodes/plant; N3= only mechanical lesioning on root system at sowing). * No data available.

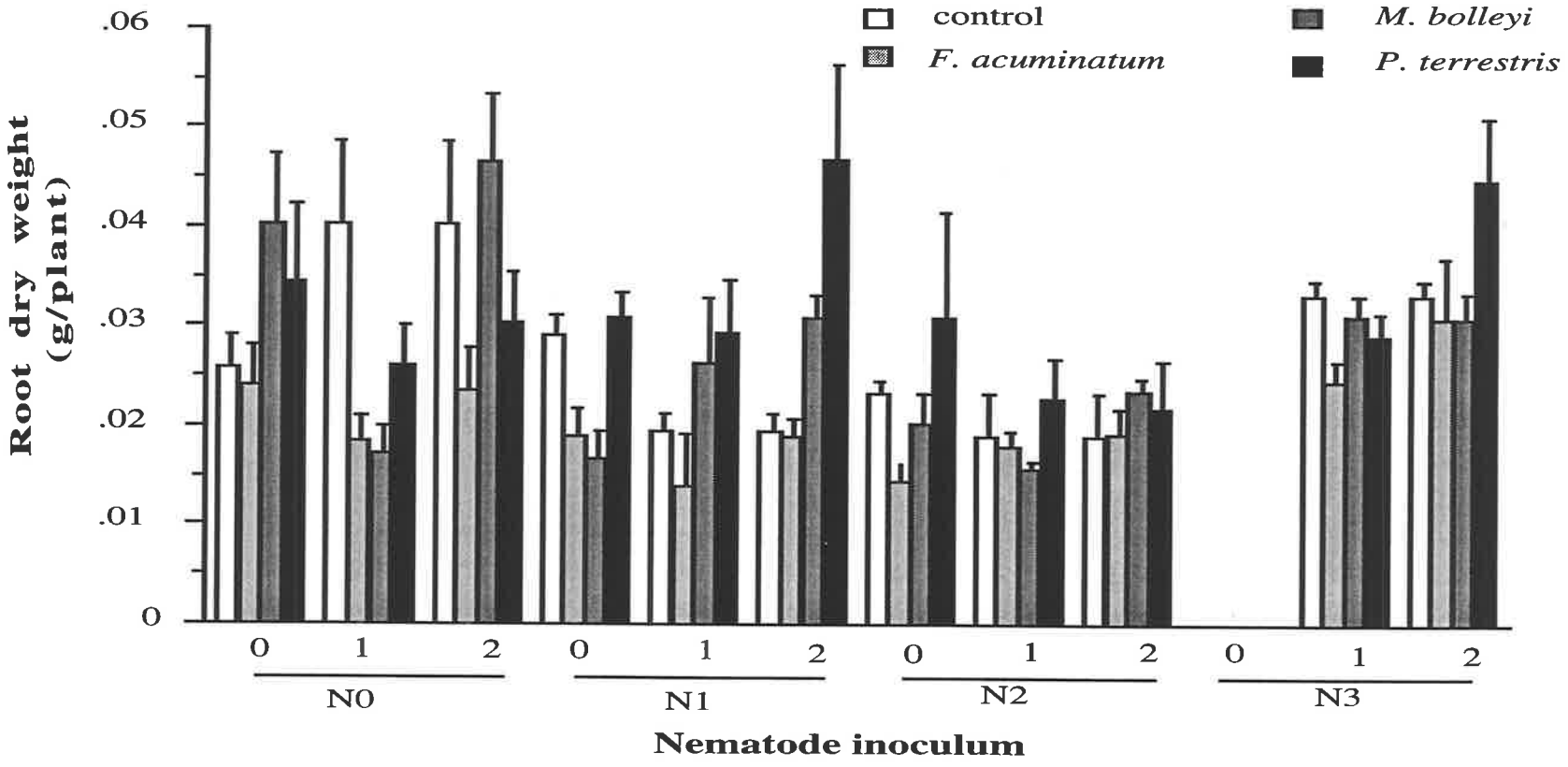
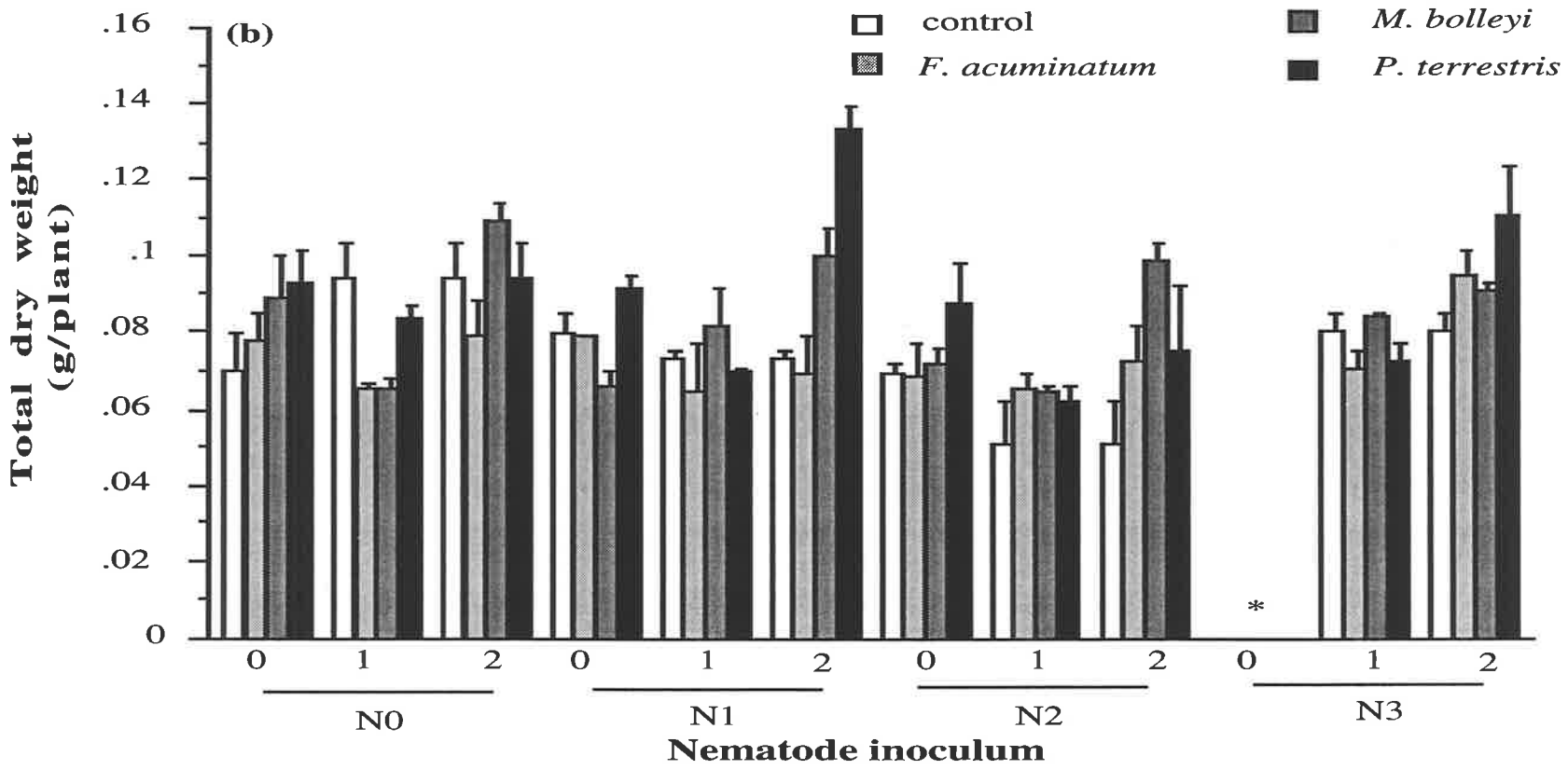


Figure 6.6 Effect of three way interaction between nematode*fungus*inoculation time on total dry weight/plant of wheat cultivar Machete 35 days after sowing. (**Inoculation time:** 0= fungus at sowing, nematode two weeks later; 1= fungus and nematodes at sowing; 2= nematode at sowing, fungus two weeks later. **Nematode inoculum:** N0= no nematodes added; N1= 1500 nematodes/plant; N2= 15000 nematodes/plant; N3= only mechanical lesioning on root system at sowing). * No data available.



Combination of *M. bolleyi* with either 1500 or 15000 nematodes/plant decreased root dry weight by 29% or 31% compared to the effect of fungus alone, but did not differ from the nematode alone at either level (Figure 6.5). Inoculation time had significant effects on root dry weights as well as on shoot or total dry weights. Root dry weight of plants inoculated with 15000 nematodes and *M. bolleyi* at sowing decreased by 38% compared to those inoculated with the lower nematode number or by 60% compared to the controls (no fungus or nematode added), but differed from fungus or nematode alone at the same density (Figure 6.5).

Plants inoculated with both *P. terrestris* and the nematode (15000/plant), produced 21% less roots than those inoculated with 1500/plant and 42% compared to control (no fungus or nematodes added) (Figure 6.5).

Total dry weight of plants was also affected by nematode, fungus or inoculation time. Fungi alone or in combination with nematodes either increased or did not change total dry weight of plants (Figure 6.6).

Total dry weight of plants inoculated with 1500 or 15000 nematodes/plant decreased by 22% or 46%, respectively, compared to control (no nematode or fungus added) (Figure 6.6). With mechanical wounding and the fungi, there was no significant effect on total dry weights compared to the control (no fungi added or those that had no fungi and nematodes) (Figure 6.6).

6.4 Discussion

Nematode movement through soil is dependent on the soil type, possibly host and the activity of other soil micro-organisms. Soil-borne fungi may influence the attraction and the penetration rate of the nematodes by modifying the rhizosphere. Plant exudates are an important factor in the rhizosphere which can attract many pathogens to the plant or *vice versa*. The presence of *M. bolleyi* or *P. terrestris* significantly increased the penetration rate of *P. neglectus*. As the inoculum of fungus on millet seed (dried) and nematodes in liquid was added to the soil at sowing, it is likely that the nematodes entered the plant

immediately, followed by fungal hyphae. However, activity of fungi in the lesioned parts of roots together with the nematodes within roots may alter plant exudates which are later distributed in the rhizosphere.

For a long time it was thought that wounding caused by nematodes was responsible for increasing the susceptibility of plants to invasion by other pathogens. For example, Westerlund *et al.* (1974) observed that *Fusarium oxysporum* f. sp. *ciceri* may require wounding for efficient infection of chickpeas. However, suceptibility of a host to fungal pathogens may not always be localised to the site of nematode infection, but systemic physiological changes may occur in the host that are favourable to fungal pathogens. Bowman and Bloom (1966), and later Faulkner *et al.* (1970), found that susceptibility of a nematode infested host to fungal pathogens is tranlocatable through the plant. Plants inoculated with *Meloidogyne incognita* in one part of the root system and *Fusarium oxysporum* f. sp. *lycopersici* in another part showed wilting even though the nematode and fungus were added to separate parts of the root system. Root exudates in the rhizosphere, therefore, may attract more nematodes and fungi to the host and increase root damage.

The results of these experiments showed that root exudates were attractive for all fungi, but after fungus establishment some were attractive to nematodes and some were not. *M. bolleyi* or *P. terrestris* infected plants were significantly attractive for *P. neglectus,* whereas *F. acuminatum* infected plants were not. This was particularly seen at higher nematode inoculum. Whereas *M. bolleyi* or *P. terrestris* increased penetration rates by 20% or 22%, respectively, *F. acuminatum* reduced penetration by 18% compared to the control.

As the inoculum level of the nematode increases, competition amongst the nematodes to penetrate the root would also increase. With more nematodes entering the roots, more lesions resulted, which in turn would allow for greater fungal infection. This may be particularly true for non-pathogenic or weakly pathogenic fungi that normally are not able to penetrate root tissues.

In the case of *F. acuminatum* infested plants, which seemed to have no attraction for the nematodes, the number of nematodes penetrating roots decreased. This could be due to several factors, such as different anti-nematode chemicals (Gommers, 1981) produced by fungus or fungus infected plants. However, the mechanisms responsible for repulsion have not been fully identified and needs further investigation.

Fungi used in this experiment are known to be non-pathogenic or only slightly pathogenic to cereals. A combination of these fungi and the nematode in naturally infested soil from the field increased root lesion rating (Chapter 5). The fungi also decreased root dry weight in the presence of the nematode. However, in natural soil, there are many other micro-organisms which may influence the results. Therefore, for this experiment, sterilised sandy-loam soil was used.

The presence of *M. bolleyi* or *P. terrestris* alone or in combination with the nematode increased root lesions on wheat. From the data presented, it is clearly evident that fungi independent of the nematode can cause severe damage to the root system if the level of inoculum is high. However, *F. acuminatum* alone did not cause any damage to the roots, but the combination of all fungi with *P. neglectus* increased root lesions in wheat, suggesting that nematode, fungus or their combination can change plant physiology to be favourable for both nematode and fungus. Nematodes and fungi then can then multiply faster and extend the lesions on the roots. As *F. acuminatum* was not attractive to nematodes, there were also fewer lesions present on the root system. The decline in lesioning could also be due to a lower number of nematodes within the root system.

It is possible that the roots infected with *F. acuminatum* did not favour nematode reproduction. However, with mechanical lesions on the root prior to planting there were no significant increases in the extent of lesioning. Thus, the role of nematodes in lesioning of the roots is not only mechanical but some chemical changes must also be occurring. For example, healthy plants were more attractive to *P. terrestris* than mechanically injured plants. Although there were only fifteen punctures on the root surface, which may not be comparable to the number of lesions caused by nematodes, the

size of these lesions and therefore the amount of root exudates leaking from them would be greater than that caused by nematodes. In this experiment, the effect of lesioning on the attraction of nematodes to the roots was not examined. It would be interesting to investigate whether increased plant exudate in the rhizosphere resulting from injury may lead to higher nematode response.

The presence of fungi also favoured nematode reproduction. This was supported by previous experiments where the number of *P. neglectus* increased in the presence of some fungi (such as *M. bolleyi* or *P. terrestris*). The degree of root lesioning caused by these fungi and the nematode could be due to the increased nematode numbers. As the number of nematodes in the roots increased, the extent of root lesions also increased. The relationship between *P. neglectus* and fungi may be due to the following:

1. Nematodes create openings in roots for fungi to enter. Physiological alterations by the nematode are known to improve the nutrient status of the host for fungal pathogens (Golden and Van Gundy, 1975). Soon after fungal establishment, fungi can independently grow and, with the assistance of nematodes, may change root physiology or biochemistry to favour nematode multiplication and development.

2. Nematodes may utilise fungi as a source of energy which could lead to enhanced reproduction of nematodes. Alternatively, chemicals produced solely by fungi or as a result of the plant and fungus interaction may aid in nematode reproduction. As shown in Plate 6.2a, nematodes in roots infected with *M. bolleyi* multiplied extensively and produced many eggs. Plate 6.2b shows *P. neglectus* and hyphae of *M. bolleyi* in the same root system. However, where nematodes are present within cortical cells, fungal hyphae are not apparent. It is suggested that the nematode may be able to digest fungus hyphae within a localised area and then use it as a source of food. However, there is no evidence in the literature that *Pratylenchus* species feed on fungal hyphae. Therefore, the hypothesis that the nematode may feed on fungi requires further investigation.

Plant dry matter was significantly affected by fungus or nematode inoculum or by their

association. While mechanical lesions on the root surface and the presence of fungi had no significant effect on plant dry matter compared to the healthy plant, plants inoculated with fungi or nematodes showed significantly less growth. Reduction in plant dry matter was significantly correlated with the amount of lesioning on the root system and with the number of nematodes/plant or nematodes/g dry root.

The amount of lesioning on the roots caused by a pathogen alone is not always a good way to assess the effect on growth and development of a plant. This is particularly true when an experiment is conducted in a glasshouse where the conditions are optimal for disease development. Therefore, root lesioning could appear more quickly than in the field, but plants may still have a chance to recover. In this situation, plant growth may be stimulated. Although root lesioning caused by *M. bolleyi* or *G. graminis* alone was relatively higher compared to when both pathogens were present, the reduction in plant dry matter was significant only when both pathogens were combined, or with nematodes alone. However, fungus alone stimulated plant growth. This may cause problems in natural field conditions, particularly late in the season where root systems suffer from severe damage due to nematode and/or fungus attack. At this stage, when plants require more water and nutrients, roots can not support the extra growth and as a result tillers will die or produce no grain.

The time of inoculation of the fungus or nematodes may also influence the nematode-fungus interaction. Sequential or simultaneous inoculation of the pathogens may effect the type of interaction, synergistically or antagonistically (Zacheo, 1993).

Migratory nematodes like *Pratylenchus* spp. generally feed for a relatively short time and move from one feeding site to another (Zacheo, 1993). The damaged cells subsequently become necrotic (Plate 4.3). According to Canto-Saenz (1985), plants with resistance to nematodes show several forms of incompatibility after nematode attack, of which hypersensitivity of cells is the most common. Necrotic cells are frequently found around the area of the nematode head in resistant plants (Evans and Haydock, 1993). In

the penetration experiments reported here, within seven days of nematode inoculation, necrotic cells appeared around nematodes in the highly susceptible wheat variety Machete, and damaged cells were also found. It is therefore suggested that all plant varieties infected by nematodes may produce necrotic cells in response to nematode damage, but the degree and timing of the cell response may differ between susceptible and resistant plants.

The hypersensitive reaction of a susceptible plant to nematode attack may be due to longer feeding at a site, or cessation of nematode movement for some reason, allowing plant defence systems to react against the nematode. In a resistant host, this reaction would occur much faster, so the nematode cannot escape from the necrotic cells.

Simultaneous or sequential inoculation of plants with *M. bolleyi* or *P. terrestris* increased severity of lesioning on the root system. With pre-inoculation of plants with *P. neglectus*, two weeks prior to fungus inoculum, only *F. acuminatum* showed an increase in root lesion rating but, with *M. bolleyi* or *P. terrestris* , lesions significantly decreased. This could have been due to production of a chemical, or (according to Golden and Van Gundy, 1975) improvement in the nutrient status of the host by nematodes while feeding. This would increase pathogenicity of *F. acuminatum* to wheat, and/or a fungus-plant interaction may favour nematode penetration. Therefore, after both pathogens establish in the plant, they may favour each other and increase disease rating.

The short duration of the experiment (five weeks), may not have been an adequate time for some fungi such as *M. bolleyi* or *P. terrestris* to interact with the nematode and for lesions to develop. There is evidence that nematodes, particularly root lesion nematodes, predispose roots to some fungal pathogens and not to others (Litter and Head, 1967). The question remains as to whether the role of lesion nematodes is merely mechanical predisposition of plants to fungal attack, or physiological changes alone, and/or both mechanical and physiological means.

Wounding roots with a knife did not increase wilt caused by *F. oxysporum* f. sp.

tracheiphilum race 1 in soyabean cultivar Cobb, but the disease was more severe in the presence of the nematodes *Belonolaimus longicaudatus* and *P. brachyurus* (Sumner and Mintons, 1987). From data presented here, it is also evident that mechanical wounding of wheat roots does not enhance fungal infection. Furthermore, with mechanical lesioning, less infection by fungus occurred compared to the uninjured plants.

On the other hand, plants inoculated with nematodes favoured infection by *M. bolleyi* or *P. terrestris* and significantly extended lesions on the root system. The results, however, suggest that the role of nematodes is more than simple wounding. The involvement of a physiological change in the plant and/or chemicals which may be produced by fungi is quite possible.

Further investigation is required to determine the mechanism(s) of interactions between nematodes and fungi, as well as the role of both *P. neglectus* and soil-borne fungi in relation to plant damage. Activity of nematodes may increase the level of carbohydrates in the cells, or there may be some change in total amino acids which would then increase total protein in the plant. Also, some chemotoxins may be produced as a result of fungus-plant, nematode-plant, fungus-nematode interactions, or all three components (fungus-nematode-host), may be involved.

Chapter 7

Fungal interactions with *Pratylenchus neglectus* or *P. thornei*

7.1 Introduction

Both *P. neglectus* and *P. thornei* are root pathogens of many crops including cereals, legumes and pastures. Wheat roots in particular suffer damage from both species of root lesion nematode. In South Australia, Nicol (1991) found that under aseptic laboratory conditions, *P. thornei* can cause significant damage to cereals, particularly wheat. *P. thornei,* however, is not as common as *P. neglectus* in South Australia (Nicol, 1996).

Association of numerous soil-borne fungi with wheat roots infected with *P. neglectus* in the field (Chapter 3), and the results of glasshouse interaction tests (Chapter 4), indicated that fungi associated with the nematode are responsible for increased damage to wheat. *Microdochium bolleyi* and *Fusarium acuminatum* are commonly found in association with the damaged roots of nematode infected plants (Chapter 3; Vanstone, 1991). Presence of fungi increased root lesion rating of wheat as well as increasing nematode numbers in the root system (Chapter 4).

Due to the presence of both species of *Pratylenchus* in South Australia, an aseptic interaction test (Experiment 1) under growth room conditions was undertaken to investigate interaction between the two most frequent soil-borne fungi, *M. bolleyi* and *F. acuminatum,* and *P. neglectus* or *P. thornei.* This was done in collaboration with Ms. J. M. Nicol, Department of Crop Protection, Waite Campus, The University of Adelaide.

Experiment 2 involved *P. neglectus* only in combination with *M. bolleyi* or *F. acuminatum.* The aim of this experiment was to determine from what stage of plant growth interaction occurs and lesions on the root appear. The third experiment, involving *P. neglectus* and/or *M. bolleyi* or *F. acuminatum,* was undertaken in a controlled

environment to determine the effect of inoculation time on the fungus-nematode interaction. Four inoculation times were used: nematode at sowing, fungus two weeks later; nematode at sowing, fungus three weeks later; nematode at sowing, fungus four weeks later; fungus and nematode at sowing.

7.2 Methods

7.2.1 Experiment 1

Surface-sterilised Machete wheat seeds were germinated and selected as described in the General Methods. Sandy soil was steam pasteurised at 70°C for 40 minutes, then air dried for 72 hours and sieved through a 2mm sieve.

7.2.1.1 Fungal and nematode inoculum

Fungal inoculum of *M. bolleyi* and *F. acuminatum* was prepared on millet seed (General Methods). Plastic pots of 300ml capacity with no drainage holes were used. Fungal inoculum of *F. acuminatum* or *M. bolleyi* on millet seeds was added to the soil at 1% w/w in two layers. One pre-germinated Machete seed was sown in each cup at a depth of 1.5cm.

P. thornei and *P. neglectus* were extracted from carrot cultures as described in the General Methods. The nematodes were added in a volume of 1ml using a truncated pipette, at densities of 0, 2000, 6000 or 12000/plant, around each plant. Sterile distilled water was added for the control (no nematode treatment).

7.2.1.2 Experimental design and harvest

The experiment was a split plot design with six replicates. There were two harvest times (main plots), seven and ten weeks, two nematode species (*P. neglectus* and *P. thornei*) at four different initial densities (0, 2000, 6000 and 12000 nematodes/plant), and two fungi (*M. bolleyi* and *F. acuminatum*) at only one density. Plants were grown in a controlled temperature room at 23°C with a twelve hour photoperiod.

Plants were harvested seven and ten weeks after inoculation. The soil was gently washed from the root system. Nematodes were extracted over a period of four days using the mister extraction method and were counted. At each harvest time the root lesions were scored from 0-5 (0= healthy roots and 5= complete lesioning of whole root system) as in the General Methods, and the number of tillers/plant (excluding the main tiller) were counted. Dry weight of shoots and roots was recorded after drying at 80°C for three days. A 2cm root segment from each treatment was sampled and fixed in FAA preservative for staining nematodes and fungi.

Data were transformed, where the original analysis showed heterogeneity of variance, using either the log transformation, $\log_e (x+1)$, or square root transformation ($\sqrt{x+0.5}$).

7.2.2 Experiments 2 and 3

These experiments were similar to Experiment 1, but involved only *P. neglectus*. Experiment 2 was set out as a factorial split plot design and Experiment 3 was set out as a completely randomised design. There were three replicates for Experiment 2 and six replicates for Experiment 3. Inoculum of both fungi, *M. bolleyi* or *F. acuminatum* on millet seed, was added to the soil in two layers at sowing. In Experiment 2 3000 *P. neglectus* were added to each pot next to the seedling soon after planting and 6000 nematodes/plant was used in Experiment 3.

Plants from Experiment 2 were harvested at four, six, eight and ten weeks after inoculation. At each harvest time, roots were scored for lesions as for previous experiments. Nematodes were extracted from the whole root system over a period of four days and counted. Dry weight of shoots and roots was recorded as for Experiment 1.

Experiment 3 was also conducted in a controlled environment (20°C with twelve hour day length and light intensity of 65 µEinsteins). Fungus inoculum of *M. bolleyi* or *F. acuminatum* on millet seed was added to the soil in two layers. There were four different inoculation times: fungus and nematode at sowing (T0); nematode at sowing, fungus

two weeks later (T1); nematode at sowing, fungus three weeks later (T2); nematode at sowing, fungus four weeks later (T3).

Control pots with no nematodes received the same quantity of distilled water. Plants were harvested ten weeks after sowing, and roots and shoots were processed as for previous experiments. Nematodes were also extracted from the root system over four days using the mist chamber and counted.

7.3 Results

7.3.1 Experiment 1

The analyses of variance for all measurements are shown in Table 7.1.

Table 7.1 Summary of analyses of variance for the effect of interaction between *F. acuminatum, M. bolleyi* and *P. neglectus* or *P. thornei* on the extent of root lesioning, number of nematodes/plant, number of tillers/plant, shoot and root dry weights, total dry weight of plants and nematode multiplication rate for wheat cultivar Machete, 49 days after sowing.

Source	df	MS RL	P	MS N/p	P	MS tillers	P	MS dws/p	P	MS dwr/p	P	MS tdw/p	P	MS MR	P
Block	5	0.44		4958		8.46		0.16		0.39		0.99		9.53	
Harvest	1	14.86	**	107248	**	2.04	ns	16.73	**	0.85	ns	9.85	**	362.82	**
Block × Harvest	5	0.04	-	5587	-	0.77	-	0.05	-	0.17	-	0.36	-	5.94	-
Nematode species (nemtyp)	1	0.27	ns	8054	ns	0.07	ns	0.05	*	0.39	*	0.18	ns	14.33	ns
Nematode density (nemden)	3	53.54	**	152367	**	0.85	ns	0.13	**	0.57	**	1.32	**	28.25	*
Fungus	2	9.01	**	341	ns	62.68	**	3.46	**	1.27	**	5.97	**	0.16	ns
Harvest × nemtyp	1	0.23	ns	1274	ns	0.35	ns	0.01	ns	0.35	*	0.59	*	2.75	ns
Harvest × nemden	3	1.25	**	4495	ns	0.23	ns	0.04	*	0.10	ns	0.08	ns	3.03	ns
Harvest × fungus	2	1.97	**	18273	**	0.99	ns	0.14	**	1.15	**	1.20	**	40.20	**
Nemtyp × fungus	2	0.12	ns	2587	ns	1.57	ns	0.001	ns	0.35	*	0.38	*	10.00	ns
Nemden × fungus	6	0.77	**	1491	ns	0.88	ns	0.02	ns	0.44	**	0.67	**	7.94	ns
Nemtyp × nemden	3	0.11	ns	7421	ns	0.59	ns	0.01	ns	0.03	ns	0.02	**	9.62	ns
Nemtyp × nemden × fungus	6	0.17	ns	1179	ns	0.32	ns	0.01	ns	0.06	ns	0.12	ns	4.43	ns
Harvest × nemden × fungus	6	0.38	ns	3708	ns	0.32	ns	0.01	ns	0.34	**	0.35	**	5.13	ns
Harvest × nemtyp × fungus	2	1.25	**	9524	*	0.60	ns	0.01	ns	0.15	ns	0.27	ns	30.78	*
Harvest × nemtyp × nemden	3	0.46	ns	9878	*	0.33	ns	0.006	ns	0.11	ns	0.14	ns	14.89	ns
Residual	197	0.22		2678		0.56		0.01		0.07		0.11		9.36	

** significant at P= 0.01 * significant at P= 0.05 ns= not significant P= Probability.
RL= root lesioning; N/p= nematodes/plant; tillers= tillers/plant; dws/p= shoot dry weight/plant; dwr/p= root dry weight/plant; tdw/p= total dry weight/plant; MR= nematode multiplication rate.

Tiller number: With or without nematodes, plant tillering increased where fungus was present (Figure 7.1).

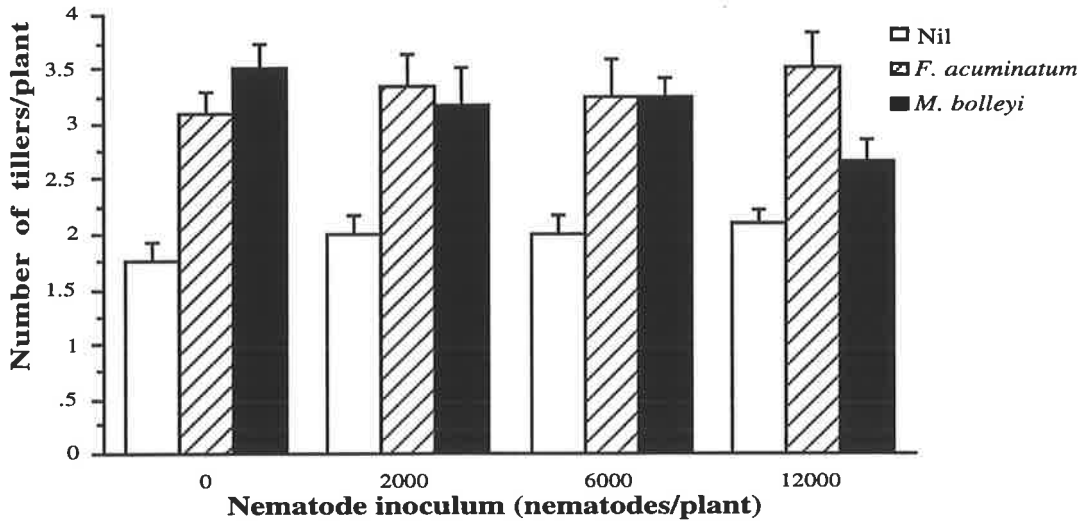


Figure 7.1 Effect of nematode-fungus interaction on number of tillers/plant for Machete wheat. Data are means for both nematode species.

Root lesion rating: The amount of lesioning on the root system increased with increase in initial nematode inoculum for both *P. neglectus* and *P. thornei* (Figure 7.2a). Further increase in root lesion rating resulted when either *F. acuminatum* or *M. bolleyi* were also present. At the second harvest time (ten weeks after sowing) there was also a further significant increase in root lesion rating (Figure 7.2b). Although at harvest one there was no significant interaction between nematode and fungus on root lesion rating, at harvest two, with the presence of fungi, root lesion rating increased significantly compared to the control (no fungus inoculum added) (Figure 7.2c).

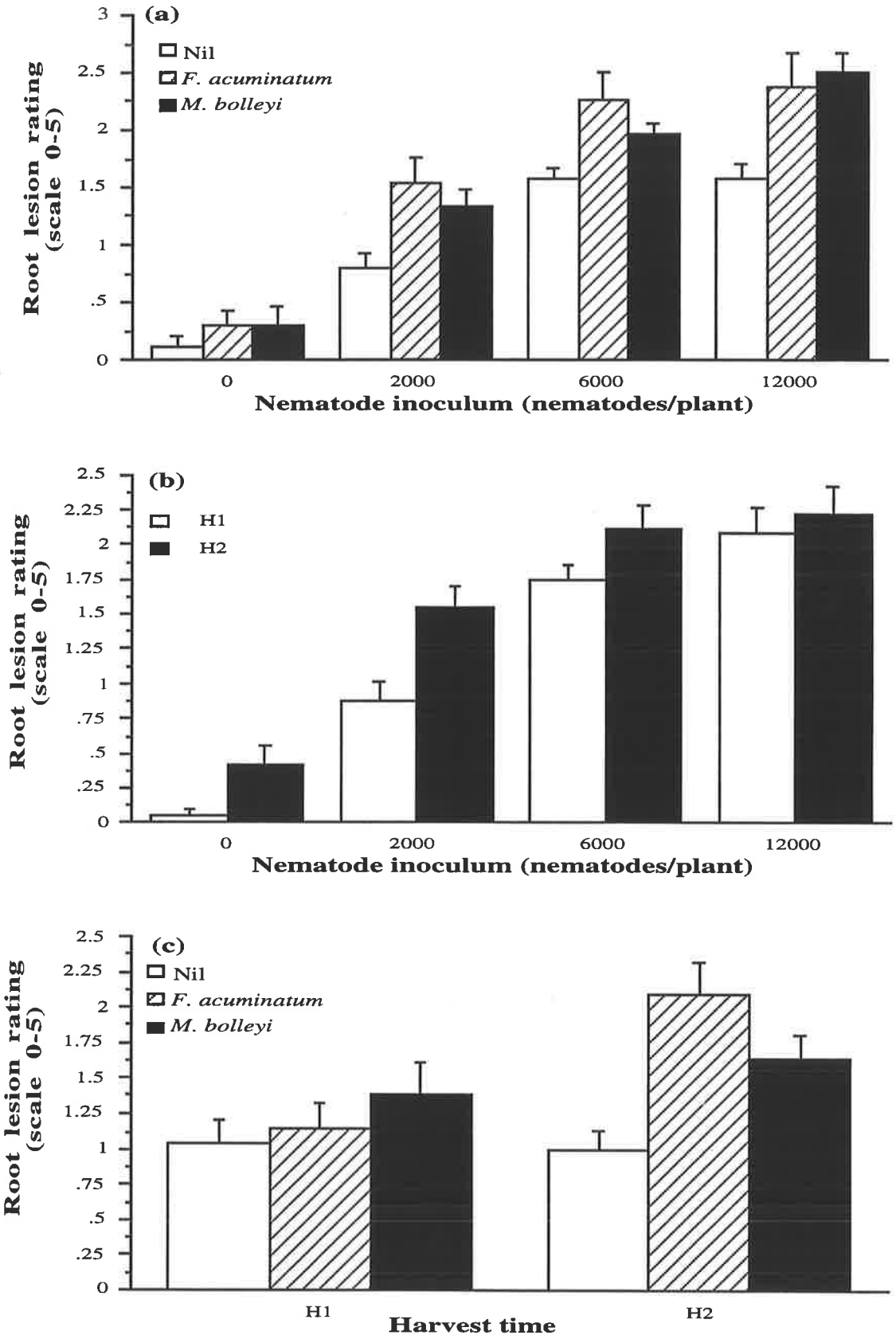


Figure 7.2 (**a**) Effects of nematode-fungus, (**b**) nematode-harvest time, and (**c**) harvest time-fungus interactions on root lesion rating of wheat cultivar Machete. H1= plants harvested seven weeks after sowing, H2= plants harvested ten weeks after sowing. Data are means for both nematode species.

Nematode number: There were no significant differences between the two nematode species for number of nematodes, both nematodes/plant and nematodes/g dry root, so data are shown together. As the initial inoculum density increased, the final number of nematodes extracted from the root system increased (Figure 7.3). This was statistically significant (P=0.001).

Number of nematodes/plant was significantly (P=0.01) affected by a 2-way interaction between harvest time and nematode inoculum and shown in Figure 7.3a. There were two 3-way interactions (harvest × nematode species × fungus and harvest × nematode species × nematode density) significant for nematodes/plant (Table 7.1). Overall, number of *P. neglectus* extracted from root systems was 20% greater than the number of *P. thornei* extracted. This difference was not statistically significant.

At the first harvest time (seven weeks after sowing) number of nematodes/plant for plants inoculated with *F. acuminatum* increased by 100% compared to the control (no fungus added). With *M. bolleyi,* however, nematode numbers/plant increased by only 16% compared to the control (no fungus added) (Figure 7.3b). At the second harvest time (ten weeks after sowing), both nematodes/plant and nematodes/g dry root had decreased where either *F. acuminatum* or *M. bolleyi* was present. Nematode numbers/plant for plants inoculated with *F. acuminatum* or *M. bolleyi* decreased by 34% compared to the nematode alone. This decrease also occurred for nematodes/g dry root.

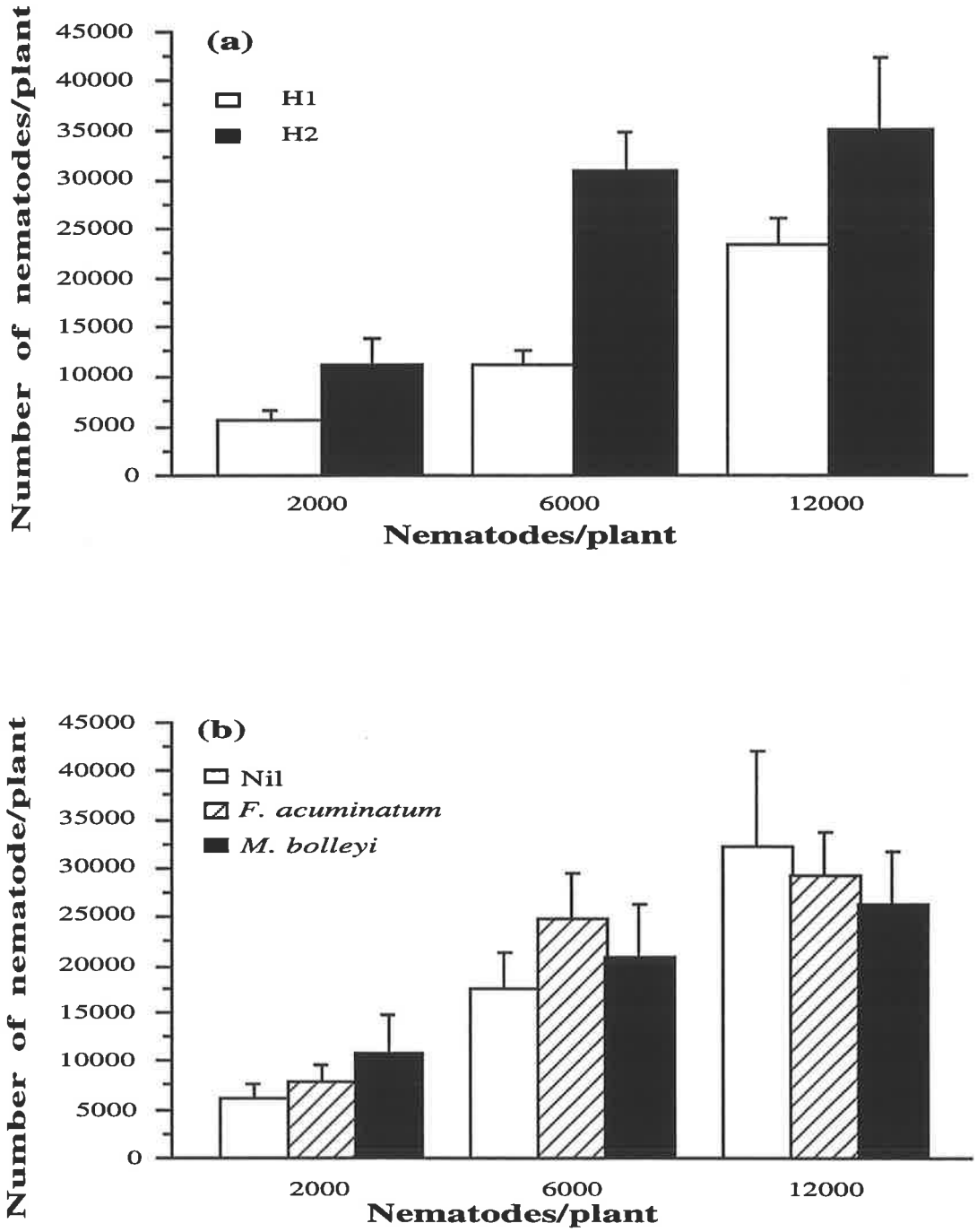


Figure 7.3 The effect of (**a**) nematode × harvest time and (**b**) nematode × fungus interaction on the number of nematodes extracted from roots of wheat cultivar Machete. H1= Harvest 1, H2= Harvest 2. Data are means for both nematode species.

Plant dry matter: There were two 2-way interactions (harvest × nematode density and harvest × fungus) significant for shoot dry weight and a 3-way interaction significant for root dry weight (Table 7.1). Total dry weight was also significantly affected by the 3-way interaction (Table 7.1). As the nematode inoculum density increased, root and shoot dry weights significantly (P=0.05) decreased.

Root dry weight did not significantly differ between the first and second harvest times for plants inoculated with *F. acuminatum* or the control (no fungus added), but plants inoculated with *M. bolleyi* showed a 33% reduction in root dry weight at the second harvest time compared to the first (Figure 7.4).

At the first harvest (seven weeks after sowing), root dry weight of plants inoculated with 6000 or 12000 nematodes/plant, regardless of fungus inoculum, decreased by 41% or 29%, respectively, compared to the control (no fungus or nematode added). With 2000 nematodes/plant, root dry weight increased by 8% compared to the control (no fungus or nematode added) (Figure 7.4).

Fungus inoculum, however, also had a significant effect on root dry weight when combined with the nematode. *F. acuminatum* in combination with 2000 nematodes/plant increased root dry weight by 30% compared to when fungus was applied alone. With 6000 or 12000 nematodes/plant, root dry weight decreased by 31% or 11%, respectively, compared to the effect of fungus alone (Figure 7.4). *M. bolleyi* in combination with 2000 or 12000 nematodes/plant also decreased root dry weight by 24% or 37%, respectively, compared to when fungus alone was added.

At the second harvest (ten weeks after sowing), both fungus and nematode inoculation had a significant effect on root dry weight. With 2000, 6000 or 12000 nematodes/plant, root dry weight decreased by 12%, 12% or 22.5%, respectively, compared to the control (no nematodes added). *F. acuminatum* had a greater effect on root dry weight at the second than the first harvest time. A 31% or 25% reduction in root dry weight occurred for plants inoculated with *F. acuminatum* and 2000 or 12000 nematodes/plant,

Figure 7.4 Effect of harvest-nematode density-fungus interaction on root dry weight/plant of wheat cultivar Machete. H1= plants harvested at seven weeks after sowing, H2= plants harvested at ten weeks after sowing. N0= no nematodes added, N2= 2000 nematodes/plant, N6= 6000 nematodes/plant, N12= 12000 nematodes/plant.

