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GENETIC VARIATION FOR TOLERANCE AND RESISTANCE TO *PRATYLENCHUS NEGLECTUS* .

by

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SUMMARY

A major problem in the production of agricultural crops, including wheat, is the damage caused by destructive plant parasitic nematodes. Among these are species of cyst and root-knot nematodes and the root lesion nematode (*Pratylenchus* spp.). The genus *Pratylenchus* has a worldwide distribution, attacking a wide diversity of plants. *P. neglectus* has been recognised in South Australia since 1956 and its association with fungi in cereal root disease has been repeatedly reported.

Root lesion nematode infection is associated with leaf yellowing, which reduces plant photosynthesis and grain yield. There are reports that in nematode infested soil, well fertilised crops are usually less affected and crop losses can be reduced by adding extra fertiliser, compensating for the reduced nutrient uptake caused by the nematode attack. Plants more efficient in nutrient uptake and less sensitive in expressing the yellow lower leaves would be less affected by the nematode.

Two experiments were conducted in the glasshouse, using different methods to reduce the nematode population and different fertiliser regimes to compensate for yield losses caused by the nematode. These experiments, with small modifications, were repeated under field conditions over two years at two sites. Several more experiments were also conducted in glasshouse, inoculating pasteurised soil with aseptic nematode population and applying different fertiliser regimes to assess the effect of the nematode and on expression of yellow leaf symptom in absence of other microorganisms and to investigate the effect of plant genotype on developing the symptom.

Nematodes increased the severity of leaf yellowing for intolerant genotypes. Controlling nematodes by either nematicide application or soil freezing resulted in greener plants. The yellow leaf symptom was strongly related to nitrogen deficiency and, to a lesser extent, phosphorus deficiency. N+P fertiliser could reduce yellow leaf symptoms on plants growing in nematode infested soil. Uptake of P, K, Ca, Mg, Mn, Na and S was facilitated by nitrogen application. Nitrogen had a nematicidal effect,

with calcium nitrate reducing the number of nematodes.

The wheat variety Molineux appears inherently more susceptible to leaf yellowing than most other commercial wheat varieties. Janz and Spear were more efficient in absorbing nitrogen compared to Molineux, as they showed a higher concentration of N in their shoots. Molineux was also less efficient in absorbing phosphorus from the soil. Some derivatives of Molineux were greener than Molineux, both in the nematode infested soil and in nematode free soil, indicating a relatively simple inheritance of this trait. Genotypes with a higher root growth rate were greener and had a significantly greater shoot fresh weight.

Yellow leaf symptoms cannot be used in a breeding program as a measure of tolerance to nematode invasion. But, these symptoms in the paddock are often an indication of *Pratylenchus* attack, resulting in unhealthy root systems. Although application of nitrogen and phosphorus could be recommended to farmers to alleviate leaf yellowing and overcome yield loss caused by the root lesion nematode, a high rate of nitrogen is required to cause satisfactory nematode reduction.

The most economical method of nematode control is the growing of resistant varieties. These varieties would result in better yields on nematode infested soil. To screen varieties for resistance to the nematode, experiments were conducted in the glasshouse in controlled temperature waterbaths, using pasteurised soil and aseptic *P. neglectus* for inoculation. The optimum time of sampling was examined using different wheat genotypes.

It was demonstrated that in pot experiments with 650 g soil, a population around 500 nematodes is adequate to differentiate resistant varieties. Terminating pot experiments at around eight weeks after sowing and inoculation and counting number of nematodes per plant was adopted to rank host plants for resistance.

Before resistant wheat varieties had been identified, the resistance of species related to wheat was investigated. Durum and triticale varieties demonstrated a lower nematode multiplication rate than wheat varieties. All lines with rye chromosomal material demonstrated a comparatively low number of nematodes compared to the wheats. Abacus, consisting of rye and tetraploid wheat (durum) genomes, could be used either as a rotational crop or as a donor parent to incorporate *P. neglectus* resistance gene(s) into commercial wheat varieties. Fortunately, there appears to be sufficient resistance to *P. neglectus* in wheats such as Persia 20 and Virest, exotic materials from Iran and Italy respectively.

In a study of the mechanisms of resistance, resistant varieties (Persia 20 and Virest wheat and Abacus triticale) showed non-significantly lower nematode penetration than Spear, the susceptible check variety. The time of moulting and commencement of egg laying was delayed in the resistant genotypes compared to the susceptible. Nematodes in resistant plants were surrounded by dead cells which could be based on plant tissue hypersensitivity to nematode infection. In resistant plants, a higher proportion of those nematodes entering the roots failed to establish feeding sites.

Due to limited time for this thesis, the genetics of resistance to *P. neglectus* was investigated in F_2 populations. Based on data transformation, the genetics of resistance of crosses of (Virest x Frame could be interpreted based on a single gene, that of (Virest x Barunga) either one or two genes, and that of (Virest x Tatiara) two genes.

Resistant plants should also be tested for their level of tolerance, as resistance combined with tolerance in the same cultivar is the best approach to controlling nematodes. Transferring the gene or genes from Virest or Persia 20, although is easier than that of ryes or triticales, however, as both are non-adapted varieties considerable backcrossing to adapted recurrent parents will be necessary. Assessing the genetics of resistance in F_3 families or double haploid lines is more accurate. Locating gene(s) on

chromosomes and mapping it with known gene(s) will facilitate the screening of resistant plants.

STATEMENT

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

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

Mohammad Farsi

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PUBLISHED PAPERS FROM THIS THESIS

1- Farsi, M., A. J. Rathjen, J. M. Fisher and V. A. Vanstone. 1993. Effects of root lesion nematode (*Pratylenchus* spp.) on the nutrition and growth of wheat. Proceedings, 10th Australian Plant Breeding Conference, Gold Coast, Queensland, April, 1993.

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CHAPTER 1

GENERAL INTRODUCTION

The human population is predicted to reach between eight and ten billion early in the 21st century. The main objective for most scientists working on agricultural plants will be to increase the supply of food during the next 30-40 years, just to keep pace with population growth. Wheat, rice and maize together provide about 75% of calories and half the protein requirements of humankind (Johnson, 1982).

As a leading global food source, wheat will become even more important, underscoring the need to rapidly and continuously increase its production. Greater wheat production can be achieved in two ways : either by expanding the area sown, or by improving the yield per unit of area . As the former is impracticable, increased wheat production must come from higher yields so the requirement of high-yielding varieties with adequate grain quality will remain the principal objectives of wheat breeders. Significantly more wheat could be made available for consumption by reducing the enormous pre and post-harvest losses that occur worldwide. Among these, reducing the losses caused by various species of nematodes will be important.

Wheat is recorded as a host of about 26 species of nematodes belonging to eleven different genera (Goodey *et al.*, 1965). Among these, species of *Heterodera*, *Meloidogyne* and root lesion nematodes (*Pratylenchus* spp.) are prominent.

The degree of damage to a particular crop is influenced by the tolerance of the crop itself, the nematode species, the level of infestation and the favourability of the environmental conditions. Severe damage may occur if the nematode population density is high and the variety sown is intolerant and susceptible to the nematode species present.

On the basis of information from different countries, the genus *Pratylenchus* has about 66 species with a worldwide distribution. Infestation with these pose internationally important nematode problems on a great diversity of plants including alfalfa (Griffin and Gray, 1990), chickpea (Vanstone, 1991), sorghum, maize (Jordaan and Van-Rooyen, 1989), strawberry (Sandoval *et al.*, 1992) barley (Van Gundy *et al.*, 1974) and wheat (Van Gundy *et al.*, 1974; Vanstone, 1991).

The presence of two species of *Pratylenchus* has been reported in the roots of Australian wheat crops, *P. thornei* (Sher and Allen), and *P. neglectus* (Rensch) Filipjev and Schumans Stekhoven (synonym: *P. minyus* Sher and Allen) (Colbran and McCulloch, 1965; Baxter and Blake, 1968; Kimpinski *et al.*, 1976; Vanstone, 1991). The effect of the first of these nematodes was documented on Darling Downs of Queensland in 1981 (Thompson *et al.*, 1981), when the second wheat crop yielded far less than the first. Further studies revealed that the poor second wheat cop was infested with root lesion nematode, and that these also seriously affect wheat crops in Northern New South Wales.