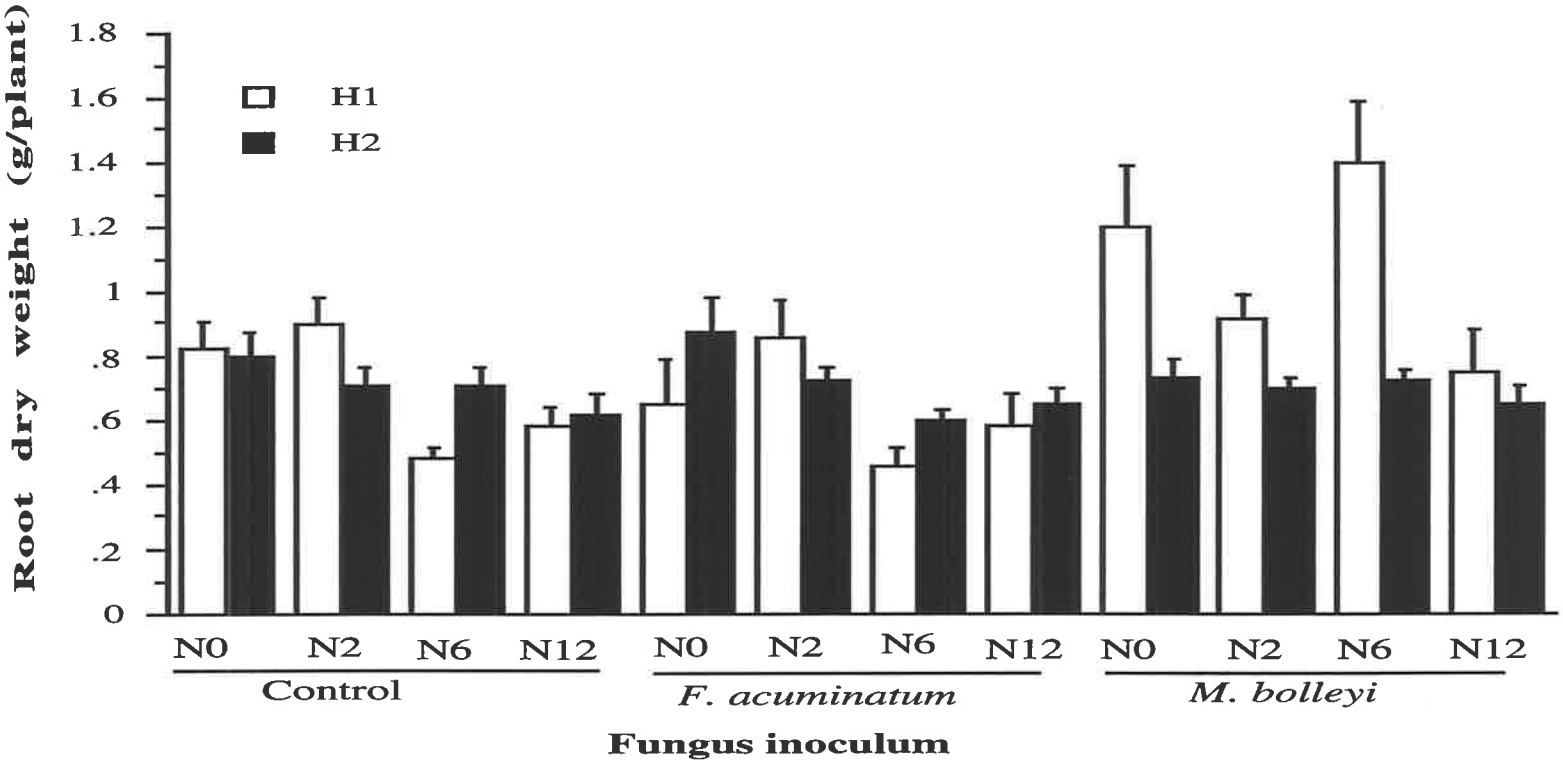
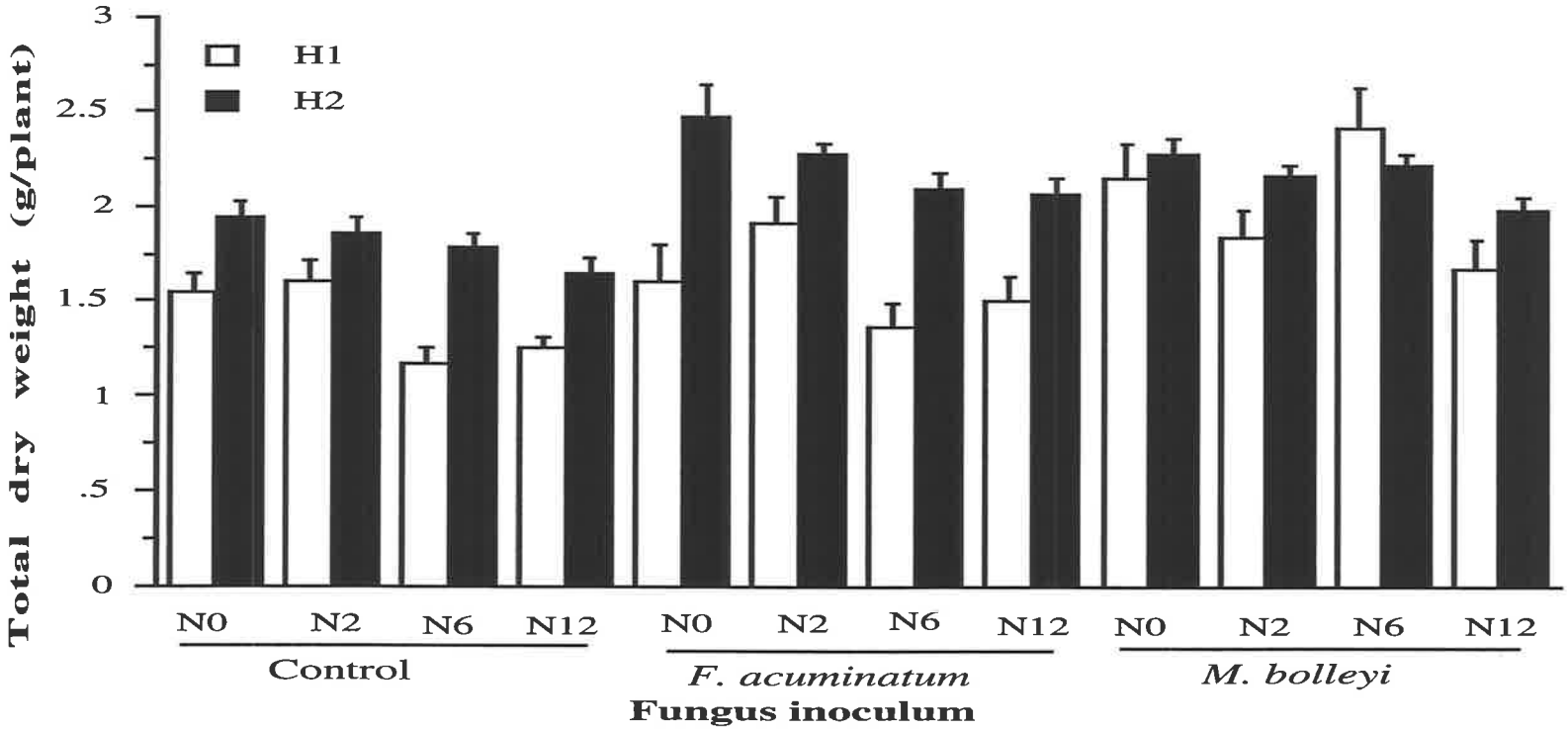


Figure 7.5 Effect of harvest-nematode density-fungus interaction on total dry weight/plant of wheat cultivar Machete. H1= plants harvested at seven weeks after sowing, H2= plants harvested at ten weeks after sowing. N0= no nematodes added, N2= 2000 nematodes/plant, N6= 6000 nematodes/plant, N12= 12000 nematodes/plant.



respectively. *M. bolleyi* too, in combination with 2000 or 12000 nematodes/plant decreased root dry weight by 5% or 11%, respectively, compared to the effect of fungus alone (Figure 7.4). The significant 3-way interaction between harvest × nematode density × fungus for total plant dry weight is shown in Figure 7.5.

7.3.2 Experiment 2

The analyses of variance for all measurements are shown in Table 7.2. Nematode inoculum was successful as is indicated by the very significant differences between nematode numbers in different nematode treatments.

Tiller numbers/plant: Number of tillers/plant was not significantly affected by fungus and nematode inoculum or by harvest time and there were no interaction effects.

Root lesion rating: Root lesion rating was affected by fungus × nematode, fungus × harvest or nematode × harvest interactions (Table 7.2). The 2-way interactions between fungus and nematode, fungus and harvest or nematode and harvest are shown in Figure 7.6. In the presence of *F. acuminatum* or *M. bolleyi* and *P. neglectus*, root lesion rating increased by 126% or 140%, respectively, compared to the fungus alone (Figure 7.6a).

Table 7.2 Summary of analyses of variance for the effect of harvest time (four, six, eight or ten weeks after sowing) on the interaction between *F. acuminatum, M. bolleyi* and *P. neglectus* on the extent of root lesioning, number of nematodes/plant and nematodes/g dry root, shoot and root dry weights, total dry weight of plants and nematode multiplication rate for wheat cultivar Machete.

Source	df	MS RL	P	MS N/p (log)	P	MS N/g (log)	P	MS dws/p	P	MS dwr/p	P	MS tdw/p	P	MS MR	P
Block	2														
Harvest	3	4.15	***	1.03	***	.14	**	2.10	***	.29	***	3.77	***	66.21	***
Fungus	2	12.35	***	.07	***	.08	***	.12	***	.0015	ns	.14	***	7.84	***
Nematode (nem)	1	21.67	***	46.12	***	113.67	***	.05	***	.03	***	.15	***	336.56	***
Fungus × nematode	2	1.10	***	.07	***	.08	***	.003	ns	.0024	ns	.01	*	7.84	***
Fungus × harvest	6	.44	**	.07	***	.10	***	.02	***	.0028	ns	.03	***	13.87	***
Nematode × harvest	3	.31	*	1.03	***	.14	***	.04	***	.0025	ns	.04	***	66.21	***
Fungus × harvest × nem	6	.13	ns	.07	***	.10	***	.004	ns	.01	***	.02	**	13.87	***
Residual	40	.11		.004		.01		.0034		.0014		.0038		.30	

*** significant at P= 0.001 ** significant at P= 0.01 * significant at P= 0.05 ns= not significant P= Probability.
RL= root lesioning; N/p (log)= nematodes/plant; N/g (log)= nematodes/g dry root; dws/p= shoot dry weight/plant; dwr/p= root dry weight/plant; tdw/p= total dry weight/plant; MR= nematode multiplication rate.

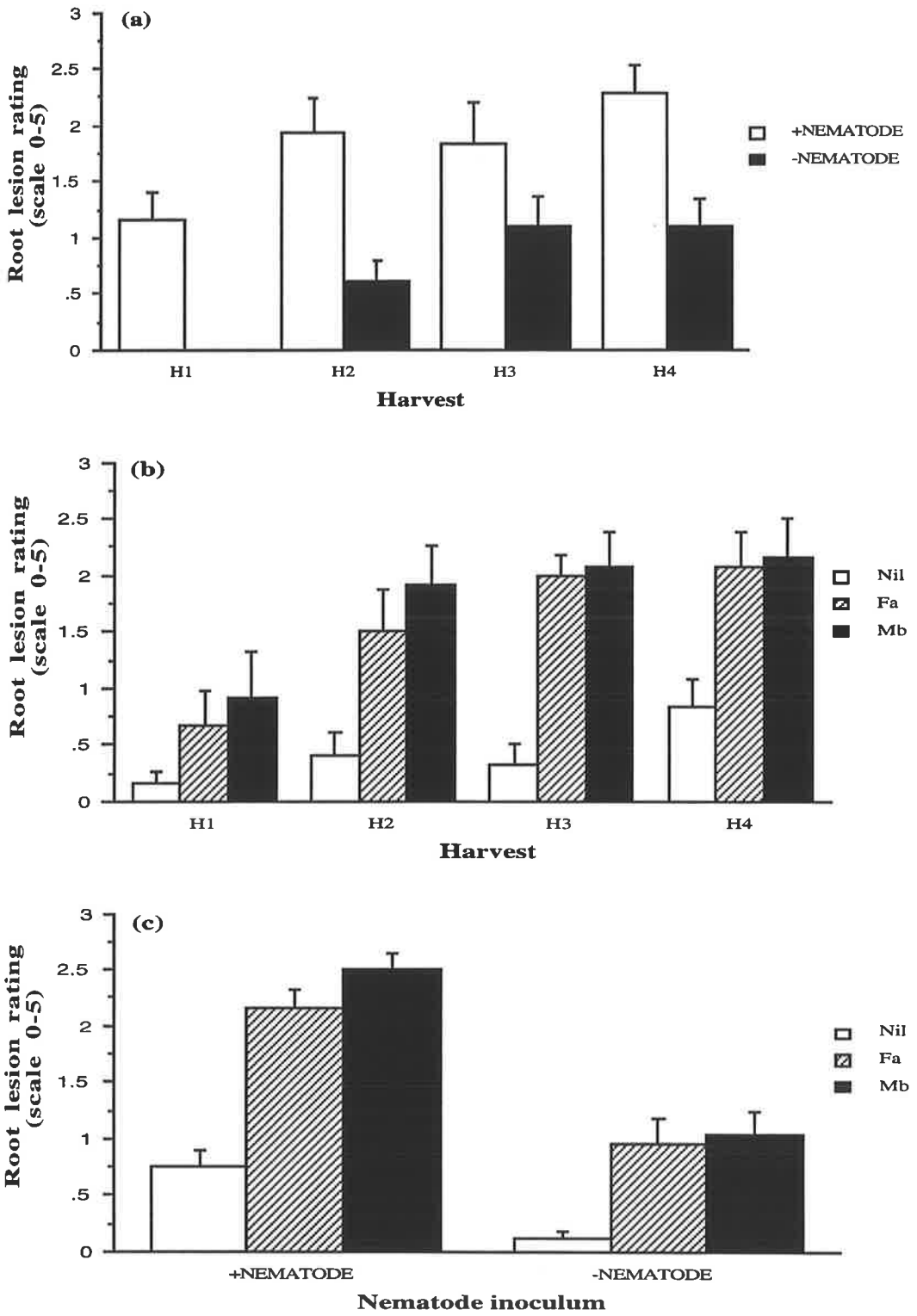


Figure 7.6 Effect of 2-way interaction between **(a)** *P. neglectus* and harvest time, **(b)** fungus and harvest time and **(c)** fungus and *P. neglectus* on the root lesion rating of wheat cultivar Machete. H1= harvest four weeks after inoculation, H2= harvest six weeks after inoculation, H3= harvest eight weeks after inoculation and H4= harvest ten weeks after inoculation. Nil= no fungus added, Fa= *Fusarium acuminatum* and Mb= *Microdochium bolleyi*.

Harvest time also had a very significant effect (P=0.001) on the root lesion rating. The significant 2-way interaction between harvest time and fungus is illustrated in Figure 7.6b. At the first, second, third and fourth harvest times, both *F. acuminatum* and *M. bolleyi* increased root lesion rating by 294%, 257%, 506% and 150% or 441%, 357%, 369% and 161%, respectively, compared to the control (no fungus added) at the same harvest time (Figure 7.6b). Nematode density and root lesion rating increased significantly over the duration of the experiment.

Nematode numbers: Number of nematodes/plant and nematodes/g dry root were affected by all main treatments and their possible combinations (Table 7.2). At the first harvest (four weeks after sowing) only 48% of the initial nematode inoculum was extracted from roots of the control plants (no fungus added). However, with *F. acuminatum* or *M. bolleyi*, 79% or 99% of nematodes, respectively, were extracted from the plants.

At the second, third or fourth harvest time, nematodes/plant (regardless of fungus inoculum) increased by 113%, 610% or 1200%, respectively, compared to the initial inoculum level. With *F. acuminatum,* at the third or fourth harvest times, nematodes/plant decreased by 54%, 42%, respectively, compared to the control (no fungus added) (Figure 7.7a). However, with *M. bolleyi*, number of nematodes/plant at the third harvest increased by 76% but decreased by 61% at the fourth harvest when compared to the control (no fungus added) (Figure 7.7a). Number of nematodes/g dry root showed similar results to nematodes/plant.

Nematode multiplication rate (final nematode numbers extracted from roots/initial inoculum level) also showed a significant increase in the presence of either *F. acuminatum* or *M. bolleyi.* The significant (P=0.05) 3-way interaction between fungus, nematode and harvest time for multiplication rate is shown in Figure 7.7b.

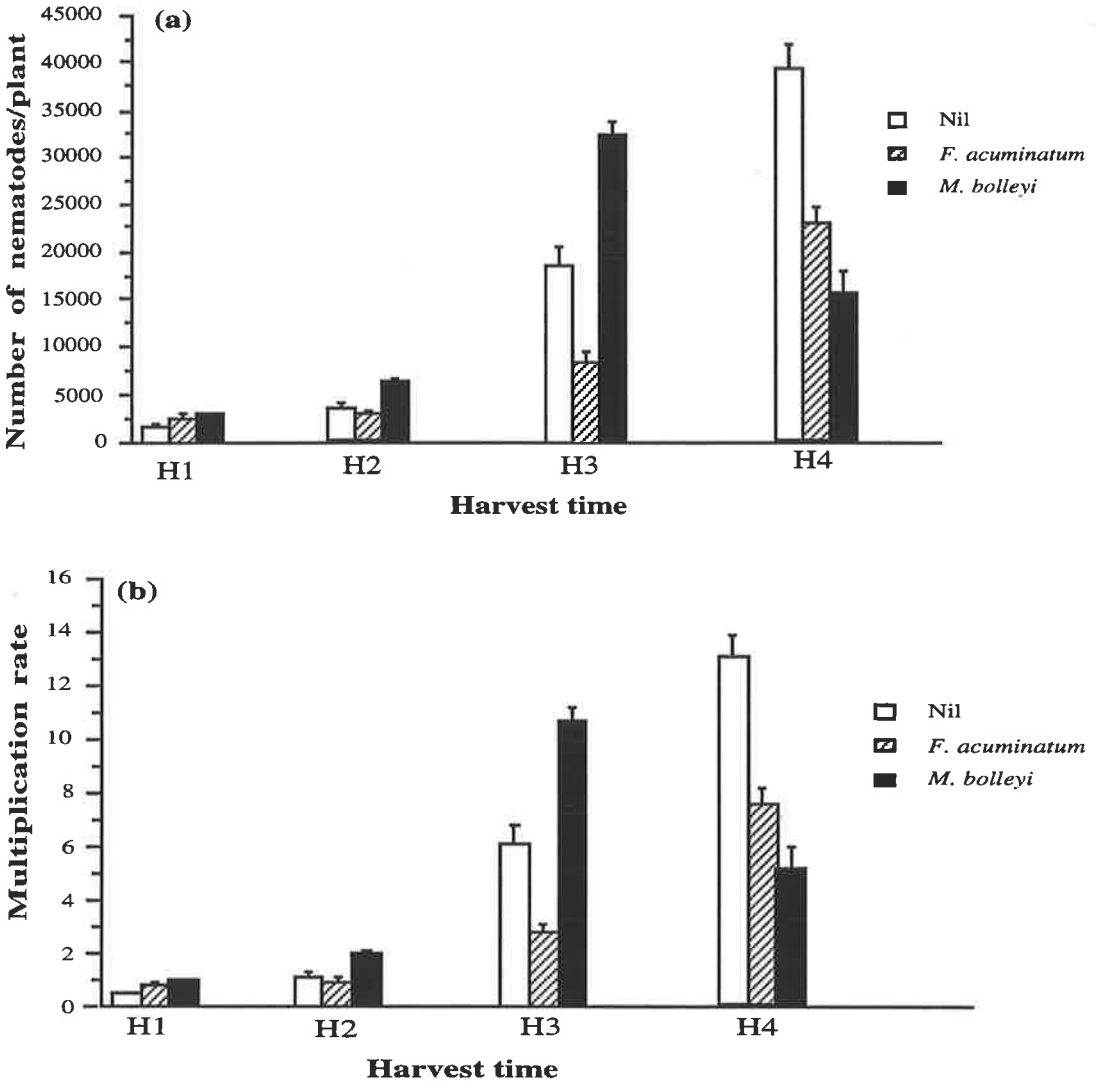


Figure 7.7 Effect of 3-way interaction between fungus and harvest time on **(a)** number of nematodes/plant and **(b)** multiplication rate of *P. neglectus* on wheat cultivar Machete. H1= harvest four weeks after inoculation, H2= harvest six weeks after inoculation, H3= harvest eight weeks after inoculation and H4= harvest ten weeks after inoculation.

Plant dry matter: Shoot weight was significantly affected by the two 2-way interactions (fungus × harvest and nematode × harvest), whereas root dry weight was affected by only a 3-way interaction between fungus, nematode and harvest time (P=0.001) (Table 7.2).

Both *F. acuminatum* and *M. bolleyi* caused increase in shoot dry weight when compared to the control (no fungus added). Unlike fungi, nematode inoculation decreased production of shoots significantly. At the first, second, third or fourth harvest, shoot dry weight decreased by 25%, 13%, 2% or 21%, respectively, compared to when

nematodes were not added (Figure 7.8).

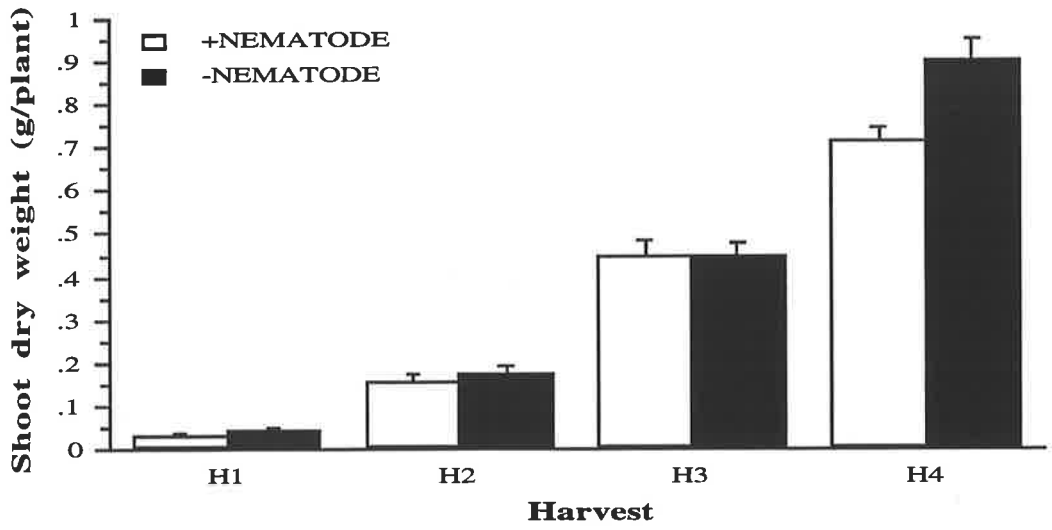


Figure 7.8 Effect of nematode-harvest time on shoot dry weight of Machete wheat over time. H1= harvest four weeks after inoculation, H2= harvest six weeks after inoculation, H3= harvest eight weeks after inoculation and H4= harvest ten weeks after inoculation.

Fungus inoculum had no significant effect on root dry weight, but nematode or harvest time had a significant effect (P=0.001) on the production of roots. The significant (P=0.001) 3-way interaction between fungus, nematode and harvest time for root dry weight is shown in Figure 7.9a. Nematodes alone at either the first, second or fourth harvest decreased production of roots by 20%, 7% or 37%, respectively, compared to the control (no nematodes added) (Figure 7.9a). However, at the third harvest time (eight weeks after sowing), root dry weight increased by 17% compared to the control.

With the combination of *F. acuminatum* and *P. neglectus*, a further decrease in root dry weight occurred. At the first, second or third harvest time, root dry weight decreased by 33%, 41% or 34%, respectively, compared to the effect of fungus alone (Figure 7.9a). At the fourth harvest, however, *F. acumintaum* in combination with the nematode caused a 14% increase in root dry weight compared to the fungus alone.

M. bolleyi in combination with the nematode also had a significant effect on root dry weight. At the second, third or fourth harvest time, root dry weight of plants inoculated with *M. bolleyi* and the nematode decreased by 33%, 27% or 22%, respectively,

compared to the effect of fungus alone or control (no fungus or nematode added) (Figure 7.9a). Overall, regardless of the fungus effect, the nematodes decreased production of roots by 15%.

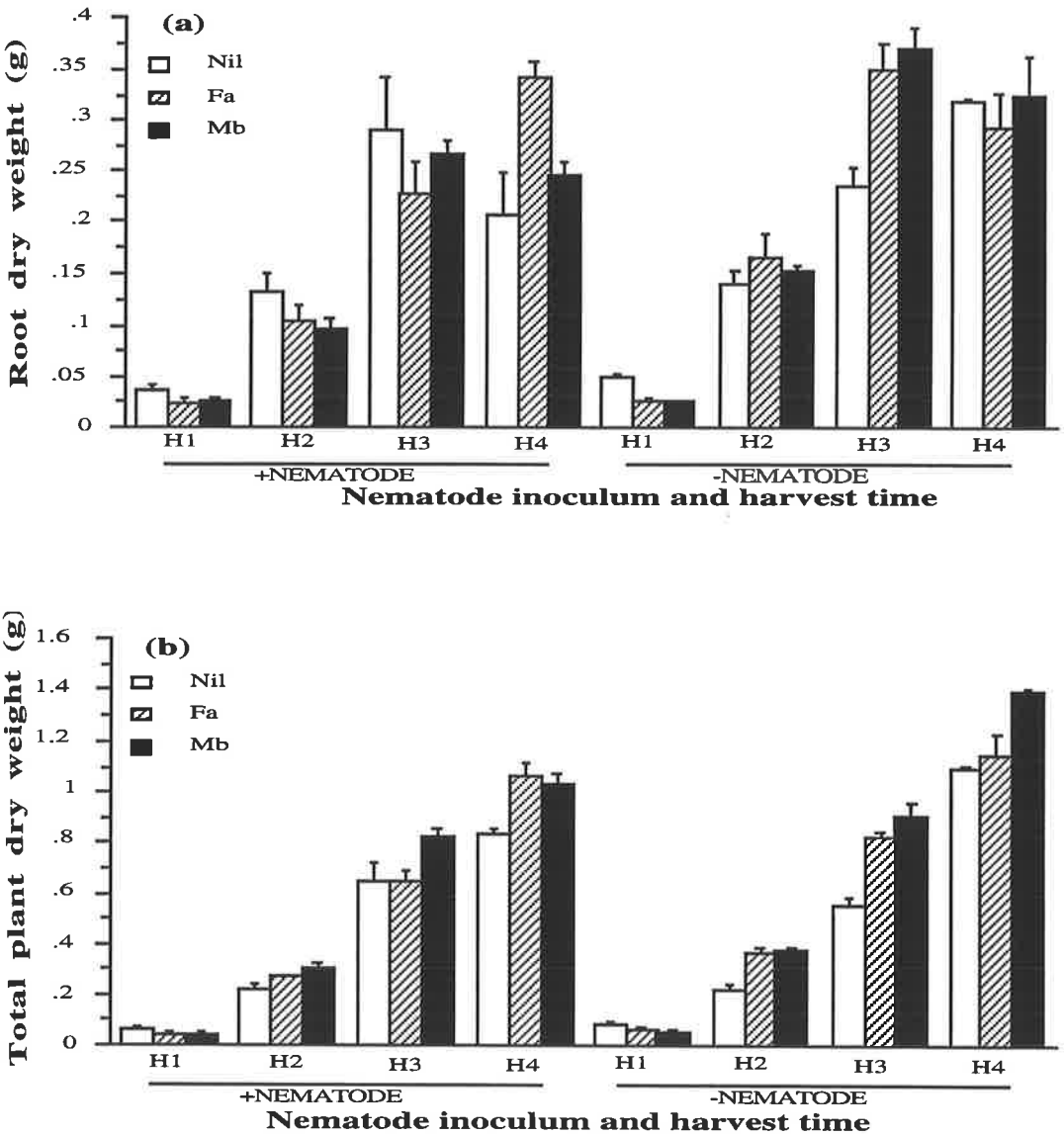


Figure 7.9 Effect of 3-way interaction between fungus, nematode and harvest time on **(a)** root dry weight/plant and **(b)** total plant dry weight of wheat cultivar Machete. H1= harvest four weeks after inoculation, H2= harvest six weeks after inoculation, H3= harvest eight weeks after inoculation and H4= harvest ten weeks after inoculation. Nil= no fungus added, Fa= *Fusarium acuminatum* and Mb= *Microdochium bolleyi*.

Similarly, total dry weight of plants was affected by nematode and fungus inoculation as well as by harvest time. At the fourth harvest, the nematodes alone caused a 24%

reduction in root dry weight compared to when nematodes were not added (Fingure 7.9b). Also, at this harvest, both *F. acuminatum* and *M. bolleyi* in combination with the nematode decreased total dry weight by 8% and 26%, respectively, compared to when fungus was applied alone (Figure 7.9b).

7.3.3 Experiment 3

The analyses of variance for all measurements are shown in Table 7.3. Root lesion rating, nematode number/plant or nematodes/g dry root, nematode multiplication rate and shoot dry weight were significantly affected by all main treatments (fungus, nematode or inoculation time). Root dry weight, however, was only affected by nematode inoculum or inoculation time and not by fungus (Table 7.3). A 2-way interaction between nematode and fungus was significant for root lesion rating.

***Tiller number*:** Number of tillers/plant was significantly affected by the interaction between fungus and inoculation time (Table 7.4). Presence of either *F. acuminatum* or *M. bolleyi* increased number of tillers/plant, but a significant increase resulted when *M. bolleyi* was added to the pots. Highest number of tillers was produced when both fungus and nematode inoculum were applied at sowing. However, with late application of fungus (nematode first, fungus four weeks later), the lowest number of tillers resulted (Table 7.4). This reduction was 34% compared to when both fungus and the nematode were applied at sowing.

Root lesion rating: Root lesion rating was significantly affected by the 2-way interaction between nematode and fungus (Table 7.3). Presence of the nematode increased root lesion rating by up to 98% compared to the control (no nematodes added) (Table 7.5). With fungi too, in the presence of either *F. acuminatum* or *M. bolleyi,* the amount of lesions increased by 28% and 30%, respectively, compared to the control (no fungus inoculum added). Different inoculation times also had a significant effect on root lesion rating. Simultaneous inoculation of both fungus and nematode resulted in the highest root lesion rating.

Table 7.3 Summary of analyses of variance for effect of interaction between *Microdochium bolleyi* and/or *Fusarium acuminatum* and *P. neglectus* on extent of root lesioning, number of nematodes/plant, shoot and root dry weight of plants, number of tillers/plant and nematode multiplication rate for wheat cultivar Machete, 70 days after sowing.

Source	df	MS RL	P	MS N/p	P	MS dws/p	P	MS dwr/p	P	MS tillers	P	MS MR	P
Nematode (nem)	1	123.76	***	1.82E11	***	.05	*	.63	**	.25	ns	5062	***
Fungus (fun)	2	1.52	***	9.82E9	***	.78	***	.10	ns	2.79	***	273	***
Time	3	.23	*	1.31E10	***	.55	***	.14	*	6.27	***	365	***
Nematode × fungus	2	1.28	***	9.82E9	***	.01	ns	.12	ns	.06	ns	273	***
Fungus × time	6	.07	ns	3.63E9	***	.14	***	.09	ns	1.96	***	101	***
Nematode × time	3	.11	ns	1.31E10	***	.05	ns	.01	ns	.15	ns	365	***
Nem × fun × time	6	.13	ns	3.63E9	***	.02	ns	.10	ns	.08	ns	101	***
Residual	120	.07		4.02E8		.05		.05		.25		11	

*** significant at P= 0.001 ** significant at P= 0.01 * significant at P= 0.05 ns= not significant P= Probablibity.
RL= root lesioning; N/p= nematodes/plant; dws/p= shoot dry weight/plant; dwr/p= root dry weight/plant; tillers= tillers/plant; MR= nematode multiplication rate.

Table 7.4 Effect of nematode-fungus interaction on the number of tillers/plant 70 days after sowing in a pasteurised soil under controlled environment conditions. Values in the 3-way table are the average of six single plant blocks.

	N0				N6000			
Fungus	T0	T1	T2	T3	T0	T1	T2	T3
Nil	1.83	1.83	1.83	1.83	1.83	1.83	1.83	1.83
F. acuminatum	2.83	2.00	1.67	1.83	2.50	2.00	1.83	1.5
M. bolleyi	3.67	2.00	2.00	1.83	3.33	2.17	1.83	1.67

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD at P= 0.05).

					LSD
Fungus	Nil	*F. acuminatum*	*M. bolleyi*		
	1.83	2.02	2.31		*0.20*
Nematode	N0	N6000			
	2.10	2.01			*ns*
Inoculation time	T0	T1	T2	T3	
	2.67	1.97	1.83	1.75	*0.23*
Fungus	Nil	*F. acuminatum*	*M. bolleyi*		
Nematode					
N0	1.83	2.08	2.38		*ns*
N6000	1.83	1.96	2.25		
Inoculation time	T0	T1	T2	T3	
Fungus					
Nil	1.83	1.83	1.83	1.83	
F. acuminatum	2.67	2.00	1.75	1.67	*0.40*
M. bolleyi	3.50	2.08	1.92	1.75	
Inoculation time	T0	T1	T2	T3	
Nematode					
N0	2.78	1.94	1.83	1.83	*ns*
N6000	2.56	2.00	1.83	1.67	

Inoculation time: T0= fungus and nematode at sowing; T1= nematode at sowing, fungus two weeks later; T2= nematode at sowing, fungus three weeks later; T3= nematode at sowing, fungus four weeks later. N0= no nematodes added, N6000= 6000 nematodes/plant.

Table 7.5 Effect of nematode-fungus interaction on the root lesion rating (scale 0-5) of wheat cultivar Machete 70 days after sowing in a pasteurised soil under controlled environment conditions. Values in the 3-way table are the average of six single plant blocks .

	N0				N6000			
Fungus	T0	T1	T2	T3	T0	T1	T2	T3
Nil	0.00	0.00	0.00	0.00	1.5	1.5	1.5	1.5
F. acuminatum	0.00	0.00	0.00	0.00	2.42	2.17	2.08	1.92
M. bolleyi	0.33	0.00	0.00	0.00	2.00	2.17	2.17	1.67

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD at P= 0.05).

					LSD
Fungus	Nil	*F. acuminatum*	*M. bolleyi*		
	0.75	1.07	1.04		*0.10*
Nematode	N0	N6000			
	.03	1.88			*0.08*
Inoculation time	T0	T1	T2	T3	
	1.04	0.97	0.96	0.85	*0.12*
Fungus	Nil	*F. acuminatum*	*M. bolleyi*		
Nematode					
N0	0.00	0.00	0.08		*0.15*
N6000	1.50	2.15	2.00		
Inoculation time	T0	T1	T2	T3	
Fungus					
Nil	0.75	0.75	0.75	0.75	
F. acuminatum	1.21	1.08	1.04	0.96	*ns*
M. bolleyi	1.17	1.08	1.08	0.83	
Inoculation time	T0	T1	T2	T3	
Nematode					
N0	0.11	0.00	0.00	0.00	*ns*
N6000	1.97	1.94	1.92	1.69	

Inoculation time: T0= fungus and nematode at sowing; T1= nematode at sowing, fungus two weeks later; T2= nematode at sowing, fungus three weeks later; T3= nematode at sowing, fungus four weeks later. N0= no nematodes added, N6000= 6000 nematodes/plant.

In contrast, at inoculation time three, where fungus was added to the pots four weeks after nematode inoculum, the lowest amount of lesioning was recorded (Table 7.5). A significant 2-way interaction between nematode and fungus is shown in Table 7.5. A 28% increase in root lesion rating occurred when *F. acuminatum* was combined with 6000 *P. neglectus* at sowing compared to the effect of nematodes alone at the same density or a 100% increase compared to fungus alone. *M. bolleyi* too, in combination with 6000 nematodes/plant, increased root lesion rating by 25% compared to the nematode alone or 100% compared to the effect of fungus alone (Table 7.5).

***Number of nematodes*:** All main treatments, as well as their interactions, were significant for nematode numbers/plant (Table 7.3). The 3-way interactions between fungus, nematode and inoculation time for nematode numbers/plant, nematodes/g dry root or multiplication rate of *P. neglectus* are illustrated in Figure 7.10. *M. bolleyi* or *F. acuminatum* increased nematode numbers by 58% and 52%, respectively, compared to the nematode alone (Figures 7.10a and 7.10b). Similarly, nematode multiplication rate (final/inital number) was increased in the presence of *F. acuminatum* or *M. bolleyi* compared to in the absence of fungus (Figure 7.10c).

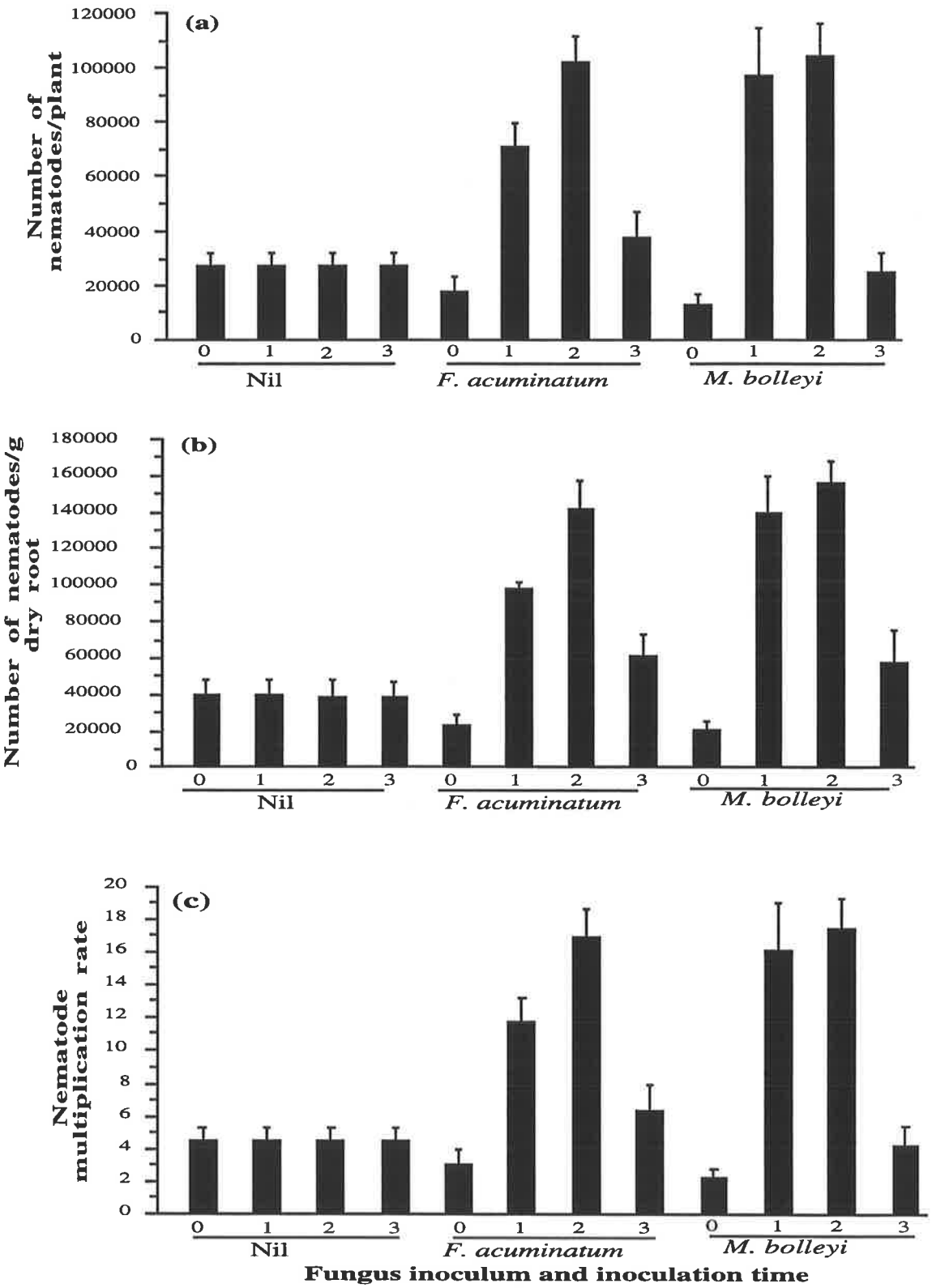


Figure 7.10 Effect of interaction between nematode × fungus × inoculation time on **(a)** number of nematodes/plant, **(b)** number of nematodes/g dry root and **(c)** nematode multiplication rate of wheat cultivar Machete 35 days after sowing. (**Inoculation time:** T0= fungus and nematode at sowing; T1= nematode at sowing, fungus two weeks later; T2= nematode at sowing, fungus three weeks later; T3= nematode at sowing, fungus four weeks later).

***Plant dry matter*:** Root and shoot dry weights were significantly affected by nematode and inoculation time. Fungus inoculum only affected shoot dry weight (Table 7.3). The 3-way treatment means as well as 1 and 2-way interactions for shoots and roots are shown in Tables 7.6 and 7.7.

Root dry weight of plants inoculated with 6000 nematodes decreased by 19% compared to the control (no nematodes added) (Table 7.6). This reduction was statistically significant (P= 0.01). Inoculum of *F. acuminatum* or *M. bolleyi,* however, had no significant effect on dry weight of roots (Table 7.6).

Different fungus inoculation time also had a significant effect on root dry weight. Root production of plants inoculated with either fungus increased when fungus was applied two weeks after nematodes (Table 7.6). Root dry weight, however, decreased when fungus was applied to the pots four weeks after nematode inoculum (Figure 7.6).

Shoot dry weight was also affected by all main treatments as well as by a 2-way interaction between fungus and inoculation time (Table 7.7). With simultaneous inoculation of fungus and nematode, shoot dry weight increased significantly compared to the control (no fungus added) (Table 7.7). However, as the fungus inoculum was added to the pots later (two, three or four weeks after the nematode), shoot dry weight was decreased compared to when fungus was applied with nematodes at sowing (Table 7.7).

Table 7.6 Effect of nematode-fungus interaction on the root dry weight (g/plant) of wheat cultivar Machete 70 days after sowing in a pasteurised soil under controlled environment conditions. Values in the 3-way table are the average of six single plant blocks.

	N0				N6000			
Fungus	T0	T1	T2	T3	T0	T1	T2	T3
Nil	0.73	0.73	0.73	0.73	0.72	0.72	0.72	0.72
F. acuminatum	0.74	1.25	0.83	0.72	0.75	0.72	0.73	0.61
M. bolleyi	0.96	0.72	0.86	0.69	0.61	0.68	0.66	0.47

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD at P= 0.05).

					LSD
Fungus	Nil	*F. acuminatum*	*M. bolleyi*		
	0.73	0.79	0.71		*ns*
Nematode	N0	N6000			
	0.81	0.68			*0.07*
Inoculation time	T0	T1	T2	T3	
	0.75	0.81	0.76	0.66	*0.11*
Fungus	Nil	*F. acuminatum*	*M. bolleyi*		
Nematode					
N0	0.74	0.89	0.81		*ns*
N6000	0.72	0.70	0.61		
Inoculation time	T0	T1	T2	T3	
Fungus					
Nil	0.73	0.73	0.73	0.73	
F. acuminatum	0.75	0.99	0.78	0.66	*ns*
M. bolleyi	0.78	0.70	0.76	0.58	
Inoculation time	T0	T1	T2	T3	
Nematode					
N0	0.81	0.90	0.81	0.71	*ns*
N6000	0.69	0.71	0.71	0.60	

Inoculation time: T0= fungus and nematode at sowing; T1= nematode at sowing, fungus two weeks later; T2= nematode at sowing, fungus three weeks later; T3= nematode at sowing, fungus four weeks later. N0= no nematodes added, N6000= 6000 nematodes/plant.

Table 7.7 Effect of nematode-fungus interaction on the shoot dry weight (g/plant) of wheat cultivar Machete 70 days after sowing in a pasteurised soil under controlled environment conditions. Values in the 3-way table are the average of six single plant blocks.

	N0				N6000			
Fungus	T0	T1	T2	T3	T0	T1	T2	T3
Nil	1.07	1.08	1.10	1.07	1.14	1.12	1.16	1.14
F. acuminatum	1.57	1.47	1.23	1.13	1.51	1.38	1.35	1.17
M. bolleyi	1.62	1.28	1.13	1.08	1.51	1.38	1.39	1.04

(3-way interaction not significant)

1 and 2-way treatment means (with appropriate LSD at P= 0.05).

					LSD
Fungus	Nil	*F. acuminatum*	*M. bolleyi*		
	1.11	1.35	1.31		*0.05*
Nematode	N0	N6000			
	1.24	1.27			*0.04*
Inoculation time	T0	T1	T2	T3	
	1.41	1.29	1.22	1.11	*0.06*

Fungus	Nil	*F. acuminatum*	*M. bolleyi*		
Nematode					
N0	1.08	1.35	1.28		*ns*
N6000	1.14	1.35	1.33		
Inoculation time	T0	T1	T2	T3	
Fungus					
Nil	1.11	1.14	1.09	1.11	
F. acuminatum	1.54	1.42	1.29	1.15	*0.10*
M. bolleyi	1.56	1.33	1.26	1.06	
Inoculation time	T0	T1	T2	T3	
Nematode					
N0	1.42	1.28	1.15	1.10	*0.08*
N6000	1.39	1.30	1.29	1.12	

Inoculation time: T0= fungus and nematode at sowing; T1= nematode at sowing, fungus two weeks later; T2= nematode at sowing, fungus three weeks later; T3= nematode at sowing, fungus four weeks later. N0= no nematodes added, N6000= 6000 nematodes/plant.