The presence of *P. neglectus* (Vanstone, 1991; J. M. Fisher, pers. comm.) has been reported widely throughout South Australia, affecting cereals, wheat, barley and oats and rotational crops including medic and chickpea. The presence of *P. neglectus* has been recognised in South Australia since 1956 (J. M. Fisher, pers. comm.) and its association with fungi in cereal root disease in South Australia has been reported by Kimpiski (1972), Stynes (1975), Patel (1983) and Vanstone, (1991). Studies by V. A Vanstone at the Waite Agricultural Research Institute clearly identified *P. neglectus* as one of the major contributors to the reduction by pests and diseases in wheat yield in southern Australia (Vanstone, 1991). When examining wheat roots, she identified *P. neglectus* in unrotted portions of root cortices adjacent to the lesioned or rotted areas. It was also noted that roots from non-fumigated areas containing nematodes had poor lateral root development compared to those from fumigated areas where extensive laterals had developed.

Plant reaction varies greatly depending on the species and genotype of both nematode and host. Necrotic cortical reactions to *Pratylenchus* spp. have been proven aseptically (Mountain and Petrick, 1959; Pitcher *et al.*, 1960; Townshend, 1963). The nematode can be a pathogen either by itself or by association with other microorganisms. Enzymes secreted by the nematode, by destroying plant tissue, assist in the infection of plants by other pathogens. The direct effect of *Pratylenchus* involve both physical disruption of the host as well as physiological stress caused by the hydrolytic enzymes produced by the nematode which react with plant materials to produce phenolic substances (Mountain, 1965).

The presence of root lesion nematode is associated with yellow leaf symptoms, which cause a reduction in plant photosynthesis and as a result a lower plant yield. On a global basis yield losses caused by root lesion nematode have been estimated at 13.7% in chickpea, 12% in groundnut, 11.8% in pearl millet, 13.2% in pigeonpea and 6.9% in sorghum (Sasser and Freckman cited by Sharma and McDonald, 1990). The effect of nematodes on wheat yield, investigated by application of nematicides, indicated they can reduce the yield by up to 1.6 t/ha. On the Darling Downs, intolerant wheat varieties lost up to 0.5 t/ha following infection by *P. thornei* (Thompson *et al.*, 1981). The problem becomes more serious when wheat is cultivated as a monoculture so that up to 50% yield losses have been observed in these circumstances (Doyle *et al.*, 1987). Nevertheless the response of all cultivars is not equal and some varieties yield more in nematode-infested paddocks than others, therefore it would appear that selection of tolerant varieties is important in breeding for sowing into nematode-infested soils (Van Gundy *et al.*, 1974).

In nematode-infested soils, well fertilised crops are usually less affected, but leave more nematodes in the soil for the next year (Van Gundy *et al.*, 1974). There are reports from many countries that crop losses from plant parasitic nematodes can be reduced by adding extra fertiliser (Muller and Gooch, 1982). The reduction in uptake of nutrients caused by nematode attack can be compensated for by applying additional fertiliser near to the root zone, so that the remaining roots can absorb more nutrients (Stirling, 1989).

The most promising and most economical method of nematode control is by growing of resistant varieties. Resistant varieties ultimately result in better yields on nematode infested soil as the nematode populations are kept at a low level. If a crop is to be grown in a heavily infested soil, then the choice of a tolerant variety will lessen the economic loss. Unfortunately tolerant varieties, unless resistant, will generally result in large populations remaining in the soil after the crop has been harvested.

Much of the work undertaken with *P. neglectus* in South Australia is being modelled on that with *Heterodera avenae*, the cereal cyst nematode. In South Australia the cereal cyst nematode has been recognised for decades as the most economically important problem on cereals including wheat and barley (Davidson and Se, 1930). The yield loss caused by the nematode has been estimated at up to 50% (Fisher, 1987). By the devoted efforts of plant breeders and nematologists at the Waite Agricultural Research Institute and the South Australian Department of Agriculture (O'Brien and Fisher, 1974; Asiedu *et al.*, 1990) and other workers in Victoria, the problem has now been somewhat reduced. Breeding programs have resulted in the release and widespread cultivation of resistant varieties of wheat (Rathjen *et al.*, 1989 and 1993; Brown and Young, 1982), barley (Sparrow, 1987) and oats (Barr *et al.*, 1988).

While resistance to many root lesion nematodes including *P. neglectus* (Marull *et al.*, 1990; Davis *et al.*, 1983) and *P. thornei* (J. P. Thompson, pers. comm.) has been recorded, there have been few attempts to breed for resistance. No commercial wheat varieties resistant to *P. neglectus* are available in Australia and no sources of resistance are known in *Triticum aestivum*.

Often, there is insufficient resistance in adapted wheats and resistance has to be sought in varieties that are poorly adapted to the area in which the breeding program is being carried out. In the absence of resistant varieties in *T. aestivum*, one of the potential and most favourable sources of resistance for plant breeding are the related species and genera closely

related to the species which the breeder is trying to improve. This area of research has been extensively developed with *T. aestivum* and its relatives.

This thesis reports attempts to reduce yield losses caused by the nematode *P. neglectus* either through the short term solution of applying appropriate fertilisers, or the long term development of resistant varieties.

CHAPTER 2

REVIEW OF LITERATURE

2.1 WHEAT

2.1.1 Importance

Bread wheat (*Triticum aestivum*) is grown across a wide range of environments around the world as it now has the broadest adaptation of any of the cereal crop species. More land is devoted to the production of wheat than to any other commercial crop. Wheat is the primary food grain among the cereals consumed directly by humans, and its production is greater than any other crop, including rice, maize and potato. In the temperate zone, wheat supplies more nourishment for people than any other food source of carbohydrates in the majority of countries (Leonard and Martin, 1963). Wheat and wheat flour are used by a wide range of food and non-food industries.

2.1.2 Distribution and climate requirements

Wheat is grown over a wide range of precipitation and temperature conditions mostly between latitudes 25 and 60° N and 27 and 40° S (Kenet, 1975). However, wheat can be and is grown beyond these limits even in latitudes less than 15° as a cool-season crop (Khalifa *et al.*, 1977). The minimum temperature requirement for growth is about 3 to 4°C, the optimum temperature is about 25°C, and the maximum is about 30 to 32°C (Briggle, 1980). Wheat grows best on well-drained soils from sea level to about 3000 m above sea level. In some tropical countries, wheat is being grown from 2000 to 3200 m and it has been reported at 4270 to 4570 m in Tibet (Percival, 1921). It can be grown in most locations where annual precipitation ranges from 230 to 1750 mm; about three fourths of the land area used for wheat production receives an average of between 375 and 875 mm annually (Leonard and Martin, 1963; Kenet, 1975). While wheat can be grown on residual moisture alone in some areas, the seasonal distribution of precipitation is a critical factor in

many production environments. Especially since the green revolution in the 1960's, increasing areas of wheat have been grown under irrigation.

Agriculture provides a significant proportion of Australia's income with wheat comprising one of Australia's chief export commodities. With annual production of about fourteen million tonnes of wheat, Australia is a major growing region. Wheat is grown in the moderate rainfall areas of New South Wales, Victoria, South Australia, Western Australia and Queensland. It is a crop of major importance to South Australia, which provides around 14% of Australian production. Some parts of Australia including the south west of Western Australia and the southern part of South Australia have a Mediterranean type environment (Gibbon, 1981), characterised by their mild, wet winters and hot, dry summers. Annual rainfall varies considerably and ranges between 275 and 900 mm with more than 65% of annual rain falling during winter (Hamblin *et al.*, 1987; Buddenhagen, 1990). The length of growing season depends on the locality and season and ranges from five to eight months (Prescott and Thomas, 1949).

2.1.3 Production in the future

Efforts to increase wheat production were made especially from the 1960's through the 1980's and most of this success, both in developed and developing countries, is the direct result of investments in wheat research.

In many developing countries, wheat yields can be doubled or tripled by irrigating, controlling weeds and pathogens more effectively and by applying fertiliser and other inputs. The second and longer term avenue for raising yields is through improvement of the wheat plant itself through manipulation of its genetic material.

In the genetic improvement of crop plants, breeders are constantly looking for new sources of germplasm. Cultivated species often lack the genes required by the plant breeder, particularly genes for disease and pest resistance. In many genera, included in *Triticeae,*

which comprise a polyploid complex, related species provide an important source of genes for transfer to cultivated species (Knott and D' vorak, 1976).

There are over 30,000 varieties of the genus *Triticum*, and all can be grouped into three distinct species derived from separate original ancestors differing in chromosome numbers. Common or bread wheat, *T. aestivum*, is a hexaploid (2n = 42) with three genomes, believed to have arisen from the hybridisation of the diploid species with different genomes (A, B and D) each having 14 chromosomes (Kenet, 1975). It is generally believed that these three genomes have been derived from a single ancestral species having seven pairs of chromosomes, so that the genetic contents of these three genomes are similar (Hart, 1987). A number of studies have shown that the chromosomes within these three genomes in wheat not only are related to each other, but also to other species within the *Triticinae*, for example *Secale cereale, Aegilops comosa, Ae. umbellulata* and *Agropyron* (Sears, 1958; Athwal and Kimber, 1972; Shepherd, 1973). Wheat breeders now use these related genera to transfer desirable genes from these wild relatives into wheat (Knott, 1987; Gale and Miller, 1987). Wheat- rye translocation lines carrying the rye arm 1RS have been extensively used in wheat breeding programs around the world, and now many widely-grown and high-yielding wheat cultivars carry one or other of these translocations. Lines carrying the 1BL.1RS translocations have been found to have several agronomically desirable characters including increased disease resistance, broad adaptation, tolerance to stress and high grain yield (Rajarma *et al.*, 1984).

There are, however, several reports that a wide range of problems arise in interspecific hybridisation and interspespecific transfer of genes. Progress is often slow and difficult. The alien chromosome segment should be as small as possible, otherwise the introduction of an alien chromosome segment may result in undesirable duplication and linkage of genes. Many of the cultivars carrying a 1RS translocation produce grain with serious quality defects (Pena *et al.*, 1990) and the removal of the deleterious genes, linked to a desired gene, depends on the homology of donor and recipient genomes. Nevertheless, outstanding examples of successful exploitation of stem rust resistance derived from related species of

bread wheat can be demonstrated (Roelfs, 1988). McIintosh (1991) has published a comprehensive review of alien genetic transfers into wheat from different species of tetraploid wheat, *Agropyron* and *S. cereale* improving the resistance of bread wheat to diseases.

2.1.4 Root characteristics

The root system of wheat consists of primary (seminal) and secondary (crown) roots, both of which produce lateral root branches and root hairs. The initials for the seminal roots are present in the seed before germination. Seminal roots are believed to be temporary structures supplying water and nutrients at the seedling stage and remaining important until about stemming (Sallans, 1942). However it is known that in Australia these roots remain essential, especially for water supply, until maturity (A. J. Rathjen, pers. comm.). Crown roots, which are larger than seminal roots, arise adventitiously from the main stem and tillers. Crown roots are produced throughout the life of the plant and in favourable growing conditions are the main means of water and nutrient uptake (Schuurman and de Boer, 1970).

Roots have a polyarch vascular pattern, with seven to eleven protoxylem ridges. Small roots have a single large central metaxylem element; larger roots a sclerified pith parenchyma. The phloem consists of slender strands of two or four sieve tube elements between adjacent protoxylem ridges. A single layered pericycle just outside of the protoxylem and phloem strands delimits the central cylinder, also called the stele. Pericycle cells become sclerified in older parts of a root (Lersten, 1987). The endodermis, a single layer of cells just external to the pericycle, is the innermost cortical layer. It has thickened inner tangential and radial walls and a thin outer tangential wall, a feature of unknown significance found in varying degrees among grasses.

The root hair contains a giant central vacuole, and the majority of the cytoplasm forms an extremely thin layer next to the wall; the nucleus and a large aggregation of hyaloplasm remain in the tip of the root hair (Mauseth, 1988). Root hairs are tubular extensions of the

root epidermal cells and occur as a result of lateral cell growth. They arise in the region of maturation just beyond the zone of elongation. Their growth can increase the area of the outer surface of the epidermal cell by two to ten times. Root hairs function is to facilitate absorption by the regular epidermal cells; altering the environment immediately adjacent to the root (the rhizosphere) (Weatherley, 1975; Tinker, 1976). Their respiration produces the energy necessary for cation exchange releasing positively charged ions from the negatively charged soil particles. Also, they secrete mucigel, which may be important for changing the surface properties of soil micelles, either by coating them with a humid film directly or by encouraging the growth of microbes that in turn can alter soil properties and mineral availability (Rovira, 1979).

Without root hairs, there would be little direct contact between the root surface and soil surface in either large or small pores. Therefore, root hairs allow exploitation of the numerous soil pores which the root axis itself cannot reach. Nutrients are absorbed much more rapidly by regions with root hairs than without.

The cortex between the exodermis and the endodermis is typically a rather uniform mass of parenchyma tissue rich in starch. Some epidermal cells originating from the cortex generate root hairs, which greatly increase the absorbing surface area (Lersten, 1987). Lateral roots also grow through the cortex and epidermis of the parent root (McCully, 1975). Cortex tissues are able to store some ions such as P and transport them in marginal conditions (Fitter, 1989).

2.1.5 Root function

Nutrient uptake by the plant is a complex process because it is affected by external factors such as soil properties or nutrient availability, by the flux of water through the system and by the organisation within the root. The root operates as a system for absorption of ions from the soil and for supply of ions to the shoot. Absorption is a property primarily associated with the outer cell membranes. Within the root, ions can be accumulated to high concentrations in cell vacuoles or pass in the symplasm into the stele and eventually to the xylem. So, the task of roots is important and critical in determining whether plants have a normal growth rate and satisfactory yield production (Pitman, 1972).

2.1.6 Root growth

Grasses are among a small number of plant families in which the root cap has its own initial, so the cap is sharply distinct from the rest of the root. The root proper has a subapical meristem (Clowes, 1961) in which a central cluster of cells (the irregular, semicircular zone just above the root cap) has an extremely low rate of division. This zone is called the inactive centre or quiescent centre. The tipmost 4.5 mm of each wheat root is the zone of growth, and mitosis is rather uniformly distributed in the distal 2.0 mm except for the quiescent centre (Hejnowicz, 1959).

The epidermis/cortex arises from peripheral cells of the subapical meristem, whereas the central vascular cylinder arises from a central portion. Differentiation occurs gradually away from the meristem. Some epidermal cells then generate root hairs, 170 to 350 μm in length, which greatly increase the absorbing surface area. The short-lived root hairs probably exist for one to three weeks, and are replaced at about the same rate that they die off, so root hairs are maintained as an almost constant zone near the root tip.

Lateral (branch) roots are initiated in the pericycle, opposite a phloem strand and between two protoxylem ridges. These proliferating pericycle cells later form a typical root organisation, make vascular connections with the parent root, and eventually grow through

the cortex and epidermis of the parent root to emerge into the soil (Carson, 1974; Russell, 1977). Roots are able to grow compensatorily, that is, if part of the root system is restricted, the rest will grow faster, or if one root enters a region that is especially favourable, it will greatly increase its growth rate, while the rest slow down (Carson, 1974; 1975; Russell, 1977).

The root epidermis, which is palisade-like near its tip, secretes a firmer mucilage than the root cap. Two chemically different layers have been detected (Miki *et al.*, 1980). Phenolic substances in the outer layer may protect against certain pathogens in the soil. Epidermal cells gradually become flat and tabular and cease to secrete mucilage as the root elongates within the first 2 mm behind the root tip (Miki *et al.*, 1980). Specific substances excreted from the roots attract some nematodes such as cereal cyst nematode and stimulate the eggs to be hatched (Beane and Perry, 1983).

2.1.7 Pests and diseases of the root

Wheat is susceptible to several genera of parasitic nematodes (Butler, 1961). Among these are species of *Heterodera*, *Meloidogyne* and the root lesion nematode (*Pratylenchus* spp.). Histopathological studies of lesions caused by *Pratylenchus* spp. have been made by various authors on several crops (Shafiee and Jenkins, 1963; Pitcher, 1965; Bhatt, 1986; Baxter and Blake, 1968).

2.2 ROOT LESION NEMATODE

2.2.1 Systematics

Root lesion nematodes are microscopic eelworms belonging to the genus *Pratylenchus*, and occur in agricultural soils the world over (Dropkin, 1989). Root lesion nematodes are classified as obligate parasites, since they need a living host to complete their life cycle. There are more than 66 species in the genus *Pratylenchus*, first characterised by Filipjev in 1963 (cited by Loof, 1978).