7.4 Discussion

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 responds.

Mechanical injuries caused by nematodes as they penetrate within or feed on root tissues together with physiological and/or biochemical changes induced or produced by nematodes, provide potential sites for fungal infection or directly enhance the invasion and development of pathogenic fungi. However, mechanical wounding does not always promote fungal penetration within root tissues (Chapter 6). There is strong evidence, particularly with the sedentary nematodes such as root knot nematodes, that physiological and/or biochemical changes predispose the host to fungal pathogens (Prot, 1993).

Certain fungi (*M. bolleyi* and *F. acuminatum*) and nematodes (*P. neglectus* and *P. thornei*) invade cortical tissues. *M. bolleyi* has been found in cortical and epidermal cells (Kirk and Deacon, 1987a,b). However, *M. bolleyi* has been reported to cause damage and plant growth reductions (Domsch and Gams, 1968; Chapter 6) and was suggested to be the primary fungal coloniser on cereals in the eastern prairies of Canada (Sturz and Bernier, 1989). In the results reported, here, *M. bolleyi* alone at 1% w/w did not cause severe root damage but in combination with either nematode species (*P. neglectus* or *P. thornei*) significantly increased root lesion rating. Therefore, it is unlikely that *M. bolleyi* is the primary fungal coloniser on wheat crops but is one of the early fungal colonisers on plants infected by *P. neglectus*.

F. acuminatum has similar pathology to *M . bolleyi*, penetrating epidermal cells, but hyphae are rarely observed to enter wounds directly (Sturz and Bernier, 1985). *F. acuminatum* is considered to be a weakly virulent pathogen causing the most damage when adverse environmental conditions, such as drought stress, persist (Hill and Blunt, 1994). In the experiments reported here it was also found that the fungus is not a major pathogen in its own right but could be considered as a potential pathogen in interaction with the root lesion nematodes, causing extensive damage to the root system of wheat.

However, both fungi are found at high levels in late July and August in South Australian cropping regions (Chapter 3; Vanstone, 1991) where the number of nematodes is also high.

The fungal component of the interaction in the disease complex generally has real effects on nematode populations (Powell, 1971). In general, it is believed that populations of root lesion nematodes increase in the presence of some root-rotting fungi (Chapter 4; Vrain, 1987; Hasan, 1988). *P. thornei* and *P. neglectus* appeared to increase in number and multiplication rate in the presence of fungi after seven weeks. This increase has previously been reported for eggplant and tomato infected with *Verticillium* and *P. penetrans* (Mountain, 1954). Also Faulkner and Skotland (1965) observed increased reproduction of *Pratylenchus* on peppermint in the presence of *Verticillium dahliae*.

After ten weeks, however, *P. neglectus* with the fungus significantly declined in number and multiplication 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 or may simply reflect nematode species sepecific behaviour. It has been well documented that the juveniles of *Pratylenchus* spp. do not invade roots already rotted by fungi, and are known to migrate out of extensively damaged tissues (Corbett, 1972; Dropkin, 1989).

Although the dominant species of *Pratylenchus* in South Australian soil is *P. neglectus*, the closely related species, *P. thornei,* also occurs widely. Importantly, mixed populations of these two species are evident (Nicol, 1996). *P. thornei* also positively interacted with *M. bolleyi* or *F. acuminatum* in a similar way to *P. neglectus*. Both fungi in combination with *P. thornei* increased root lesion rating and number of nematodes within roots of Machete wheat. However, it has been reported that nematode species with closely related biology and feeding behaviour may present differences in ability to predispose a plant to infection by the same fungus (Prot, 1993).

Numbers of nematodes are found to increase with increasing nematode density while the multiplication rate declines. As shown in Figure 7.2, increases in nematode number and density increased the severity of root lesioning, with the degree of severity increasing with time. Root damage was greater at high initial nematode densities (6000 and 12000 nematodes/plant) with both *M. bolleyi* and *F. acuminatum*. However, severely rotted roots contained fewer nematodes. Others also reported that number of *Pratylenchus* spp. declines with a high level of infection by fungi or other nematodes (Bhatt, 1986; Chandel and Sharma, 1989).

The growth parameters measured for wheat cultivar Machete were all significantly affected by both *P. thornei* and *P. neglectus,* regardless of fungal inoculum. However, the degree of root lesioning and increase in number of nematodes or multiplication rate of both nematodes were even higher when fungus inoculum was also added. In general, increasing nematode density, with or without a fungal combination, was found to decrease the growth of roots, shoots and the summation of these. However, there was some evidence that at low initial densities (2000 nematodes/plant) plant growth was stimulated, particularly up to seven weeks. This was also reported by Nicol (1991). However, because reproduction of root lesion nematodes occurs continuously throughout the growing season in the presence of a host, it is likely that with time, damaging densities would over-ride this stimulatory effect as appeared to have happened by week ten.

Plant growth and number of tillers were stimulated in plants inoculated with fungi alone or in combination with nematodes at both harvest times. Fungi did not significantly affect root dry weight, except for *M. bolleyi*. This fungus had significantly stimulated root growth at the first harvest, in comparison with the control or *F. acuminatum*. However, by ten weeks, there was no difference between the control, *F. acuminatum* or *M. bolleyi*. The medium used for growing these fungi (millet seed) was rich in nutrients which could also have stimulated plant growth. This made it difficult to determine whether increased plant growth was due to the effect of fungus alone or increased nutrition. It might also affect the interaction between nematodes and fungi tested. Thus,

further work is required to establish a method of inoculation with less risk of nutritional side-effects.

Prot (1993) notes that nematode species with closely related biology and feeding habits may have different abilities to predispose a plant to infection by the same fungus. 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*. However, by harvest two, after ten weeks, there was no distinction between the root dry weight of wheat plants inoculated with either *P. thornei* or *P. neglectus*.

Overall, the total dry weight was stimulated in the presence of fungi with or without nematodes at both harvest times. 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. However, this inoculation experiment with *P. neglectus* and *P. thornei* demonstrates that there is an interaction between both nematodes and root-rotting fungi investigated.

Further investigation of the interaction between both nematode species and/or root-rotting fungi is needed to determine the effect of different environmental conditions such as temperature, moisture and soil texture on their interaction. Cultivars known to be resistant to *P. thornei* (Thompson and Clewett, 1989) were not resistant to *P. neglectus* (Farsi, 1995). Therefore, it is important to demonstrate the possible reasons for one species of nematode dominating the population in some areas, in order to develop efficient strategies for control of these nematodes and to develop resistant varieties.

Sequential inoculation of fungus and nematode may have a significant effect on a given interaction. It was concluded in Chapter 5 that pre-inoculation of some fungi may change the interaction between nematode and fungus from synergistic to antagonistic. Synergistic interactions, where the combined effect of nematode and fungus is more than the sum of the individual pathogens, usually occur due to the role of nematodes in favouring fungal infection. However, antagonistic interaction occurs when combinations

of plant parasitic nematodes and soil-borne fungi result in less plant damage, compared to the sum of the individual damage. In Chapter 6 it was found that pre-inoculation with fungus, particularly *M. bolleyi* and *F. acuminatum,* did not effect fungus-nematode interactions, whereas when the nematode was applied two weeks before the fungus the result was different.

Root lesion nematodes, particularly *P. neglectus,* are among the species of nematode penetrating host roots in very early stages of growth (Benedict and Mountain, 1956). Considering that nematodes are able to modify the rhizosphere through root secretions of infected plants, and the fact that weakly pathogenic fungi in soil may become pathogenic in the presence of nematodes, it appears that the infection sequence of the two pathogens (nematodes and fungi) which occurs automatically in nature is important. Furthermore, the feeding process of *Pratylenchus* spp. as well as all other plant-parasitic nematodes produces a wound of some kind in the host (Taylor, 1979), providing ready avenues of entry for other pathogens.

From the results of Experiment 2, it was concluded that inoculation of plants with fungus two to three weeks after nematode inoculum results in higher nematode numbers being recovered from roots and extensive lesioning to the root system. Although this type of interaction may not always occur in nature, there are several reports indicating this kind of synergistic interaction in controlled glasshouse conditions (Powell, 1979). Pre-inoculation of plants with nematodes four weeks before inoculation with the fungus, however, did not alter disease rating caused by *M. bolleyi* or *F. acuminatum* on Machete wheat. Golden and Van Gundy (1975) noted that five to six weeks following invasion, *R. solani* had extensively colonised nematode-induced giant cells.

The second experiment in this Chapter was designed to investigate the effect of time on nematode-fungus interaction. Harvest time had a significant effect on the interaction between *M. bolleyi* or *F. acuminatum* and *P. neglectus*. Root lesion rating was visible as early as four weeks after inoculation with fungus and nematode in controlled growth chamber conditions. At six, eight and ten weeks after sowing there were progressive

increases in root lesion rating but the number of nematodes extracted from roots of Machete wheat declined after week six. It is suggested that under optimum conditions, severity of damage increased over a short period of time.

This could be true under natural conditions in the field. Early in the season, where soil temperature is low and available moisture in the soil is high, the fungus or nematodes merely infect the plant but the extent of lesioning is not severe. However, late in the season particularly when soil temperature rises, the degree of lesioning on the roots increases very rapidly (Chapter 3) making disease diagnosis easier.

From the results of this study it was concluded that a period of six to eight weeks after inoculation of fungus (*M. bolleyi* or *F. acuminatum*) and *P. neglectus* on wheat is recommended to assess a nematode-fungus interaction. By this time it is assumed that most of the nematode inoculum has penetrated the roots, and at least one generation of nematodes has been produced. Two to three weeks after nematodes were inoculated onto the plant, it is also assumed that a considerable amount of root exudate modified by nematode activity is present in the rhizosphere influencing fungus activity. It is also possible that due to the infection of plants with nematodes plants become more susceptible and more attractive to fungi. Although at ten weeks after inoculation of fungus and nematodes the root lesion rating was high, the number of *P. neglectus* recovered from roots declined. This may affect interpretation of results. Therefore, it is appropriate to consider a period of about seven weeks to study a nematode-fungus interaction under controlled conditions.

Chapter 8

Effect of soil temperature on the nematode-fungus interaction

8.1 Introduction

P. neglectus is a root pathogen in its own right (Chapters 5, 6 and 7). The nematode is widespread in South Australia, and association of soil-borne fungi with the nematode is well known (Powell, 1971). Numerous fungal-nematode interactions have been reported since the initial observation of Atkison in 1892. Association of soil-borne fungi with damaged wheat roots from the field is well documented (Chapter 3; Harris and Moen, 1985b; Vanstone, 1991). Non or weakly parasitic soil-borne fungi in the presence of nematodes can cause considerable damage to the host plant.

Microdochium bolleyi, *Fusarium acuminatum*, *Pyrenochaeta terrestris* (weakly parasitic fungi) or *Pythium irregulare* (a root parasitic fungus) are the most common fungi found in association with the damaged roots of nematode infected wheat plants (Chapter 3; Vanstone, 1991). Presence of fungi increased root lesion rating, and nematode numbers, and some fungi in combination with the nematode decreased root dry weight of wheat grown in glasshouse conditions (Chapter 4).

Of the abiotic environmental factors influencing pathogenicity of nematodes and other pathogens, temperature seems to be the most important. The effect of soil temperature on nematode-fungus interactions has been studied on various crops (Wallace, 1973).

In view of the distribution of *P. neglectus* throughout the South Australian wheat belt, several interaction tests in controlled temperature waterbaths under glasshouse conditions were undertaken to investigate the effect of soil temperature on the nematode-fungus interaction. These experiments were conducted at the CSIRO Division of Soils, Adelaide, South Australia.

Experiment 1 involved *P. neglectus* at four densities and/or *F. acuminatum* at two levels. Experiments 2 and 3, involving *P. neglectus* at the same densities as for Experiment 1 and/or *M. bolleyi*, or *P. irregulare* at only one inoculum level, were undertaken at the same temperatures as Experiment 1 in glasshouse conditions to determine the effect of temperature and inoculation times on fungus-nematode interaction.

A fourth experiment (Experiment 4) involved several wheat varieties and a triticale ranging from susceptible to moderately resistant to *P. neglectus* (Farsi, 1995; V. A. Vanstone, personal communication) (Table 2.2). A weakly pathogenic fungus, *M. bolleyi,* which showed a significant interaction with *P. neglectus* (Chapters 4 and 6), was used in this experiment.

8.2 Methods

8.2.1 Experiments 1-3

Surface sterilised Machete seeds were germinated and selected as described in the General Methods. Steam pasteurised sandy loam soil from Avon was used for this study.

Fungal inoculum of *F. acuminatum*, *M. bolleyi* and *P. irregulare* were prepared on millet seed (General Methods). Plastic pots of 300ml capacity were used. Fungal inoculum of *F. acuminatum* or *M. bolleyi* on millet seeds was added to the soil at 1% w/w in two layers. Inoculum of *P. irregulare* on millet seeds was added to the soil at 0.1% w/w. One pre-germinated Machete seed was sown in each cup at a depth of 1.5cm. Plastic beads were added to the top of all pots to prevent loss of soil moisture and, to some degree, help maintain soil temperature.

P. neglectus was extracted from carrot cultures as described in the General Methods. The nematodes were added in a volume of 1ml, at the densities of 0, 1000, 5000 and 10000/plant, around each plant. Control pots with no nematode inoculum received the same volume of distilled water.

Two inoculation times were used: nematode at sowing, fungus two weeks later; fungus and nematode at sowing. These inoculation times were chosen based on the results of the aseptic interaction experiment (Chapter 6, Experiments 1 and 3), where pre-inoculation of fungi had no significant effect on fungus-nematode interaction.

The experiments were set up as a completely randomised design with five replicates. Plants were harvested seven weeks after inoculation. There were four nematode densities (0, 1000, 5000 and 10000/plant), with fungus inoculum in each experiment (excluding Experiment 1, where there were two fungus densities) at only one density (no fungus and plus fungus). Plants were grown in controlled temperature water tanks at three different temperatures (15°C, 20°C or 25°C) in a glasshouse with a 25°C air temperature.

At harvest, soil was gently washed from the root systems. Nematodes were extracted over a period of four days using the mister extraction method and counted. At each harvest time, root lesions were scored from 0-5 (0= healthy roots and 5= complete lesioning of whole root system) as described in the General Methods, and the number of tillers/plant (excluding the main tiller) was counted. Dry weight of shoots and roots was recorded after drying at 80°C for three days. A 2cm root segment from each treatment was sampled and fixed in FAA preservative for staining nematodes and fungi.

8.2.2 Experiment 4

A genetic study of resistance to *P. neglectus* has been carried out (Farsi, 1995). His results suggest that some degree of resistance to *P. neglectus* occurs in some Australian and overseas wheat cultivars. In particular, some triticale varieties are resistant to *P. neglectus* (Farsi, 1995; Vanstone *et al.*, 1995). However, due to the important role of soil-borne fungi and evidence that some fungi can break resistance of crops to nematodes, an experiment was carried out to investigate the effect of fungi in combination with the nematode on nematode resistant varieties.

Experiment 4 involved a comparison between a range of wheat varieties (susceptible to moderately resistant to *P. neglectus*), and Abacus (triticale) to determine the influence of varietal reaction on the interaction between nematode and fungi.

8.2.2.1 Methods

Due to the limited availability of seeds of all varieties tested, only two soil temperatures (20°C or 25°C) were used in this experiment.

Pot size, soil, seed germination, sowing depth, inoculation, harvest time and measurements were as for other experiments outlined in this Chapter.

8.3 Results

8.3.1 Experiment 1

A summary of analyses of variance for all measurements is shown in Table 8.1. The 3-way interactions between fungus, nematode or temperature were significant for most of the measured characters (Table 8.1). Data for inoculation time is not presented in the results for all measurements, except where it was necessary.

***Tiller number*:** Plant tillering was significantly affected by temperature or by the two 2-way interactions between nematode and fungus or fungus and inoculation time. At high soil temperature (25°C), number of tillers/plant increased by 47% compared to those at 15°C (Figure 8.1a). There was no difference between 15°C or 20°C. A 72% reduction in the number of tillers/plant was recorded when fungus inoculum at either level was added to the pots two weeks after nematodes compared to when fungus was applied at sowing (Figure 8.1b).

***Root symptoms*:** Soil temperature had a major effect on the root lesion rating. While there was no significant effect on root lesion rating at low soil temperature (15°C or 20°C), at 25°C root lesion rating significantly increased compared to either 15°C or 20°C.

A significant 3-way interaction between nematode, fungus and temperature is illustrated in Figure 8.2. Inoculation timing, however, had no significant effect on root lesion rating.

With nematode inoculum levels of 1000, 5000 or 10000 nematodes/plant, root lesion rating increased by 82%, 90% or 92%, respectively, compared to the control (no nematodes added) (Figure 8.2).

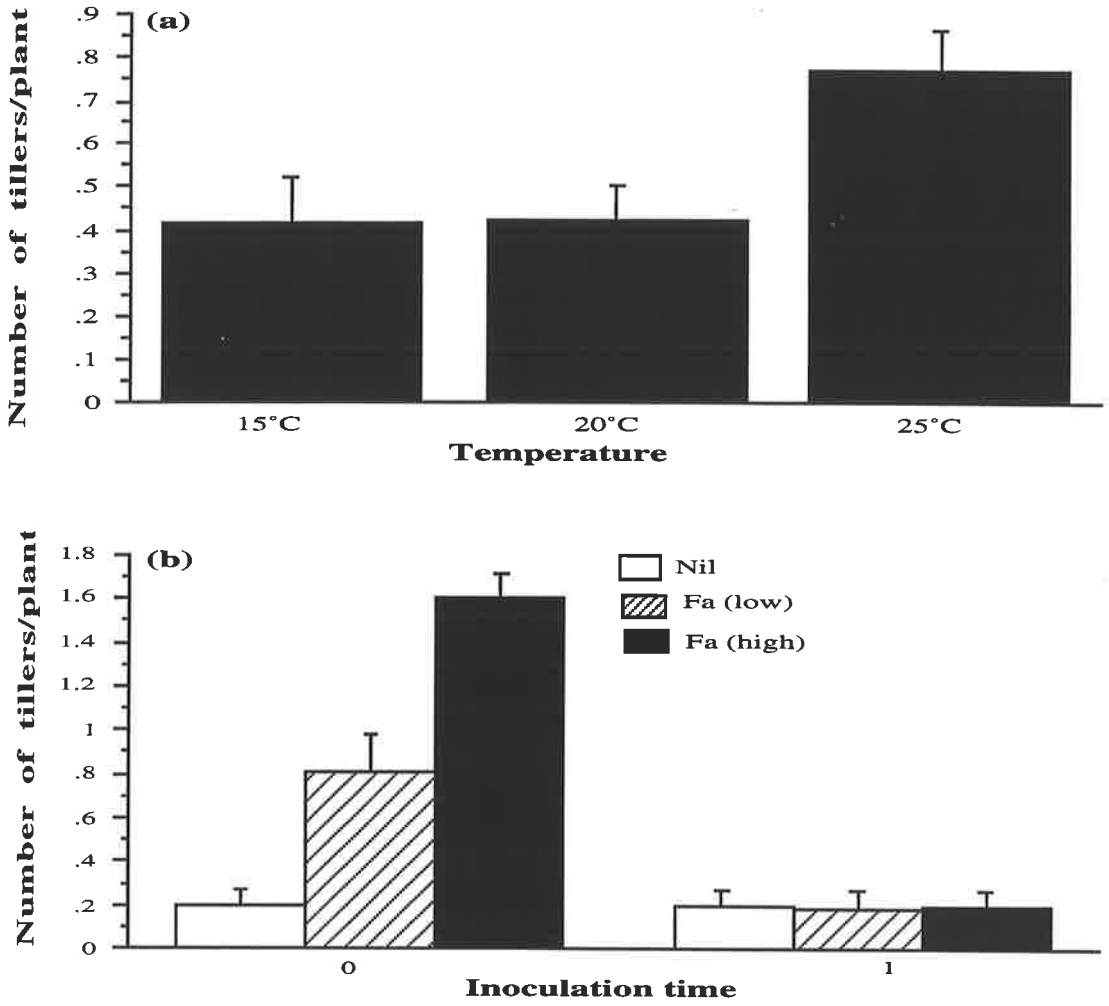


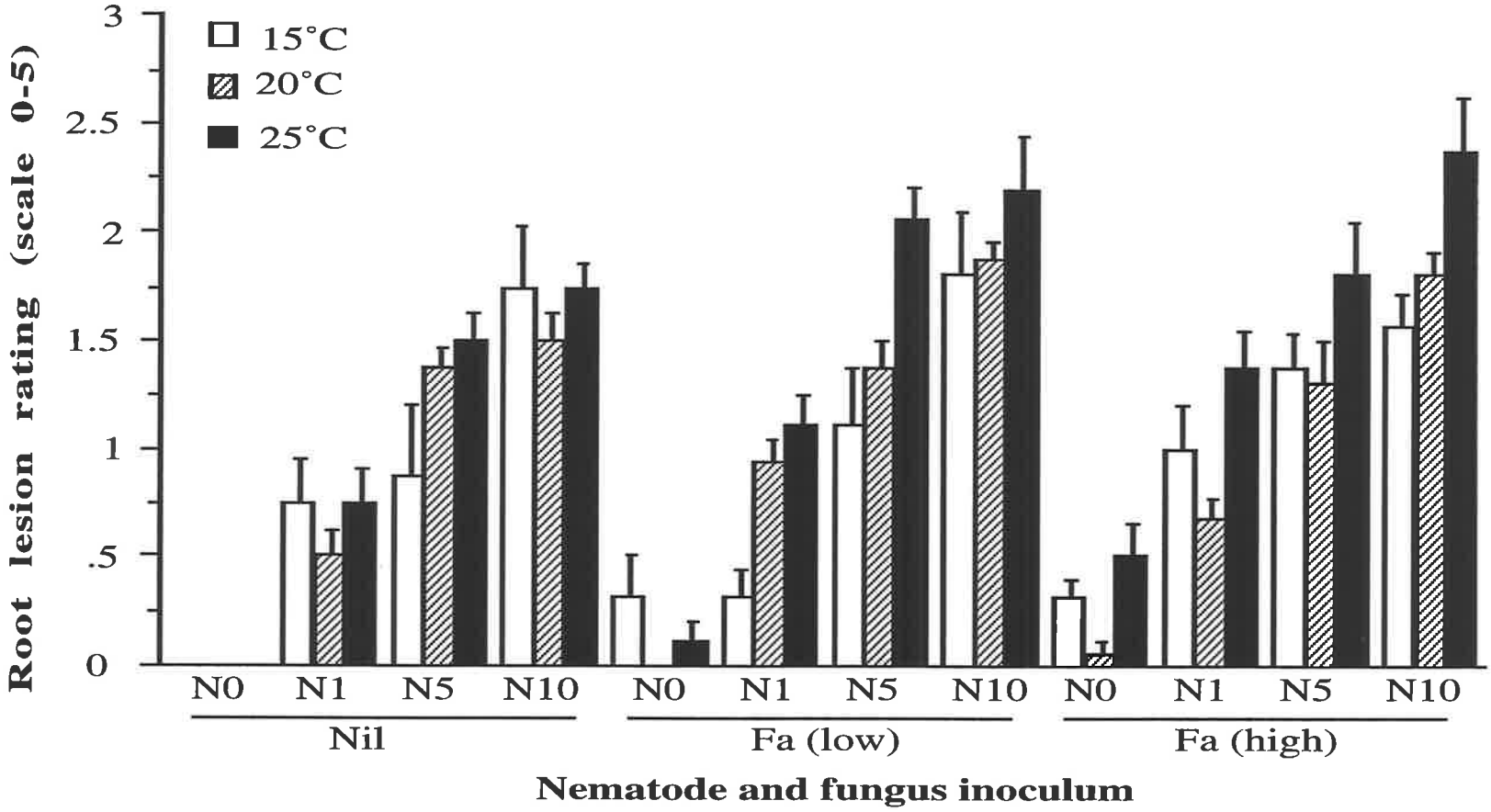
Figure 8.1 Plant tillering affected by **(a)** nematode inoculum and **(b)** fungus × inoculation time interaction. **Inoculation time:** 0= fungus and nematode at sowing, 1= nematode at sowing, fungus two weeks later. Fa (low)= *F. acuminatum* added at 0.5% w/w, Fa (high= *F. acuminatum* added at 1% w/w.

Table 8.1 Summary of analyses of variance for the effect of interaction between *Fusarium acuminatum* and *P. neglectus* on the extent of root lesioning, number of nematodes/plant and nematodes/g dry root, shoot and root dry weights, total dry weight of plants, number of tillers and multiplication rate of *P. neglectus* for wheat cultivar Machete, 49 days after sowing (Experiment 1).

Source	df	MS RL	P	MS N/p	P	MS N/gdr	P	MS dws/p	P	MS dwr/p	P	MS tdw/p	P	MS tiller	P	MS MR	P
Temperature (temp)	2	4.02	***	6.57E8	***	6.06E10	***	.222	***	.114	***	.165	***	3.90	***	37.6	***
Fungus (fun)	2	2.10	***	1.69E8	***	1.02E10	***	.068	***	.005	ns	.107	***	11.79	***	3.2	*
Nematode (nem)	3	39.41	***	4.25E9	***	1.50E11	***	.018	***	.005	***	.047	***	.55	ns	120.6	***
Time	1	3.94	***	6.02E6	ns	3.36E9	*	.289	***	.034	***	.523	***	49.00	***	1.7	ns
Temp × fungus	4	.46	*	2.09E8	***	1.56E10	***	.012	***	.009	**	.040	***	.58	ns	1.9	ns
Temp × nematode	6	.46	*	2.85E8	***	2.21E10	***	.004	*	.002	ns	.009	ns	.19	ns	17.2	***
Nematode × fungus	6	.11	ns	1.74E8	***	7.14E9	***	.003	ns	.008	**	.020	**	1.21	**	5.5	***
Temp × time	2	.64	*	6.84E7	*	1.60E9	ns	.009	**	.014	**	.033	**	.25	ns	3.4	*
Fungus × time	1	.01	ns	1.78E8	**	7.97E9	**	.018	***	.007	ns	.003	ns	.25	***	4.4	*
Nematode × time	3	.13	ns	5.85E7	*	2.70E9	*	.005	*	.001	ns	.006	ns	7.13	ns	1.7	ns
Temp × fungus × nem	12	.33	*	2.50E8	***	1.21E10	***	.005	**	.003	ns	.010	*	.03	ns	5.5	***
Temp × fungus × time	2	.11	ns	1.74E8	***	1.20E10	***	.005	ns	.002	ns	.008	ns	.48	ns	3.3	*
Temp × nem × time	6	.57	**	1.66E7	ns	6.08E8	ns	003	ns	.002	ns	.009	ns	.94	ns	.5	ns
Temp × fungus × nem	3	.001	ns	2.71E8	***	2.07E10	***	.001	ns	.002	ns	.005	ns	.65	ns	4.2	**
Temp × fun × nem × time	6	.020	ns	1.79E8	***	1.59E10	***	.004	ns	.002	ns	.010	ns	.35	ns	5.5	***
Residual	228	.20		2.33E7		1.00E9		.002		.003		.006		.41		1.2	

*** significant at P= 0.001 ** significant at P= 0.01 * significant at P= 0.05 ns= not significant P= Probability.
RL= root lesioning; N/p= nematodes/plant; N/gdr= nematodes/g dry root; dws/p= shoot dry weight/plant; dwr/p= root dry weight/plant; tdw/p= total dry weight/plant; tiller= tiller/plant; MR= nematode multiplication rate.

Figure 8.2 Effect of a 3-way interaction between fungus × nematode × temperature on root lesion rating of wheat cultivar Machete 49 days after sowing. **Nematode inoculum:** N0= no nematodes, N1= 1000 nematodes/plant, N5= 5000 nematodes/plant and N10= 10000 nematodes/plant. **Fungus inoculum:** Nil= no fungus added, Fa (low)= *F. acuminatum* at 0.5% w/w, Fa (high)= *F. acuminatum* at 1% w/w.



***Nematode number*:** Number of nematodes/plant or nematodes/g dry root were significantly affected by the 3-way interactions between nematode, fungus and temperature (Table 8.1). Nematode inoculum was successful, as is indicated by the very significant differences between nematode numbers in inoculated and uninoculated treatments. As nematode inoculum level increased, number of nematodes/plant or nematodes/g dry root also increased.

Once again, temperature had a greater effect on number of nematodes extracted from the root system than other treatments. As temperature increased, number of nematodes extracted from roots also increased. At 25°C, nematode numbers from plants inoculated with *F. acuminatum* (1% w/w) increased by 332% compared to 15°C, and by 165% compared to 20°C (Table 8.2).

Number of nematodes/g dry root showed a pattern similar to numbers/plant, therefore, data for nematodes/g dry root was not presented.

Multiplication rate of *P. neglectus* was another factor which was affected by different treatments (fungus, nematode, inoculation time or temperature) (Table 8.3). The significant 3-way treatment means for nematode multiplication rate are presented in Table 8.3. At 25°C and in the presence of *F. acuminatum*, the reproduction rate of *P. neglectus* significantly increased compared to those at 15°C. However, at 15°C or 20°C with increases in the nematode inoculum levels, multiplication rate of the nematode decreased (Table 8.3).

Table 8.2 Effect of nematode-*F. acuminatum* (Fa) interaction on the number of nematodes/plant (log) 49 days after sowing in a pasteurised sandy soil under controlled glasshouse conditions. Values in the 3-way table are the average of eight single plant blocks.

	Nil			Fa (low)			Fa (high)		
Nematode	15°C	20°C	25°C	15°C	20°C	25°C	15°C	20°C	25°C
N1000	3.14	3.45	3.64	2.77	3.58	3.52	3.03	3.67	3.45
N5000	3.61	4.02	4.04	3.92	4.03	4.26	3.56	3.75	3.98
N10000	4.26	3.83	3.83	4.03	4.06	4.38	3.82	4.12	4.55
3-way interaction.									LSD 5%= 0.22

1 and 2-way treatment means (with appropriate LSD at P= 0.05 level).

				LSD
Fungus	Nil	Fa (low)	Fa (high)	
	2.82	2.88	2.83	*ns*
Nematode	N1000	N5000	N10000	
	3.35	3.91	4.12	*0.08*
Temperature	15°C	20°C	25°C	
	2.66	2.88	2.99	*0.07*
Nematode	N1000	N5000	N10000	
Fungus				
Nil	3.41	3.89	3.97	
Fa (low)	3.29	4.07	4.16	*0.12*
Fa (high)	3.38	3.76	4.16	
Tempratrure	15°C	20°C	25°C	
Fungus				
Nil	2.75	2.83	2.88	
Fa (low)	2.68	2.92	3.04	*0.13*
Fa (high)	2.60	2.88	3.00	
Nematode	N1000	N5000	N10000	
Temperature				
15°C	2.95	3.72	3.99	
20°C	3.59	3.91	4.04	*0.13*
25°C	3.52	4.11	4.34	

Fa (low)= *F. acuminatum* added at 0.5% w/w, Fa (high= *F. acuminatum* added at 1% w/w. N1000= 1000 nematodes/plant, N5000= 5000 nematodes/plant, N10000= 10000 nematodes/plant.

Table 8.3 Effect of nematode-*F. acuminatum* (Fa) interaction on nematode multiplication 49 days after sowing in a pasteurised sandy soil under controlled glasshouse conditions. Values in the 3-way table are the average of eight single plant blocks.

	Nil			Fa (low)			Fa (high)		
Nematode	15°C	20°C	25°C	15°C	20°C	25°C	15°C	20°C	25°C
N1000	1.46	3.26	4.61	0.76	4.47	3.47	1.51	5.56	2.97
N5000	0.93	2.21	2.22	2.25	2.72	3.89	1.01	1.6	2.07
N10000	2.29	0.69	1.00	1.22	1.47	2.79	0.87	1.42	3.76
3-way interaction.									LSD 5%= 1.16

1 and 2-way treatment means (with appropriate LSD at P= 0.05 level).

				LSD
Fungus	Nil	Fa (low)	Fa (high)	
	1.56	1.92	1.69	*ns*
Nematode	N1000	N5000	N10000	
	3.12	2.10	1.80	*0.42*
Temperature	15°C	20°C	25°C	
	1.00	1.99	2.29	*0.36*
Nematode	N1000	N5000	N10000	
Fungus				
Nil	3.11	1.79	1.32	
Fa (low)	2.90	2.95	1.82	*0.67*
Fa (high)	3.35	1.42	2.02	
Tempratrure	15°C	20°C	25°C	
Fungus				
Nil	1.17	1.54	1.96	
Fa (low)	1.06	2.16	2.54	*ns*
Fa (high)	0.85	2.04	2.20	
Nematode	N1000	N5000	N10000	
Temperature				
15°C	1.20	1.49	1.29	
20°C	4.66	1.99	1.29	*0.73*
25°C	3.50	2.83	2.82	

Fa (low)= *F. acuminatum* added at 0.5% w/w, Fa (high= *F. acuminatum* added at 1% w/w. N1000= 1000 nematodes/plant, N5000= 5000 nematodes/plant, N10000= 10000 nematodes/plant.

***Dry matter*:** One, two or 3-way treatment means for root and shoot dry weights are presented in Tables 8.4 and 8.5.

In the presence of *F. acuminatum*, root dry weight increased regardless of nematode inoculum. Both levels of fungus inoculum and nematodes at 1000, 5000 or 10000/plant increased shoot dry weight.

Inoculation time also had a significant effect on plant growth. When fungus inoculum was applied to the soil two weeks after nematode inoculum, a 16% reduction in shoot dry weight resulted compared to simultaneous inoculation of fungus and nematode (Table 8.4).

Unlike shoots, root dry weight decreased in the presence of nematodes or at high soil temperature. At higher soil temperature (25°C), root dry weight was reduced by 25% compared to 15°C or 20°C (Table 8.5).

Fungus alone had no significant effect on the production of roots. However, two 2-way interactions between nematode and fungus or temperature and time were significant for root dry weight (Table 8.5).

Nematodes alone at high density significantly reduced root dry weight. A 15% decrease in root dry weight resulted when fungus was applied with 5000 *P. neglectus* compared to nematodes alone at the same density, or a 24% reduction compared to when fungus was applied alone (Table 8.5).

Likewise, shoot and root dry weights were reduced when fungus was applied two weeks after nematodes. This decrease was significant (P=0.01) (Tables 8.4 and 8.5).

Total dry weight of plants was also affected by all main treatments and by two 2-way interactions between nematode and fungus or temperature and inoculation time (Table 8.1).

Table 8.4 Effect of nematode-*F. acuminatum* (Fa) interaction on the shoot dry weight (g/plant) of wheat cultivar Machete 49 days after sowing in a pasteurised sandy soil under controlled glasshouse conditions. Values in the 3-way table are the average of eight single plant blocks.

	Nil			Fa (low)			Fa (high)		
Nematode	15°C	20°C	25°C	15°C	20°C	25°C	15°C	20°C	25°C
N0	0.124	0.239	0.237	0.183	0.229	0.262	0.221	0.229	0.323
N1000	0.150	0.244	0.313	0.181	0.280	0.305	0.241	0.331	0.298
N5000	0.198	0.265	0.297	0.217	0.241	0.280	0.236	0.317	0.278
N10000	0.115	0.250	0.255	0.202	0.234	0.268	0.242	0.305	0.293
3-way interaction was significant								LSD 5%= 0.043	

1 and 2-way treatment means (with appropriate LSD at P= 0.05 level).

					LSD
Fungus	Nil	Fa (low)	Fa (high)		
	0.224	0.240	0.276		*0.012*
Nematode	N0	N1000	N5000	N10000	
	0.233	0.265	0.260	0.247	*0.015*
Temperature	15°C	20°C	25°C		
	0.202	0.266	0.286		*0.013*
Nematode	N0	N1000	N5000	N10000	
Fungus					
Nil	0.200	0.236	0.253	0.207	
Fa (low)	0.225	0.256	0.246	0.235	*0.025*
Fa (high)	0.258	0.290	0.277	0.280	
Temperature	15°C	20°C	25°C		
Fungus					
Nil	0.147	0.249	0.276		
Fa (low)	0.196	0.246	0.279		*0.021*
Fa (high)	0.235	0.295	0.298		
Nematode	N0	N1000	N5000	N10000	
Temperature					
15°C	0.178	0.199	0.221	0.201	
20°C	0.231	0.293	0.276	0.265	*0.027*
25°C	0.282	0.304	0.283	0.276	

Fa (low)= *F. acuminatum* added at 0.5% w/w, Fa (high= *F. acuminatum* added at 1% w/w. N0= no nematodes added, N1000= 1000 nematodes/plant, N5000= 5000 nematodes/plant, N10000= 10000 nematodes/plant.

Table 8.5 Effect of nematode-*F. acuminatum* (Fa) interaction on the root dry weight (g/plant) of wheat cultivar Machete 49 days after sowing in a pasteurised sandy soil under controlled glasshouse conditions. Values in the 3-way table are the average of eight single plant blocks.

	Nil			Fa (low)			Fa (high)		
Nematode	15°C	20°C	25°C	15°C	20°C	25°C	15°C	20°C	25°C
N0	0.225	0.279	0.216	0.301	0.275	0.237	0.291	0.316	0.260
N1000	0.233	0.243	0.212	0.222	0.245	0.214	0.262	0.250	0.169
N5000	0.225	0.287	0.214	0.240	0.227	0.147	0.239	0.252	0.136
N10000	0.182	0.193	0.168	0.219	0.215	0.144	0.242	0.269	0.155

3-way interaction not significant.

1 and 2-way treatment means (with appropriate LSD at P= 0.05 level).

					LSD
Fungus	Nil	Fa (low)	Fa (high)		
	0.225	0.224	0.237		*ns*
Nematode	N0	N1000	N5000	N10000	
	0.274	0.227	0.214	0.202	*0.019*
Temperature	15°C	20°C	25°C		
	0.246	0.255	0.187		*0.017*
Nematode	N0	N1000	N5000	N10000	
Fungus					
Nil	0.249	0.229	0.242	0.181	
Fa (low)	0.271	0.227	0.205	0.193	*ns*
Fa (high)	0.289	0.227	0.209	0.222	
Temperature	15°C	20°C	25°C		
Fungus					
Nil	0.223	0.250	0.203		
Fa (low)	0.245	0.240	0.185		*0.026*
Fa (high)	0.259	0.272	0.180		
Nematode	N0	N1000	N5000	N10000	
Temperature					
15°C	0.287	0.240	0.237	0.221	
20°C	0.292	0.246	0.249	0.232	*ns*
25°C	0.242	0.196	0.156	0.153	

Fa (low)= *F. acuminatum* added at 0.5% w/w, Fa (high= *F. acuminatum* added at 1% w/w. N0= no nematodes added, N1000= 1000 nematodes/plant, N5000= 5000 nematodes/plant, N10000= 10000 nematodes/plant.

8.3.2 Experiment 2

The summary of analyses of variance for all measurenents is shown in Table 8.6.

***Tiller number*:** Plant tillering was significantly affected by fungus inoculum and temperature, as well as by three 2-way interactions between nematode and fungus, nematode and inoculation time or fungus and temperature (Table 8.7). At high soil temperature (25°C), number of tillers/plant increased by 33% compared to those at 15°C. There was a significant difference between soil temperatures of 20°C and 25°C. The tiller number of plants grown at 25°C increased by 34% compared to those grown at 20°C. *M. bolleyi* increased plant tillering by 34% compared to the control (no fungus inoculum added) (Table 8.7).

There was no significant difference in the number of tillers/plant at either fungus inoculation time or between nematode inoculum levels.

Root lesion rating: Root lesion rating was significantly affected by fungus, nematode, inoculation time or temperature. There were also five 2-way interactions and one 3-way interaction (fungus × nematode × inoculation time) significant for root lesion rating (Table 8.8). Nematode inoculum at all densities (1000, 5000 or 10000 nematodes/plant) increased root lesion rating by 89%, 94% or 96%, respectively, compared to the control (no nematode added). Fungus inoculum also increased lesions on roots by 49% compared to the control (no fungus added) (Table 8.8).

Simultaneous inoculation of fungus and nematodes decreased root lesion rating by 17% compared to when fungus was added two weeks after nematodes (Table 8.8).

Temperature was another main treatment which significantly affected root lesion rating. Root lesioning increased with increase in soil temperature. At 20°C or 25°C, root lesions increased by 33% or 61%, respectively, compared to those at 15°C (Table 8.8).

Table 8.6 Summary of analyses of variance for the effect of interaction between *Microdochium bolleyi* and *P. neglectus* on extent of root lesioning, number of nematodes/plant and nematodes/g dry root, shoot and root dry weights, total dry weight of plants, number of tillers/plant and nematode multiplication rate for wheat cultivar Machete, 49 days after sowing (Experiment 2).

Source	df	MS RL	P	MS N/p	P	MS N/gdr	P	MS dws/p	P	MS dwr/p	P	MS tdw/p	P	MS tillers	P	MS MR	P
Nematode (nem)	3	26.64	***	1.17E10	***	3.98E10	***	.011	ns	.058	***	.067	**	.56	ns	238.3	***
Fungus (fun)	1	17.20	***	1.10E9	***	3.33E9	***	3.107	***	.027	ns	3.717	***	62.01	***	62.1	***
Time	1	1.38	**	3.49E8	***	2.66E9	**	.078	**	.027	ns	.198	**	.01	ns	11.4	*
Temperature (temp)	2	11.16	***	9.69E9	***	2.44E10	***	1.445	***	.037	***	3.869	***	32.27	***	534.0	***
Nematode × fungus	3	1.28	***	2.64E8	***	5.43E8	*	.016	ns	.034	**	.073	**	1.49	*	21.2	***
Nematode × time	3	.51	**	8.40E7	**	9.08E8	**	.009	ns	.013	ns	.013	ns	1.29	*	5.3	ns
Fungus × time	1	1.38	**	1.60E8	**	1.70E9	**	.078	**	.027	ns	.198	**	.01	ns	8.9	ns
Temp × nematode	6	1.35	***	2.55E9	***	6.48E9	***	.011	ns	.023	*	.043	*	1.04	ns	73.8	***
Fungus × temp	2	.51	*	3.16E8	***	9.58E8	**	.227	**	.031	*	.423	***	2.92	**	17.9	**
Temp × time	2	.05	ns	4.89E7	ns	4.21E8	ns	.016	ns	.494	ns	.015	ns	.20	ns	6.9	ns
Time × fun × nem	3	.51	**	7.21E7	*	8.45E8	**	.009	ns	.013	ns	.013	ns	1.29	*	.4	ns
Nem × fun × temp	6	.16	ns	1.10E8	***	3.97E8	*	.009	ns	.010	ns	.010	ns	.62	ns	1.1	***
Nem × time × temp	6	.11	ns	1.37E7	ns	2.61E8	ns	.011	ns	.010	ns	.017	ns	.89	ns	7.9	ns
Temp × fun × time	2	.05	ns	4.49E7	ns	4.12E8	ns	.016	ns	.494	ns	.015	ns	.20	ns	1.1	ns
Temp×fun×nem×time	6	.11	ns	1.83E7	ns	3.18E8	ns	.011	ns	.010	ns	.017	ns	.89	ns	.4	ns
Residual	192	.14		2.19E7		1.79E8		.010		.009		.019		.53		1.0	

*** significant at P= 0.001 ** significant at P= 0.01 * significant at P= 0.05 ns= not significant P= Probability.
RL= root lesioning; N/p= nematodes/plant; N/gdr= nematodes/g dry root; dws/p= shoot dry weight/plant; dwr/p= root dry weight/plant; tdw/p= total dry weight/plant; tillers= tillers/plant; MR= nematode multiplication rate.