It has been reported that the roots of wheat are invaded by *P. thornei* (Fortuner, 1977; Clewett and Thompson, 1985), *P. neglectus* (Kimpinski, 1972; Townshend and Anderson, 1976: Vanstone, 1991), *P. crenatus* (Loof, 1978), *P. mediterraneus* (Corbett, 1983), *P. pinguicaudatus* (Corbet, 1970), and *P. zeae* (Colbran and McCulloch, 1965). Direct losses from root lesion nematodes on wheat are incompletely assessed (Wiese, 1987); however, they have been implicated as an important component of root rot complexes (Mountain, 1954; Vanstone, 1991; Taheri *et al.*, 1994).

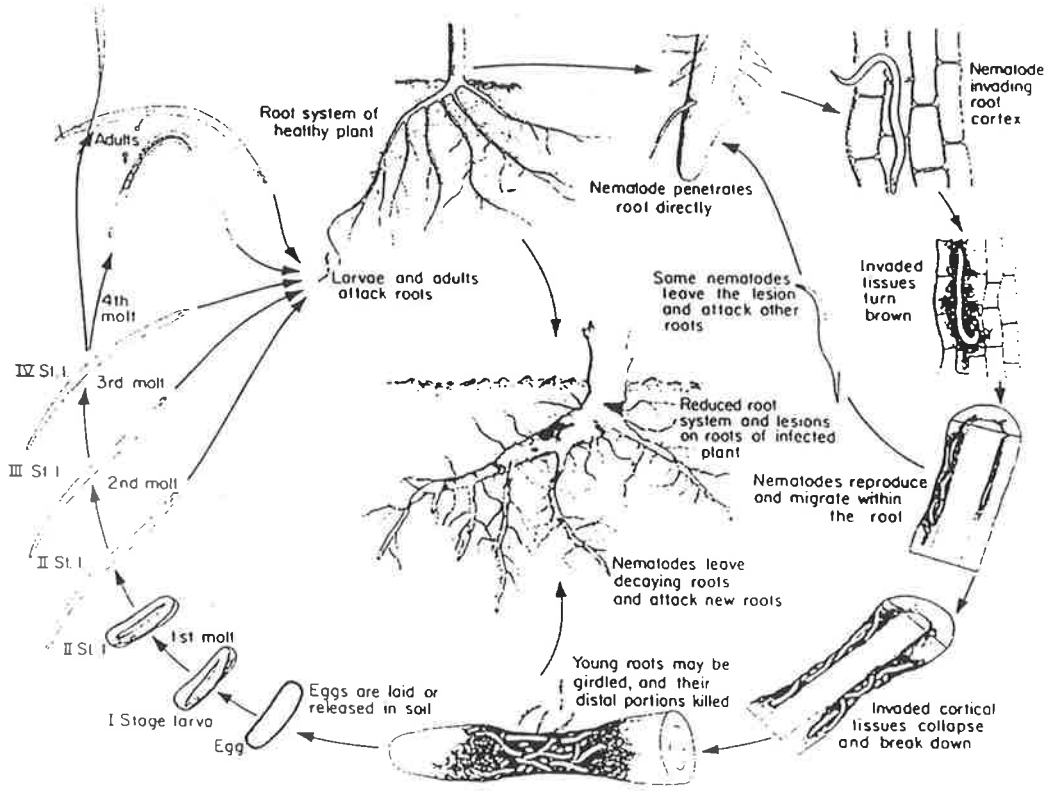
Corbett(1970) describes *P. neglectus* in the following way:
"*P. minyus* (*neglectus*), head with two annules, first annule convex. Lateral field four, five or six lines, inner two lines frequently broken, or may be replaced by oblique striae. Tail usually curving ventrally, with rounded smooth tip. Spear with rounded basal knobs cupped anteriorly. Post vulval sac approximately one vulval body-width long."

2.2.2 Life cycle of root lesion nematode

Lesion nematodes are obligate and migratory endoparasites of plants; that is, they can enter and move within the root tissue as well as leave the infected root to attack and move within other roots (Figure 2,1). The nematodes congregate along the roots, primarily in the region of root hair production, and it is suggested that the nematodes are attracted to the host roots (Jenkins and Taylor, 1976; Zunke, 1990a). The nematode moves between soil and roots

and eggs are deposited within the root or in the soil when female nematodes are outside the root (Dropkin, 1989; Farsi *et al.*, 1994b). Penetration of the root by the nematode is performed by the stylet puncturing the wall of the epidermal cell several times until the wall is torn, after which the nematode pushes its way through the opening (Jenkins and Taylor, 1976). Each infection cavity is usually invaded by several nematodes and they continually enlarge these cavities by feeding on cortical cells (Zunke, 1990; Thompson, in press).

Figure 2.1 Disease cycle of the root lesion nematode *Pratylenchus* sp. (From Agrios, 1988).



Males of *P. neglectus* have been reported, but are rare (Townshend and Anderson, 1976; Taheri, pers. comm.) and are not necessary for reproduction (Corbet, 1970). These nematodes survive in the absence of growing hosts in infected roots or in the soil as eggs, larvae, or adults. In a paddock kept as a weed-free fallow for four years, the numbers of viable *P. thornei* decreased to about one tenth, but still some nematodes could be recovered (Thompson, 1989). As the soil dries, *Pratylenchus* probably becomes anhydrobiotic like other nematode species (Demeure and Freckman, 1981 cited by Thompson, 1989) and has a considerable capacity to survive in the absence of hosts (Townshend and Anderson,

1976). Females lay their eggs singly or in small groups inside infected roots (Figure 2.1). The eggs hatch inside the roots, or in the soil. The first larval stage and the first moult occur in the egg. The emerging second-stage larva enters the roots or moves about in the soil, developing into the subsequent larval stages and the adult (Agrios, 1988).

A *Pratylenchus* generation, depending on the host and temperature, is completed in 20 to 50 days when conditions are favourable for reproduction (Thompson, 1989). At 20°C, *P. neglectus* reproduced after 28 days in wheat (cv. Machete) (Vanstone and Nicol, 1993). The ambient temperature in relation to the thermal developmental threshold of the nematode determines the length of the life cycle. Temperature influences feeding rates and the physiology of host plants, including resistance to plant parasites (Carter, 1982).

The reproductive rate of *P. neglectus* is density dependent. The reproductive index (the ratio of final to initial density) was greater on alfalfa plants at an inoculum level of 1000 nematodes per plant than at 5000 or 10,000 nematodes per plant. The highest reproduction occurred at 30°C with the rate being positively associated with increased temperature (Griffin and Gray, 1990). In an experiment conducted by Griffin (1991) for assessing the pathogenicity of four *P. neglectus* populations on alfalfa, nematode reproductive index decreased as inoculum density increased. A negative linear relationship was found between the number of *P. penetrans* invading the roots and inoculum densities greater than 200 nematodes per plant, while at densities below 200, the percentage entering the roots increased as the inoculum level increased (Mauza and Webster, 1982).

2.2.3 Distribution

The genus *Pratylenchus* has a worldwide distribution (ranging from the temperate zones to the tropical zones) and parasitises a wide variety of field crops.

In an investigation of the distribution of cereal *Pratylenchus* in England, Corbet (1970) reported that *P. neglectus* was commonest and occurred in all soil types. It was

accompanied by *P. thornei* in heavier loam and clay soils. In Australia *P. thornei* is also associated with heavy soils (Thompson, 1989). Grandison (1972) also reported a high number of *P. thornei* in clay soils in the Adelaide Metropolitan area.

P. neglectus has been reported on alfalfa roots in North America (Griffin and Gray, 1990), on maize (*Zeae. mays* L.) in the Republic of South Africa (Jordaan and Van-Rooyen, 1989), and in the Ferrara area of Emilia-Romagna in Italy (Tacconi *et al.*, 1988), on wild cereals in the Karakum mountains, Tyakursk territory of the Turkmen USSR (Shagalina *et al.*, 1988), on strawberry (Sandoval Hermandez *et al.*, 1992) and wheat (Van Gundy, 1974) in Mexico and in Finland (Kurppa, 1988) and in Australia (J. M. Fisher, pers. comm.; de Beer, 1965; Kimpinski, 1972; Kimpinski *et al.*, 1976; Vanstone, 1991).

Larso (cited by Van Gundy *et al.*, 1974) stated that the preferred hosts of *P. thornei*, another species of root lesion nematode which is widespread in Australia, were wheat, *Agrostis* sp., *Trifolium repens* (clover), and *Crotalaria juncea*. Other good hosts included *Medicago hispida* (alfalfa), *T. resupinatum*, *T. subterraneum*, *Hordeum vulgare* (barley), *Avena sativa* (oats) and *Secale cereale* (rye). Moderate hosts included *Z. mays* (corn), *M. falcata*, *Glycine max* (soybean) and *Vicia atropurpurea*. Poor or non-hosts included *Lotus corniculutus*, *T. pratense*, *Fragaria californica*, *Phlox drummondi*, *Helianthus* sp., *Nicotiana* sp., *Beta vulgaris*, *Allium cepa* and *Citrullus vulgaris*. Greco *et al.* (1988) stated that chickpea, faba bean, lentil, annual medic, pea and vetch are hosts of *P. thornei* with very high nematode infestations found in their roots.

P. thornei has been found at a depth of up to 120 cm in Queensland, with greatest numbers often at 30-60 cm, particularly after fallow (Doyle *et al.*, 1987). The nematode population is generally higher in the surface 30 cm than at 30-60 cm depth, while the populations of some species such as *P. hamatus* were detectable even at 120 cm deep with highest populations in the surface 60 cm of the soil profile (McKenry, 1989a). In South Australian soil, the nematodes *P. thornei* and *P. neglectus* were found mostly in the top 10 cm with few below 30 cm depth (S. P. Taylor, pers. comm.).

2.2.4 Penetration

Soil physical factors are important in determining migration and penetration of nematodes (Van Gundy and Stolzy, 1963). Free soil moisture, a characteristic of soil texture, has an important role in nematode movement. Nematodes move in the water films around soil particles. Lack of water immobilizes them and reduces their ability to find the roots of host plants (Kimpinski, 1972; Vanstone and Nicol, 1993). The ability of nematodes to survive lack of water seems to be higher in heavier than lighter soils, but root penetration of *P. neglectus* is greater in sandy soils than in heavier soils under normal conditions (Vanstone and Nicol, 1993). Wallace (1968) suggested that the optimum movement of nematodes occurs when the soil particle size is one-third the length of the nematode which makes sand a more suitable medium than clay.

Some investigators believe that root necrosis of host cells is caused by simple mechanical injury. However, Baxter and Blake (1968) found no reproduction of nematodes in the presence of necrosis, so they concluded a host-response phenomenon is necessary to cause necrosis in addition to mechanical and chemical effects of *Pratylenchus* spp. In studies carried out by Bhatt (1986) under aseptic conditions, when nematode and fungus were inoculated together at the early seedling phase, it was observed that *P. thornei* preferred maturing tissues for penetration more than growing points or root apices. The nematodes moved into the cortex where they fed and reproduced. Necrosis of cortical cells followed the path of the nematodes, but discolouration, granulated cytoplasm and hypertrophied nuclei could be observed in the adjacent cells. Usually two to three cell layers on each side of the nematode tunnels were affected, but sometimes the lesion involved over half the circumference of the root. As feeding of the nematode continued through cortical tissues, cell walls broke down and cavities appeared in the cortex with their walls lined with brown deposits.

Many nematodes enter at the first lesion (Zunke, 1990a). The females lay eggs in the cortical tissues and frequently eggs, juveniles and adults congregate in the cortex. After hatching from eggs, the nematodes feed on parenchymatous cells and move within the cortex enlarging the lesion. Some nematodes leave the lesion and emerge from the root causing new infection points on the same or other roots. The large necrotic lesions in the cortex are readily invaded by fungi and bacteria which cause rotting and break down of the root tissues around the point of infection and subsequent death of the distal part of the root. The above ground part of the plant becomes stunted and chlorotic, showing symptoms of water and nutrient deficiencies (Mountain, 1954; Pitcher, 1965).

All plant-parasitic nematodes are armed with a stylet capable of penetrating or rupturing plant cells or, perhaps, of forcing a passage between them (Mountain, 1954 and 1961). All wound in some degree, either by a simple micro-puncture or by rupturing or separating cells. They may thereby either introduce a pathogen on their body or aid a pathogen already present on the plant surface. For instance, *P. penetrans* aids the penetration of root rotting fungi of peach (Mountain and Patrick, 1959) and strawberry (Morgan and McAllan, 1962). All nematodes must modify the host in order to derive nourishment from it. They must first wound it and then, usually, use their salivary secretions to pre-digest or otherwise prepare the cell contents for ingestion. The preparatory process may cause necrosis, as with *P. penetrans* (Mountain, 1954) or may have no apparent effect as in *P. neglectus* (Pitcher, 1965). But, the necrosis might be a function of the host reaction rather than any difference between the nematode species (A. J. Rathjen, pers. comm.).

When Egyptian clover (*T. alexandrinum*) seedlings were inoculated with *P. penetrans* in a pot experiment, the dense root-hair zone was the preferred zone of penetration of females, males and third-stage larvae. An elliptical yellow-coloured lesion developed as nematodes penetrated the epidermis and fed in the cortex. Dark brown cells appeared in the centre of the lesion. At 20°C, females penetrated the root earlier and in greater numbers than males or third-stage larvae. Penetration was inversely proportional to tissue age (Abdel-Hadi and Ghorab, 1987).

Baxter and Blake (1968) observed *Pratylenchus* in the cortex of wheat lying parallel to the long axis of the root. First the nematodes withdrew the cell cytoplasm, and consequently the cell walls disintegrated and cavities formed in the cortex. The nematodes preferred to invade roots where there was already an opening caused by nematodes or other microorganisms. The cavities were enlarged continually by nematodes feeding on peripheral cells and the cells adjacent to cavities showed necrosis. When the pathogenecity of *P. thornei* was examined in the absence of other microorganisms, it was observed the nematode could invade wheat roots and destroy parenchyma cells to form cavities in the cortex.

While some investigators believe that *Pratylenchus* do not feed on root hairs (Kurpa and Vrain, 1985), others have observed *P. penetrans* feeding on root hairs of various host plants (Zunke, 1990b). When Zunke (1990b) inoculated rape seedlings (*Brasica napus* cv. Akela), oil radish (*Raphamus sativus* var. oleiformis), tobacco (*Nicotiana tabacum* cv. Samaun) and potatoes (*Solanum tuberosum* cv. Hansa) grown in aseptic nutrient agar, he observed that the majority of nematodes moved directly to the root hair region in all species. After the nematodes made contact with the root hairs, they started rubbing the root hair surfaces with the lip region and then probing it with the stylet. The stylet was inserted slowly into the root hair with increasingly deeper thrusts. After the stylet was finally inserted to a length of approximately 2 µm, the nematode salivated for a few minutes, and then food was taken up by nematode pumping (Zunke, 1990b).

Although there is evidence that *Pratylenchus* spp. do not enter vascular tissues (Krusberg, 1963; Kimpinski, 1972), contrasting evidence was obtained in an experiment carried out by Khan (1992) on *P. brachyurus* in some vegetable crops. Histopathological examination of serial sections of roots of susceptible plants revealed that penetration and multiplication of the nematode was not confined to the cortex alone, but extensive damage was found in the stellar region with production of cavities in the phloem parenchyma and xylem vessels.

2.2.5 Chemical exudates of nematodes

Plant-nematode biochemical interactions are primarily responsible for plant injury, and mechanical damage and withdrawal of food from plants by nematodes is generally significant. Chemicals secreted or excreted by nematodes into plants are certainly involved in symptom production, and presumably impair overall physiology of plants (Krusberg, 1963). Root lesioning is a result of interactions between the feeding enzymes excreted by the nematode and host materials. However, the degree of necrosis may not be directly related to the amount of plant damage (Mountain and Patrick, 1959; Baxter and Blake, 1968).

In an experiment carried out by Mountain and Patrick (1959), it was observed that the death of peach root cells occurs rapidly and apparently in advance of the penetrated area. They suggested that amygdalin is hydrolysed in the invaded root portions by *P. penetrans* which results in the release of HCN and benzaldehyde, toxic in peach root tissue. The hydrolysis of amygdalin is performed by β-glucosidase secreted by *P. penetrans* (Mountain and Patrick, 1959). Krusberg (1963) showed that when cut stem ends were placed in *Pratylenchus*-infected tissue extracts, the healthy leaves of the same plant became curled and discoloured.