Table 8.7 Effect of nematode-*M. bolleyi* interaction on the number of tillers/plant of wheat cultivar Machete 49 days after sowing in a pasteurised soil under controlled glasshouse conditions. Values in the 3-way table are the average of ten single plant blocks.

	Nil			*M. bolleyi*		
Nematode	15°C	20°C	25°C	15°C	20°C	25°C
N0	1.8	2.0	2.6	2.7	2.6	4.0
N1000	1.6	1.6	2.8	2.3	2.4	4.3
N5000	1.4	1.8	1.8	2.6	2.6	4.1
N10000	2.0	1.6	2.8	2.7	2.3	3.4

3-way interaction not significant.

1 and 2-way treatment means (with appropriate LSD at P= 0.05 level).

					LSD
Fungus	Nil	*M. bolleyi*			
	1.98	3.0			*0.18*
Nematode	N0	N1000	N5000	N10000	
	2.6	2.5	2.38	2.46	*ns*
Temperature	15°C	20°C	25°C		
	2.13	2.11	3.22		*0.22*
Nematode	N0	N1000	N5000	N10000	
Fungus					
Nil	2.1	2.0	1.6	2.1	*0.36*
M. bolleyi	3.1	3.0	3.1	2.8	
Temperature	15°C	20°C	25°C		
Fungus					
Nil	1.7	1.7	2.5		*0.31*
M. bolleyi	2.5	2.4	3.9		
Nematode	N0	N1000	N5000	N10000	
Temperature					
15°C	2.2	1.9	2.0	2.3	
20°C	2.3	2.0	2.2	1.9	*0.44*
25°C	3.3	3.5	2.9	3.1	

N0= no nematodes added, N1000= 1000 nematodes/plant, N5000= 5000 nematodes/plant, N10000= 10000 nematodes/plant.

Table 8.8 Effect of nematode-*M. bolleyi* interaction on the extent of root lesion rating (0-5) of wheat cultivar Machete 49 days after sowing in a pasteurised soil under controlled glasshouse conditions. Values in the 3-way table are the average of ten single plant blocks.

	Nil			*M. bolleyi*		
Nematode	15°C	20°C	25°C	15°C	20°C	25°C
N0	0.00	0.00	0.00	0.50	0.00	0.30
N1000	0.10	0.20	0.60	0.50	1.02	1.05
N5000	0.40	0.60	1.40	0.80	1.50	2.20
N10000	0.70	1.10	1.60	1.25	1.85	2.60

3-way interaction not significant.

1 and 2-way treatment means (with appropriate LSD at P= 0.05 level).

Fungus	Nil	*M. bolleyi*			LSD
	0.55	1.09			*0.09*

Nematode	N0	N1000	N5000	N10000	
	0.05	0.58	1.15	1.52	*0.13*

Temperature	15°C	20°C	25°C		
	0.47	0.78	1.22		*0.11*

Nematode	N0	N1000	N5000	N10000	
Fungus					
Nil	0.00	0.30	0.80	1.13	*0.18*
M. bolleyi	0.12	0.86	1.50	1.90	

Temperature	15°C	20°C	25°C	
Fungus				
Nil	0.30	0.47	0.90	*0.16*
M. bolleyi	0.65	1.09	1.54	

Nematode	N0	N1000	N5000	N10000	
Temperature					
15°C	0.02	0.30	0.60	0.97	
20°C	0.00	0.61	1.05	1.47	*0.23*
25°C	0.15	0.82	1.80	2.10	

N0= no nematodes added, N1000= 1000 nematodes/plant, N5000= 5000 nematodes/plant, N10000= 10000 nematodes/plant.

Nematode numbers: Nematode inoculum was successful as, with increases in nematode density, number of nematodes extracted from roots increased (P=0.001). *M. bolleyi* also increased nematode numbers in roots by 28% compared to the control (no fungus inoculum added) (Table 8.9). Different fungus inoculation times (at sowing or two weeks later) had a significant effect on both nematode numbers/plant or nematodes/g dry root. When *M. bolleyi* was applied to the soil two weeks after sowing, number of nematodes increased by 16% compared to when both were added at sowing.

There was a very significant three way interaction between nematode, fungus and teperature on the number of nematodes and nematode multiplication rate (Table 8.6). The means for the different combinations of treatments are shown in Tables 8.9 and 8.10.

Higher temperature led to greater multiplication rates of *P. neglectus*. Higher initial nematode inoculum led to greater nematode numbers but the multiplication rate declined as expected with the higher inoculum. The presence of *M. bolleyi* also led to increases in nematode numbers and multiplication rates. The combination of all three at the highest levels gave a 70 fold increase in nematode numbers compared to all three at the lowest level.

Each variable alone had quite significant effects over and above the interaction effects. The presence of *M. bolleyi* increased the number of nematodes. Higher temperatures led to an eight fold increase in nematode numbers when averaged over all nematode inoculum levels, with or without *M. bolleyi*.

As expected, inoculation time was important and showed the same results as in previous experiments (Chapters 6 and 7).

Table 8.9 Effect of nematode-*M. bolleyi* interaction on the number of nematodes/plant 49 days after sowing in a pasteurised soil under controlled glasshouse conditions. Values in the 3-way table are the average of ten single plant blocks.

	Nil			*M. bolleyi*		
Nematode	15°C	20°C	25°C	15°C	20°C	25°C
N1000	998	2114	6170	1021	3472	12843
N5000	4868	11416	31018	5339	21010	35735
N10000	7608	20503	48630	6820	34029	66648
3-way interaction is significant.						LSD 5%= 4110

1 and 2-way treatment means (with appropriate LSD at P= 0.05 level).

				LSD
Fungus	Nil	*M. bolleyi*		
	11110	15576		*1186*
Nematode	N1000	N5000	N10000	
	4437	18231	30707	*1679*
Temperature	15°C	20°C	25°C	
	3332	11568	25131	*1453*
Nematode	N1000	N5000	N10000	
Fungus				
Nil	3094	15767	25580	*2373*
M. bolleyi	5779	20695	35833	
Temperature	15°C	20°C	25°C	
Fungus				
Nil	3369	8508	21454	*2055*
M. bolleyi	3295	14628	28807	
Nematode	N1000	N5000	N10000	
Temperature				
15°C	1010	5104	7214	
20°C	2793	16213	27266	*2906*
25°C	9506	33376	57639	

N1000= 1000 nematodes/plant, N5000= 5000 nematodes/plant, N10000= 10000 nematodes/plant.

Table 8.10 Effect of nematode-*M. bolleyi* interaction on the multiplication rate of *P. neglectus* 49 days after sowing in a pasteurised soil under controlled glasshouse conditions. Values in the 3-way table are the average of ten single plant blocks.

	Nil			*M. bolleyi*		
Nematode	15°C	20°C	25°C	15°C	20°C	25°C
N1000	1.00	2.11	6.17	1.02	3.47	12.84
N5000	.97	2.28	8.08	1.07	4.20	7.15
N10000	.76	2.05	4.86	.68	3.40	6.66
3-way interaction is significant.						LSD 5%= 1.40

1 and 2-way treatment means (with appropriate LSD at P= 0.05 level).

				LSD
Fungus	Nil	*M. bolleyi*		
	2.36	3.38		*0.40*
Nematode	N1000	N5000	N10000	
	4.44	3.96	3.07	*0.57*
Temperature	15°C	20°C	25°C	
	.69	2.19	5.72	*0.49*
Nematode	N1000	N5000	N10000	
Fungus				
Nil	3.09	3.78	2.56	*0.81*
M. bolleyi	5.78	4.14	3.58	
Temperature	15°C	20°C	25°C	
Fungus				
Nil	.68	1.61	4.78	*0.70*
M. bolleyi	.69	2.77	6.66	
Nematode	N1000	N5000	N10000	
Temperature				
15°C	1.01	1.02	.72	
20°C	2.79	3.24	2.73	*0.99*
25°C	9.51	7.61	5.76	

N1000= 1000 nematodes/plant, N5000= 5000 nematodes/plant, N10000= 10000 nematodes/plant.

***Dry matter*:** One, two or 3-way treatment means for root dry weights are presented in Table 8.11. Fungus, inoculation time and temperature, as well as two 2-way interactions between fungus and inoculation time or fungus and temperature, were significant for shoot dry weight (Table 8.6).

Nematode inoculum affected root production significantly. This decrease was similar at either level of nematode inoculum compared to the control (no nematodes added) (Table 8.11). However, fungus inoculum or inoculation time had no significant effect on root dry weight.

Fungus alone had no significant effect on the production of roots. However, three 2-way interactions between nematode and fungus, nematode and temperature or temperature and fungus were significant for root dry weight (Table 8.11).

Nematodes alone significantly reduced root dry weight at the high density. A 13% decrease in root dry weight resulted when plants were inoculated with 10000 *P. neglectus* compared to when nematode and fungus were applied at the same density or a 20% reduction compared to when fungus was applied alone (Table 8.11).

Soil temperature also significantly affected production of roots. Regardless of fungus inoculum, root dry weight increased with increase in soil temperature. At 25°C, root dry weight increased by 22% compared to those at 15°C. A further 10% increase occurred when fungus inoculum was also added (Table 8.11). Plants inoculated with fungus at 25°C showed a 33% increase in root dry weight compared to those at 15°C. Overall, at higher soil temperature (25°C) root dry weight was reduced by 28% compared to 15°C, or at 20°C, it was reduced by 11% (Table 8.11).

Root dry weight of plants inoculated with 1000, 5000 or 10000 nematodes/plant at 25°C decreased by 17%, 13% or 13%, respectively, compared to the control (no nematodes added) at the same temperature.

Table 8.11 Effect of nematode-*M. bolleyi* interaction on the root dry weight (g/plant) of wheat cultivar Machete 49 days after sowing in a pasteurised soil under controlled glasshouse conditions. Values in the 3-way table are the average of ten single plant blocks.

	Nil			*M. bolleyi*		
Nematode	15°C	20°C	25°C	15°C	20°C	25°C
N0	0.458	0.547	0.694	0.516	0.590	0.746
N1000	0.487	0.531	0.599	0.426	0.541	0.592
N5000	0.516	0.511	0.590	0.465	0.436	0.660
N10000	0.425	0.502	0.556	0.445	0.451	0.704

3-way interaction not significant.

1 and 2-way treatment means (with appropriate LSD at P= 0.05 level).

					LSD
Fungus	Nil	*M. bolleyi*			
	0.535	0.556			*0.021*
Nematode	N0	N1000	N5000	N10000	
	0.592	0.530	0.529	0.513	*0.033*
Temperature	15°C	20°C	25°C		
	1.133	1.357	1.572		*0.029*
Nematode	N0	N1000	N5000	N10000	
Fungus					
Nil	0.566	0.539	0.539	0.495	
M. bolleyi	0.617	0.520	0.520	0.567	*0.048*
Temperature	15°C	20°C	25°C		
Fungus					
Nil	0.472	0.523	0.610		
M. bolleyi	0.463	0.530	0.676		*0.041*
Nematode	N0	N1000	N5000	N10000	
Temperature					
15°C	0.487	0.457	0.490	0.435	
20°C	0.569	0.536	0.473	0.427	*0.058*
25°C	0.720	0.595	0.625	0.630	

N0= no nematodes added, N1000= 1000 nematodes/plant, N5000= 5000 nematodes/plant, N10000= 10000 nematodes/plant.

Shoot dry weights were significantly affected by fungus, inoculation time or temperature but not by nematode inoculum (Table 8.6). The data for shoot dry weight is not presented.

Total dry weight of plants also was affected by all main treatments and there were four significant 2-way interactions between nematode and fungus, fungus and inoculation time, fungus and temperature or nematode and temperature (Table 8.6).

8.3.3 Experiment 3

Summary of analyses of variance for all measurements are shown in Table 8.12. Main treatments (fungus, nematode, inoculation time or temperature) were significant for number of nematodes/plant or nematodes/g dry root, multiplication rate of nematode or shoot dry weight (Table 8.12).

***Tiller number*:** Plant tillering was not significantly affected by fungus, temperature, inoculation time or by two 2-way or 3-way interactions (data not presented).

Root lesion rating: Significant 3-way treatment means between nematode, fungus and temperature are shown in Table 8.13. *P. irregulare* had no overall affect on root lesion rating but there was an interaction with *P. irregulare* and temperature at 20°C and 25°C.

Table 8.12 Summary of analyses of variance for the effect of interaction between *Pythium irregulare* and *P. neglectus* on the extent of root lesioning, number of nematodes/plant and nematodes/g dry root, shoot and root dry weights, total dry weight of plants and nematode multiplication rate for wheat cultivar Machete, 49 days after sowing (Experiment 3).

Source	df	MS RL	P	MS N/p	P	MS N/gdr	P	MS dws/p	P	MS dwr/p	P	MS tdw/p	P	MS MR	P
Nematode (nem)	3	27.61	***	238.34	***	272.75	***	.021	*	.001	ns	.018	ns	158.40	***
Fungus (fun)	1	.02	ns	.55	***	.80	***	.072	**	.009	ns	.030	ns	47.23	***
Time	1	.03	ns	.05	ns	.09	*	.046	**	.033	*	.001	ns	.42	ns
Temperature (temp)	2	2.70	***	12.01	***	10.81	***	.022	*	.765	***	.951	***	269.47	***
Nematode × fungus	3	.17	ns	.14	***	.20	***	.008	ns	.017	ns	.022	ns	10.72	***
Nematode × time	3	.30	*	.05	*	.07	**	.003	ns	.008	ns	.015	ns	1.46	ns
Fungus × time	1	3.09	***	.04	ns	.07	*	.046	**	.033	*	.001	ns	.02	ns
Temp × nematode	6	.52	***	1.86	***	1.73	***	.009	ns	.002	ns	.010	ns	37.48	***
Fungus × temp	2	10.61	***	.17	***	.21	***	.034	**	.016	ns	.063	*	11.54	***
Temp × time	2	1.23	***	.03	ns	.03	ns	.011	ns	.004	ns	.004	ns	4.17	*
Time × fun × nem	3	.16	ns	.05	*	.08	**	.003	ns	.008	ns	.015	ns	1.65	ns
Nem × fun × temp	6	.86	***	.07	***	.05	*	.015	*	.006	ns	.018	ns	5.83	***
Nem × time × temp	6	.46	***	.07	***	.06	**	.002	ns	.003	ns	.007	ns	1.25	ns
Temp × fun × time	2	1.16	***	.05	*	.04	ns	.011	ns	.004	ns	.004	ns	4.13	*
Temp×fun×nem×time	6	.10	ns	.08	***	.07	**	.002	ns	.003	ns	.007	ns	1.29	ns
Residual	192	.11		.02		.02		.006		.009		.018		1.18	

*** significant at P= 0.001 ** significant at P= 0.01 * significant at P= 0.05 ns= not significant P= Probability.
RL= root lesioning; N/p= nematodes/plant; N/gdr= nematodes/g dry root; dws/p= shoot dry weight/plant; dwr/p= root dry weight/plant; tdw/p= total dry weight/plant; MR= nematode multiplication rate.

Table 8.13 Effect of nematode-*P. irregulare* interaction on the root lesion rating of wheat cultivar Machete 49 days after sowing in a pasteurised sandy soil under controlled glasshouse conditions. Values in the 3-way table are the average of ten single plant blocks.

	Nil			*P. irregulare*		
Nematode	15°C	20°C	25°C	15°C	20°C	25°C
N0	0.00	0.0	0.00	0.40	.05	.25
N1000	0.50	1.25	0.30	0.50	0.37	1.25
N5000	1.10	2.05	1.00	0.85	0.90	2.05
N10000	1.45	2.20	1.40	1.25	1.65	2.20
3-way interaction is significant.						LSD 5%= 0.20

1 and 2-way treatment means (with appropriate LSD at P= 0.05 level). LSD

Fungus	Nil	*P. irregulare*			
	0.96	0.98			*ns*
Nematode	N0	N1000	N5000	N10000	
	0.16	0.70	1.32	1.69	*0.12*
Temperature	15°C	20°C	25°C		
	0.75	1.09	1.05		*0.10*
Nematode	N0	N1000	N5000	N10000	
Fungus					
Nil	0.08	0.68	1.83	1.68	*ns*
P. irregulare	0.23	0.71	1.27	1.70	
Temperature	15°C	20°C	25°C		
Fungus					
Nil	0.76	1.44	0.67		*0.14*
P. irregulare	0.75	0.74	1.44		
Nematode	N0	N1000	N5000	N10000	
Temperature					
15°C	0.20	0.50	0.97	1.35	
20°C	0.15	0.81	1.47	1.92	
25°C	0.12	0.77	1.52	1.80	

N0= no nematodes added, N1000= 1000 nematodes/plant, N5000= 5000 nematodes/plant, N10000= 10000 nematodes/plant.

***Nematode number*:** Data for both nematodes/plant and nematodes/g dry root were transformed to the natural $\log_e$. Number of nematodes/plant or nematodes/g dry root were affected by all main treatments as well as by most of their interactions (Table 8.12). Nematode inoculum was successful as is indicated by the very significant differences between nematode numbers in inoculated and uninoculated treatments. As nematode inoculum level increased, number of nematodes/plant or nematodes/g dry root also increased.

The effect of temperature, in general, was significant on number of nematodes extracted from root systems regardless of fungus inoculum (Figure 8.3a). However, *P. irregulare* at 25°C significantly increased number of *P. neglectus* within the roots of Machete wheat (Figure 8.3a).

Number of nematodes/g dry root also showed a similar pattern to numbers/plant (Figure 8.3b). Multiplication rate of *P. neglectus,* however, decreased with increases in initial nematode density (Figure 8.3c).

Multiplication rate of *P. neglectus* was affected by all treatments (fungus, nematode, inoculation time or temperature) as well as by most of their interactions (Table 8.12). At 20°C or 25°C, the reproduction rate of *P. neglectus* increased by 80% or 88%, respectively, compared to those at 15°C (Figure 8.3c).

Overall, plants inoculated with *P. irregulare* showed a 31% increase in nematode multiplication rate. This increase was statistically significant (Figure 8.3c). However, regardless of fungus inoculum, with increases in the nematode inoculum levels, multiplication rate of the nematode decreased by 59% at 10000 nematodes/plant compared to 1000 (Figure 8.3c). This reduction was statistically significant at P= 0.01.

***Dry matter*:** One, two or 3-way treatment means (fungus, nematode or temperature) for shoot dry weight are presented in Table 8.14.

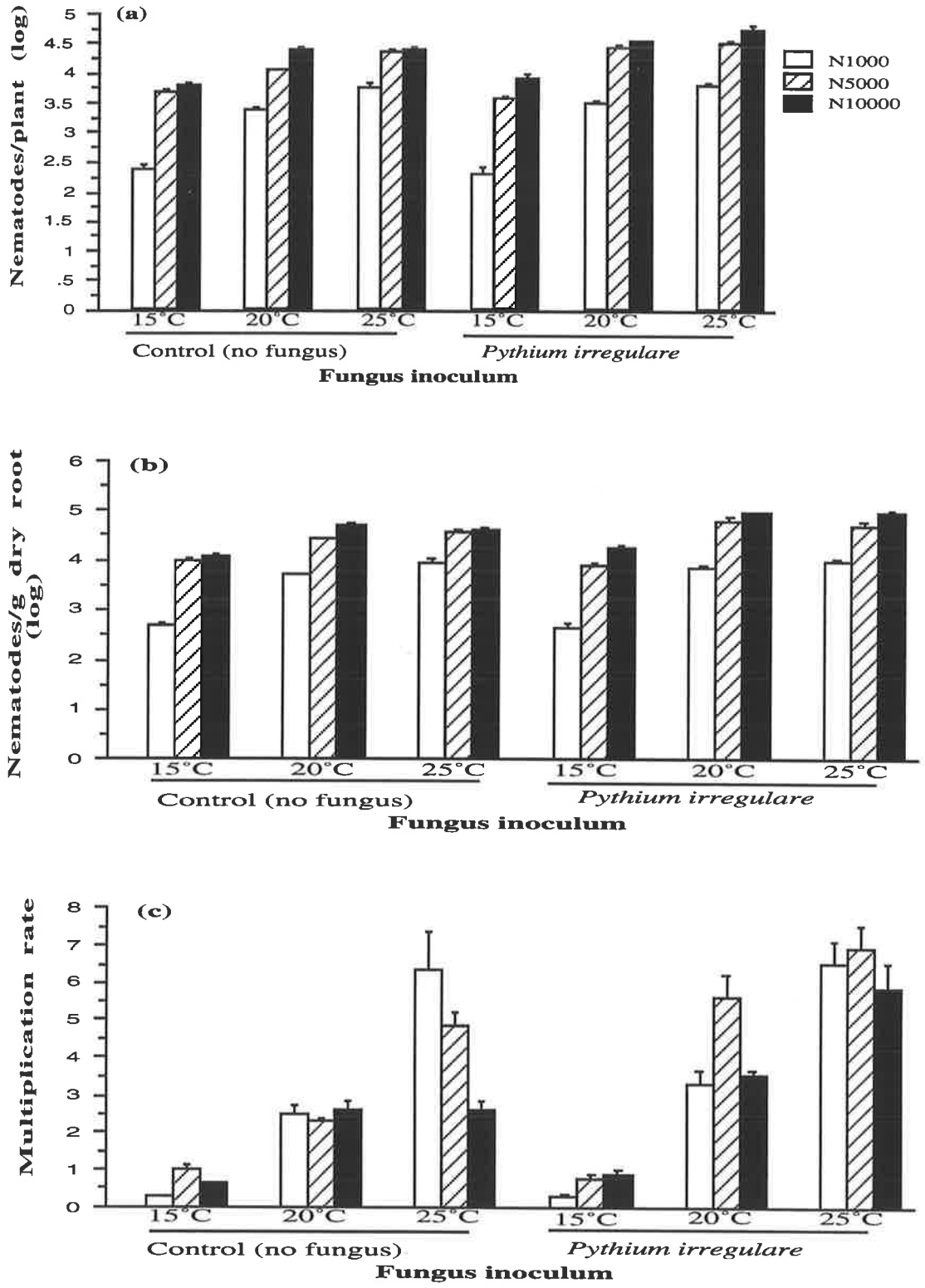


Figure 8.3 Effect of 3-way interaction between nematode, fungus and temperature on (**a**) number of nematodes/plant, (**b**) nematodes/g dry root and (**c**) nematode multiplication rate 49 days after sowing. N1000= 1000 nematodes/plant, N5000= 5000 nematodes/plant, N10000= nematodes/plant.

High soil temperature (25°C) increased production of roots by 22%. This was statistically significant at P= 0.001 (Figure 8.4).

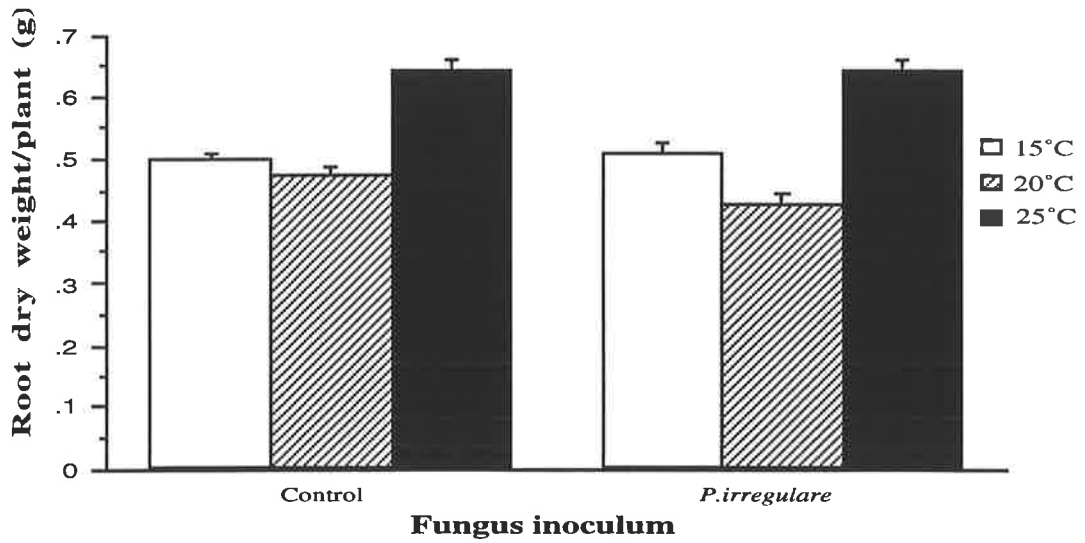


Figure 8.4 Effect of 2-way interaction between fungus and temperature on the root dry weight of wheat cultivar Machete 49 days after sowing.

Shoot dry weight, on the other hand, was affected by all main treatments and by two 2-way interactions between fungus and inoculation time or fungus and temperature (Table 8.14). Fungus alone at 25°C reduced shoot dry weight by 12% compared to the control (no fungus added) at the same temperature, or by 10% compared to those at 15°C.

Total dry weight of plants, however, was significantly affected only by temperature and by a 2-way interaction between fungus and temperature but not by any other treatment (data not presented).

Table 8.14 Effect of nematode-*P. irregulare* interaction on the shoot dry weight (g/plant) of wheat cultivar Machete 49 days after sowing in a pasteurised sandy soil under controlled glasshouse conditions. Values in the 3-way table are the average of ten single plant blocks.

	Nil			*P. irregulare*		
Nematode	15°C	20°C	25°C	15°C	20°C	25°C
N0	0.64	0.61	0.64	0.61	0.64	0.72
N1000	0.54	0.65	0.56	0.63	0.63	0.69
N5000	0.65	0.59	0.58	0.59	0.62	0.66
N10000	0.56	0.61	0.59	0.58	0.62	0.63

3-way interaction not significant.

1 and 2-way treatment means (with appropriate LSD at P= 0.05 level).

Fungus	Nil	*P. irregulare*			LSD
	0.6	0.64			*0.02*
Nematode	N0	N1000	N5000	N10000	
	0.64	0.62	0.61	0.6	*0.03*
Temperature	15°C	20°C	25°C		
	0.6	0.62	0.63		*0.02*
Nematode	N0	N1000	N5000	N10000	
Fungus					
Nil	0.63	0.58	0.61	0.58	*ns*
P. irregulare	0.66	0.65	0.62	0.61	
Temperature	15°C	20°C	25°C		
Fungus					
Nil	0.6	0.61	0.59		*0.03*
P. irregulare	0.6	0.63	0.68		
Nematode	N0	N1000	N5000	N10000	
Temperature					
15°C	0.63	0.58	0.62	0.57	
20°C	0.62	0.64	0.6	0.61	*ns*
25°C	0.68	0.62	0.62	0.61	

N0= no nematodes added, N1000= 1000 nematodes/plant, N5000= 5000 nematodes/plant, N10000= 10000 nematodes/plant.

8.3.4 Experiment 4

The summary of analyses of variance for all measurements is shown in Table 8.15.

Tiller numbers/plant: The significant 3-way interaction between temperature, fungus and cultivar for number of tillers/plant (excluding the main tiller) is shown in Figure 8.5. Generally, plant tillering for all wheat cultivars except Xiao increased at higher soil temperature. At both soil temperatures fungus inoculum increased plant tillering for all wheat cultivars and Abacus. *M. bolleyi* did not affect tiller numbers of wheat cultivar Xiao.

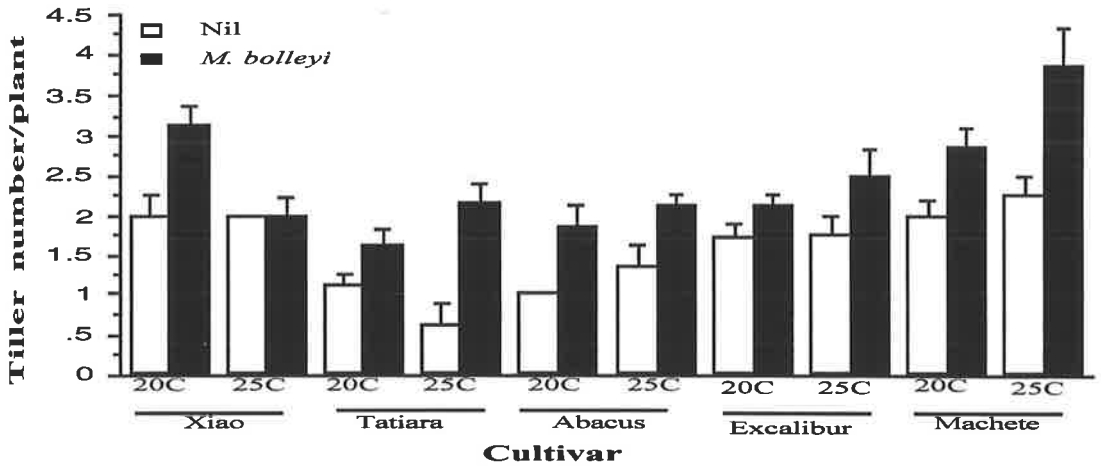


Figure 8.5 Effect of 2-way interactions between nematode (*P. neglectus*) and temperature (20°C or 25°C) on the tiller numbers of four wheat cultivars (Excalibur, Machete, Tatiara or Xiao) and one triticale cultivar (Abacus), 49 days after inoculation under controlled conditions.

Root lesion rating: With or without nematode or fungus, Abacus showed the least lesioning on its root system and Machete roots had the greatest extent of lesioning. Other cultivars including Excalibur, Tatiara and Xiao were intermediate.

Although the extent of root lesion rating on plants grown at 25°C was twice that of plants grown at 20°C, this was not statistically significant. However, a significant 2-way interaction between nematode and fungus was present for root lesion rating. Lesioning of plants inoculated with both fungus and nematode increased by 55%, 884%, respectively, compared to those inoculated with either pathogen alone (Figure 8.6).

Table 8.15 Summary of analyses of variance for the effect of interaction between *Microdochium bolleyi* and *P. neglectus* on the root lesion rating, number of nematodes/plant and nematodes/g dry root, shoot and root dry weights, total dry weight of plants, tillers/plant and nematode multiplication rate for four wheat cultivars and a triticale cultivar 49 days after sowing (Experiment 4).

Source	df	MS RL	P	MS N/p	P	MS N/gdr	P	MS dws/p	P	MS dwr/p	P	MS tdw/p	P	MS tiller	P	MS MR	P
Nematode (nem)	1	29.52	***	1.51E10	***	3.95E10	***	.132	**	.322	**	.88	***	8.90	***	604.24	***
Fungus (fun)	1	2.76	***	2.95E7	ns	2.46E8	ns	3.076	***	.144	*	4.54	***	29.57	***	1.18	ns
Cultivar (cult)	4	1.03	***	2.66E8	***	2.66E9	***	.372	***	1.277	***	.69	***	9.40	***	10.64	***
Temperature (temp)	1	3.98	***	5.34E9	***	9.89E9	***	.064	***	.616	***	.29	**	.60	ns	213.78	***
Nematode × fungus	1	.78	**	4.13E7	ns	2.97E8	ns	.022	*	.021	ns	.01	ns	.007	ns	1.65	ns
Nematode × cultivar	4	.72	***	2.27E8	***	2.43E9	***	.015	ns	.192	***	.18	**	.56	ns	9.09	***
Fungus × cultivar	4	.18	ns	1.36E8	**	4.83E8	**	.045	ns	.038	ns	.01	ns	.61	ns	5.47	**
Temp × nematode	1	1.37	***	5.02E9	***	9.17E9	***	.016	*	.116	*	.10	ns	2.30	**	200.78	***
Fungus × temp	1	.07	ns	4.24E7	ns	3.25E8	ns	.012	ns	.013	ns	.04	ns	.39	ns	1.69	ns
Temp × cultivar	4	.07	ns	1.17E8	*	9.21E7	***	.113	ns	.472	***	.33	***	1.45	**	4.68	*
Culivar × fun × nem	4	.10	ns	1.44E8	**	5.15E8	**	.024	***	.025	ns	.08	ns	.19	ns	5.79	**
Nem × fun × temp	1	.10	ns	3.81E7	ns	3.13E8	ns	.001	ns	.002	ns	.01	ns	.007	ns	1.52	ns
Nem × cult × temp	4	.02	ns	1.13E8	*	8.94E8	***	.011	ns	.058	ns	.10	ns	.87	*	4.55	*
Temp × fun × cultivar	4	.19	ns	1.54E8	**	1.77E8	ns	.015	ns	.081	*	.13	*	1.36	**	6.18	**
Temp×fun×nem×cult	4	.20	ns	1.43E8	**	1.64E7	ns	.036	ns	.045	ns	.10	ns	.78	*	5.73	**
Residual	115	.09		3.91E7		1.51E7		.018		.034		.05		.31		1.56	

*** significant at P= 0.001 ** significant at P= 0.01 * significant at P= 0.05 ns= not significant P= Probability.
RL= root lesioning; N/p= nematodes/plant; N/gdr= nematodes/g dry root; dws/p= shoot dry weight/plant; dwr/p= root dry weight/plant; tdw/p= total dry weight/plant; tiller= tillers/plant; MR= nematode multiplication rate.

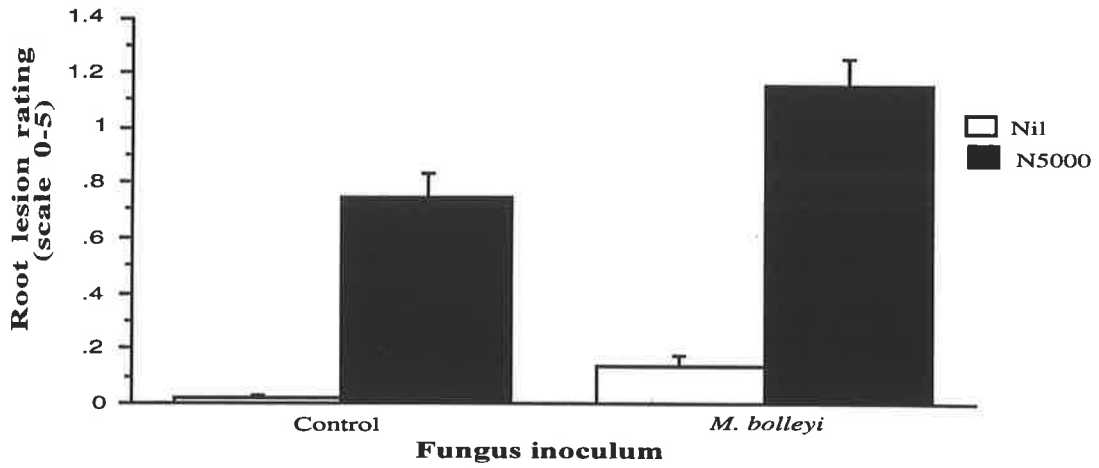


Figure 8.6 Effect of 2-way interaction between nematode (*P. neglectus*) and fungus (*M. bolleyi*) on the extent of root lesion rating of four wheat cultivars (Excalibur, Machete, Tatiara and Xiao) and one triticale cultivar (Abacus), 49 days after inoculation under controlled conditions. Nil= no nematodes added, N5000= 5000 nematodes/plant.

The significant 2-way interaction between nematode and temperature for root lesion rating is shown in Figure 8.7. Plants inoculated with 5000 nematodes/plant and grown at 25°C showed a 77% increase in root lesion rating compared to those grown at 20°C (Figure 8.7).

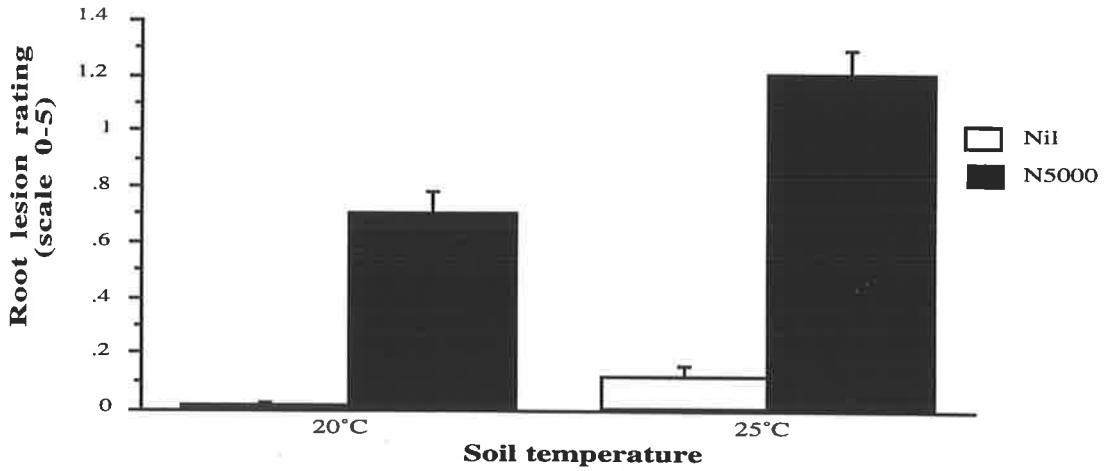


Figure 8.7 Effect of 2-way interaction between nematode (*P. neglectus*) and temperature (20°C or 25°C) on the extent of root lesion rating of four wheat cultivars (Excalibur, Machete, Tatiara and Xiao) and one triticale cultivar (Abacus), 49 days after inoculation under controlled conditions. Nil= no nematodes added, N5000= 5000 nematodes/plant.

Nematode numbers: Nematode, cultivars or temperature (main treatments) had significant effects on number of nematodes/plant or nematodes/g dry root. Cultivars Abacus and Excalibur supported the lowest number of nematodes/plant or nematodes/g dry root.

The significant 3-way interaction between nematode, varieties and temperature for number of nematodes/plant is shown in Figure 8.8. Number of nematodes/plant for varieties Abacus, Excalibur, Machete, Tatiara or Xiao, increased by 204%, 295%, 457%, 578% or 549%, respectively, compared to the initial nematode inoculum level (5000/plant) (Figure 8.8).

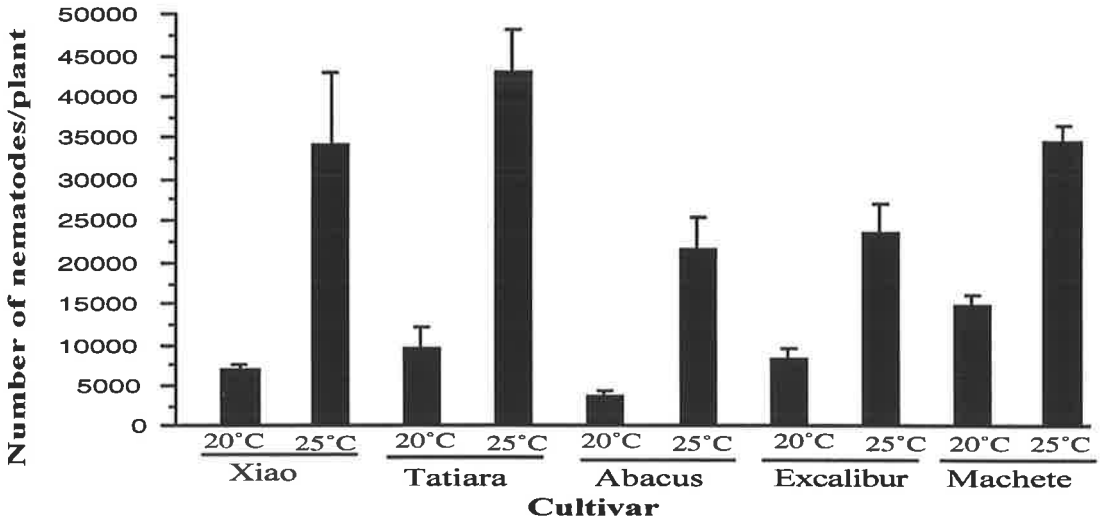
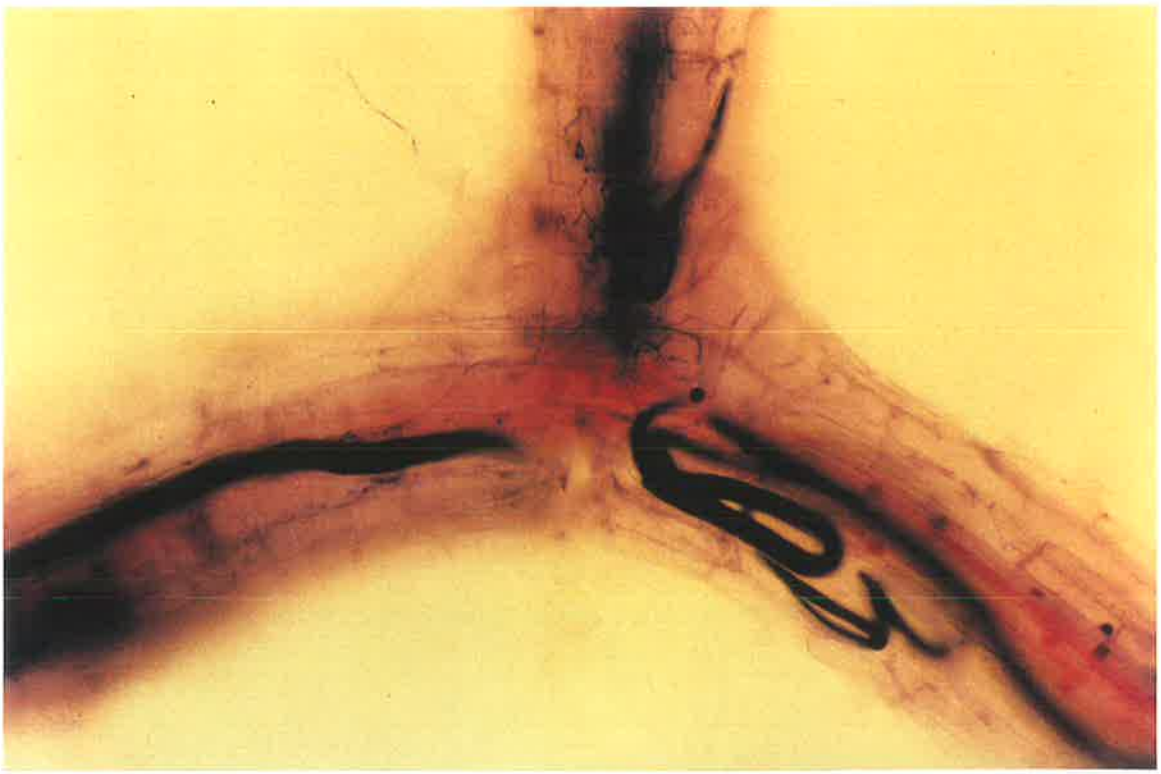


Figure 8.8 Effect of 3-way interaction between nematode (*P. neglectus*), temperature (20°C or 25°C) and cultivar on the number of nematodes/plant 49 days after inoculation under controlled conditions.

In the presence of *M. bolleyi* there was a greater increase in the number of nematodes/plant than in its absence (Figure 8.9). *M. bolleyi* did not affect number of nematodes on plants grown at 20°C. However, at 25°C, *M. bolleyi* increased number of *P. neglectus* on Abacus, Excalibur and Machete but decreased number on Xiao (Figure 8.9). Plate 8.1 shows the extensive root damage caused by *P. neglectus* and *M. bolleyi* together with a large number of nematodes and their eggs within the roots of Excalibur wheat. In general, number of nematodes/g dry root showed a similar pattern to nematodes/plant. As soil temperature increased, nematodes/g dry root also increased.

Plate 8.1 Extensive root damage caused by *P. neglectus* and *M. bolleyi* together with large number of nematodes (represented by the dark black area) and their eggs (oval shaped rods) within the roots of Excalibur wheat.



Similarly, nematode multiplication rate increased as the soil temperature increased. At 20°C, *P. neglectus* did not multiply on Abacus so the final number of nematodes/plant was much lower than the initial. At 25°C, however, the nematode multiplied on Abacus 2.15 times, compared to the initial inoculum level (Figure 8.10).