2.2.6 Symptoms

As with most soil-borne pathogens, root lesion nematodes can cause both acute and chronic damage to wheat. Acute symptoms include premature senescence, plant death, yellowing of lower leaves, and stunting (Kotcon *et al.*, 1985; Thompson, 1989), and in combination with other diseases (especially *Rhizoctonia* root rot), can result in large diseased patches within fields. Plants wilt prematurely under moisture stress, and roots show varying degrees of browning and necrosis (Thompson, 1989). Less pronounced damage, such as poor tillering, may go unnoticed, although forage or grain yields may be reduced. Symptoms of root lesion nematode appear as areas of poor growth in fields where wheat or other host plants have been grown for many years. The most general symptom of nematode disease is

a reduction in the growth rate as compared with that of a healthy plant (Pitcher, 1965). In all cases, examination of the roots for necrotic lesions, nematodes, and eggs inside roots or extraction of nematodes from root tissue is necessary for diagnosis.

Symptoms of wheat decline in soil infested with *Pratylenchus* usually appear after tillering as patchy, yellowish areas. Seldom is an entire field uniformly affected. Sometimes, young plants die and the stand is reduced, but more often tillering is reduced and only one head is produced instead of two to four per plant. Head size is sometimes reduced due to nematode infection. Nematode attack starts in the primary roots, causing the plant to be stunted and vulnerable to attack by soil fungi (Van Gundy *et al.*, 1974).

In an experiment carried out by Sher (1957) to understand the effects of *P. vulnus* on roses, plants in the treated soil series made better growth than did those in the control series throughout the experiment, and there was a very marked difference at the end of the experiment. Chlorosis of leaves began to appear in the control plants just before the first cutting and it became more severe as the experiment progressed, and at the termination of the experiment many plants in the untreated soil appeared to be dying. Plants grown in infested field soil had small root systems that appeared necrotic and devoid of feeder roots.

Pratylenchus spp. characteristically cause discoloured lesions on roots of tobacco and apple, which may girdle roots under heavy infestation, resulting in sloughing of the cortex (Krusberg, 1963). Examination of roots from infected trees showed that many feeder roots had been killed by *P. pratensis*. Large lesions or cankers were found on infected roots (Serr and Day, 1949). *P. neglectus* (Mountain, 1954) and *P. vulnus* (Lownsbery, 1956) in the absence of other organisms produced lesions on tobacco and walnut, respectively. Reduction of secondary roots and changing colour from yellow to black are the symptoms of root lesion nematode on walnut (Lownsbery, 1956). In an experiment carried out by Gao and Cheng (1992), it was observed that direct penetration and infection by *P. scribneri* in maize roots resulted in destruction of the cortex and necrosis of the roots.

P. penetrans reduced the growth of peach seedlings under otherwise optimum growing conditions for the host and the extent of stunting was associated with the size of the nematode population. Infested roots of peach seedlings showed discoloured and necrotic regions, while uninfected roots were white and apparently healthy. The cortical cells around the invaded area were definitely necrotic and contained shrunken, granular cytoplasm. *P. penetrans* can invade, colonise, and kill peach root tissue in the absence of other organisms (Mountain and Patrick, 1959).

Reduction in the growth rate of infected as compared with healthy plants is the most universal symptom of nematode disease but, paradoxically, light infestations may apparently stimulate growth, usually of the roots (Coursen and Jenkins, 1958; Peters, 1961). Stimulation of callus growth has been recorded in aseptic culture (Sandstedt and Schuster, 1963). Nematodes modify the plant substrates in two ways:

(1) they induce the production of metabolites favourable to the pathogen, which are either scarce or absent in healthy tissues;

(2) the nematodes destroy certain chemicals antagonistic to the pathogen or obstruct defence reactions by means of which the healthy plant would otherwise repel it (Pitcher, 1965).

2.3 EFFECTS OF NEMATODES ON PLANTS

2.3.1 Water absorption

Most plant nematode species attack the below ground portions of plants and some particularly disrupt root tissues and hamper water transport. Altered water transport could also affect mineral uptake and transport by roots. By invading the plant root cortex and limiting root development, nematodes may induce drought stress in the host, a factor which is thought to influence development of some plant diseases (Riedel, 1988).

Thompson *et al.* (1980a) showed that crops affected with *P. thornei* wilt prematurely in dry periods. There is evidence that *P. thornei* reduces yield of crops grown in relatively low moisture more than those grown in high moisture soils (Baxter and Blake, 1968). Bhatt (1986) noted that chickpea infected with *P. thornei* showed signs of water stress. Flowering in the inoculated plants also occurred later as compared to healthy plants. He concluded that the migratory nematodes interfered with normal water uptake and thereby the transport of synthesised hormones from the root to aerial plant parts, adversely affecting growth and development of the plant and delaying the onset of flowering.

Colonisation of the wheat root cortex by nematodes and reduction in root capability to absorb water and, in turn, nutrients from the soil induce plant stresses such as water and nutrient deficiency. Controlling the nematode population by any method would increase water use efficiency of plant roots and would reduce water stress (Amir *et al.*, 1985, cited by J. M. Nicol, 1991). Although there may be little or no reduction in yield where water is in adequate supply, it may be suggested that the main effect of nematodes on plants is in destroying the root system and reducing water absorption (Orion *et al.*, 1984).

O'Banon and Reynolds (1965), working on cotton plants infested with *Meloidogyne* spp., showed that where water was freely available through irrigation, growth response in heavily nematode infected plants was as great or greater than those of uninfected plants. Infected plants transpired as much or more water than uninfected plants. But, in conditions where

moisture content was brought to field capacity at intervals, infected roots did not develop normally, and growth was significantly reduced. This result suggests that the reduction in growth of nematode infected plants in comparison with uninfected plants is caused by limiting water absorbing efficiency, which in turn could affect the nutritional status of the plant.

To have satisfactory plant production on soils infected with root lesion nematodes, soil should be maintained close to field capacity, not only to compensate for the reduced water absorption, but also because nematode reproduction may be higher in soils with low moisture. In an experiment carried out by Orion *et al.* (1984), the highest population of *P. thornei* was observed in drought conditions and the lowest in moist conditions. Nematode population in the auxiliary irrigation treatments were extremely low. They concluded that low moisture level is a major ecological factor required for *P. thornei* build up.

2.3.2 Nutrient uptake

Root feeding nematodes impair the ability of plants to take up water and nutrients from soil, thereby causing symptoms of deficiency of these materials in the shoots. Affected crops become inefficient in uptake of nutrients like nitrogen (Thompson, 1987b) and phosphorus (Evans *et al.*, 1975), accounting for the chlorotic appearance. It is suggested that mineral deficiencies in plant tissues could be a major component of the expression of nematode disease. Alteration of the nutritional content of root tissues by nematode feeding is an important physiological mechanism influencing fungal colonisation of the host (Emamanouil and Wood, 1981).

The relationship between nematode infection and internal nutrition of plants has been demonstrated by several authors (Chitwood and Oteifa, 1952; Hunter, 1958; Nasr *et al.*, 1980; Thompson, 1987b) but there has been no consistent agreement as to the effect of nematode damage on absorption of nutrients by plants.

There are reports from many countries that crop losses from plant parasitic nematodes can be reduced by adding fertiliser to the soil (Muller and Gooch, 1982; de Beer, 1965). Reduction of the absorption rate caused by nematode attack can be compensated for by applying fertiliser close to the root zone, so that the healthy roots can absorb sufficient or more nutrients than they would in a nematode free situation (Stirling, 1989). However, even then, Thompson *et al.* (1981) showed that *P. thornei* could result in an average wheat yield reduction of 0.5 t/ha. A further complication is that fertilised crops could leave more nematodes in the soil for the next year (Thompson *et al.*, 1981).

Absorption and transport of nutrient ions and water by the roots of intact plants are complex cell processes which depend on both the plant's inherent physiology and the nutrient's availability to the roots (Trudgill *et al.*, 1975). This latter consideration is mostly a consequence of soil environmental conditions.

The composition and activity of microorganisms in the rhizosphere can also be influenced by activity of plant-parasitic nematodes on the nature of root exudates. Root exudates have a major effect on the rhizosphere community providing energy for host penetration or enhancing sclerotial formation (Van Gundy *et al.*, 1977; Riedel, 1988).

Nutritional changes in the host could be a mechanism by which susceptibility to some fungi in crops is induced by *Pratylenchus* spp. Dennis *et al.* (1989) showed that decreased cation concentration was a good indication for the number of nematodes present in the roots, but this was not true for all genotypes.