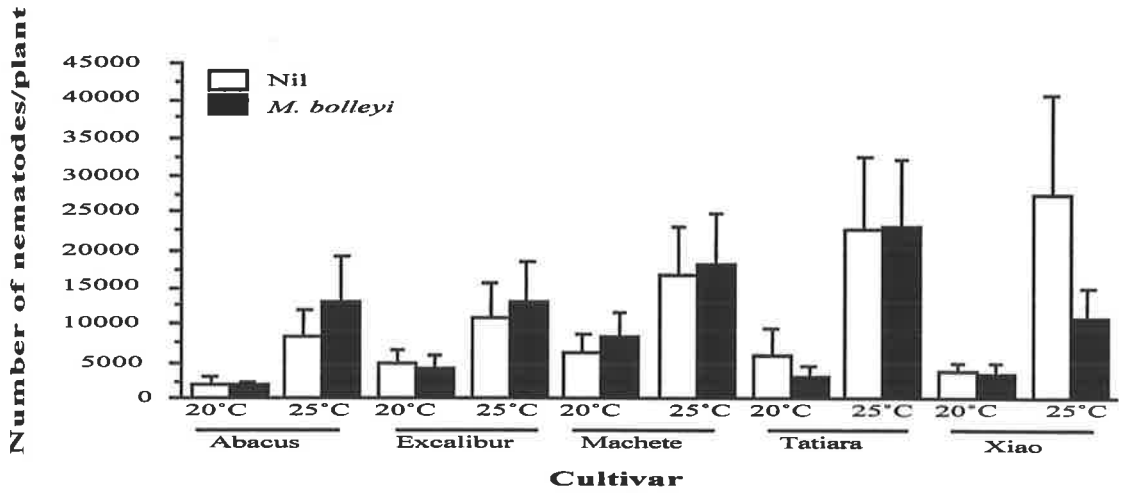


Figure 8.9 Effect of 3-way interaction between fungus (*M. bolleyi*), temperature (20°C or 25°C) and cultivar on the number of nematodes/plant 49 days after inoculation under controlled conditions.

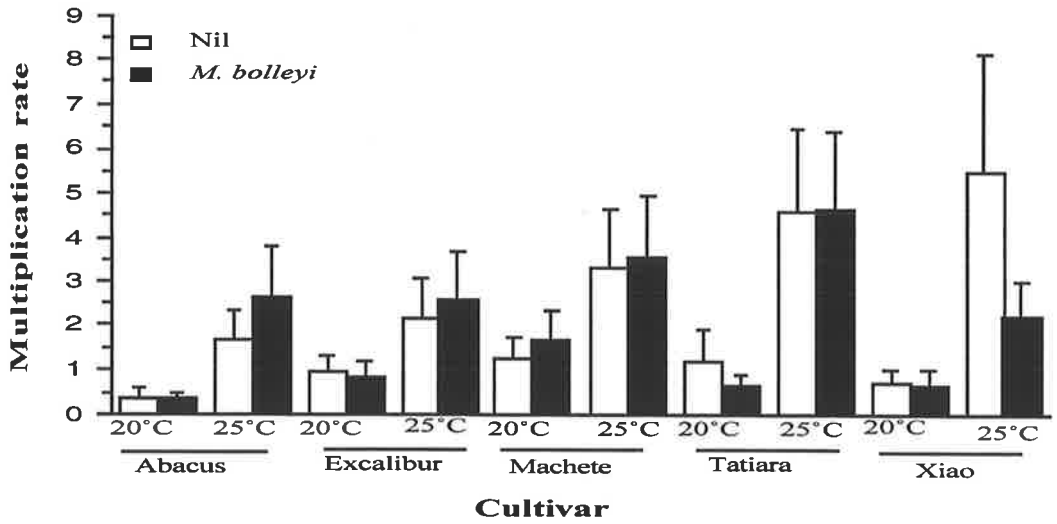


Figure 8.10 Effect of 3-way interaction between fungus (*M. bolleyi*), temperature (20°C or 25°C) and cultivar on the multiplication rate of *P. neglectus* 49 days after inoculation under controlled conditions. Nil= no fungus added.

At 20°C, only the wheat cultivar Machete allowed an increased multiplication rate (1.5 times), but for other varieties the rate was less than one. However, at 25°C, the multiplication rate for varieties Excalibur, Machete, Tatiara or Xiao were 2.37, 3.45, 4.58 or 3.70 times compared to the initial inoculum level (Figure 8.10). Again, in the presence of *M. bolleyi*, a further increase in nematode multiplication rate occurred.

Plant dry matter: The 2-way interaction between nematode and temperature as well as a 3-way interaction between nematode, fungus and cultivar were significant for shoot dry weight (Table 8.15). Root dry weight was significantly affected by the 2-way interactions between nematode and cultivar, nematode and temperature as well as a 3-way interaction between cultivar, fungus and temperature (Table 8.15).

A significant 2-way interaction between nematode and cultivar for root dry weight is shown in Figure 8.11. *P. neglectus* at 5000/plant reduced root dry weight of cultivars Abacus, Machete, Tatiara or Xiao by 12%, 18%, 9% or 27%, respectively, compared to the control (no nematode added). However, with or without nematode, root dry weight of Excalibur was not different (Figure 8.11).

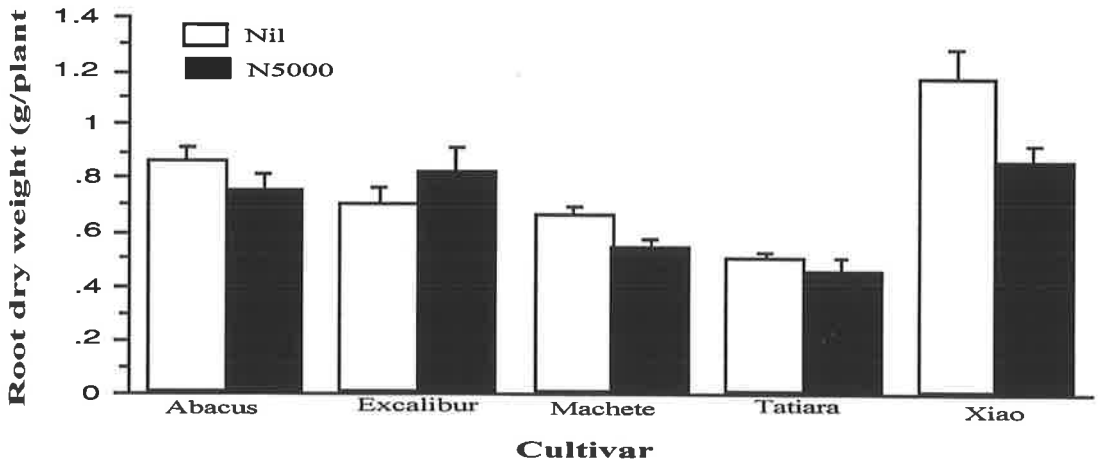


Figure 8.11 Effect of 2-way interaction between nematode (*P. neglectus*) and cultivar on the root dry weight of four wheat cultivars (Excalibur, Machete, Tatiara and Xiao) and one triticale cultivar (Abacus), 49 days after inoculation under controlled conditions. Nil= no nematodes added, N5000= 5000 nematodes/plant.

Root dry weight increased as the soil temperature increased. A significant 3-way interaction between fungus, cultivar and temperature for root dry weight is shown in Figure 8.12. Regardless of fungus inoculation, root production of cultivars Abacus, Excalibur, Machete or Tatiara at 25°C increased by 49%, 22%, 24% or 22%, respectively, compared to those grown at 20°C. In contrast, wheat cultivar Xiao produced 32% more roots at 20°C than at 25°C (Figure 8.12). Xiao is not related to the other cultivars and its growth patterns may differ from those of Australian cultivars even in the absence of pathogens.

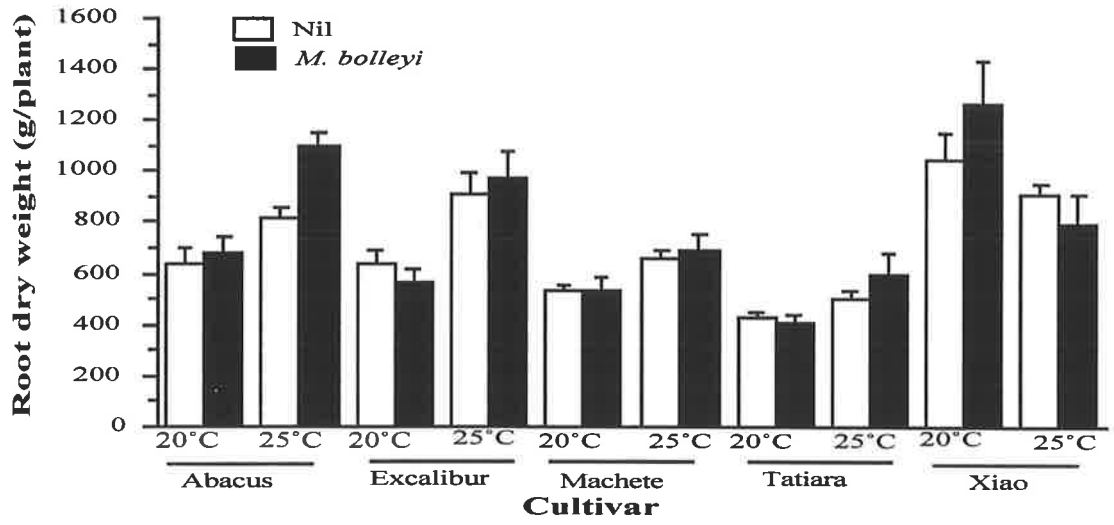


Figure 8.12 Effect of 3-way interaction between fungus (*M. bolleyi*), temperature (20°C or 25°C) and cultivar on the root dry weight of four wheat cultivars (Excalibur, Machete, Tatiara and Xiao) and one triticale cultivar (Abacus), 49 days after inoculation under controlled conditions.

While cultivars Abacus, Excalibur or Tatiara produced more roots at 25°C, the production of shoots was less at this temperature compared to those grown at 20°C. At 20°C, shoot dry weight of cultivars Abacus, Excalibur or Tatiara increased by 10%, 11% or 15%, respectively, compared to those grown at 25°C. However, at 20°C, shoot dry weight of Machete or Xiao decreased by 12% or 10%, respectively, compared to those at 25°C (Figure 8.13).

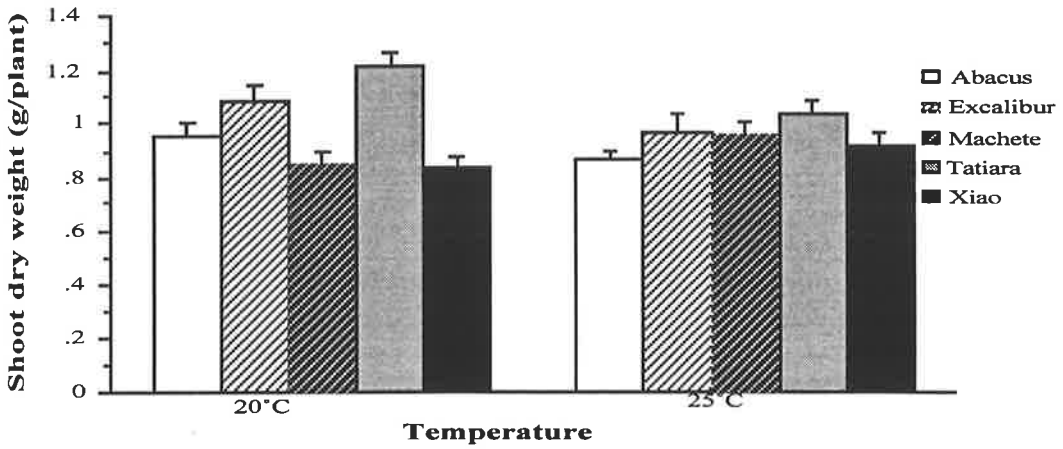


Figure 8.13 Effect of 2-way interaction between temperature (20°C or 25°C) and cultivar on the shoot dry weight of four wheat cultivars (Excalibur, Machete, Tatiara or Xiao) and one triticale cultivar (Abacus), 49 days after inoculation under controlled conditions.

Total dry weight was also significantly affected by all main treatments as well as by the 2-way interaction between nematode and varieties or temperature and varieties and by a 3-way interaction between fungus, varieties and temperature (Table 8.15).

8.4 Discussion

The interaction of three root-rot fungi of wheat, *F. acuminatum, M. bolleyi* or *P. irregulare,* with *P. neglectus* was affected by soil temperature. Of biotic and abiotic factors influencing nematodes and other pathogens, temperature is particularly important. Wallace (1973) stressed the importance of soil temperature on nematode movement, rate of development and reproduction and distribution as well as other functions.

Under the conditions of these studies, the optimum temperature for infection, growth and reproduction of *P. neglectus* was 25°C. At 15°C, the number of nematodes extracted from roots was lower than at 20°C or 25°C. However, the majority of nematodes extracted from plants grown at 20°C or 25°C were adults, whereas those extracted from plants grown at 15°C were mostly second-stage juveniles. This indicates that nematodes actually penetrated roots over the duration of the experiment, but little development had occurred before plants were harvested and roots were placed in the mist chamber for four days to extract nematodes. It is possible that during the extraction period where the temperature was around 25°C, eggs hatched.

The movement and reproduction of *P. neglectus* is greater at higher soil temperature (Benedict and Mountain, 1956; Vanstone and Nicol, 1993). Umesh and Ferris (1994) found that maximum root penetration by *P. neglectus* occurred at soil temperature of 25°C. At this soil temperature, the growth of wheat plants was also maximised.

At 15°C the disease rating was reduced and reproduction of nematodes was delayed as compared to 20°C or 25°C. At 20°C, infectivity was greater than at 15°C. However, there was no major difference between 20°C and 25°C in the amount of root damage and nematode multiplication rate.

The presence of all fungi tested (*F. acuminatum, M. bolleyi* or *P. irregulare*) increased the amount of lesioning on the root system and number of nematodes within roots. Soil temperature also had a significant effect. At 25°C, the degree of root damage was extremely high compared to 15°C. However, at 20°C, yellow leaf symptoms were observed indicating that shoot growth of plants was affected by soil temperature.

It is suggested that plants grown under optimum soil moisture and temperature conditions are able to recover from the damage caused by nematode, fungus or the combination of both organisms through producing more roots. However, such a condition may not occur in the field where soil temperature late in the season increases above 20°C, and available moisture in the soil is usually limited. In South Australia, this water stress usually coincides with maximum plant moisture requirements. Root damage caused by nematodes and associated fungi may disrupt normal water absorption and translocation. Damaged root systems and shortage of available moisture in the rhizosphere at plant maturity intensify water stress, and cause severe yield losses.

On the other hand, the effect of temperature on plants grown at 20°C with optimum soil moisture was pronounced, with general yellowing compared to those at 25°C or 15°C. Therefore, it is suggested that both soil temperature and available moisture are important in a given interaction between nematode and fungus for full expression of disease.

The number of nematodes extracted from roots increased with increase in the soil temperature and was highest at 25°C. In the absence of fungal inoculum, the multiplication rate of *P. neglectus* (final/initial density) decreased with increase in initial nematode inoculum levels. However, in the presence of fungal inoculum (*F. acuminatum*, *M. bolleyi* or *P. irregulare*), as the inoculum level of the nematode increased, reproduction rate of *P. neglectus* also increased, particularly at higher soil temperature. This very important observation indicates the significance of fungal inoculum in the soil where nematodes are also present and may interact with them. All fungi tested naturally occur in most soils, sometimes at high levels, and will readily infect roots making this result even more important and of direct implication to field situations.

The optimum temperature for movement and reproduction of nematodes depends upon host plant and nematode species as well as soil type and available soil moisture. Townshend (1972) reported that penetration of corn roots was maximised at 20°C for *P. penetrans* and at 30°C for *P. neglectus*. However, Umesh and Ferris (1992) found that the optimum temperature for development of *P. neglectus* on barley is about 25°C. The results obtained here are similar to those previously reported (Umesh and Ferris, 1992; Vanstone and Nicol 1993). However, the addition of fungal inoculum significantly increased penetration, development and multiplication rate of *P. neglectus*.

Fungi present in the soil or roots may be directly used as a source of energy for the nematode, providing a continuous food supply. Fungi may also modify the rhizosphere, attracting nematodes to the roots of the host. It is also possible that nematode and fungus infection and establishment in the roots could lead to a better environment and more nutritious substrate for nematode development and reproduction.

The majority of nematodes extracted from plants grown at 20°C or 25°C were adults, whereas most nematodes extracted from plants grown at 15°C were second-stage juveniles This indicated that even at lower soil temperature, nematodes were able to move and penetrate roots, but were slow in development or multiplication. However, under cooler conditions (and similarly in dry periods), nematodes may lay eggs for

survival, and as soon as temperature and available soil moisture increases, eggs begin to hatch and their number increases exponentially within a short period of time. Thus, conditions favourable for plant growth favour nematode movement, development and reproduction, indicating a close evolutionary relationship between wheat and *P. neglectus*.

Although at high soil temperature (25°C) maximum plant growth occurred and there were no foliar symptoms (yellow or dead leaves) compared to the plants at 20°C, root production was decreased by nematodes, or the combination of nematodes and fungus. This was positively correlated with the amount of damage to roots and numbers of *P. neglectus* in roots. Under optimum conditions for plant growth, even a small number of uninfected roots will be capable of supporting plant growth. At higher soil temperatures, availability of soil nutrients is high. Therefore, providing soil is well wetted a small quantity of healthy roots will be capable of supporting shoot growth for most, if not all, of the plant's life.

The reported resistance of the cultivars to *P. neglectus* used was unchanged by the presence of *M. bolleyi*. It was in the order which other researchers (Vanstone *et al.*, 1994; Farsi, 1995) established, that is, Abacus and Excalibur were more resistant than the other cultivars. The discrimination of resistance, as measured by nematode number, was far greater at the higher temperature. It is suggested that breeders selecting for resistance should screen at temperatures of 25°C rather than 20°C. Root lesion rating was significantly increased in all cultivars tested except Abacus when nematodes and *M. bolleyi* were combined compared with either pathogen alone, particularly at 25°C. Therefore, because there was no interaction between these cultivars and the presence or absence of *M. bolleyi*, it is worth considering the addition of *M. bolleyi* to a resistance screening procedure because of the greater (and thus easier scoring of) root lesioning.

Generally, the results of these experiments confirmed the previous findings with fungi in combination with *P. neglectus* using only Machete wheat (Chapters 4, 6 and 7).

Chapter 9

Field investigations of interaction between *Pratylenchus neglectus* and root-rotting fungi of wheat

9.1 Introduction

A number of fungi associated with root lesion nematodes have been found in the field (Chapter 3). *Microdochium bolleyi*, *G. graminis*, *Fusarium* spp. and *Pythium* spp. were the most commonly isolated fungi from lesioned root segments. The pathogenicity of these fungi alone or in combination with *P. neglectus* was investigated (Chapter 4).

The effect of factors such as fungus or nematode at different densities, soil temperature, harvest time or inoculation timing of both fungus and nematode were tested in a controlled environment glasshouse. Experiments under controlled conditions in the glasshouse and laboratory indicated a positive interaction between root lesion nematode and *M. bolleyi*, *F. acuminatum*, *P. terrestris* or *P. irregulare*. Plants inoculated with the above fungi and *P. neglectus* suffered more damage than those inoculated with either pathogen alone (Chapter 4). The fungal association also resulted in a greater number of nematodes within the root system.

However, the results obtained under controlled conditions needed to be confirmed in the natural conditions of the field, where there are numerous factors possibly effecting such interactions. Environmental factors such as temperature, moisture availability and soil texture are important in nematode-fungus interactions (Wallace, 1983; Sikora and Carter, 1987).

A number of field experiments were carried out. In 1993 and 1994, microplot experiments were conducted in the field at Roseworthy Campus (Figure 2.1) to test

nematode-fungus interaction under field conditions. In 1994, a trial was conducted at Stow (Figure 2.1) to further investigate the effect of environmental conditions on the nematode-fungus interaction.

9.2 Methods

9.2.1 Microplot experiments

Microplots were established in order to investigate interactions between the root lesion nematode and soil-borne fungi which, in glasshouse tests, showed positive results under semi-controlled conditions. A sandy soil was collected from the top 10-15cm of the field, steam pasteurised at 70°C for 40 minutes and air-dried for four days prior to use.

9.2.1.1 Seed germination and planting

Seed of wheat cultivars Machete and Spear were surface-sterilised in 2.5% sodium hypochlorite for ten minutes and washed thoroughly with sterile distilled water for one minute. Seeds were then transferred into sterile Petri dishes with moist filter paper and incubated at 4°C for two days followed by one day at 25°C to germinate.

9.2.1.2 Nematode inoculum

Aseptically grown *P. neglectus* were pipetted in 1ml of distilled water around each plant at the appropriate concentration.

9.2.1.3 Inoculation of soil with fungi

Inocula of *M. bolleyi, F. acuminatum* or *P. terrestris* were added to the soil in each microplot at 1% w/w and *P. irregulare* at 0.1% w/w. The inoculum was added to the soil in two layers.

The microplots were then transferred to the field and partially buried so that the soil surface inside the microplot was level with the soil outside the microplots. Microplots

were 1m apart. In this way, plants were exposed to the actual weather conditions but the roots were exposed to specific treatments.

9.2.1.4 1993 Microplot experiment (Experiment 1)

In 1993, a microplot experiment was conducted at Roseworthy. Two wheat varieties, Spear and Machete, were used. Twelve pre-germinated seeds of each cultivar were sown in each of 4 liter containers (20cm length, 20cm width, 15cm depth) to a depth of 1.5cm. Cultivars were selected on the basis of susceptibility to *P. neglectus* and soil-borne pathogens (V. A. Vanstone, personal communication).

The experiment was a factorial randomised block design, with two wheat varieties, four fungal treatments (*M. bolleyi*, *F. acuminatum*, *M. bolleyi*+*F. acuminatum* or nil) and two nematode treatments (with or without *P. neglectus*) to give a total of sixteen treatments, which were each replicated six times. Two thousand aseptically grown *P. neglectus* in 1.0ml of distilled water were pipetted around each plant. Each microplot contained twelve plants.

9.2.1.5 1994 microplot experiment (Experiment 2)

A further trial was conducted in 1994, in order to investigate the effect of nematode-fungus interactions using a range of nematode densities (0, 100, 500, 1500, 3000, 6000 or 12000 nematodes/plant) as well as a number of soil-borne fungi (*M. bolleyi*, *F. acuminatum*, *P. terrestris* or *P. irregulare*) at one density. Only one wheat variety (Machete) was used for this experiment. Seven pre-germinated seeds were sown in each microplot (five litre container) (30cm length, 20cm width, 15cm depth) as shown in Plate 9.1 to a depth of 1.5cm. Fertiliser was not added to the plots, nor was herbicide applied to plots or to the surrounding area. The experiment was a factorial randomised block design with four replications.

Plate 9.1 Field preparation and layout of microplot experiment at Roseworthy Campus.

Where necessary, data for both the 1993 and 1994 experiments were transformed before analyses of variance to $\log_e (x+1)$ to render variance independent of the means.

9.2.1.6 Sampling

1993: Two plants were carefully removed from each plot on July 20 and on August 18 (eight and twelve weeks after sowing) using a 6.0cm diameter metal tube (Plate 9.2). Plants were stored in plastic bags at 4°C until processing. Soil was washed from the roots under running tapwater. Disease rating (based on the number, size and severity of lesions on both seminal and crown roots) was calculated for both sample dates using a scale of 0-5 (0= healthy plant and 5= whole root system rotted) (General Methods). At the first sample date, four 1.0cm long root segments were selected at random from each set of two plants, and plated on isolation medium. Nematodes were extracted from roots in a mist chamber over four days and counted. Roots and shoots were dried and weighed.

Plots were harvested on November 26 (six months after sowing), at which time eight whole plants were removed by hand from each microplot. The following were determined: number of fertile tillers/plant and total shoot weight/plot. Finally, plants were threshed and grain yield/plot measured.

1994: One plant was sampled from each microplot on July 18 and on August 15 (eight and twelve weeks after sowing), when disease rating values for the root system were calculated, root segments plated on isolation medium, nematodes extracted from roots, and roots and shoots dried and weighed.

All methods were identical to those described above for the 1993 microplot experiment. Five whole plants from each microplot were harvested by hand on November 20, and the following were measured: total weight of plants/plot, total number of tillers/plant, number of fertile heads/plant. Finally, plants were threshed and total grain yield/plot, number of seeds/head and 1000 seed weight were measured.

Plate 9.2 Sampling method used for microplot experiment at Roseworthy Campus.

9.2.2 Field experiment 1994 (Experiment 3)

A field experiment was conducted in 1994 at Stow (Figure 2.1), in order to further investigate the effect of nematode-fungus interactions on wheat under natural conditions in the field. Before sowing, ten soil samples were collected at random from the experimental area and nematodes were extracted using Whitehead trays (General Methods). Average density of *P. neglectus* prior to sowing was 10 nematodes/g of dry soil.

Fungal inoculation: Inoculum of *M. bolleyi*, *F. acuminatum* or *P. terrestris* was prepared on millet seed, as described in the General Methods, and applied with the wheat seed at sowing at the rate of $6g/m^2$. Plots not inoculated with fungi received an equal amount of dead millet seed.

Experimental design: The experiment was a split plot randomised block design, where the main plots were chemical application (Temik® two weeks before sowing, Temik® at sowing or methyl bromide two weeks before sowing) and sub-plots were fungus inoculum (*M. bolleyi, F. acuminatum, P. terrestris* or nil). A total of sixteen treatments were replicated four times. Where necessary, data were transformed before analyses of variance to $\log_e (x+200)$ to render variances independent of the means. The wheat cultivar Machete was sown on 20 June.

Soil was fumigated with methyl bromide ($850ml/14m^2$) two weeks before sowing to reduce populations of soil-borne organisms, and was expected to allow inoculated fungi to establish on plants in the absence of other competitive soil-borne pathogens. Temik® (aldicarb) (3.75kg/ha a.i.) was applied. The early application was carried out to reduce the risk of possible Temik® effects on seed and its germination.

Sampling: Five plants were carefully dug from each plot on August 20 and on September 18 (eight and twelve weeks after sowing). Plants were processed as for previous experiments. Roots were scored for lesioning and nematodes extracted in a mist chamber over four days and counted. At the first sampling, five 1.0cm long root

segments were selected at random from each set of five plants, and plated on isolation medium.

All plots were harvested on November 15. Before machine harvesting, whole plants were removed, by hand, from two 50cm long rows of each plot. The following were measured: total weight of plants/plot, total number of tillers/plant, number of heads/plant and number of fertile heads/plant. Finally, plants were threshed and total grain yield/plot, number of seeds/head and 1000 seed weight measured.

9.3 Results

9.3.1 1993 microplot experiment (Experiment 1)

The analyses of variance for all measurements are shown in Table 9.1.

Root lesion rating: The 2-way interaction between soil treatment and fungus was significant for root lesion rating. At the first sample date (eight weeks after sowing), root lesion rating was increased where both fungus and nematodes were present compared to the effect of either pathogen alone (Figure 9.1). Further increases occurred at the second sample date (twelve weeks after sowing). The extent of root symptoms on plants inoculated with both fungus and nematode is shown in Plate 9.3.

Nematodes alone had a significant effect on root lesion rating at the first sample date but nematodes in combination with added fungi interacted to cause extensive root lesioning. Fungus inoculum or wheat varieties independently had no significant effect on the root lesion rating. At the second sample date, both fungus and nematode inoculum independently had a significant effect on the amount of lesioning on the root system which increased again dramatically when both were present. The fungus, nematodes or both together were comparable. However, there was no significant difference between wheat varieties.

Plate 9.3 Root samples collected from the 1993 microplot experiment at Roseworthy, twelve weeks after sowing.

A. Plants inoculated with *F. acuminatum* (1% w/w) and 2000 *P. neglectus*/plant.

B. Plants inoculated with *M. bolleyi* (1% w/w) and 2000 *P. neglectus*/plant.

Extensive lesioning and few lateral roots caused by the combination of nematodes and fungus.

A

B

Table 9.1 Summary of analyses of variance for the effect of nematode-fungus interaction on the root lesion rating eight and twelve weeks after sowing, number of nematodes/plant eight and twelve weeks after sowing, root and shoot dry weights (eight weeks after sowing), shoot dry weight at maturity and grain yield (g/plot) for wheat cultivars Spear and Machete in the Roseworthy microplot experiment (Experiment 1).

Source	df	MS RL (8 weeks)	P	MS RL (12 weeks)	P	MS N/p (8 weeks)	P	MS N/p (12 weeks)	P	MS dwr (8 weeks)	P	MS dws (8 weeks)	P	MS dws (maturity)	P	MS Yield	P
Block	5																
Fungus (fun)	3	3.31	***	9.95	***	9.05E	*	1.27E7	**	0.04	ns	18.62	*	20.15	ns	4.88	*
Cultivar (cult)	1	0.06	ns	0.12	ns	3.62E8	***	2.36E5	ns	0.05	ns	1.31	ns	17.51	ns	1.77	ns
Nematode (nem)	1	12.56	***	59.52	***	3.35E4	ns	3.98E8	***	0.01	ns	3.23	ns	1658.34	***	99.33	***
Fungus × cultivar	3	0.15	ns	0.15	ns	9.77E6	*	4.22E6	ns	0.02	ns	6.67	ns	20.59	ns	1.90	ns
Fungus × nematode	3	0.59	**	4.18	***	3.43E6	ns	1.05E7	**	0.03	ns	0.12	ns	49.98	**	1.97	ns
Nematode × cultivar	1	0.12	ns	0.26	ns	2.71E6	ns	6.04E5	ns	0.17	**	7.09	ns	0.84	ns	0.66	ns
Fun × nem × cult	3	0.19	ns	0.20	ns	3.55E6	ns	4.82E6	ns	0.03	ns	6.18	ns	23.59	ns	2.04	ns
Residual		0.13		0.17		3.10E6		2.32E6		0.03		6.52		12.30		1.65	

***= significant at P=0.001 **= significant at P= 0.01 *= significant at P= 0.05 ns= not significant P= Probability.
RL= root lesioning; N/p= nematodes/plant; dwr= root dry weight/plant; dws= shoot dry weight/plant; dws (maturity)= shoot dry weight/plant at maturity; Yield= grain yield/plot.

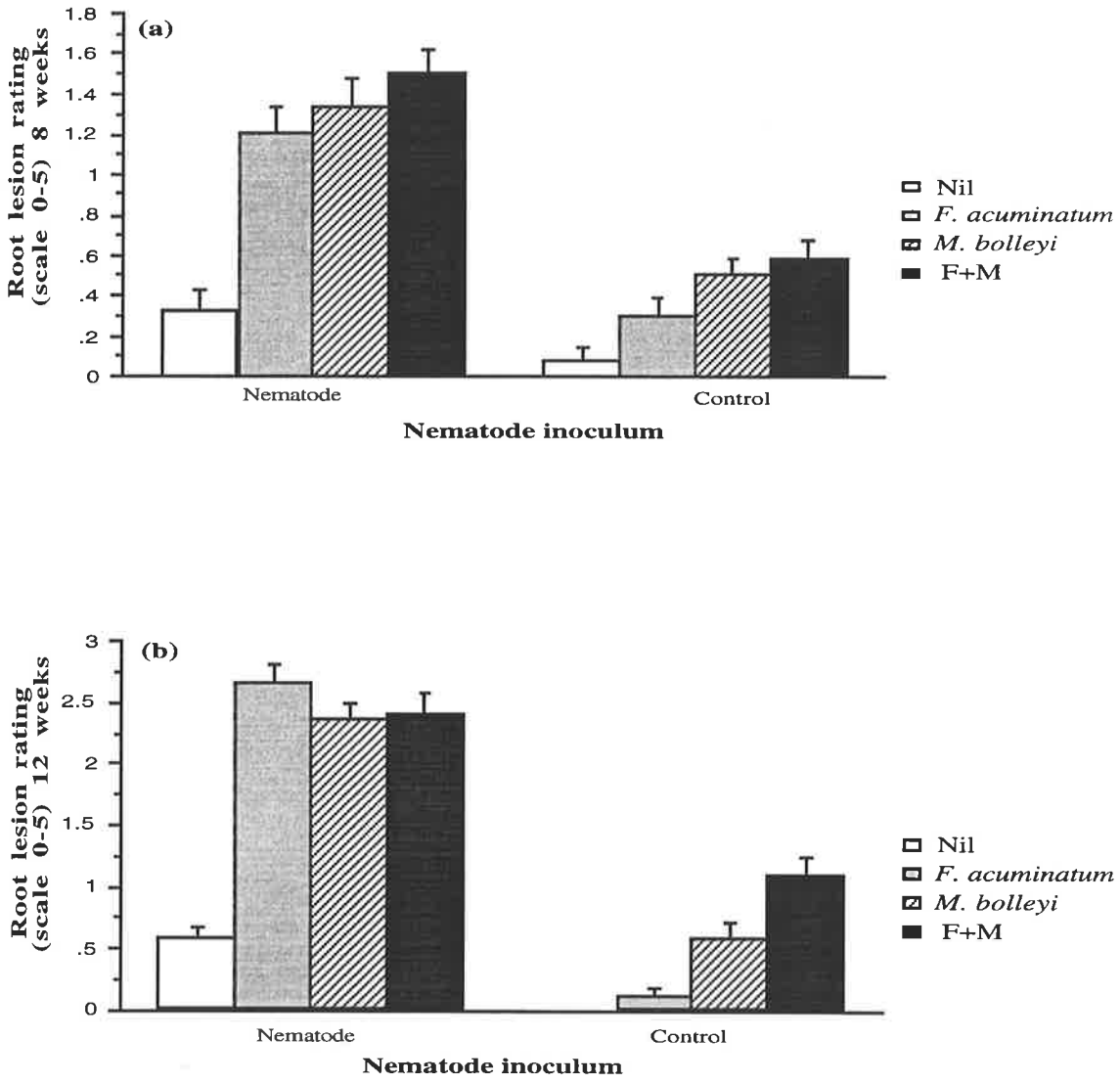


Figure 9.1 Effect of nematode-fungus interaction on root lesion rating **(a)** eight weeks and **(b)** twelve weeks after sowing. Data are means of two wheat cultivars (Spear and Machete). F+M= *F. acuminatum+M. bolleyi*.

Number of nematodes: In the presence of fungus, number of nematodes/plant or nematodes/g dry root were significantly increased (Figures 9.2 and 9.3). At the first sample date, inoculation with *M. bolleyi* or *M. bolleyi+F. acuminatum* increased nematode numbers/plant by 83% or 95%, respectively, compared to the uninoculated plants (control) (Figure 9.2). This increase was significant at P= 0.05. Further increase in the number of nematodes occurred at the second sample date.

At the second sample date (twelve weeks after sowing), a 2-way interaction between nematode and fungus was significant (P=0.01) for nematodes/plant. With *M. bolleyi* or *M. bolleyi+F. acuminatum* numbers of nematodes/plant increased by 118% or 95%, respectively, compared to the uninoculated plants (control) (Figure 9.2b).

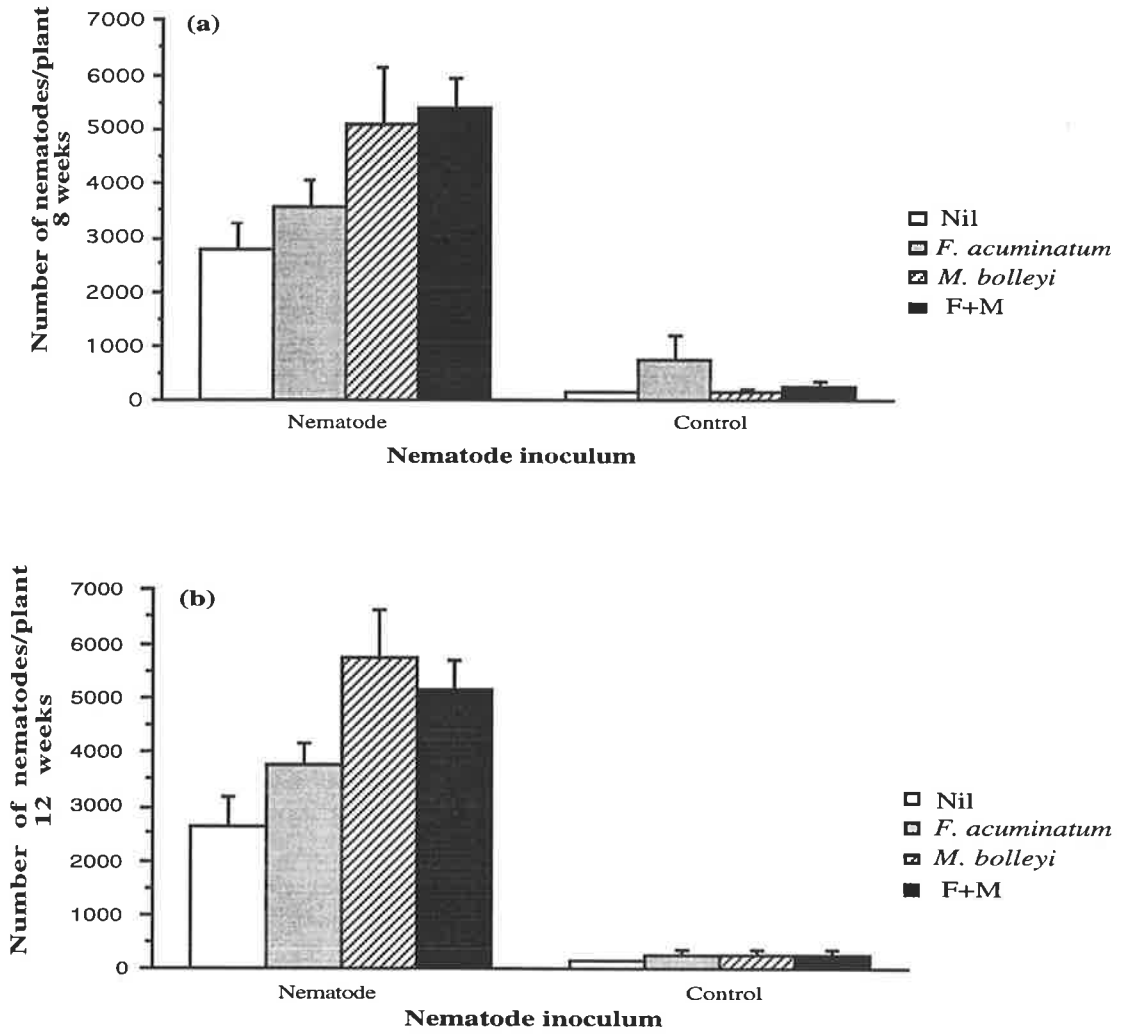


Figure 9.2 Effect of nematode-fungus interaction on number of nematodes extracted from the root system of wheat cultivars Spear and Machete (means) **(a)** eight weeks and **(b)** twelve weeks after sowing. F+M= *F. acuminatum+M. bolleyi*.

Number of nematodes/g dry root was significantly affected by fungus or nematode alone and by the 2-way interaction between fungus and cultivars. For uninoculated plants (no fungus added), Spear had 156% more nematodes than Machete. However, there were no differences between fungi and no fungus on Spear. With *F. acuminatum*, *M. bolleyi* or *M. bolleyi+F. acuminatum*, number of nematodes/g dry root for Machete

increased by 130%, 227% or 256%, respectively, compared to the corresponding control plants (uninoculated) (Figure 9.3).

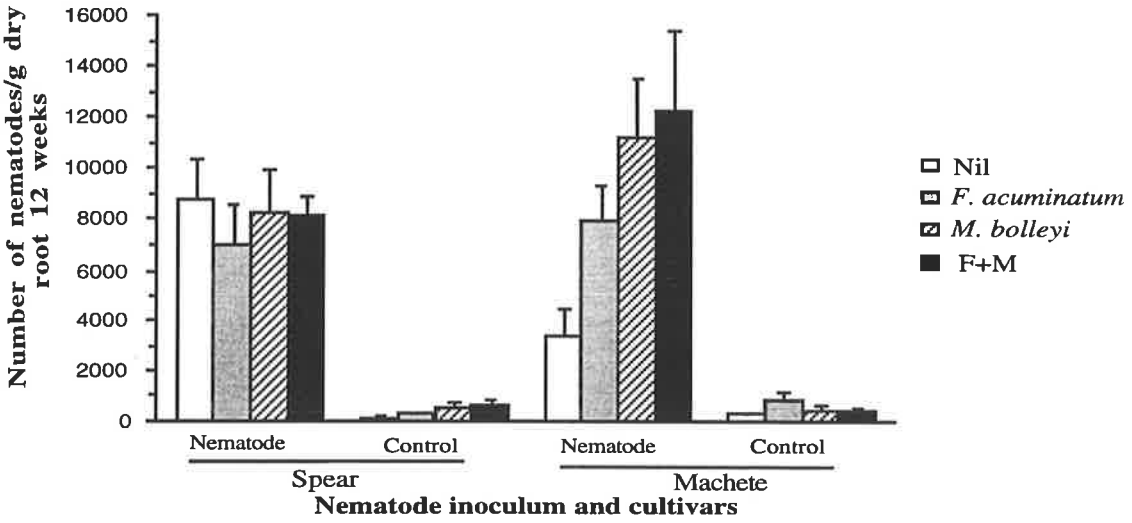


Figure 9.3 Effect of nematode-fungus interaction on number of nematodes/g dry root extracted from the root systems of wheat cultivars Spear and Machete twelve weeks after sowing. F+M= *F. acuminatum*+*M. bolleyi*.

Plant dry matter: Shoot dry weight was significantly affected by fungus inoculum but not by nematode inoculum or by their combination at the first sample date. At the second sample date none of the treatments had significant affects on shoot dry weight. Root dry weight, however, was significantly affected by a 2-way interaction between nematode and cultivar. Inoculum of *P. neglectus* at 2000/plant did not affect root dry weight of Machete, but Spear showed a 20% increase in root dry weight where *P. neglectus* was added (Figure 9.4).

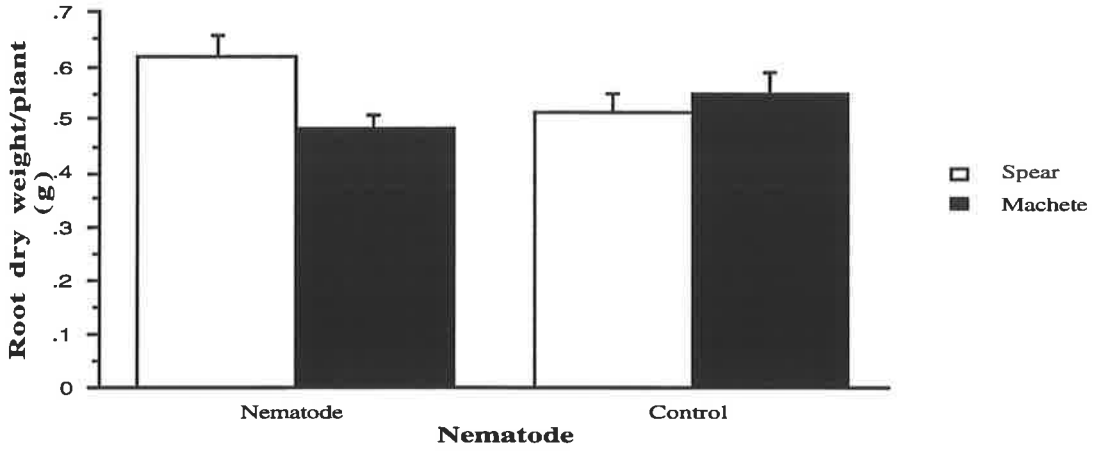


Figure 9.4 Effect of nematode-variety interaction on root dry weight/plant eight weeks after sowing in field microplots.

Shoot dry weight at maturity: Total biomass/plot was significantly affected by nematode alone or by a 2-way interaction between nematode and fungus, but not by fungus inoculum alone or by wheat varieties. *F. acuminatum*, *M. bolleyi* or *M. bolleyi*+*F. acuminatum* in combination with the nematode decreased total biomass/plot by 12%, 23% or 24%, respectively, compared to when the nematode was added alone (Figure 9.5).

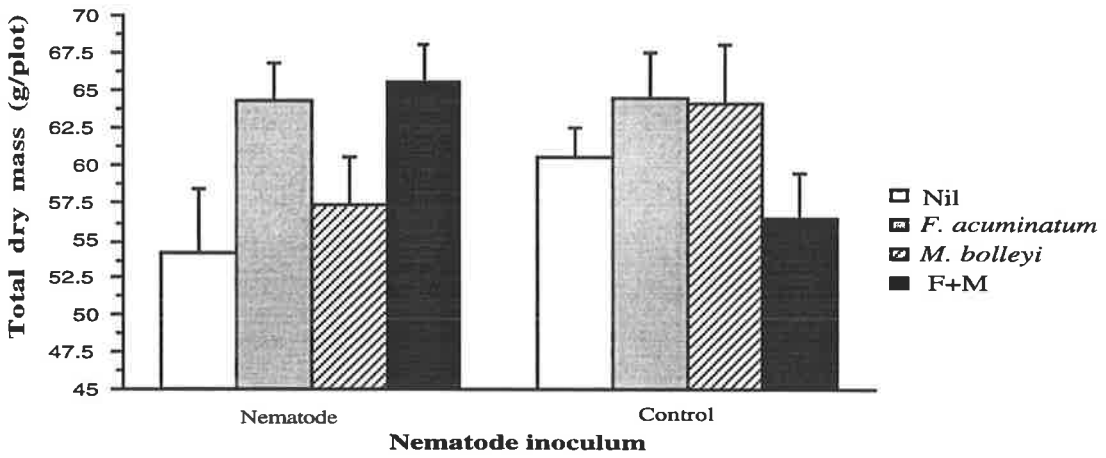


Figure 9.5 Effect of nematode-fungus interaction on total dry matter/plot. Data are means for both wheat varieties (Spear and Machete). F+M= *M. bolleyi* + *F. acuminatum*.

Grain yield: Fungus or nematode inoculation independently had significant effects on grain yield/plot, but there was no significant difference between wheat varieties (Table 9.1). There were no significant 2-way or 3-way interactions between main treatments (fungus, nematode or variety) for grain yield. With *F. acuminatum*, *M. bolleyi* or *M. bolleyi+F. acuminatum* in the absence of *P. neglectus*, grain yield increased by 11%, 7% or 8%, respectively, compared to the control (no fungus added) (Figure 9.6a). However, with nematode inoculum, grain yield was decreased by 22% compared to the control (no nematodes added) (Figure 9.6b).

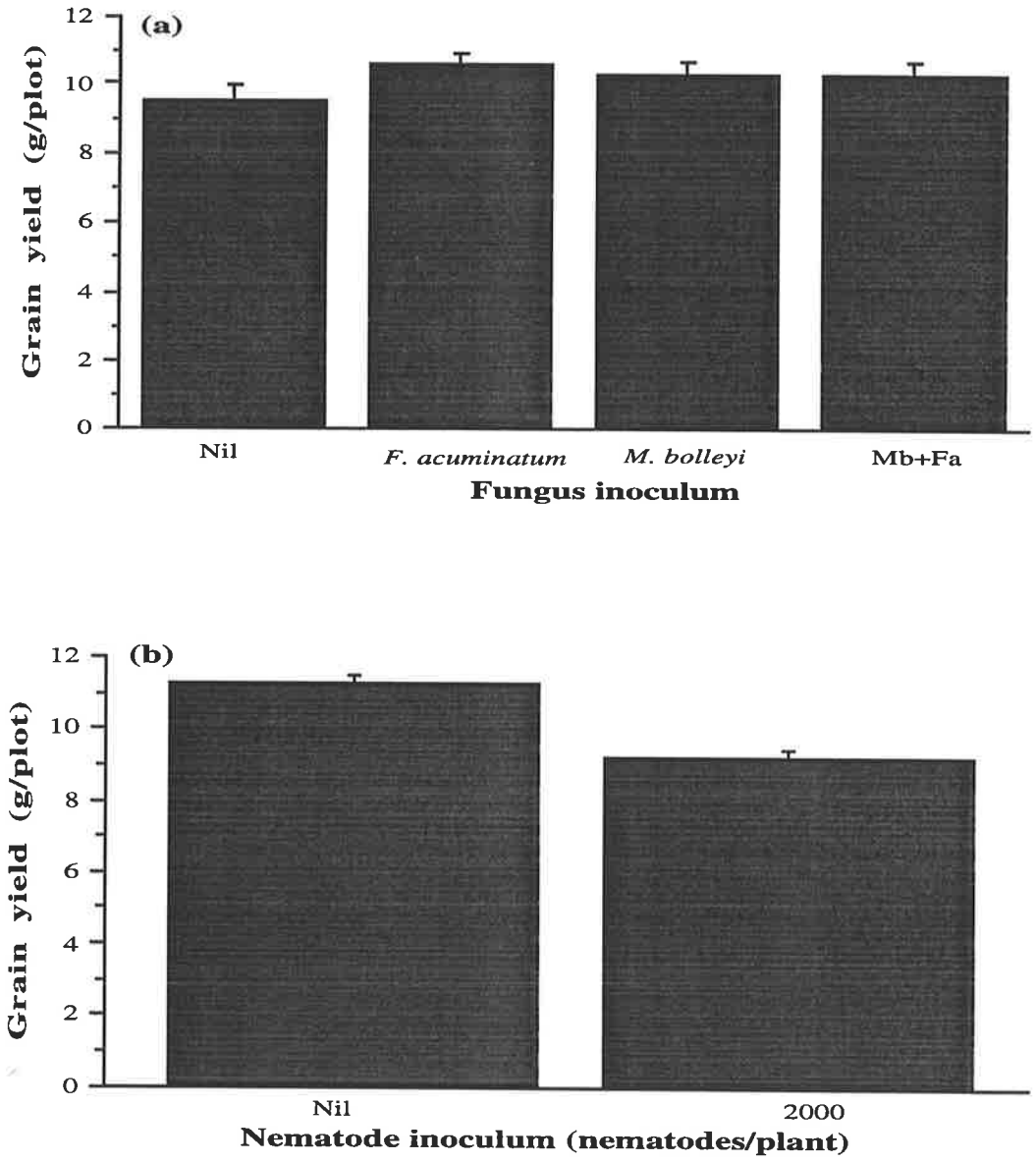


Figure 9.6 Effect of **(a)** fungus inoculum and **(b)** nematode inoculum on grain yield of wheat cultivar Machete.

9.3.2 1994 microplot experiment (Experiment 2)

The analyses of variance for all measurements are shown in Table 9.2.

Tiller number/plant: There was a significant 2-way interaction between nematode and fungus for number of tillers/plant (Figure 9.7). Either pathogen alone decreased number of tillers/plant compared to the control (no fungus or nematode added).

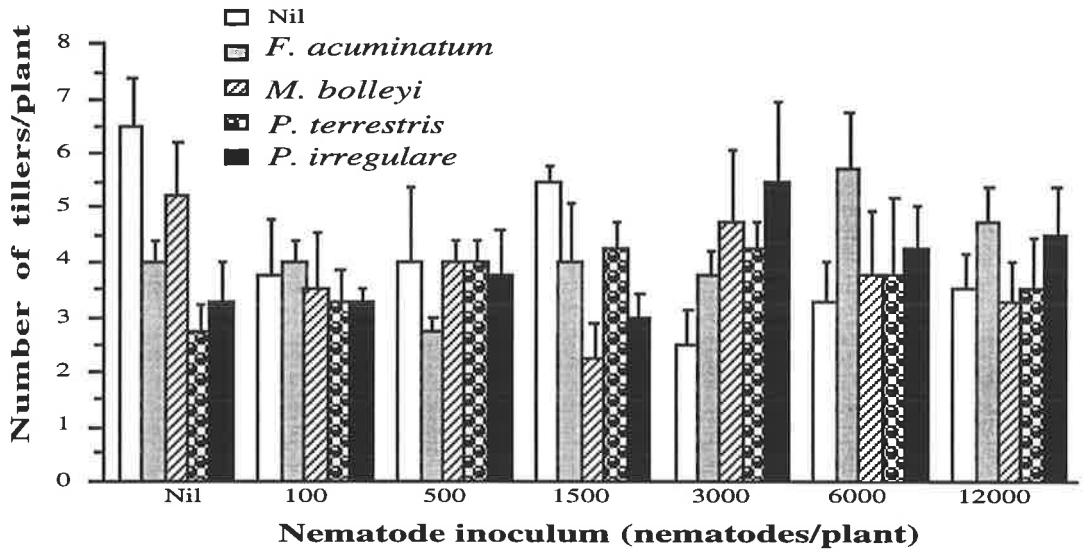


Figure 9.7 Effect of nematode-fungus interaction on number of tillers/plant of wheat cultivar Machete twelve weeks after sowing.

Root lesion rating: The extent of lesioning on the root system at the first and second sample dates (eight and twelve weeks after sowing) was greater on plants inoculated with fungi and nematodes than on those inoculated with either pathogen alone, particularly with the higher levels of nematodes (Figure 9.8).

Table 9.2 Summary of analyses of variance for the effect of nematode-fungus interaction on the extent of root lesioning eight and twelve weeks after sowing, number of nematodes/plant eight and twelve weeks after sowing, root dry weight/plant (eight weeks after sowing), shoot dry weight (12 weeks after sowing), number of tillers/plant and shoot dry weight at maturity (g/plot) for wheat cultivar Machete in the Roseworthy microplot experiment (Experiment 2).

Source	df	MS RL (8 weeks)	P	MS RL (12 weeks)	P	MS N/p (log) (8 weeks)	P	MS N/p (log) (12 weeks)	P	MS dwr (8 weeks)	P	MS dws (12 weeks)	P	MS tiller		MS Bio	P
Block	3																
Fungus	4	1.97	***	5.44	***	0.72	***	4.28	***	0.002	*	0.67	ns	1.15	ns	2.82	ns
Nematode	6	5.97	***	10.04	***	10.97	***	38.75	***	0.006	***	0.82	ns	1.62	ns	9.00	ns
Fungus × nematode	24	0.19	***	0.28	*	0.08	*	1.10	***	0.001	ns	1.77	**	4.42	*	7.61	*
Residual	105	0.07		0.16		0.05		0.29		0.001		0.76		2.71		4.62	

***= significant at P=0.001 **= significant at P= 0.01 *= significant at P= 0.05 ns= not significant P= Probablibity.
RL= Root lesion rating; N/p = Number of nematodes/plant; dwr= root dry weight/plant; dws= Shoot dry weight/plant/plant; tiller= tillers/plant; Bio= shoot dry weight/plant at maturity.

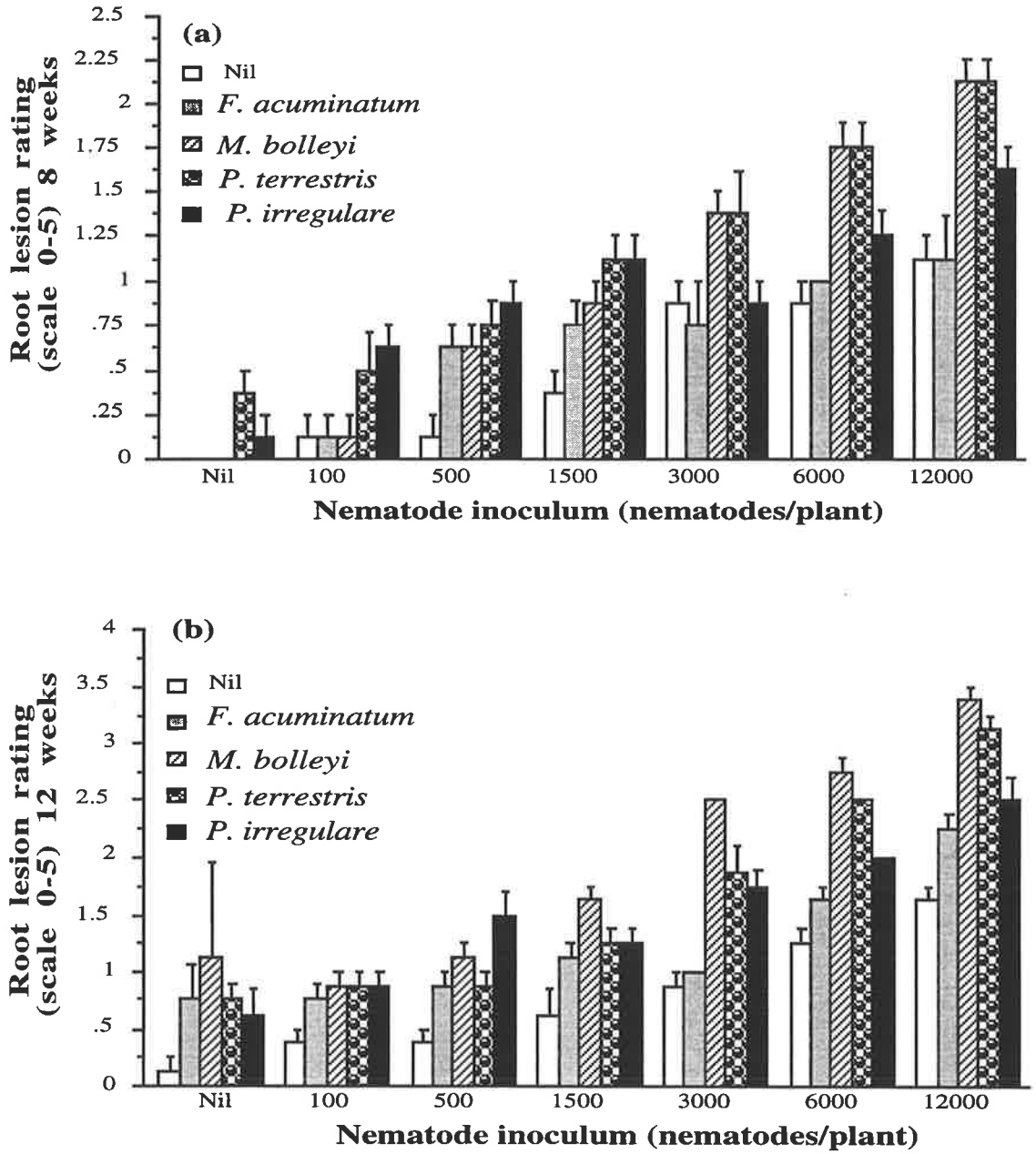


Figure 9.8 Effect of nematode-fungus interaction on the root lesion rating (**a**) eight weeks after inoculation and (**b**) twelve weeks after inoculation with fungus and nematodes.

With *F. acuminatum*, *M. bolleyi*, *P. terrestris* or *P. irregulare* in combination with 100, 500, 1500, 3000, 6000 or 12000 nematodes/plant, root lesion rating increased significantly compared to the control (no fungus or nematodes added) (Figure 9.8a). At the second sample date, further increase in the amount of lesioning on the root system occurred (Figure 9.8b).

Nematode numbers: At the first and second sample dates there was a significant 2-way interaction between nematode and fungus for nematodes/plant (Figure 9.9).

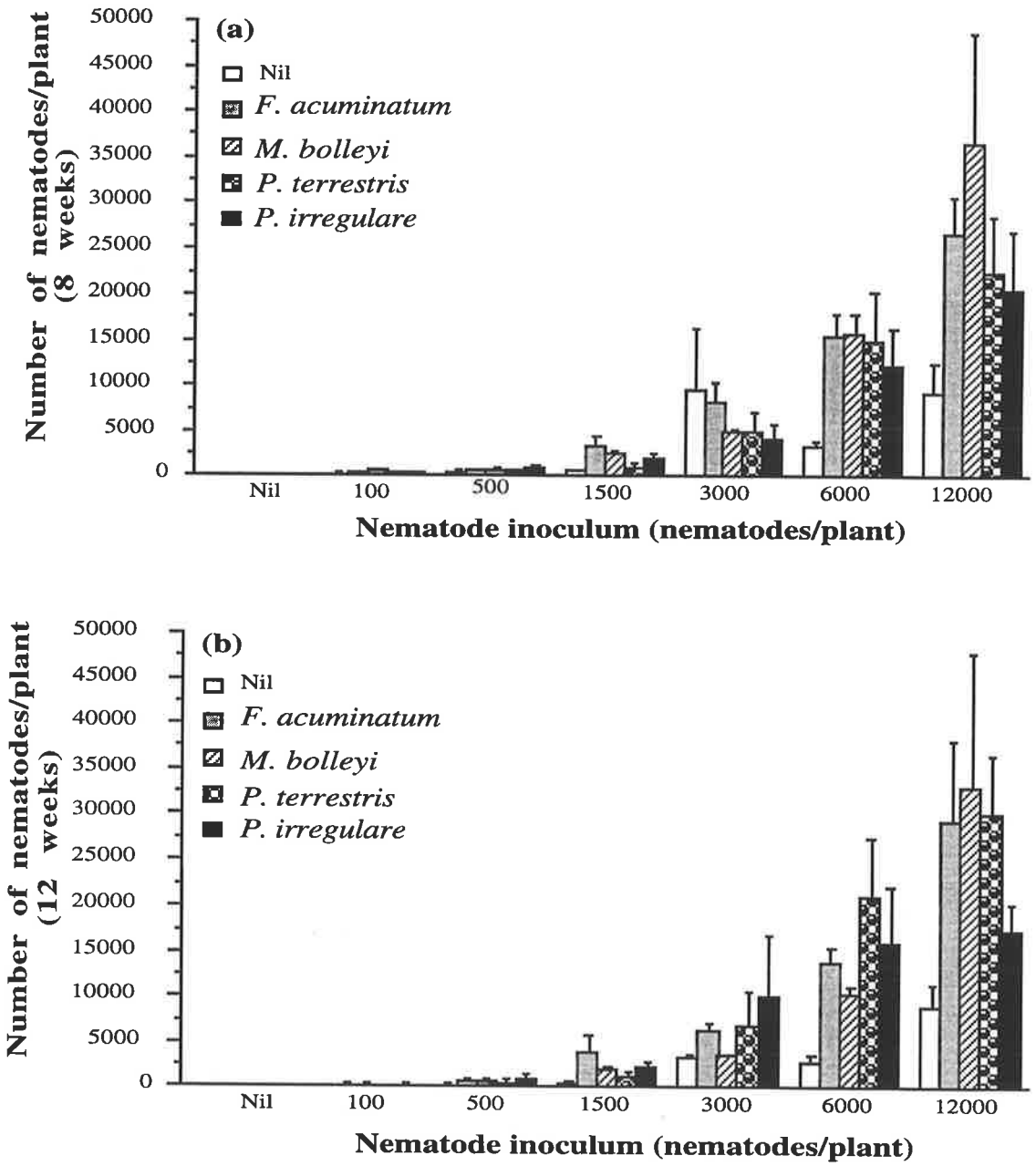


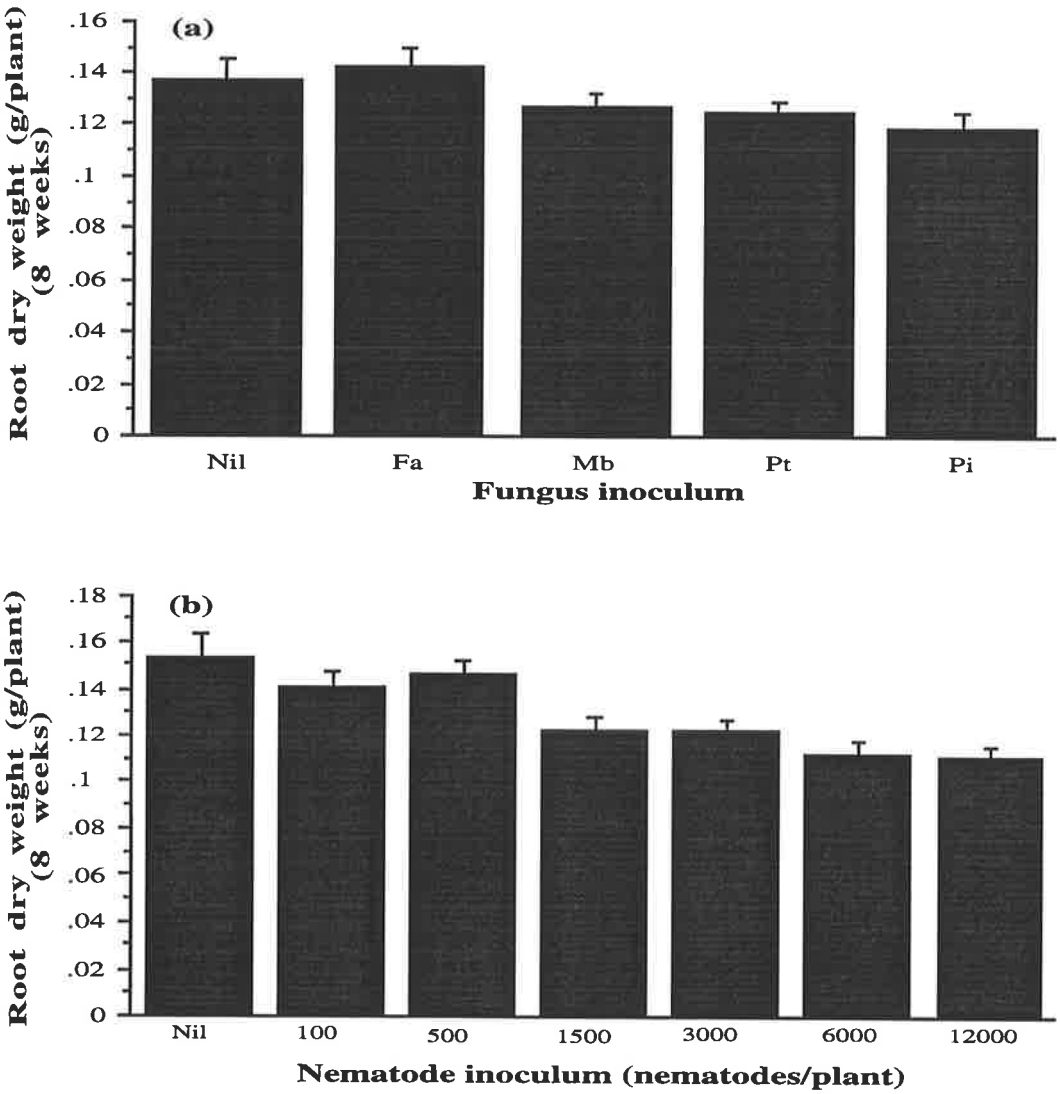
Figure 9.9 Effect of nematode-fungus interaction on the number of nematodes/plant **(a)** eight weeks after inoculation and **(b)** twelve weeks after inoculation with fungus and nematodes.

At eight weeks after sowing, number of nematodes increased significantly where fungus inoculum was added compared to the control (no fungus added). With *F. acuminatum*, *M. bolleyi*, *P. terrestris* or *P. irregulare* number of nematodes/plant increased significantly compared to the control (no fungus added) (Figure 9.9a).

As the initial inoculum density increased, nematodes/plant and nematodes/g dry root increased significantly (P=0.0001). At low initial nematode densities (100, 500 or 1500 nematodes/plant) and in the presence of fungi, number of nematodes extracted from the root system also increased significantly (Figure 9.9b). *F. acuminatum*, *M. bolleyi*, *P. terrestris* or *P. irregulare* in combination with 3000, 6000 or 12000 nematodes/plant increased number of nematodes extracted from roots compared to the control at corresponding nematode levels (Figure 9.9b).

Plant dry matter: Shoot dry weight was significantly affected by nematode and fungus interaction at the second sample date (twelve weeks after sowing) but was not affected by either treatment at the first sample date (eight weeks after sowing). However, root dry weight was significantly affected by fungus or nematode inoculum alone at the first sample date, but was not affected by either treatment at the second sample date. In the presence of *F. acuminatum*, *M. bolleyi*, *P. terrestris* or *P. irregulare* root dry weight decreased by 3%, 7%, 9% or 13%, respectively, compared to the control (no fungus added) (Figure 9.10a).

At the first sample date (eight weeks after sowing), root dry weight significantly decreased with nematode inoculum densities of 100, 500, 1500, 3000, 6000 or 12000 nematodes/plant by 8%, 4%, 20%, 20%, 27% or 27%, respectively, compared to the control (nematodes not added) (Figure 9.10b).



Fifure 9.10 Effect of **(a)** fungus and **(b)** nematode on root dry weight of Machete wheat eight weeks after sowing. Nil= no fungus or nematodes added, Fa= *F. acuminatum*, Mb= *M. bolleyi*, Pt= *P. terrestris* and Pi= *P. irregulare*.

At the second sample date (twelve weeks after sowing), shoot dry weights were significantly affected by a 2-way interaction between nematode and fungus. None of the pathogens alone had any significant effect on shoot dry weight. At 100, 500, 1500, 3000, 6000 or 12000 nematodes/plant shoot dry weight decreased by 45%, 62%, 35%, 78%, 64% or 64%, respectively, compared to the control (no fungus or nematodes added) (Figure 9.11). Shoot dry weight was significantly decreased by all fungi at 1500 nematodes/plant compared to the control (no fungus added) (Figure 9.11). However, at 3000 nematodes/plant combined with fungi, shoot dry weight increased.

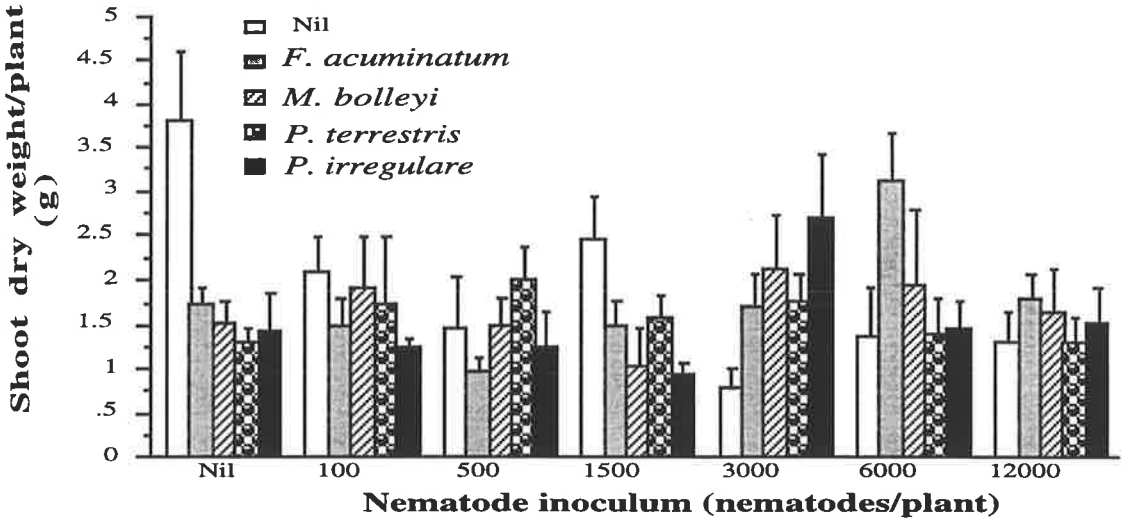


Figure 9.11 Effect of nematode-fungus interaction on shoot dry weight of wheat cultivar Machete twelve weeks after sowing.

Shoot dry weight at maturity was also significantly affected by a 2-way interaction between nematode and fungus (Figure 9.12). However, there was no significant effect on grain yield/plot.

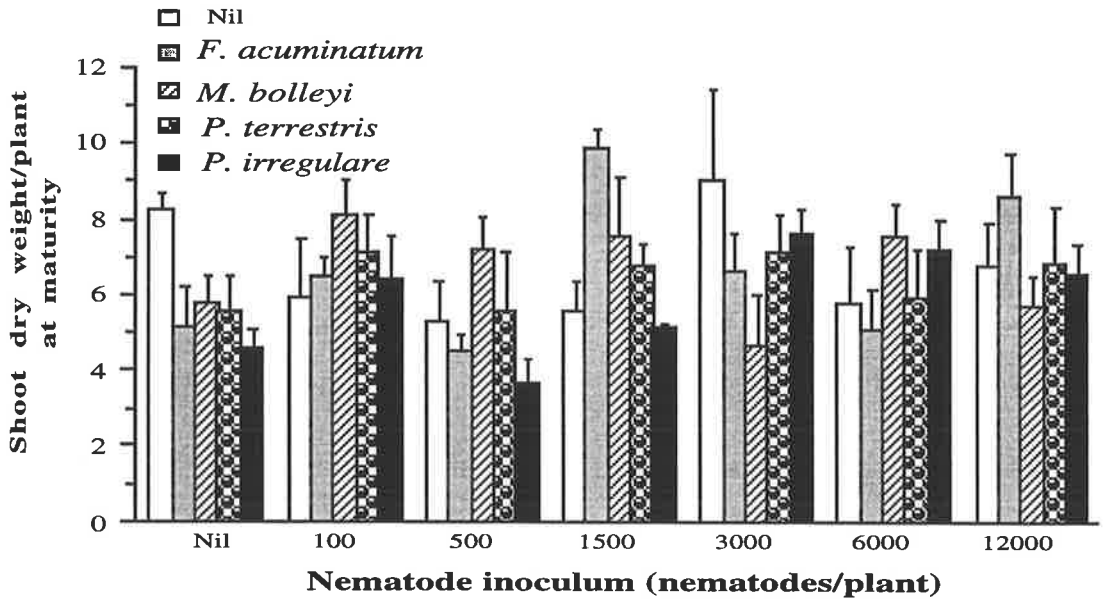


Figure 9.12 Effect of nematode-fungus interaction on shoot dry weght/plant of wheat cultivar Machete at maturity.

9.3.3 1994 Field experiment (Experiment 3)

The analyses of variance for all measurements are shown in Table 9.3.

Root lesion rating: At eight and twelve weeks after sowing, *F. acuminatum*, *M. bolleyi* or *P. terrestris* increased severity of lesioning on the root system when compared with uninoculated plots (control) (Figure 9.13). Fumigation significantly reduced root lesion rating (P=0.0001). There was little difference in disease rating values between fungi (*F. acuminatum*, *M. bolleyi* or *P. terrestris*) for either soil treatment (Temik® or methyl bromide).

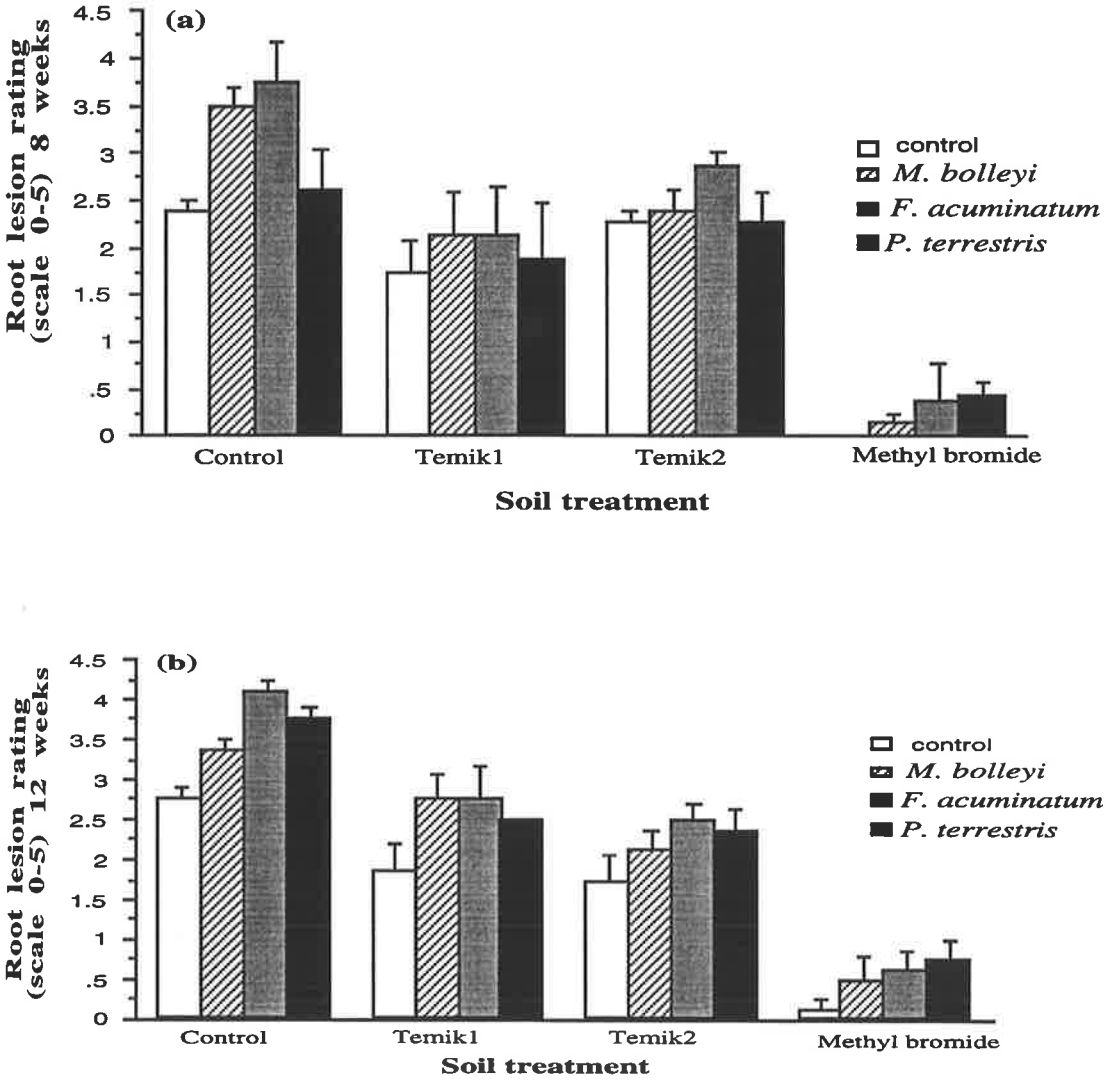


Figure 9.13 Effect of nematode-fungus interaction on the root lesion rating of wheat cultivar Machete **(a)** eight weeks after sowing and **(b)** twelve weeks after sowing. Temik1= Temik® (3.75kg/ha a.i.) applied two weeks before sowing, Temik 2= Temik® (3.75kg/ha a.i.) applied at sowing. Methyl bromide at 850ml/14m^2.

Table 9.3 Summary of analyses of variance for the effect of nematode-fungus interaction on the extent of root lesioning eight and twelve weeks after sowing, number of nematodes/g dry root eight and twelve weeks after sowing, shoot dry weight (eight weeks after sowing), shoot dry weight at maturity and grain yield for wheat cultivar Machete in the Stow field experiment (Experiment 3).

Source	df	MS RL (8 weeks)	P	MS RL (12 weeks)	P	MS N/g dr (8 weeks)	P	MS N/g dr (12 weeks)	P	MS dws (8 weeks)	P	MS dws (maturity)	P	Ms Head	P	MS Yield	P
Block	3																
Soil treatment (ST)	3	23.61	***	24.48	***	3.75E10	***	57.65	***	0.11	*	1324	*	0.19	**	100355	ns
ST × block	9	0.46		0.32		9.19E8		3.66		0.02		373		0.02		30071	
Fungus	3	1.41	*	2.32	***	1.30E9	ns	3.08	**	0.008	ns	68	ns	0.01	ns	5412	ns
Fungus × ST	9	0.34	ns	0.15	ns	1.09E9	*	0.72	ns	0.01	ns	77	ns	0.01	ns	7228	*
Residual	33	0.41		0.17		5.15E8		0.74		0.01		172		0.01		3515	

*** significant at P=0.001 ** significant at P= 0.01 * significant at P= 0.05 ns= not significant P= Probablibity.
RL= Root lesion rating; N/g dr = Number of nematodes/g dry root; dws= Shoot dry weight/plant; dws (maturity)= shoot dry weight/plant at maturity; Head= head size/g; Yield= grain yield/plot.

F. acuminatum, *M. bolleyi* or *P. terrestris* increased root lesion rating by 28%, 43% or 11%, respectively, compared to the control (uninoculated plots). *P. terrestris* apparently was not well developed at eight weeks. There was little difference between pre-application of Temik® or application of Temik® at sowing for the extent of lesioning on the roots (Figure 9.13). However, there was a significant difference between fumigated (methyl bromide) plots and other soil treatments for root lesion rating.

Overall, fumigation with methyl bromide reduced root lesion rating by 92% compared to plants grown in unfumigated plots. With pre-application of Temik® or application at sowing, root lesion rating decreased by 35% or 20%, respectively, compared to the control in corresponding untreated plots (Figure 9.13).

Inoculated plots often had higher root lesion rating than the corresponding uninoculated plots, except for plots fumigated with methyl bromide. This was true for all fungal treatments. At the second sample date, root lesion rating of plants inoculated with *F. acuminatum*, *M. bolleyi* or *P. terrestris* increased by 35%, 54% or 44%, respectively, compared to the control (no fungus added) (Figure 9.13).

All fungi added to the soil were successfully re-isolated from root samples collected from corresponding plots.

Nematode number: At the first sample date there was a significant 2-way interaction between soil treatments and fungus inoculum for nematode numbers/g dry root (Table 9.3). At the second sample date, number of *P. neglectus*/g dry root was significantly affected by fungus inoculation as well as by soil treatments alone (Table 9.3). Fumigation with methyl bromide was 100% effective in controlling the nematode population (Figure 9.14). No fungal pathogens were isolated from root samples collected from uninoculated fumigated plots.

Pre-application of Temik® or application at sowing significantly reduced nematode numbers in soil (P=0.001). At the first sample date (eight weeks after sowing), pre-application of Temik® reduced nematode numbers by 93% compared to the control (untreated soil), whereas Temik® applied at sowing reduced nematode numbers by 87% compared to the control. Methyl bromide, however, completely eradicated nematodes in the areas sampled (Figure 9.14).

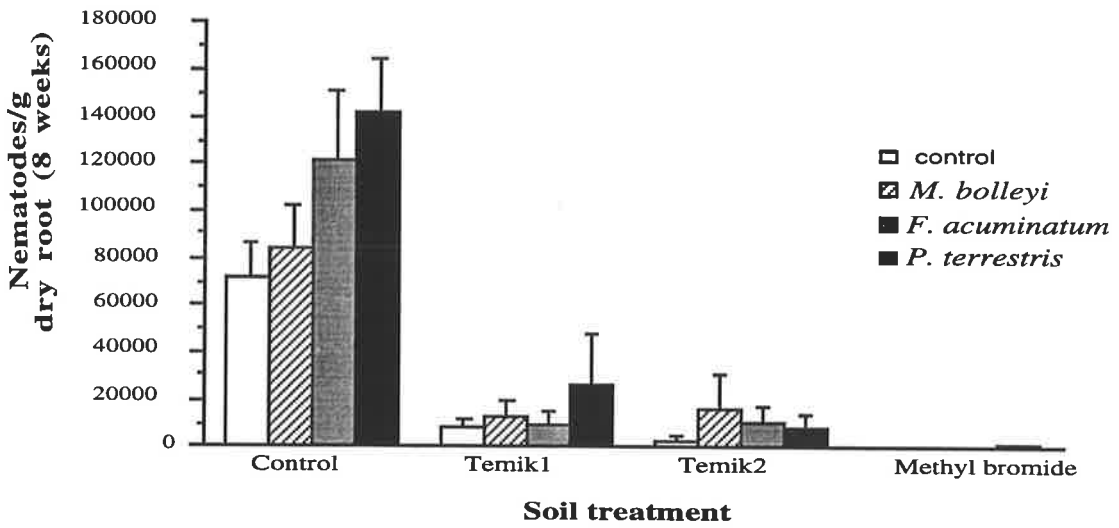


Figure 9.14 Effect of fungus-nematode interaction on number of nematodes/g dry root eight weeks after sowing for wheat cultivar Machete under field conditions. Temik1= Temik® (3.75kg/ha a.i.) applied two weeks before sowing, Temik 2= Temik® (3.75kg/ha a.i.) applied at sowing. Methyl bromide at 850ml/14m^2.

Plant dry matter: Shoot and root dry weights of plants from methyl bromide fumigated plots were far higher than for plants from other treatments (Temik® or untreated) (Figure 9.15). There were some differences in shoot dry weight between plants inoculated with either *F. acuminatum* or *M. bolleyi* and plants inoculated with *P. terrestris* or uninoculated plants. Plots inoculated with *F. acuminatum* or *M. bolleyi* showed greater shoot growth than those inoculated with *P. terrestris* or uninoculated plots. However, these differences were not statistically significant.

Overall, soil treatments significantly affected shoot dry weight (P=0.05). Pre-application of Temik® increased shoot dry weight/plant by 44% compared to the control (untreated plots), but Temik® applied at sowing did not effect production of shoots. However, soil fumigation increased shoot dry weight/plant by 227%, compared to the control (untreated plots) (Figure 9.15).

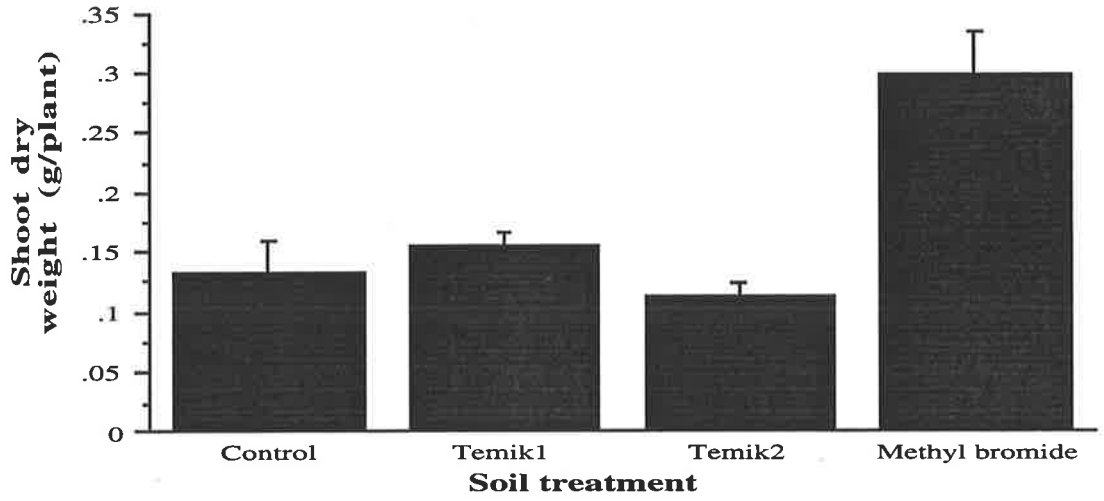


Figure 9.15 Effect of soil treatment on shoot dry weight (g/plant) of Machete wheat. Control= no soil treatment applied, Temik 1= Temik® (3.75kg/ha a.i.) applied two weeks before sowing, Temik 2= Temik® (3.75kg/ha a.i.) applied at sowing, and Methyl bromide applied two weeks before sowing. Methyl bromide at 850ml/14m^2.

Harvest: Head size (g/head) was significantly affected by nematodes and by a 2-way interaction between nematode and fungus. Pre-application of Temik® or application at sowing increased head size (g/head) by 7% and 11.6%, respectively. However, fumigation reduced head size (g/head) by 24% compared to the untreated plots (Figure 9.16).