Viglierchio (1987), investigating the elemental distribution in tissues of plants heavily infected with nematodes, came to the conclusion that elemental content of plant tissues depends on host plant species, the species of infecting nematodes, and the soil environment conditions. Inorganic fertilisers, soil type, soil pH, salinity, cation exchange capacity, oxygen concentration, and the dominant vegetation are also important (Ferris *et al.*, 1971; Johnson and Burton, 1973). Depending upon the optimum levels for growth of a particular

plant species, addition of minor elements or selected major elements to infected fields could improve growth.

The invasion of roots by migratory nematodes imposes a demand for increased nutrition to compensate for the maintenance of integrated root and shoot growth. Thus, the adverse effects of nematodes will be observed if their development interferes with root growth and overcomes the development potential of the root (Bhatt, 1986).

Nitrogen

In an experiment carried out by Van Gundy *et al.* (1974), with application of nitrogen to the soil, the grain yield of wheat in plots treated with nematicide was similar to plants in nematode infested plots. Nitrogen application gave significant increases in grain yield on both fumigated and non-fumigated plots. They concluded that adding nitrogen can compensate for yield losses caused by nematodes. In other words, nematode attack causes nitrogen deficiency.

Thompson (1987b) showed that *P. thornei* restricts nitrogen absorption by plants, as plants under nematicide treatment took up more N than did fertilised plants without nematicide. He concluded that since N mineralisation in the soil was not affected by the nematicide aldicarb, improved N uptake in wheat should be due solely to its nematicidal action. He concluded that N cycling and N experimentation in soils under wheat culture can be influenced by the presence of root lesion nematodes.

In contrast, bitter almond roots infected by root-knot nematode species contained significantly greater concentrations of N than the un-infested controls (Nasr *et al.*, 1980). Shafiee and Jenkins (1963), working on tomato roots infested by *M. incognita*, reported that N concentration was significantly increased. On the other hand, Hunter (1958) found that the concentration of N was unaffected. It has been shown by some investigators that

increasing the number of nematodes decreased the percentage of N only a little (Trudgill *et al.*, 1975).

Mian and Rodriguez-Kabana (1982), and Rodriguez-Kabana (1986) reported that the nematicidal effect of fertiliser was partly due to the production of ammonia and that their effectiveness was therefore related to N content and form.

Phosphorus

Early reports noted changes in the major nutritional elements such as phosphorus in the roots and, occasionally, in the shoots resulted from nematode infection. In sugar beet and tomato, P levels were augmented modestly by infection with certain nematodes, but not by others, whereas P levels in the roots of Brussels sprouts showed a greater augmentation by all nematode infestations (Viglierchio, 1987). Krusberg (1963) reasoned that an increase in phosphorus and organic compounds would occur due to increased synthetic activity and movement of substances to the diseased area.

Hunter (1958), working on tomato, observed no difference in the total P content of the tops of infected and uninfected plants, while the amount of P in the roots of non-infected plants was less than those in infected plants. He showed that after absorption took place, there was no apparent inhibition of translocation of P^{32} due to nematode infection. There is also evidence that *P. vulnus* did not have any important effect on the leaf content of phosphorus in roses (Sher, 1957).

On the other hand, Evans *et al.* (1975) showed that invasion of potato roots by potato cyst nematodes (*H. rostochiensis* and *H. pallida*) decreased P concentration in the haulm.

Potassium

Infected lima beans had a lower total amount of K compared to control plants (Otiefa, 1952). The amount of damage caused by nematodes was correlated with the amount of K available,

and that an increase in the application of K fertiliser compensated for the restriction of growth caused by the nematodes, whereas this treatment gave no response in non-infected plants. *P. vulnus* caused K deficiency in infested rose plants (Sher, 1957). Evans *et al.* (1975), showed that invasion of potato roots by larvae of potato cyst nematodes (*H. rostochiensis* and *H. pallida*) decreased K concentration in the haulm.

In an experiment carried out by Lownsbery (1956), it was observed that K content of the seedling leaves of plants in nematode-infested soil was less than those grown in air-dried, nematode-free soil. Low potassium content of plants infested with nematodes has been reported (Tarjan, 1950; Oteifa, 1952), and it may be that some of the plant symptoms result from potassium deficiency brought about by either the nematode's use of the element, or the effect of nematodes on potassium-absorbing capacity of roots, or both. The efficiency of potassium uptake is decreased by nematode infestation (Trudgill *et al.*, 1975). The percentage of K was decreased greatly by increasing numbers of nematodes but the different varieties showed different reaction to different nematode species. Potassium content of leaves from the untreated nematode-infested soil was less than that of leaves from air-dried nematode-free soil. The decrease in K concentration is probably the major factor limiting haulm weight, leaf expansion and final leaf area, so that must have had a major effect on the tuber yield of potato. However, other factors besides K might be important in influencing the rate of senescence of plants and hence also the tuber yield.

In contrast, Hunter (1958) found no difference in K content of tomato plant tops due to *M. incognita* infection, while all the infected plants showed somewhat higher K in their roots. Bitter almond roots infected by root-Knot nematode species contained a significantly greater concentration of K than the un-infested controls. Shafiee and Jenkins (1963), working with tomato roots infested by *M. incognita*, reported that the K concentration was significantly increased. Concentration of K was greater in the leaves of nematode-infested almond plants than in the controls (Nasr *et al.*, 1980). Chitwood and Oteifa (1952) found an increase in the K concentration in leaves of Lovell peach due to nematode infection.

Calcium

Infected lima beans had lower total Ca compared to the control plants (Otiefa, 1952). Shafiee and Jenkins (1963) also reported lower concentration of Ca in capsicum roots infested with *P. penetrans*. In contrast, *P. vulnus* increased the level of Ca in the leaves of infested compared to un-infested plants (Sher, 1957). Bitter almond roots infected by a root knot nematode species also contained a greater Ca concentration than un-infested controls. The concentration of Ca was greater in the leaves of nematode-infested almond plants than in the controls (Nasr *et al.*, 1980). Chitwood and Oteifa (1952) found an increase in the Ca concentration in the leaves of Lovell peach infected by *Meloidogyne* spp.

Magnesium

Infected lima beans contained lower total Mg as compared to control plants (Otiefa, 1952). Viglierchio (1987), working on the effect of nematodes on sugar beet, tomato and Brussel sprouts, found that Mg levels in roots were lower in infected plants regardless of the plant and nematode species, while Nasr *et al.* (1980) found no changes in Mg concentrations in almond roots. There are also reports that an increasing number of nematodes decreased the percentage of Mg only a little and that the percentage of Mg in Maris Piper infested with many *H. pallida* was less affected than in plants with many *H. rostochiensis* (Trudgill *et al.*, 1975).

In contrast, Sher (1957) observed that *P. vulnus* increased the level of Mg in the leaves of infested plants compared to un-infested plants. Hunter (1958) found no significant difference in Mg content of tomato tops due to *M. incognita* infection, but while infected roots had a significantly higher Mg content than non-infected roots, increasing nematode numbers had no further effect on Mg content.

Manganese

Leaf content of manganese was the same for both plants infected with *P. vulnus* and uninfected plants (Sher, 1957). Bitter almond roots infected by root knot nematode species contained a significantly greater concentration of Mn than the uninfested controls. Owens and Novotny (1960) found Mn in almost equal concentrations in galled and healthy roots of tomato and cucumber. Concentration of Mn was greater in the leaves of nematode-infested almond plants than in the controls (Nasr *et al.*, 1980). The contrary effect was evident with low root levels of Mn in nearly all nematode infested sugar beets, Brussel sprouts and tomatoes (Viglierchio, 1987).

Zinc

The level of zinc in leaves of infested plants was higher than those of uninfested plants (Sher, 1957). Nasr *et al.* (1980) found no changes occurred in Zn concentrations in almond roots due to nematode infection.

Iron

P. vulnus caused iron deficiency in infested plants (Sher, 1957). Root level of Fe was increased by some nematode infections in sugar beets, whereas Fe was depressed by nematode infection in tomato (Viglierchio, 1987). The Fe concentration in roots was significantly less in plants infested by both *M. javanica* and *M. incognita* than the control plants (Chitwood and Oteifa, 1952).