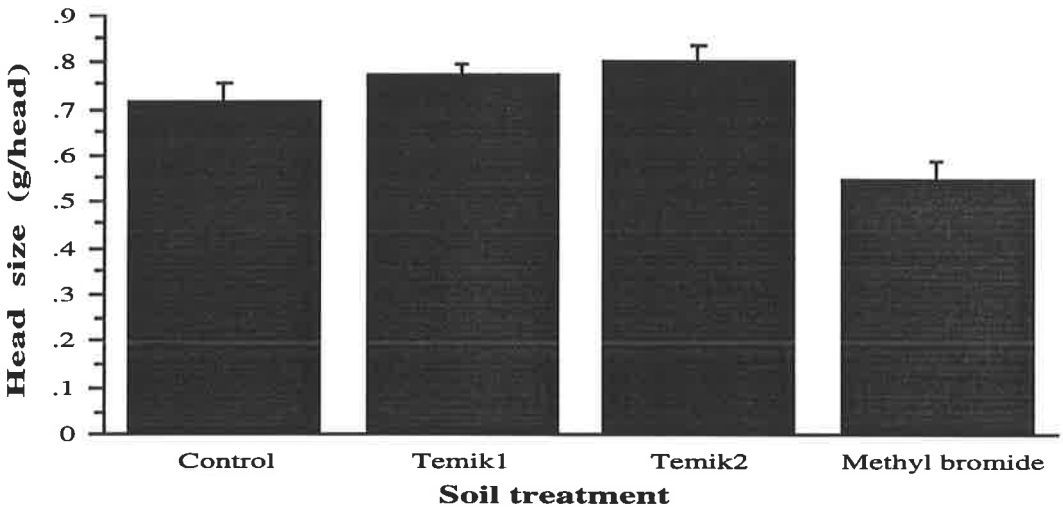


Figure 9.16 Effect of soil treatment on head size (g/head) of Machete wheat. Control= no soil treatment applied, Temik 1= Temik® (3.75kg/ha a.i.) applied two weeks before sowing, Temik® 2= Temik (3.75kg/ha a.i.) applied at sowing and Methyl bromide applied two weeks before sowing. Methyl bromide at 850ml/14m^2.

Application of Temik® either two weeks before sowing or at sowing increased seed weight/head where fungus inoculum was added.

Grain yield: Grain yield was significantly affected by soil treatment and by a 2-way interaction between soil treatment and fungi. Unfumigated plots inoculated with *M. bolleyi* had significantly (P= 0.05) less yield than those inoculated with *F. acuminatum* or *P. terrestris* or the control (uninoculated plots), even though these same plots contained fewer nematodes than those with the other fungi.

Grain yield from plots treated with either Temik® or methyl bromide inoculated with *M. bolleyi,* weighed 16% less than corresponding (uninoculated) control plots (Figure 9.17). With *F. acuminatum* or *P. terrestris,* however, grain yield increased by 9% or 8%, respectively, compared to the corresponding control plots (Figure 9.17). However, with application of Temik® at either two weeks before sowing or at sowing, or fumigation of control plots (no fungus added), grain yield was increased by 56%, 63% or 40%, respectively, compared to the corresponding untreated plots (Figure 9.17). A

significant negative correlation between number of nematodes/g dry root and grain yield is shown in Figure 9.18.

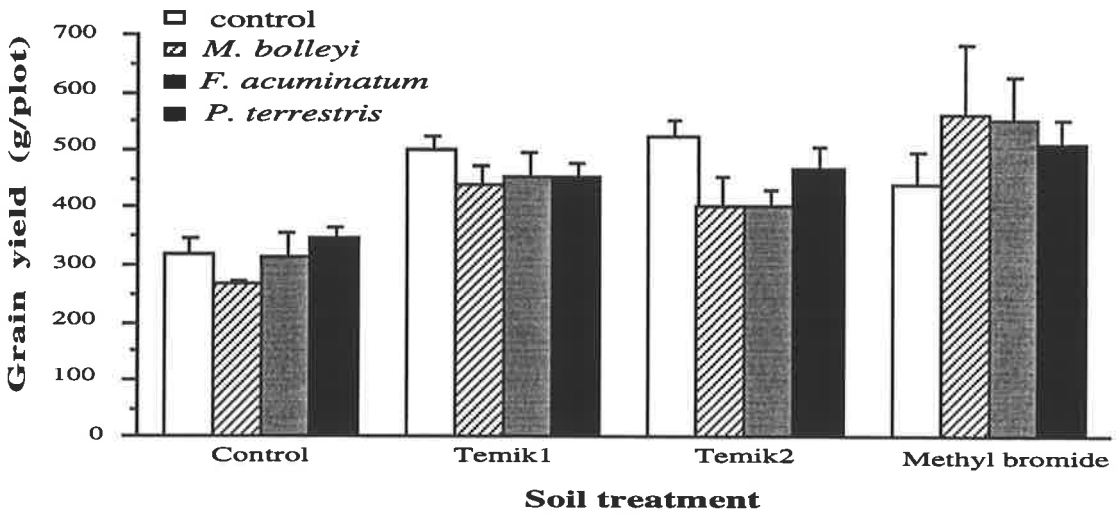


Figure 9.17 Effect of nematode-fungus interaction on grain yield of wheat cultivar Machete grown in the field. Temik1= Temik® (3.75kg/ha a.i.) applied two weeks before sowing, Temik 2= Temik® (3.75kg/ha a.i.) applied at sowing. Methyl bromide at 850ml/14m^2.

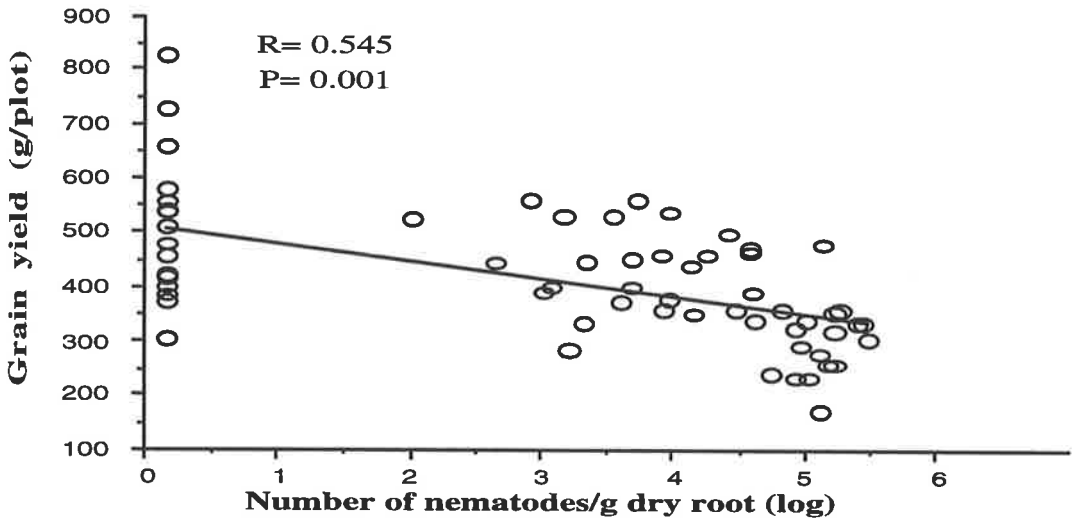


Figure 9.18 Correlation between number of *P. neglectus*/g dry root and grain yield.

9.4 Discussion

The hypothesis that *M. bolleyi, F. acuminatum, P. terrestris* or *P. irregulare* interact with *P. neglectus* was tested under semi-controlled conditions, in microplots and in a field trial. *M. bolleyi* and *F. acuminatum* occurred at high frequencies in field samples during the 1992 growing season (Chapter 3). The possibility that a combination of the three pathogens (*M. bolleyi, F. acuminatum* and/or *P. neglectus*) may be responsible for root damage under field conditions was examined.

F. acuminatum or *M. bolleyi* in combination with *P. neglectus* greatly increased root lesion rating of both wheat varieties, Spear and Machete. Combination of all three pathogens further increased root lesion rating. This result confirmed the previous findings from glasshouse experiments. It is possible, however, that positive interactions between these microorganisms are responsible for the damage seen on roots under field conditions. Using inoculation experiments, under controlled glasshouse conditions, it would be difficult to show that nematode, fungus or their combinations have any significant effect on plant growth. The results of a glasshouse experiment may not be applicable to the field as the inoculum levels of both fungus and nematode and many other factors that may influence their effect under field conditions are different.

In the field, variability in soil moisture content, temperature, nutrition and the presence of many other biotic or abiotic factors may influence a nematode-fungus interaction. More importantly, the distribution of nematode inoculum in the soil profile could be important for biological interactions between two or more organisms. Thus, considering that the results of a glasshouse experiment may not be applicable to the field and that there is large variablitiy in the field, interaction tests using microplots are recommended.

The severity of disease symptoms as well as rapid increases in nematode numbers present at eight weeks and further increases at twelve weeks after planting were good indicators of yield losses in this study. In the 1993 microplot experiment, *M. bolleyi* or *F. acuminatum* alone did not increase root lesion rating but, in combination with *P.*

neglectus (2000/plant), the root lesion rating increased significantly. Further increase occurred when all three pathogens were combined.

The results of the field microplots and field trial are in agreement with previous findings on the interaction under glasshouse conditions. The combination of *M. bolleyi* or *F. acuminatum* with *P. neglectus* significantly increased root lesion rating of wheat both in the field and glasshouse. However, the severity of lesioning was greater in the field, particularly at twelve weeks after planting. This was possibly due to the longer duration of the field trial as well as the effect of natural conditions (moisture and temperature) on the plant, nematode and fungus interactions.

Number of nematodes/plant and nematodes/g dry root were also increased where *M. bolleyi* or *M. bolleyi+F. acuminatum* were present, but *F. acuminatum* alone had no effect on number of nematodes. This may be due to the extensive root lesioning caused by the *F. acuminatum-P. neglectus* interaction. Migratory nematodes prefer freshly grown roots for penetration and feeding and will leave damaged roots in search of a fresh food source (Dropkin, 1989).

While there was no difference between wheat cultivars (Machete and Spear) in the extent of root lesioning (Experiment 1), the numbers of nematodes differed between the two cultivars. Presence of either *M. bolleyi* or *F. acuminatum* did not change number of *P. neglectus* on Spear but, in the absence of fungi, the number of nematodes increased twice as much on Spear as on Machete. However, presence of fungi had a significant effect on number of nematodes on Machete, particularly late in the season. This may explain the greater yield reduction in Machete compared to Spear in the previous field experiment (Chapter 5).

Although the overall number of nematodes which infected Spear and Machete was not significantly different, root dry weight of Machete was lower than Spear which resulted in greater yield losses at harvest. It is suggested that in the field Machete is more intolerant of *P. neglectus* than Spear, particularly where the initial number of nematodes in soil is high. Spear, however, is a variety tolerant to a wide range of root

diseases, and is also a more efficient utiliser of zinc, shortage of which is a major problem in South Australia (R. D. Graham, personal communication). It is also possible that varieties efficient in utilising zinc may also be more tolerant to *P. neglectus*. Therefore, it would be worth investigating the possible interaction between zinc or even some other micronutrients (Mn) and *P. neglectus* and/or combination of nematode and some soil-borne fungi (*M. bolleyi*, *F. acuminatum*, etc.).

In the 1994 microplot experiment, the hypothesis that the degree of interaction between nematode and fungus may differ with the different initial densities of either pathogen was tested. Again, the combination of *P. neglectus* and *F. acuminatum* increased root lesion rating significantly more than fungus did alone. However, increased root lesion rating where *M. bolleyi*, *P. terrestris* or *P. irregulare* were combined with the nematode indicated a positive interaction between *P. neglectus* and these fungi. As the inoculum density of the nematode increased with the presence of fungi, root lesion rating also increased. This indicates the importance of initial nematode numbers in the soil.

The initial densities of *P. neglectus* up to 1500/plant did not increase root lesion rating but a significant increase occurred with 3000 nematodes/plant and above. The extent of root lesioning was positively correlated with the number of nematodes extracted from roots. Higher number of nematodes in the root system resulted in more root damage. A significant increase in nematode numbers occurred in the presence of fungi. The results of this experiment were similar to those of previous glasshouse experiments. However, the initial levels of nematodes in the soil had a significant effect on the plant growth and dry matter accumulation.

Nematodes alone at lower initial densities stimulated plant growth but suppressed it at 3000 nematodes/plant and above. However, fungus alone or in combination with high levels of nematodes increased shoot dry weight, but with low nematode densities shoot dry weight decreased. It is not clear why this plant reaction to nematode and fungus occurred, but undoubtedly inoculum of *M. bolleyi* or *F. acuminatum* increased

plant growth. It is possible that root damage stimulated an over compensation in top growth. However, the medium on which the fungus inoculum was grown may have had some effect on plant growth, or may have been used by the plant as a source of nutrition. Although control plants with no fungus inoculum received a similar amount of dead millet seed, presence of the fungus may alter the seed to make it a better source of food. Vanstone (1991) found that low inoculum levels of *P. neglectus* reduced plant growth.

The results of both microplot experiments (1993 and 1994) confirmed previous findings with similar fungi in the glasshouse. The nematode inoculation technique used may influence the nematode or nematode-fungus effect on plants.

Sampling methods and the time of sampling also need to be improved or modified. High levels of nematodes, as well as other soil-borne micro-organisms, are concentrated in the top 10-15cm of the cultivated soil layer, in which root systems become heavily infested and rotted. It is well known that *Pratylenchus* spp. leave severely damaged and rotted tissues (Dropkin, 1989), and nematode numbers in the roots and surrounding soil should therefore be determined. The work of Haak *et al.* (1993) in Queensland indicated that although the highest numbers of both *P. neglectus* and *P. thornei* were in soil at depth of of 0-30cm, there were also large numbers at 30-60cm. It may therefore be essential to sample roots and surrounding soil at depths below the cultivation layer and extract nematodes from both roots and surrounding soil rather than from roots only. However, in most South Australian soils the majority of *P. neglectus* are found in the top 10-15cm of the soil (S. P. Taylor, personal communication).

The 1994 field trial at Stow confirmed all the previous findings from both microplots and glasshouse experiments. However, considerable variation between plots was observed. The 1994 growing season was relatively dry (Table 2.1), so the root damage caused by the nematodes and fungi was exacerbated. Plots inoculated with fungus generally showed greater root damage than uninoculated plots.

Fungus inoculum in Temik® treated plots did not increase the severity of root lesion rating. This probably was due to low initial nematode numbers in the soil and roots. Soil from Stow averaged 10 nematodes/g which would be considered high. However, even low numbers of nematodes in roots caused considerable damage to the root system late in the season. This indicated that conditions favouring plant growth (temperature and moisture) also favoured nematode reproduction and development.

Due to considerable variation within treatments and between plots in the field, as well as the effect of climatic, biotic and abiotic factors existing in nature, it is difficult to interpret the results obtained from field experiments, and caution must be taken. However, it was clear that results from the field agreed with all the previous findings in the glasshouse and laboratory. Thus, it should be possible to develop controlled environment tests to study differences in resistance and tolerance to *Pratylenchus* spp. in different wheat genotypes.

Thus, it can now be concluded that the interaction between the root lesion nematode, *P. neglectus,* and the two most frequent soil-borne fungi (*M. bolleyi* or *F. acuminatum*), is synergistic on wheat roots. The nematode seems to play a key role in wounding roots and modifying root physiology and the rhizosphere, favouring fungal infection.

It is important to mention that although Temik® reduced number of nematodes in the root, root lesion rating was still high. This suggests that even low numbers of nematodes in soil and roots enable fungi to cause considerable damage to the roots where conditions favour both fungus and nematode. The other possiblity is that the fungi were pathogenic even without nematodes. However, plants seem to tolerate this low nematode level and natural levels of soil-borne fungi in the soil, and the final yield was not affected.

It is known that Temik® has some side effects on plant growth (Fisher, 1993) and may also affect populations of other soil micro-organisms that could be beneficial in biological control or as competitive agents to some major root pathogens. The hypothesis that Temik® may have some side effects, particularly in stimulating plant

growth, was tested under field conditions. Application of Temik® two weeks before sowing did increase growth rate of wheat early in the season. However, at maturity, there was no difference between pre-application of Temik® or its application at sowing. The grain yield of Machete was a little higher (but not significantly) where Temik® was applied at sowing with the seed. Numbers of nematodes were lower in root samples from plots treated with Temik® two weeks before sowing. These opposing effects may have prevented the stimulating reaction of Temik® from being demonstrated. However, the hypothesis that Temik® may have some side effects should be tested in controlled environmental conditions to eliminate the effects of other microorganisms.

Chapter 10

Investigation *in vitro* of the mechanisms of interaction between *Pratylenchus neglectus* and root-rotting fungi of wheat

10.1 Introduction

Nematode, host and other pathogens (fungi, bacteria, viruses, etc.) are the three main components in all interactions of plant parasitic nematodes with other plant pathogens (Khan, 1993). Each component may independently play a different role. The role of nematodes in a nematode-fungus interaction has been studied by several researchers (Bergeson, 1972; Pitcher, 1978; Taylor, 1979). Plant parasitic nematodes predispose some plants to fungal pathogens and/or increase plant susceptibility to other pathogens. Nematodes not only cause mechanical damage to plants, but also initiate physiological and/or biochemical changes in the host which may play an important role in the nematode-fungus interaction.

Root exudates are known to attract the motile stage of fungal pathogens (Zentmyer, 1961), and Zacheo (1993) observed that weakly pathogenic fungi can cause considerable damage once they gain entry into plant roots in the presence of feeding nematodes. Aseptic glasshouse and field experiments with *F. acuminatum*, *M. bolleyi*, *P. terrestris* or *G. graminis* in combination with *P. neglectus* indicated that there is a significant positive interaction between the above mentioned fungi and *P. neglectus* (Chapters 6 and 10). Plants inoculated with *M. bolleyi*, *G. graminis* or *F. acuminatum* resulted in high nematode numbers within the root system (Chapters 5 and 7). Stained roots infected with both *P. neglectus* and *M. bolleyi* (Plate 6.2) revealed that those cells containing nematodes contained no fungal hyphae, whereas fungal hyphae were present in adjacent cells.

The observed positive interaction between the root-rotting fungi and *P. neglectus* is further examined in this chapter. Two aseptic experiments were conducted in the laboratory on agar medium, using *F. acuminatum*, *M. bolleyi* or *G. graminis*. The aim of the first experiment (Experiment 1) was to determine whether *P. neglectus* can feed on fungal hyphae. The second experiment (Experiment 2) was conducted to examine the role of the host in the interactions.

10.2 Methods

10.2.1 Experiment 1

Fungal inoculum: Inoculum of *F. acuminatum* or *M. bolleyi* on millet seed and inoculum of *G. graminis* on ryegrass seed was grown as stock cultures on PDA. Water agar (1.5% WA) medium was prepared as described in the General Methods, and plastic Petri dishes (9cm diameter) were filled with 20ml of 1.5% WA medium by using a sterile 20ml syringe. The medium was allowed to cool for 30 minutes in a sterile laminar flow cabinet. The fungal stock cultures on PDA were then sub-cultured onto 1.5% WA and all plates were then incubated at 25°C for three days.

Nematode inoculum: *P. neglectus* was obtained from carrot cultures. Under sterile conditions in a laminar flow cabinet, eggs were separated from juveniles and adults using different sizes of sieves (40µm, 30µm and finally 20µm), and washed in sterile distilled water ten times. Eggs were surface sterilised in a 1% streptomycin sulfate and penicillin-G solution for two hours, then washed five times with sterile distilled water and incubated at 25°C. Every day the freshly hatched second-stage juveniles were collected and kept at 4°C. By day five, the majority of fertile eggs had hatched.

Three hundred second stage juveniles were used as inoculum for each fungal plate, and juveniles were placed around the edge of the fungal colony and incubated at 25°C for four weeks. Observations were made every second day, and nematode activity and percentage of dead nematodes recorded.

The experiment was set out as a completely randomised design. All treatments (*F. acuminatum, M. bolleyi, G. graminis* alone or in combination with *P. neglectus*) were replicated ten times.

10.2.2 Experiment 2

Surface-sterilised Machete wheat seeds were pre-germinated and selected as described in the General Methods. Fifty millilitre test tubes were autoclaved at 121°C for 20 minutes and filled with 20ml of 1.5% WA. One pre-germinated healthy seed was placed in each tube. Three days after sowing, 3mm diameter disks of mycelium of *F. acuminatum*, *M. bolleyi* or *G. graminis* on PDA medium were added to each tube. Aseptically grown *P. neglectus* from carrot cultures (all stages) were inoculated at 1000 nematodes/plant in 50µl of sterile distilled water around each plant. The control plants received 50µl of sterile distilled water. The experiment consisted of the following eight treatments:

Single treatments:

Treatment 1 - Plants inoculated with *P. neglectus*.

Treatment 2 - Plants inoculated with *F. acuminatum*.

Treatment 3 - Plants inoculated with *M. bolleyi*.

Treatment 4 - Plants inoculated with *G. graminis*.

Treatment 5 - Uninoculated plants (Nil).

Combination treatments:

Treatment 6 - Plants inoculated with *F. acuminatum* and *P. neglectus*.

Treatment 7 - Plants inoculated with *M. bolleyi* and *P. neglectus*.

Treatment 8 - Plants inoculated with *G. graminis* and *P. neglectus*.

All treatments were replicated three times and tubes were incubated at 20°C in a growth chamber with a 12 hour photoperiod in a completely randomized design.

Collection of root diffusates: Two weeks after inoculation with fungi and/or nematodes, plants were harvested, and their roots were washed with sterile distilled water under sterile conditions in a laminer flow. To obtain root diffusates, whole root systems were incubated in 10ml of sterile distilled water at 25°C for 30 hours. Thereafter, roots were removed from the liquid and stained for fungus and/or nematode detection. The diffusates were filter-sterilised by passing them through a 0.2µm millipore filter and stored at 4°C.

Attraction test: Water agar medium (1.5% WA) was prepared as described in the General Methods. Petri dishes (9cm diameter) were filled with 20ml of cooled 1.5% WA. After solidification, four 2mm disks were cut with a sterilized cork borer and removed (Plate 10.1). Wells were equally spaced on the Petri dish, 0.5cm away from the rim.

To ensure that a good concentration gradient was established before nematodes were added to the test plates, the speed of liquid diffusion into the agar was investigated by placing 50µl of food colouring (Queen Food Colours) in each well (one colour per well) of a sample plate (Plate 10.1). Observations were made every 20 minutes, and after three hours food colours had evenly diffused into the agar in a 1.0cm radius around the well.

On test plates, aliquots of 50µl diffusates from the different treatments were added to the wells, and wells were refilled with another 50µl of diffusate one hour later. Nematodes were added a further three hours later.

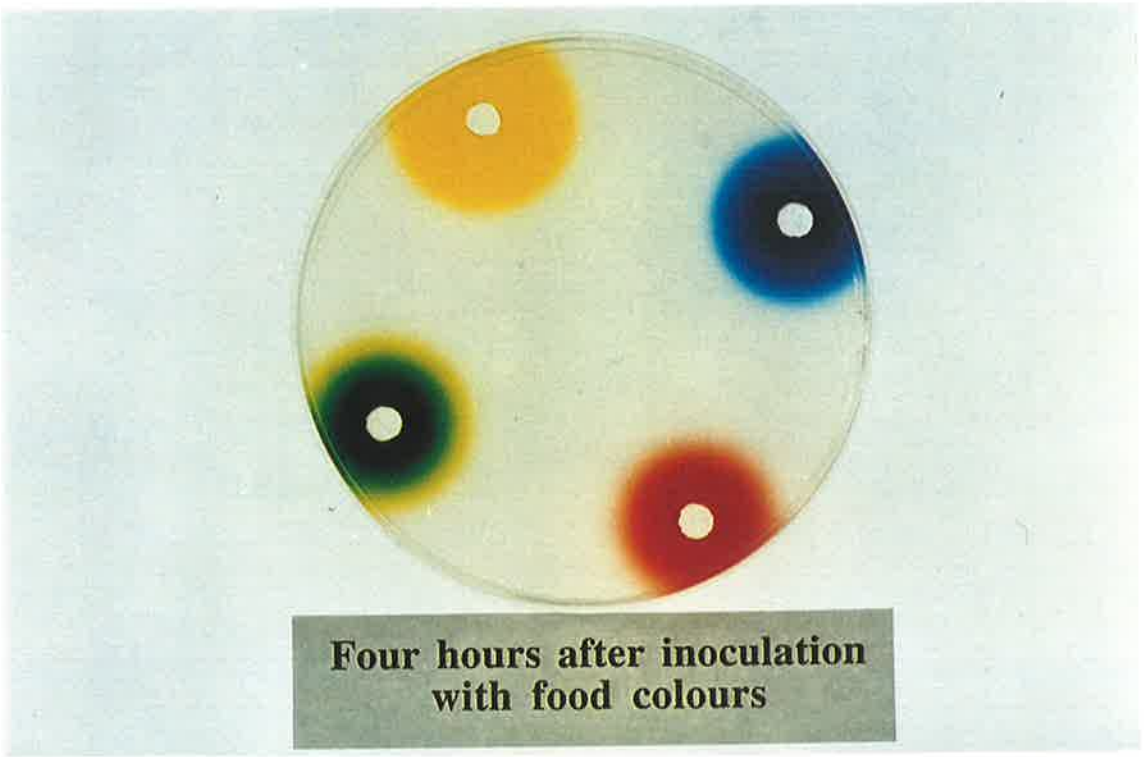
Arrangement of treatments: Each Petri dish contained four wells and every well had a different treatment corresponding to four different treatments. All test plates contained the control plant root diffusate, the plant-nematode root diffusate, one of the three plant-fungus root diffusates and the corresponding plant-fungus-nematode root diffusate.

Plate 10.1 Diffusion of food colours into the agar.

A. Four hours after inoculation with 100µl of food colour.

B. 24 hours after inoculation with 100µl of food colour.

A


Four hours after inoculation
with food colours

B


24 hours after inoculation
with food colours

The nematode inoculum for the Petri dish assay was obtained as described in Section 10.2.1. The sterile larvae were placed in sterile distilled water for five hours at 25°C and then used for the attraction experiment. One hundred and seventy-five *P. neglectus* suspended in 50µl of sterile distilled water were placed in the centre of each Petri dish and the drop was then allowed to evaporate in a laminar flow cabinet. The experiment was set out as a factorial split plot design with eight replications. The number of nematodes that had moved from the introduction point towards the test solutions was recorded after 4, 17 and 24 hours.

At the first two observation times (four or seventeen hours after inoculation), nematodes within a 1.0cm radius around the wells were counted. However, at the third observation time after 24 hours, nematodes were counted within two rings. The rings were 1.0cm and 2.0cm from the original point of nematode inoculum in the centre of the plate.

Data for the attraction test were subjected to analyses of variance, and were statistically significant, graphed as equivalent means.

The remaining root diffusates were used for determination of total mass, total carbohydrate content, total and individual amino acid content. The total carbohydrate and total amino acid contents of root diffusates were kindly performed by Dr. C. F. Jenner of the Department of Plant Science. For total carbohydrate content, a portion (0.1ml) of the root exudates was added to 0.9ml of water followed by 1.0ml of 5% aqueous phenol. A jet of concentrated sulphuric acid (5.0ml) was pumped into the centre of the sample and the optical density was measured at 490nm when the tubes had cooled using a Turne Spectrophotometer. The assay was calibrated with a standard curve of sucrose (5 to 70 µg per tube) and the data were expressed as equivalent to sucrose.

10.3 Results

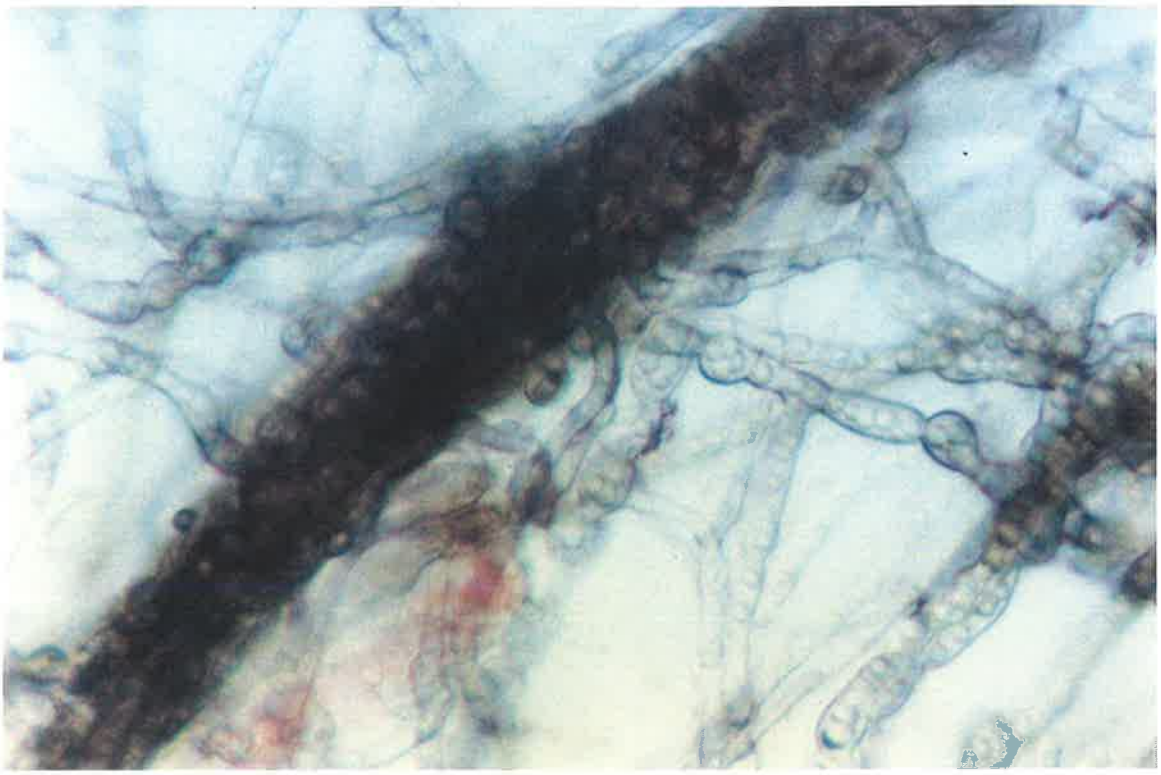
10.3.1 Experiment 1

Two days after fungal culture plates had been inoculated with nematodes, the majority of larvae were alive in all treatments (*F. acuminatum*, *M. bolleyi*, *G. graminis* or nematode alone). At the second observation, four days later, a large percentage of nematodes had been attracted to the centre of the *F. acuminatum* or *M. bolleyi* colonies. About 50% of nematodes on the *F. acuminatum* plates were dead. On the *G. graminis* plates, nematodes were distributed evenly over the plate and the majority were alive. One week after inoculation, almost 90% of the nematodes in the *F. acuminatum* culture were dead. Nematode mortality in the other cultures was lower, 70% for *M. bolleyi* and 50% for *G. graminis*. In the control plates without fungus, about 30% of the nematodes were dead. However, due to the relatively large number of nematodes added to each plate (300 nematodes/plate) and movement of nematodes within the agar as well as mass production of mycelium of fungus, it was not possible to count the exact number of nematodes in each plate. Therefore, plates were scored (as a percentage) for nematode numbers (living or dead).

Two weeks after inoculation, a further increase in nematode mortality was observed in all treatments. At that time, nematodes on *F. acuminatum* had the highest mortality and those on *G. graminis* the lowest compared to the control (no fungus added). Four weeks after inoculation, none of the fungal isolates contained live nematodes, and no development from second to third stage juvenile had occurred in either treatment or in control plates with no fungus inoculum.

Dead nematodes were penetrated by hyphae of *F. acuminatum* or *M. bolleyi,* which presumably used the nematodes as a food source (Plate 10.2). Hyphae of *G. graminis,* however, did not penetrate dead nematodes.

Plate 10.2 Dead nematode body that has been colonised by *F. acuminatum* in agar test.



10.3.2 Experiment 2

At the first observation time (four hours after nematode inoculation), on plates with *G. graminis* infected root diffusate, most nematodes were attracted to the secretions of plants that had been inoculated with *G. graminis* and nematodes compared to either pathogen alone (Figure 10.1). Few nematodes were seen around the well with root secretions from healthy plants. A small number of nematodes were attracted to the secretions of plants inoculated with fungus alone.

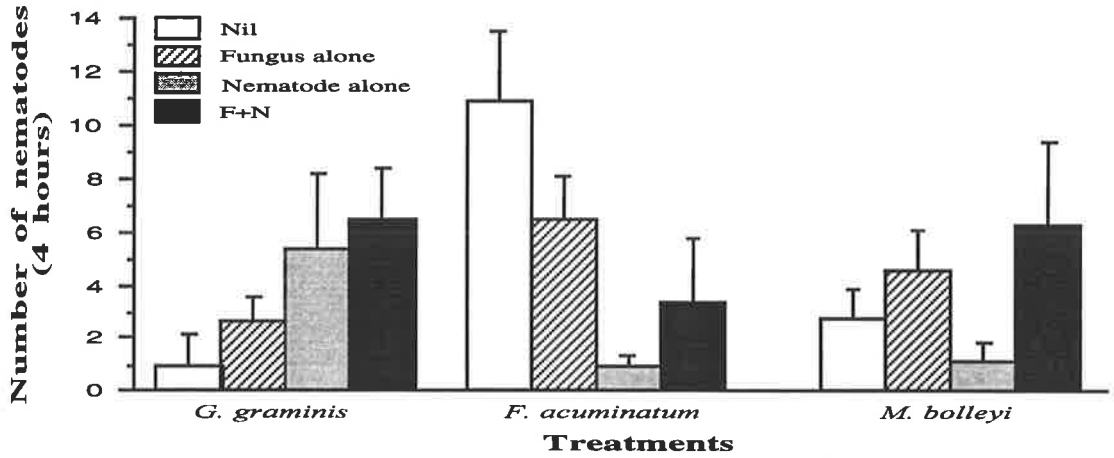


Figure 10.1 Effect of root secretions of plants infected by three different fungi (*G. graminis*, *F. acuminatum* or *M. bolleyi*) and/or *P. neglectus* on nematode attraction four hours after inoculation with nematodes. **Nil=** root diffusate of healthy plant, **Fungus=** root diffusate of plant roots infected with fungus, **Nematode=** root diffusate of plant roots infected with nematode, **F+N=** root diffusate of plant roots infected with the combination of fungus and nematode.

On plates with root exudates from *F. acuminatum* infested plants, the majority of nematodes were attracted to the control (healthy plant secretions). Fungus or nematodes alone or their combination (fungus plus nematodes), attracted 42%, 90% or 75% fewer nematodes, respectively, compared to the control (healthy plant secretions) (Figure 10.1). Root exudates of *M. bolleyi* infected plants alone or in combination with *P. neglectus* attracted 40% or 127% more nematodes, respectively, than the control (root exudates of healthy plant). Root exudates from plants infected with nematodes alone attracted 59% fewer nematodes than the control (Figure 10.1).

At the second observation, seventeen hours after nematode inoculation, exudates from *G. graminis* or *M. bolleyi* infected roots attracted more nematodes than exudates from *F. acuminatum* infected roots (Figure 10.2). Exudates from roots infected with *G. graminis* or *P. neglectus* alone or the combination of fungus and nematode attracted 31%, 15% or 85% more nematodes, respectively, compared to the control (healthy plant) (Figure 10.2). Once again, there was no difference between treatments in plates with *F. acuminatum* as the fungal pathogen. However, root exudates of plants infected with *M. bolleyi* alone or in combination with *P. neglectus* attracted more nematodes than the control. There were as many as 325% or 225% more nematodes around wells with secretions from plant roots infected with *M. bolleyi* or *M. bolleyi+P. neglectus*, respectively, compared to the control (healthy plant secretions) (Figure 10.2).

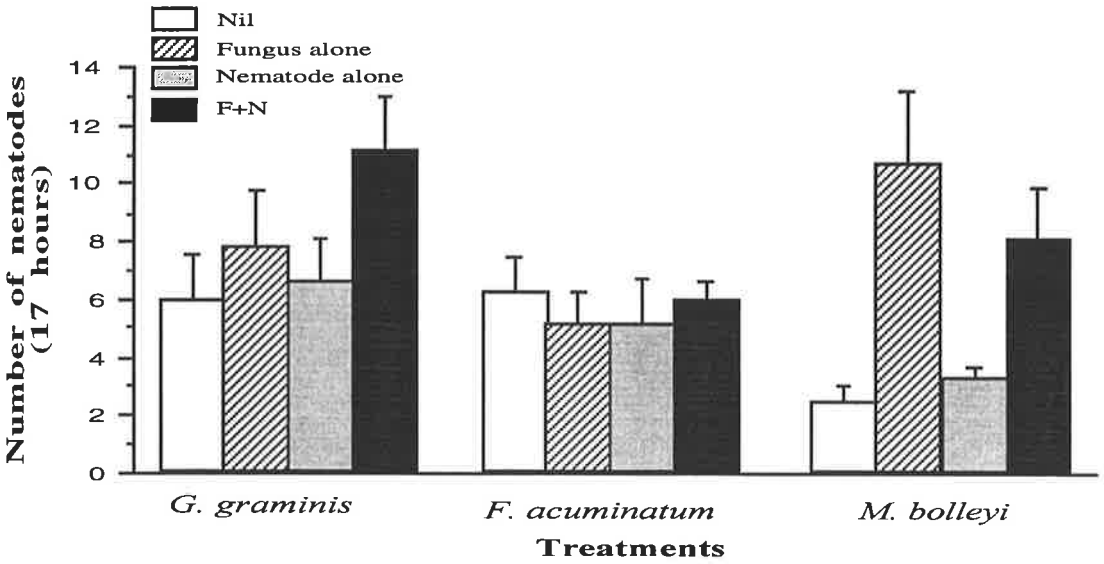


Figure 10.2 Effect of root secretions from plants infected by fungus and/or *P. neglectus* on nematode attraction seventeen hours after inoculation with nematodes. **Nil**= root diffusate of healthy plant, **Fungus**= root diffusate of plant roots infected with fungus, **Nematode**= root diffusate of plant roots infected with nematode, **F+N**= root diffusate of plant roots infected with the combination of fungus and nematode.

As described in section 10.2.2, 24 hours after nematode inoculation nematodes were counted in two rings. Within the first ring (1.0cm from the original point of nematode inoculum), secretions from roots inoculated with *G. graminis* alone or in combination

with *P. neglectus* attracted 116% or 121% more nematodes than the control (healthy plant) (Figure 10.3a). With *F. acuminatum* alone, there were 23% fewer nematodes attracted than to the control (healthy plants). Secretion from roots inoculated with *M. bolleyi* alone or the combination of *M. bolleyi* and *P. neglectus* attracted 40% and 14% more nematodes, respectively, compared to the control (healthy plant secretions).

In the second ring, however, secretion of plant roots infected with *G. graminis* alone or with *G. graminis* plus nematodes attracted 122% or 160% more nematodes, respectively, compared to the control, whereas secretion from roots infected by nematodes alone attracted only 40% of nematodes compared to the control (Figure 10.3b). With *F. acuminatum* there were again no differences between treatments, although *F. acuminatum* alone attracted 14% fewer nematodes than the controls (healthy plants) (Figure 10.3b). *M. bolleyi* alone or in combination with *P. neglectus* attracted 95% or 65% more nematodes, respectively, compared to the control. Total number of nematodes attracted to plant secretions after 24 hours is shown in Figure 10.3c.

10.3.2.1 Root exudate analyses

These analyses were not replicated, so it was not possible to statistically analyse the data.

Total dry mass: There was a considerable amount of variation in total dry mass between different root exudates. Exudates from *G. graminis*, *G. graminis*+*P. neglectus* or *M. bolleyi* infected roots had the lowest amount of dry mass/ml of root exudate (Figure 10.4). *P. neglectus* infected roots alone had the highest dry mass/ml of root exudate compared to exudates from the control plants (healthy). However, there was no difference between *F. acuminatum*, *F. acuminatum*+*P. neglectus* or *M. bolleyi*+*P. neglectus* and the controls (healthy plant) (Figure 10.4).

Total mass of root exudates of plants inoculated with *G. graminis*, *G. graminis*+*P. neglectus* or *M. bolleyi* decreased by 20%, 33% or 33%, respectively, compared to the control (no fungus added) (Figure 10.4). With *P. neglectus* alone, total dry mass increased by 40% compared to the control (healthy plants).

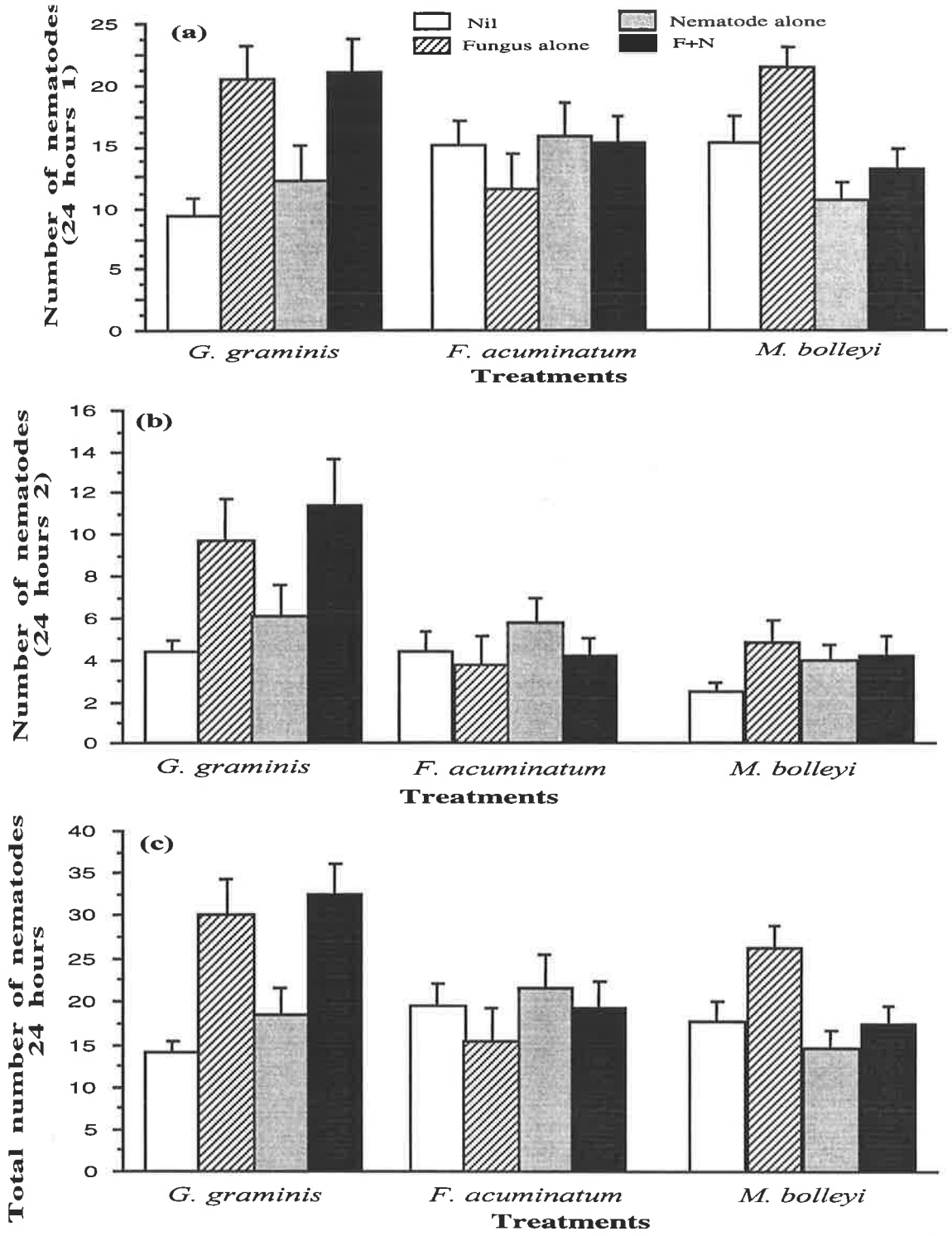


Figure 10.3 Effect of root secretions of plants infected by fungus and/or *P. neglectus* on nematode attraction twenty four hours after inoculation with nematodes **(a)** in the first ring (1cm from the original point of nematode inoculum), **(b)** in the second ring (2cm from the original point of nematode inoculum) and **(c)** total number of nematodes after twenty four hours. **Nil=** root diffusate of healthy plant, **Fungus=** root diffusate of plant roots infected with fungus, **Nematode=** root diffusate of plant roots infected with nematode, **F+N=** root diffusate of plant roots infected the with combination of fungus and nematode.

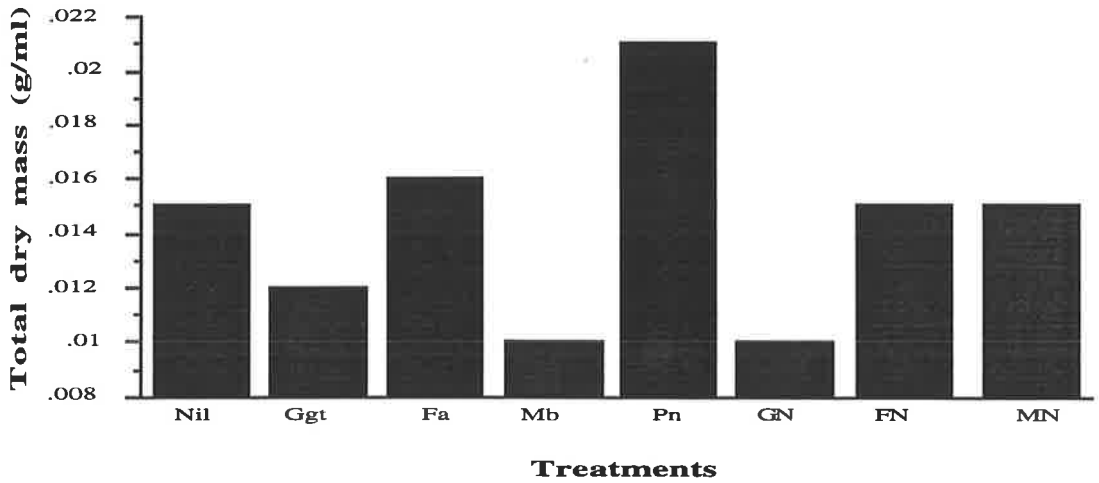


Figure 10.4 Effect of root infection with fungus and/or *P. neglectus* on total dry mass of root exudates. Nil= healthy plant, Ggt= *G. graminis*, Fa= *F. acuminatum*, Mb= *M. bolleyi*, Pn= *P. neglectus*, GN= *G. graminis+P. neglectus*, FN= *F. acuminatum+P. neglectus*, MN= *M. bolleyi+P. neglectus*.

Total carbohydrate content: Root secretions from healthy plants contained the lowest amount of carbohydrate. *G. graminis*, *F. acuminatum*, *M. bolleyi* or *M. bolleyi+P. neglectus* contained 211%, 177%, 133% or 200% more carbohydrate, respectively, compared to the uninfected plants (control) (Figure 10.5). Other treatments, including *P. neglectus* alone, *G. graminis+P. neglectus* or *F. acuminatum+P. neglectus,* were intermediate, with 90%, 94% or 77% more carbohydrate, respectively, compared to the control (Figure 10.5).

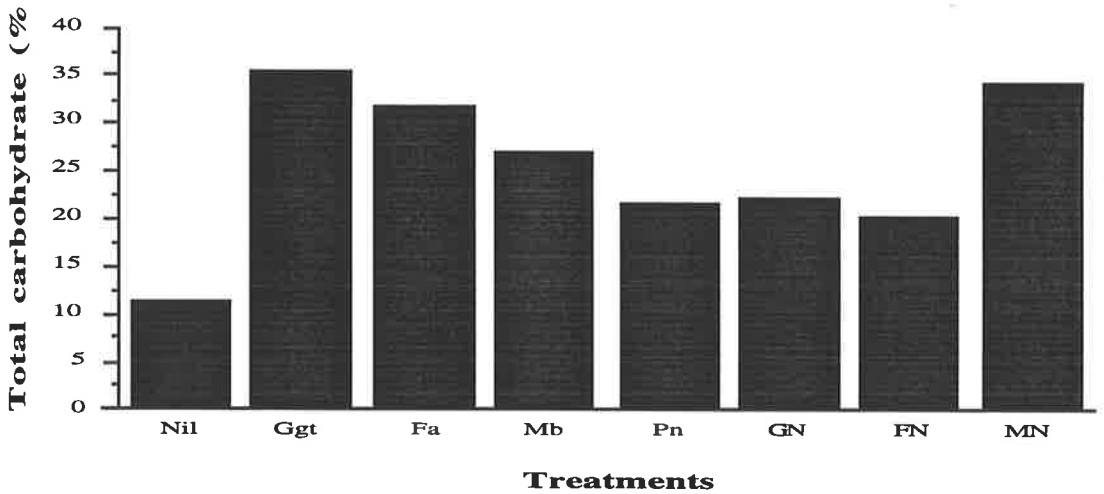


Figure 10.5 Effect of fungus and/or *P. neglectus* on total carbohydrate of root exudates. Nil= healthy plant, Ggt= *G. graminis*, Fa= *F. acuminatum*, Mb= *M. bolleyi*, Pn= *P. neglectus*, GN= *G. graminis+P. neglectus*, FN= *F. acuminatum+P. neglectus*, MN= *M. bolleyi+P. neglectus*.

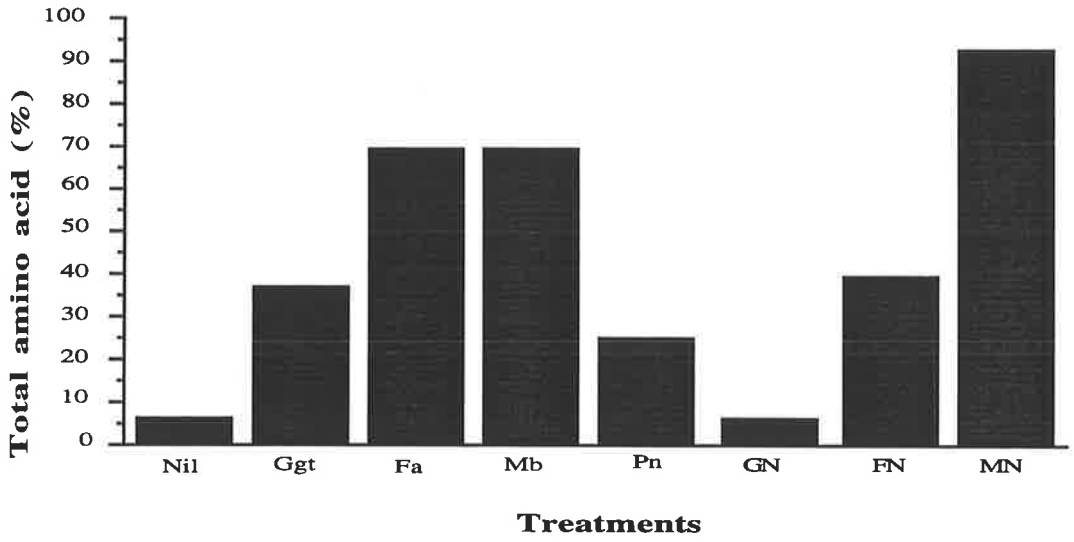


Figure 10.6 Effect of fungus and/or *P. neglectus* on total amino acid content of root exudates. Nil= healthy plant, Ggt= *G. graminis,* Fa= *F. acuminatum*, Mb= *M.bolleyi*, Pn= *P. neglectus*, GN= *G. graminis+P. neglectus*, FN= *F. acuminatum+P. neglectus*, MN= *M. bolleyi+P. neglectus.*

Total amino acids: Root exudates of healthy plants (uninoculated) contained very low amounts of amino acids. Roots infected with *F. acuminatum*, *M. bolleyi* or *M. bolleyi+P. neglectus* contained the highest amount of amino acid (Figure 10.6). Roots infected with *F. acuminatum* contained ten times more amino acid than control (healthy plants) and *M. bolleyi* or *M. bolleyi+P. neglectus* contained ten or fourteen times, respectively, more than the control. Roots infected with *G. graminis*, *P. neglectus* or *F. acuminatum* contained five, three or 5.4 times more amino acid than the controls which were relatively less than other treatments (Figure 10.6). The treatment *G. graminis+P. neglectus* contained a very low amount of amino acid.

The amount of different amino acids in root secretions of plants inoculated with *G. graminis*, *F. acuminatum*, *M. bolleyi* or control (uninoculated) plants is listed in Table 10.1. All plants infected with fungi contained a high level of Alanine, Glutamic Acid, Glycine, Threonine and Valine compared to the control plants (no fungus added) whereas none contained Arginine or Cysteine. Glutamine, Histidine, Leucine, Lysine,

Phenylalanine, Tryptophan and Tyrosine were detected in most treatments, but were in very low concentrations.

Overall, *M. bolleyi* infected plants contained the lowest amount of all amino acids compared to the other two fungi, *G. graminis* or *F. acuminatum* infected plants, but still contained more than control plants with no fungus inoculum. *F. acuminatum* infected plants contained higher amounts of Alanine and Valine than *G. graminis* or *M. bolleyi*. For all other amino acid contents there were no large differences between *G. graminis* and *F. acuminatum*.

Table 10.1 Amino acid contents (nmol/ml) of root secretions from plants infected with *G. graminis, F. acuminatum* or *M. bolleyi* and healthy plants (Nil).

Amino acid	Fungus			
	G. graminis	*F. acuminatum*	*M. bolleyi*	Nil
Alanine	9.3	15.6	3.4	0
Arginine	0	0	0	0
Asparagine	5.4	2.3	2.2	**1.4**
Aspartic Acid	4.5	2.9	**1.5**	0
Cysteine	0	0	0	0
Glutamine	**0.9**	0	**0.7**	0
Glutamic Acid	9.1	7.2	2.2	0
Glycine	6.2	6.6	**1.6**	0
Histidine	**1.1**	0	0	0
Isoleucine	4.1	5.1	**1.2**	0
Leucine	4.2	4.8	**1.2**	0
Lysine	0	**0.4**	**0.3**	0
Methionine	**0.9**	1.9	**0.5**	**0.4**
Phenylalanine	**1.0**	**1.0**	**0.3**	0
Proline	3.0	**1.4**	**0.8**	0
Serine	6.7	3.6	1.8	0
Threonine	5.4	6.9	**1.7**	0
Tryptophan	**0.3**	**0.3**	**0.1**	0
Tyrosine	**0.9**	**0.5**	0	0
Valine	6.2	9.0	1.8	**0.2**

Table values- nmol/ml

Values in bold type fall below the calibrated range, which is 1.8 nmol/ml

10.4 Discussion

Plant parasitic nematodes cause mechanical wounding as they penetrate or feed within root tissues. Feeding and migration of root parasitic nematodes, as well as growth and development of fungal pathogens, may induce physiological, biochemical changes or both in their hosts. According to Mai and Abawi (1987), physiological changes induced in the host could be direct effect of parasitism or the indirect result of stresses imposed on the host. Evidence found in Chapter 6 and shown in Plate 6.2 supported the hypothesis that *P. neglectus* may feed on fungal hyphae. However, the results obtained from the agar test suggest that *P. neglectus* do not feed on fungal mycelia. All the second-stage juveniles introduced to the cultures of three fungi (*G. graminis*, *F. acuminatum* or *M. bolleyi*) grown on agar had died one week after inoculation into the agar. This result indicates that, at least under the conditions of this experiment, juveniles of *P. neglectus* can not feed or develop on hyphae of any of the test fungi.

It is suggested that, due to the optimal conditions provided for fungus growth (agar medium and a temperature of 25°C), a high concentration of chemicals (chemotoxins, etc.) produced by the fungus may have prohibited nematodes feeding and developing on fungal hyphae. A similar observation was evident in Chapter 6, where roots heavily infected with *Pyrenochaeta terrestris* or *F. acuminatum* resulted in death of some nematodes and their eggs. This may support the above suggestion that a high concentration of fungal hyphae would result in high production of chemicals both in agar or root tissues that may be toxic to nematodes. Therefore, it is suggested that, due to the obvious differences between the root environment and agar medium, the hypothesis that *P. neglectus* may feed on fungal hyphae while both are in the roots of a host is still possible and yet to be investigated in detail.

It is also possible that in a complex of interactions between plant, nematode and/or fungus, nematodes have the chance to feed on fungi and use them as a source of energy. This may be one reason why the number of *P. neglectus* increased in the presence of some fungi. The complex medium of roots after fungal and/or nematode infection may provide a rich source of nutrients for nematode and/or fungal reproduction and

development. It is indeed difficult to study this complex medium, but it is a highly interesting area in which to study attraction of plant roots for plant pathogenic microorganisms.

Interaction between host plant, nematode and/or fungus may result in production of some attractive substances that then leak into the rhizosphere and make roots attractive or non-attractive to a certain species of nematode or fungus and to complexes of micro-organisms. Results obtained from the attraction test reported in this chapter provide evidence that substances produced in the plant after infection by some fungi, *P. neglectus* and/or the combination of both fungus and nematode infection, is attractive to *P. neglectus*.

Root exudates are known to have positive influences in the rhizosphere as they can attract fungi (Zentmyer, 1961) or serve as nutrient substrates (Rovira, 1965). They can also inhibit microbial activity in the rhizosphere (Buxton, 1958) that may influence disease development.

Exudates of *G. graminis* or *M. bolleyi* infected plants were more attractive to *P. neglectus* than root exudates of *F. acuminatum* infected plants. It is now possible to explain why the combination of *M. bolleyi* and *P. neglectus* resulted in increased nematode numbers, whereas with *F. acuminatum* number of nematodes did not increase (Chapter 6). It is difficult at this stage to conclude the reason for this behaviour, but it is clear that plants infected with different fungi may produce different substances that could be attractive to nematodes or not. Although *F. acuminatum* infected plants may not be strongly attractive to *P. neglectus*, the combination of these two pathogens resulted in extensive root lesioning of wheat.

While *P. neglectus* responded to all fungi tested, the degree of attractiveness as measured by the number of nematodes attracted to the test fungi (*G. graminis*, *F. acuminatum* or *M. bolleyi*) varied. Four hours after nematodes were introduced to test plates, root exudates from *G. graminis* and *M. bolleyi* infected plants attracted more nematodes than *F. acuminatum* infected plants. At this stage, most nematodes in the *F.*

acuminatum test plates were attracted to the root exudates from healthy plants, which could be due to the unattractiveness of root exudates from *F. acuminatum* infected plants.

Twenty four hours after addition of root exudates of *F. acuminatum* infected plants, there were more nematodes around test wells than at four hours. This may be related to the loss of a concentration gradient as the test materials diffused throughout the assay plates. However, the opposite result was obtained with the other two fungi (*G. graminis* or *M. bolleyi*). Twenty four hours after test materials had been added into agar plates, fewer nematodes were recovered from around the wells of test plates, which could be for the same reason as suggested above. Similarly, Klink *et al.* (1970) reported that the majority of *Neotylenchus linfordi* were attracted to *Gliocladium roseum* colonies four hours after introduction of nematodes, but not after twenty hours.

Total dry mass, total carbohydrate content or total amino acid content of root exudates of plants infected by either fungus and/or nematode did not provide enough information to propose a logical pattern of how these substances attract nematodes. However, individual amino acid contents showed that uninfected plants (control) contained none of fifteen amino acids detected in the other treatments. Thus it is clear that infected plants leaked more exudates that contained substances which might be attractive for nematodes. Root exudates from plants infected with *F. acuminatum* that showed less attraction to *P. neglectus* contained higher levels of Alanine and Valine than other fungi. These two particular amino acids may not be very attractive to *P. neglectus*.

According to Hale *et al.* (1971), among substances in the root exudates, amino acids are known to be soluble, readily diffusing into the soil. Generally, it is known that *Pratylenchus* infected plants contain a high concentration of amino acids and nitrates (Sudakova, 1978). In this study, either nematode or fungus infested roots resulted in a higher concentration of different amino acids which indeed could influence microbial activity in the rhizosphere community. As the results of the attraction assay showed, the concentration of different amino acids in roots infected by different pathogens differed, which then resulted in different reactions to nematode attraction. *F. acuminatum* infected

plants, which were less attractive to the nematode, contained a high level of Alanine and Valine compared to the other treatments. This could be the possible reason they were not attractive to *P. neglectus*. However, the exudates of *F. acuminatum* infested plants may not be necessarily non-attractive to the nematode, but may diffuse faster into the agar. Therefore, nematodes can reach them at the point where they were introduced to the agar. It is, therefore, important to have a better understanding of the role of root exudates and particularly amino acids leaking into the soil from plants infected with either fungal or nematode pathogens. Further investigation of the role of root exudates in the rhizosphere and mechanisms of interaction between *P. neglectus* and root-rotting fungi of wheat is needed.

Chapter 11

General discussion

11.1 Discussion

This study confirmed that *P. neglectus* in association with several fungal species are likely to contribute to root disease of wheat and other cereals. Therefore, it was appropriate to consider that root damage to wheat in South Australia can be caused by the interaction between the root lesion nematode and these root-rotting fungi when they are present together.

The fungi most frequently associated with diseased wheat roots infected by *P. neglectus* at the Stow and Palmer field sites sampled during the 1992 growing season (Chapter 3) were the same as those previously reported by Fedel-Moen and Harris (1987) and Vanstone (1991). However, the difference was that samples were taken from soil known to be infected with *P. neglectus,* and this was confirmed by extracting the nematode from all samples throughout the survey. Presumably, as *Pratylenchus* spp. are now known to occur in almost all crops and to be common in South Australia, Fedel-Moen and Harris (1987) and Vanstone (1991) took their samples from areas that were also infested with root lesion nematodes.

M. bolleyi and *F. acuminatum* in combination with *P. neglectus* are partly responsible for the observed root damage of wheat, since the frequency of these species increased as the number of *P. neglectus* increased late in the season while the severity of crown root damage also increased (Chapter 3). These fungi could be a potential agent for interaction with *P. neglectus,* as both the fungi and the nematode are widely distributed in South Australia.

Considering that nematodes are able to modify the rhizosphere through root secretions of infected plants that could be attractive for some soil-borne fungal pathogens (Van Gundy *et al.*, 1977), and the fact that weakly pathogenic fungi may become more pathogenic in the presence of nematodes (Powell, 1979), it is important to consider the role of weakly pathogenic fungi, particularly when breeding *P. neglectus* resistant cultivars. The population and number of weakly pathogenic fungi such as *M. bolleyi* and *F. acuminatum* in soil is high (Chapter 3) and *P. neglectus* is also widespread in the soil (Nicol, 1996). Furthermore, the feeding process of *Pratylenchus* spp., as well as all other plant-parasitic nematodes, produces wounds to the host roots (Taylor, 1979), which would provide a ready avenue of entry for other pathogens. Thus, it is important to understand the role of the nematode in modifying root biochemistry and/or the rhizosphere in a given nematode-fungus interaction.

The results of the fungal survey and related nematode numbers together suggest that these organisms (nematode and fungi) have similar effects to each other in producing and expanding root lesions on the plant. These effects are particularly important late in the season when plants need more nutrients and higher water uptake, which are affected by nematode damage.

Soil type is particularly important for nematode movement and attraction to roots. In the field *P. neglectus* is most commonly found in sandy soil (Nicol, 1996). In support, sandy loam soil was the best medium for maximum root penetration by the nematode and fungus in laboratory tests (Chapters 4 and 6). Considering the fact that soil in South Australian wheat growing areas is frequently sandy loam, *P. neglectus* could be a very important pest of several crops.

The results obtained under controlled glasshouse conditions, indicated that *M. bolleyi*, *F. acuminatum*, *Pyrenochaeta terrestris*, *Pythium irregulare* and *Rhizoctonia solani* are positively associated with *P. neglectus* which could cause greater damage to the plant. Presence of these fungi increased disease rating of wheat roots and increased the number of *P. neglectus* in the roots (Chapters 4 and 6). Besides two

major fungal root pathogens, *R. solani* and *P. irregulare*, the other fungi mentioned above are known to be minor pathogens in their own right. However, in both pasteurised and sterilised soils, *M. bolleyi* and *P. terrestris* alone caused considerable damage to the roots of Machete wheat, suggesting that the fungi have potential to cause cereal root disease. Thus, the nematode seems to modify root physiology, biochemistry or the rhizosphere in ways that favour fungus penetration and development which then allow nematode development and multiplication within the roots.

Under natural conditions in the field, combinations of *M. bolleyi*, *F. acuminatum* and/or *P. neglectus* cause severe damage to wheat roots and reduce yield (Chapter 9). Complimentary results were obtained with *Pythium irregulare*, *Pyrenochaeta terrestris*, *R. solani* or *G. graminis* alone or in combination with *P. neglectus* under field conditions and under controlled glasshouse conditions. In all cases greater root damage was found when both nematode and fungus were present, than either alone. These findings indicate that *P. neglectus* is usually associated with some root-rotting fungi, which together cause extensive damage to the roots of wheat. Thus, it is possible that some catastrophic crop losses involving nematodes are not merely the result of the coincidental occurrence of several factors, but because of interactions between them (Wallace, 1983). Furthermore, information on such interactions is useful in determining appropriate control measures which take into account both the nematode and the fungi.

The relationship between *G. graminis* and *P. neglectus* or *G. graminis* and *R. solani* was negative in both glasshouse and field studies (Chapter 5). Pre-infection of plants with *P. neglectus* reduced severity of *G. graminis* infection. Both *G. graminis* and *P. neglectus* seem to be very sensitive to competition for penetration of roots. Neither seems to prefer penetrating roots already damaged by another pathogen.

This negative interaction could be explained in three ways. Firstly, competition for infection sites may occur, and the first pathogen in the root may inhibit invasion

by the other. Secondly, physiological and biochemical changes in the host caused by one pathogen may not favour infection by the other pathogen. Thirdly, one pathogen may modify the rhizosphere in a way that may affect the development of organisms antagonistic to the other pathogen. Therefore, it is important to understand the different biology of the disease complex. Further investigation is required to understand the role of nematodes in relation to *G. graminis* infection and the interrelationship between *R. solani* and *G. graminis*.

Although *R. solani* was not isolated from field samples in 1992, the fungus cannot be disregarded as a cause of root damage, as there is some indication that this species could be associated with *P. neglectus* (Chapters 4 and 5). Under certain conditions, *R. solani* alone (and particularly in combination with *P. neglectus*) can cause severe root disease. It is widespread and damaging when populations of pathogenic strains increase to the level at which "bare patch" occurs in the field (Kerr, 1955; de Beer, 1965).

Nematode populations within roots of wheat declined as root rotting increased in severity, as *Pratylenchus* spp. vacate damaged roots in search of a fresh food source (Corbett, 1972; Dropkin, 1989). Thus, diagnosis of the causative agent of the disease becomes more difficult as the severity of damage increases over the growing season. This has further implications on sampling methodology for both fungus and nematode. In order to take account for the movement of nematode and fungus, it is suggested sampling of both root and soil would be more appropriate than either alone.

Mechanical wounding of the surface of roots in the presence of fungi did not increase disease rating. However, combination of fungus and *P. neglectus* resulted in severe lesioning of roots. These results suggest that association of *P. neglectus* and root-rotting fungi which are responsible for root damage of wheat is more than just physical damage by the nematode allowing fungi to penetrate.

It is important to note that the results presented from the laboratory work were conducted under standardised conditions. These included different inoculum densities of both fungus and nematode in addition to the inoculation time of these and the temperature at which the experiments were conducted. As a consequence the data may not be strictly comparable to the field. In all cases (nematode and fungus together or either alone) the damage to plants was greater at higher soil temperature (25°C). This is a common phenomenon with *Pratylenchus* spp. and has been verified by field studies (Pattison, 1993) and other laboratory work (Van Gundy *et al.*, 1974; Nicol, 1991), where the nematode multiplication and damage is greater as the temperature increases.

11.2 Critique and suggestions for further work

The medium used for growing fungi, especially weakly pathogenic fungi, could be modified. The following problems associated with demonstrating the effects of soil-borne fungi on nematode-fungus interaction were identified, particularly in the laboratory and glasshouse experiments:

1. ***Nematode inoculum***. Uneven nematode distribution in the soil and establishment of the nematodes in the roots may not result in full expression of the nematode-fungus interaction. Therefore, it seems essential to modify the inoculation technique. The current method of nematode inoculation is to add a known number of nematodes in suspension to the base of each plant. Use of naturally infested soil or inoculum mixed through the soil may be more effective.

The technique should maximise the chance of nematode infection of the majority of roots, which will result in more lesions on the root surface that could be used by fungi to enter the plant. This may result in stronger nematode-fungus interaction. The nematode inoculation technique used here may not have been optimal, and may require modification. One possible way could be to use nematodes from carrot cultures to inoculate susceptible wheat varieties, in soil in pots, and to allow them to multiply. The infected soil plus infested root segments could then be used as an

inoculum source and mixed with pot soil. This method of inoculating plants seems to be more efficient and successful.

2. ***Using realistic nematode or fungus inoculum densities***. This was a problem when inoculating with a suspension of nematodes next to the pre-germinated seed. To ensure that root segments are not infested with other soil-borne pathogens, pasteurised soil should be used to build up nematode inoculum with all fungi tested in this study, but is particularly difficult with weakly pathogenic fungi such as *M. bolleyi* or *F. acuminatum*. All densities of *G. graminis* used were relatively high (in terms of damage caused), and because of this the fungus tended to dominate. *R. solani* inoculum was adequate, but *Pythium irregulare* inoculum seemed to be relatively low compared to the natural level of fungus inoculum.

3. ***Medium used for growing the fungus, especially weakly pathogenic fungi such as* M. bolleyi *or* F. acuminatum.** The medium used for *R. solani* and *G. graminis* was adequate, as low numbers of propagules were sufficient to infect plants. However, minor pathogenic fungi, particularly *M. bolleyi* and *F. acuminatum*, required a large amount of inoculum to cause damage or to allow establishment of the fungus on the host plant.

The medium used for growing these fungi (millet seed) was rich in nutrients, enabling the fungus to survive a long time in the soil. Assuming the nematode is the co-pathogen, the fungus may not have colonised roots of the host, particularly if there were few wounds caused by un-even distribution of nematodes. Further confusion was found with the increased plant growth in the presence of fungi. It is difficult to distinguish whether the fungus caused this stimulation in growth or the degraded millet seed provided an additional nutrient source to the soil related to the undegraded dead millet seed used in the control. This might also affect the interaction between nematodes and fungi tested. Thus, further work is required to establish a sufficient and applicable inoculation technique for both nematodes and fungi.

4. ***Field experiments had considerable variation within and between the plots in terms of number of nematodes and size of fungal populations.*** This could be solved by artificial inoculation of both fungus and nematode using microplots. Another approach could be to manipulate the natural populations using different cultivars of plants ranging in susceptibility to both fungus and nematode. Furthermore, appropriate statistical designs with a sufficient number of replicates, preferably at least ten, and more than two independent variables, together with several densities of nematode and fungus should be used. Also, it is important to search for an appropriate experimental site with relatively high numbers and even distribution of *P. neglectus* (ideally more than five nematodes/g of soil) but free from other major nematode pathogens such as *Heterodera avenae*.

5. ***Sampling method is also important in evaluating the damage caused by nematodes and fungi and by their interaction.*** Therefore, developing a scoring system to evaluate plant response to the nematode-fungus interaction and particularly the time intervals for sampling should be further investigated.

From the results of this study, scoring root lesioning and counting nematode numbers in the roots appear to be the best way to assess cultivars for resistance and/or tolerance. Root dry weight may be useful but from the cultivar examined in this study, shoot dry weight is not (Chapters 8 and 9). Root lesion rating is a useful measure of root damage, as it can be attributable to both the nematode and the fungus. Combination of fungus (*F. acuminatum* or *M. bolleyi* in particular) and nematode under controlled environmental conditions would produce more visible lesions on the root system in a shorter experimental period (six to seven weeks), aiding in scoring root lesioning. Because of this, lesioning is a very useful parameter to use in screening for tolerance to nematode, fungus or the combination of both.

6. ***Other environmental factors.*** Soil moisture regime at the seedling stage of a crop varies from season to season and region to region in South Australia. This is the time when initial nematode infection is occurring and could affect penetration. This

variable was not studied in this thesis but represents a further avenue of research which could explain some field interactions between nematode and fungus and disease levels.

The study of nematode-fungus interaction as a feature of complexity in crop systems is critical for several reasons. The evidence concerning break down in plant resistance by either nematodes or fungi suggests that breeding programs need to develop cultivars which remain effective under a disease complex involving both fungi and nematodes. In addition, fungus-nematode interactions should be considered in developing appropriate integrated control measures for disease complexes. Nematodes are only one of many biotic and abiotic factors in the soil ecosystem. Therefore, it is important to have a better understanding of the relationship between nematodes and other factors (biotic and abiotic) particularly fungi in the root ecosystem (Wallace, 1983, 1987) to develop an effective strategy for breeding and control of nematodes in a short or long term program.

Appendix A

Morphometrics of South Australian population of *Pratylenchus thornei* and *P. neglectus* males and females

Paper submitted to *Australasian Plant Pathology* for publication.

Morphometrics of South Australian population of *Pratylenchus thornei* and *P. neglectus* males and females

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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.

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.

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.

Descriptions

Pratylenchus thornei

(Figures 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.

TABLE I

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 Medium 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	-	-	-					

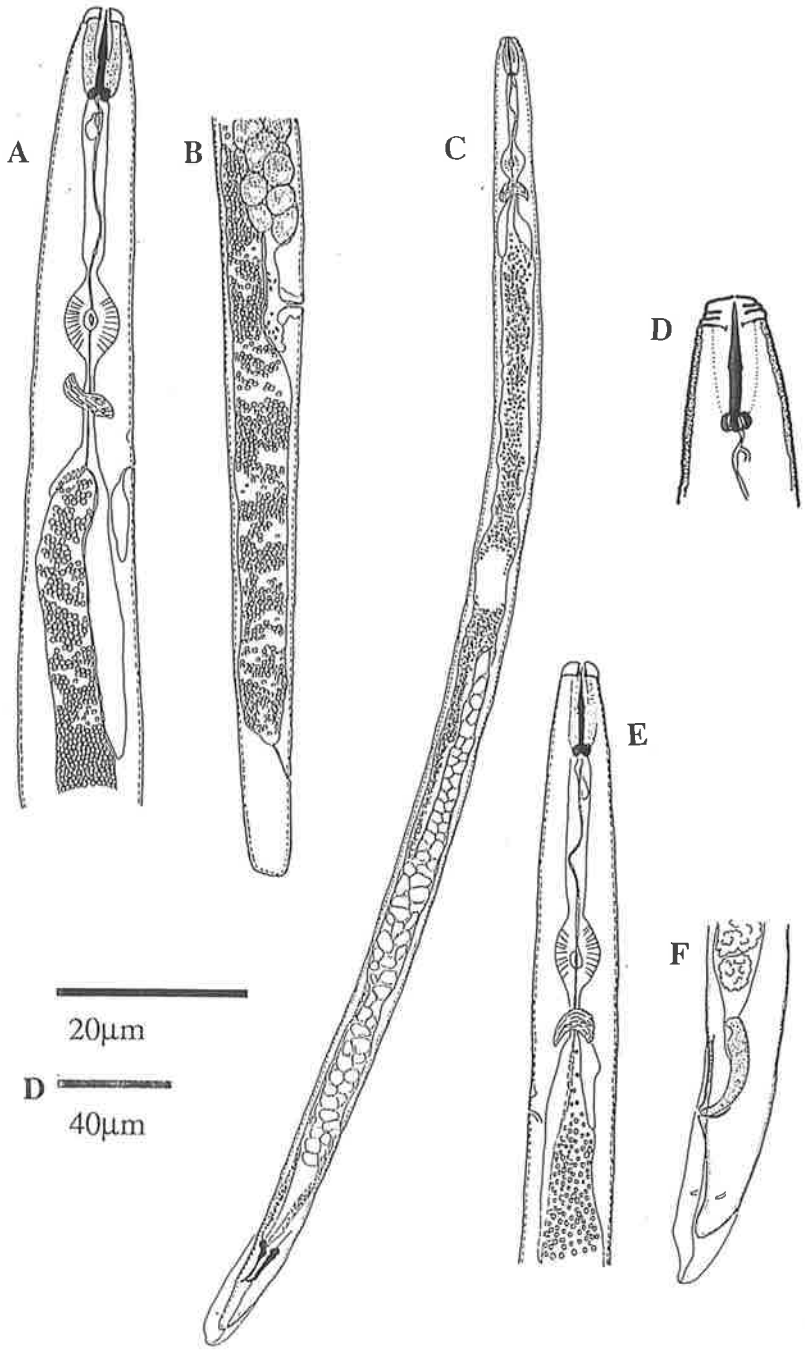

Fig. 1. *Pratylenchus thornei*. A, oesophageal region female; B, tail region female; C, entire male; D, head end female; E, oesophageal region male; F, tail region male.

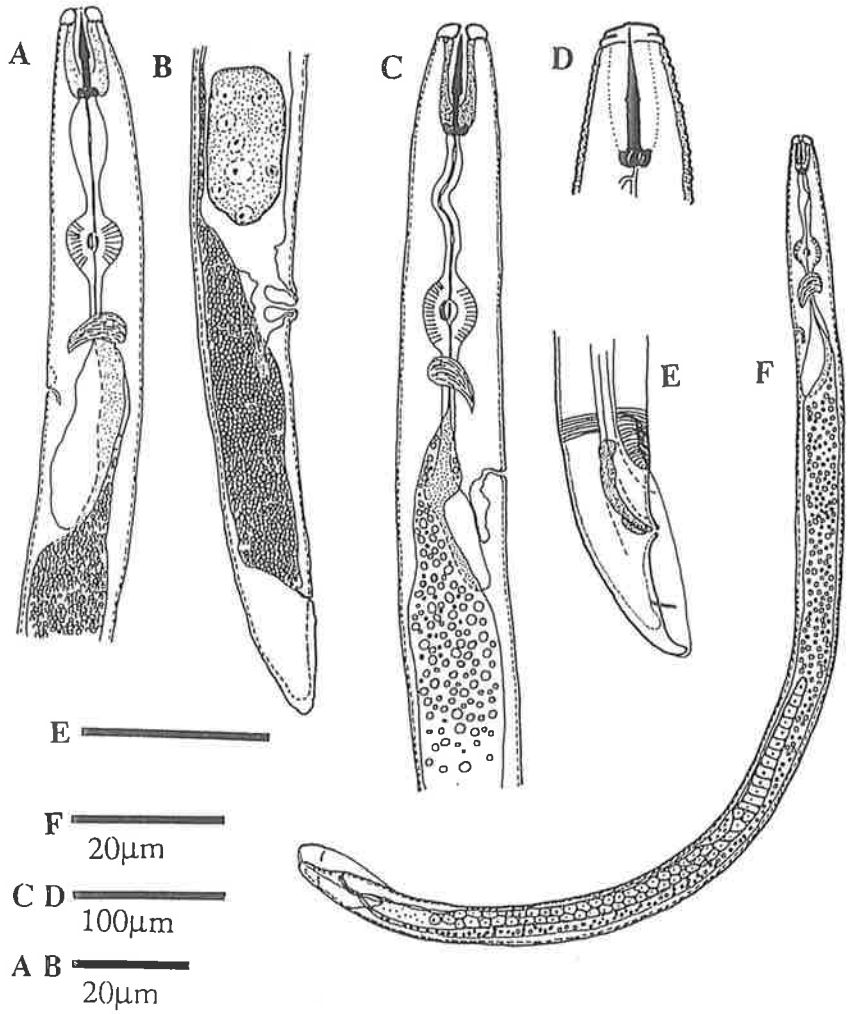

Fig. 2. *Pratylenchus neglectus*. A, oesophageal region female; B, tail region female; C, oesophageal region male; D, head end female; E, tail region male; F, entire male.

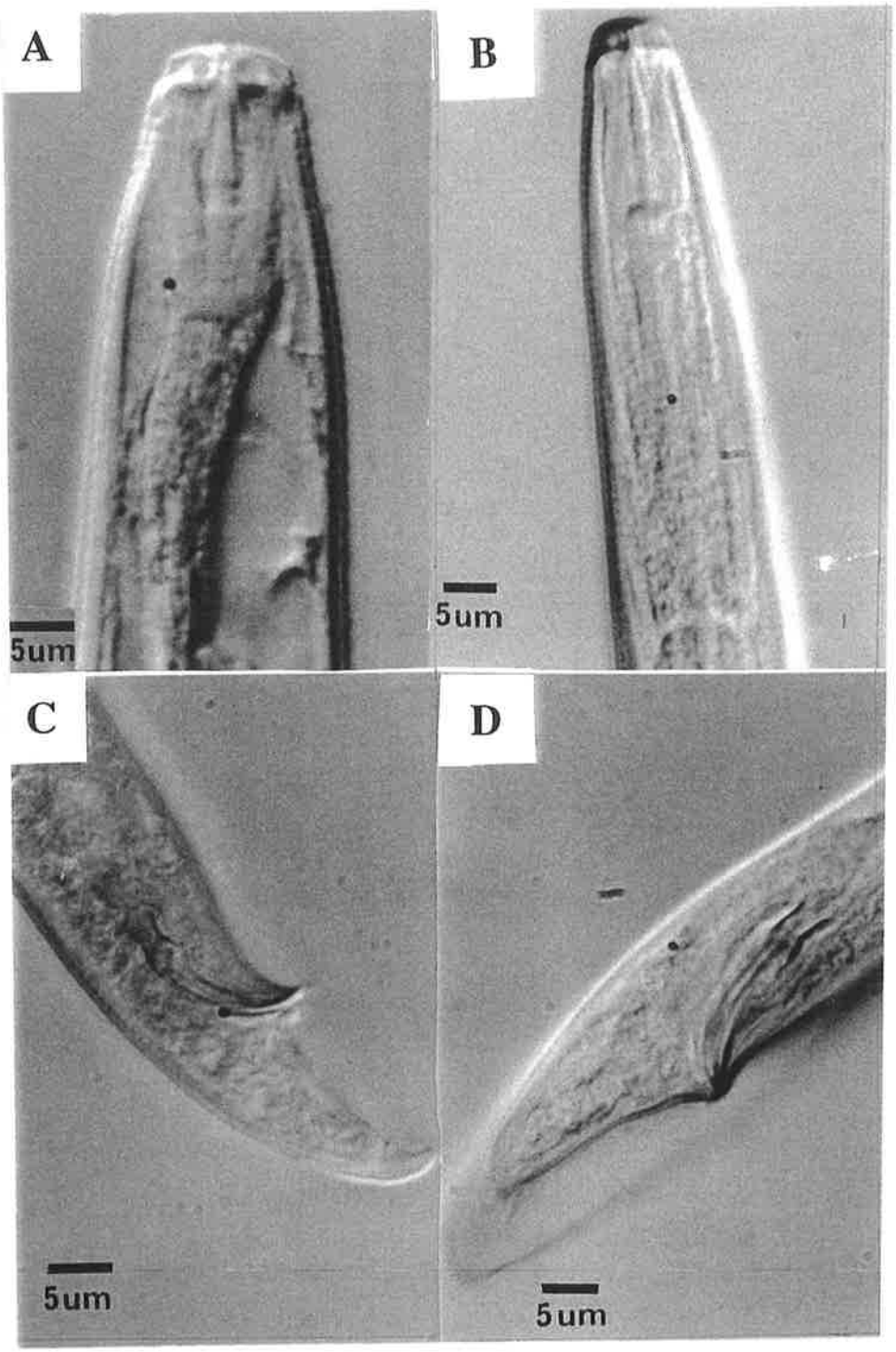

Fig. 3. Light microscopy of *Pratylenchus thornei* and *Pratylenchus neglectus*. A, head *P. neglectus* male; B, head *P. thornei* female; C, tail *P. neglectus* male; D, tail *P. thornei* male.

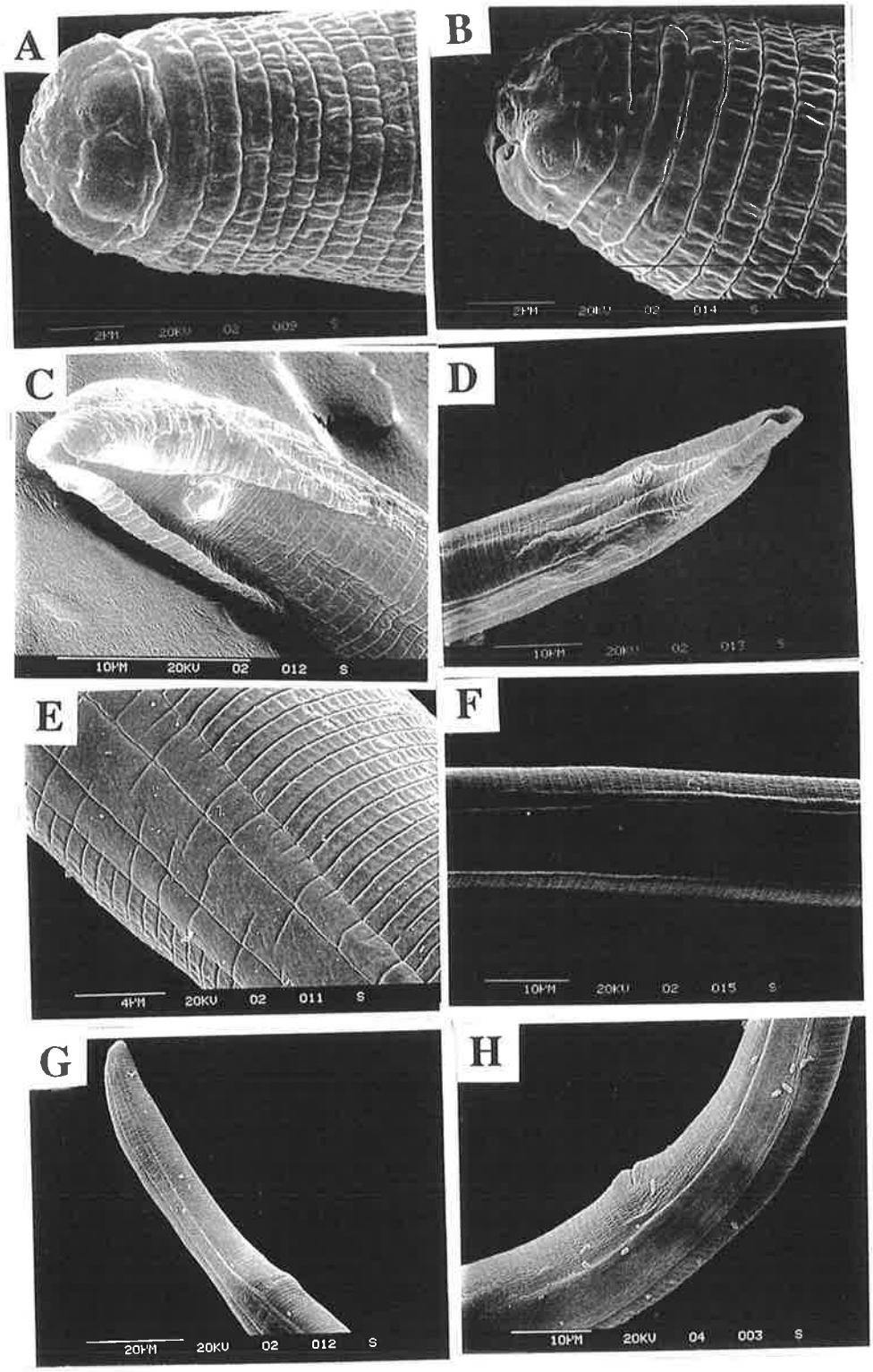

Fig. 4. SEM micrographs of *Pratylenchus thornei* and *Pratylenchus neglectus*. A, head *P. neglectus* male; B, head *P. thornei* male; C, tail *P. neglectus* male; D, tail *P. thornei* male; E, lateral field *P. neglectus* male; F, lateral field *P. thornei* female; G, vulva *P. neglectus* female; H,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

Pratylenchus neglectus

(Figures 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 (1.3-2.3µm). 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.

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 (Figure 1), but in *P. neglectus* they are typically indented on the anterior surfaces and less robust (Figure 2). The spicule length is longer in Australian specimens of *P. thornei* than *P. neglectus* (Table 1, Figure 3), as previously documented by Loof (1960) and Sher and Allen (1953). The caudal alae could be used to distinguish the two species (Figure 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, Figures 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 (Figures 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 (Figure 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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