Sodium

The leaf content of sodium was the same for both *P. vulnus* infected plants and uninfected plants (Sher, 1957). Concentration of Na in the haulm dry matter of potatoes increased greatly as the number of nematodes increased, suggesting that this element was taken up to compensate for the decrease in K, in order to maintain the cation concentration (Van Gundy and Martin, 1961; Jenkins and Malek, 1966).

2.3.3 Nodulation

Plants of the family Leguminoseae are able to fix atmospheric nitrogen in nodules on their roots. Nematodes can affect the nodulation of plants and therefore nitrogen fixation. Ali and Trabulsi (1981) showed reduced nodulation in the presence of *M. incognita* on cowpea and Singh and Redy (1981) on French bean. *P. penetrans* reduced nodule number and size, and decreased nitrogenase activity. Nitrogenase activity was significantly reduced in susceptible clones of alfalfa (Basigalup *et al.*, 1988). An average reduction of 50% in nodule number by *P. neglectus* on medics has been reported in South Australia, but the size of individual nodules remained unaffected (Vanstone *et al.*, 1993a).

There are, however, reports that *P. thornei* did not affect nodulation on peas (Green and Hornsey, 1981) and white clover (Taka and Raski, 1969). There are also reports of positive effects of nematodes on nodulation. Townshend (1984) showed that *Pratylenchus* spp., *M. hapla* and *H. dihystera* increased nodulation when the nematode populations increased. However, it is believed that since root lesion nematodes feed on root hairs, and root hairs are the point of entry of the infective threads from rhizobia, the reports of positive effects of root lesion nematode on nodulation seem unlikely to be a common response (A. J. Rathjen, pers.comm.).

2.3.4 Root growth

Dry root weights of apple seedlings were reduced when the soil was inoculated with *P. penetrans*. Plant height, root weight and root score were significantly reduced when *P. penetrans* plus *Bacillus subtilis* or *P. penetrans* plus fungi and bacteria were present in the soil (Utkhede *et al.*, 1992). Magistad and Olivera (1934) showed that the average total length and the number of roots were decreased by the nematode. Trudgill *et al.* (1975) also showed a reduction in root weight and shoot weight/root weight ratio by nematode infestation. Peterson (cited by Edmunds and Mai, 1966) stated that while the presence of *P. penetrans* significantly increased shoot weight of alfalfa, it had no effect on root weight.

Plants infected with *P. penetrans* had lower top weight but higher root weight than those of non-infected plants. Stimulation and proliferation of roots resulting from infection by nematodes has been described as the "mossy root" phenomenon (Mountain and Boyce, 1958). A positive correlation was found between dry root weight of peach and the number of nematodes present in the roots (Potter *et al.*, 1984).

2.3.5 Top growth

The effect of nematode attack on the plant depends on the initial population of nematodes in the soil (Mauza and Webster, 1982). *P. neglectus* at 1000 nematodes per plant did not have any substantial effect on shoot growth of any cultivar of alfalfa tested in the greenhouse, but when the inoculum density was increased to 5000 or 10,000 per plant, shoot growth of all three cultivars of alfalfa was significantly reduced. Root growth of alfalfa was affected similarly to shoot growth (Griffin and Gray, 1990). Top and root growth of alfalfa was also retarded by *P. penetrans* (Mauza and Webster, 1982).

Tomato infested with *P. scribneri* was used as a model to examine growth and carbohydrate partitioning. Root and shoot growth were reduced by nematode infection. Total carbohydrates per unit leaf area and per gram of root tissue was lower in the inoculated plants. Regression coefficients for the root/shoot ratio and for carbohydrates were highly significant (Anwar and Azad, 1988).

Paradoxically, stimulated growth may be caused by a light infestation of nematodes (Pitcher, 1965). This might be due to production of more root hairs in order to compensate for the damage caused by nematodes or by other pathogens.

2.3.6 Yield

Yield reduction in *Pratylenchus* infected plants is the final manifestation of a wide range of interacting physiological processes. Disruption in water absorption and changes in mineral

content due to nematode damage are factors mainly affecting photosynthesis and in turn dry matter and yield. An 85% loss in yield from *Pratylenchus* spp. in a sensitive wheat variety has been recorded in Queensland (Thompson and Clewett, 1988). Substantial increases in wheat yield, for example 2t/ha, have been obtained by treatment with nematicide, especially Temik® (aldicarb), before or at planting (Thompson *et al.*, 1980c). The reduction of wheat yields by *Pratylenchus* has been estimated by Orion *et al.* (1984) and Amir *et al.* (1985), (cited by Nicol, 1991) as 50-70% and 40-90%, respectively. The severe disease caused by *Pratylenchus* spp. reduced yield of wheat by 30 to 50% (Huang *et al.*, 1990).

A significant negative correlation was observed between the haulm fresh weight of potato and the log number of nematodes per gram of root, and leaf area was also negatively affected by nematodes (Trudgill *et al.*, 1975). However, there is a report that the tuber yield of potatoes is hardly affected by *P. neglectus* when it is present in the soil alone (Scholte and s'Jacob, 1989). A reduction in potato tuber yield of 27 to 46% has been reported (Olthof, 1989a; Orion and Shlevin, 1989). Shafiee and Jenkins (1963) showed that in pepper plants infected by *P. penetrans*, the difference in weights of both tops and roots between infected and uninfected plants was significant.

There are several records, however, where nematode infection has apparently stimulated plant growth. Coursen and Jenkins (1958) showed that in tall fescue infected with *P. projectus*, the average number of tillers produced on plants inoculated with 100, 5000 or 10,000 nematodes was 17, 16 and 29 compared with 13 on the uninfected controls (Apt and Koike, 1962). A stimulatory effect was observed on the dry weight accumulation of peppermint inoculated with *P. thornei* (Faulkner and Skotland, 1964). At rates of 40, 200 or 1000 nematodes per pot, the dry weights of inoculated plants exceeded the control by 17, 25 and 23%, respectively, after 104 days. When 5000 nematodes were used as the inoculum, plant dry weights were reduced by 21% after the same period. The percentage of dry matter in the haulms of potato was increased by nematodes from 8 to 11%, and this

increase was accompanied by a corresponding decrease in percentage ash in the haulm dry matter (Trudgill *et al.*, 1975).

However, Sundarababu and Vadivelu (1990), working on the pathogenic effect of *Pratylenchus*, *Helicotylenchus* and *Haplolaimus* on *Jasminum sambac,* reported that plant growth was reduced by all three nematodes at 100 nematodes/pot or more. Inoculating alfalfa with nematodes resulted in about a 25% increase in shoot weight compared with the non-inoculated controls (Petersen *et al.*, 1991). An increase in plant weight of celery due to *P. penetrans* infection has been reported (Edmunds and Mai, 1966) and a similar increase in Kentucky bluegrass has been recorded (Troll and Rohde, 1966). It is suggested that certain nematodes stimulate plants to produce higher than normal concentrations of growth regulators in plant tissues, and this can cause alterations in the dry matter distribution between plants and roots (Dropkin, 1989).

2.4 ASSOCIATION OF NEMATODES WITH OTHER MICROORGANISMS

Plant response to other microorganisms or pathogens may be influenced by the plant reaction to nematode injury. The plant reaction depends on species and strain of both nematode and host.

Plant parasitic nematodes promote the ingress of soil-borne plant pathogens into their host plants. Nematode vectors of viruses serve dual functions of disseminating viral particles and inserting viral particles into host cells. Bacterial plant pathogens require natural openings or wounds for ingress into their host. Wounds occurring during the colonisation of cortical root tissues by nematodes have been suggested as the mechanism explaining nematode-fungus interactions in root-rot disease. The interaction between the host, the parasite and the environment is complex and constantly changing. The relative influence of each component on the others is difficult to assess because of their interwoven nature and our lack of technology to monitor such influences accurately. Effectiveness of control strategies for crop pests can be enhanced through a better understanding of these relationships.

Infection by fungi is promoted by nematodes which assist fungi to evade the natural defences of the root by opening invasion channels (Perry and Evert, 1983; Storey and Evans, 1987). In an experiment conducted by McGuidin and Rouce (1990) it was observed that the combination of *P. penetrans* and *Verticillium dahliae* reduced the weight of potato tubers, whereas the nematode or fungus alone had no effect on yield. Microplots infested with both the nematode and a low level of *V. dahliae* resulted in 14% less yield than microplots infested with a low level of *V. dahliae* alone. *P. penetrans* alone at 24-79 nematodes per 100 cm^3 of soil did not affect the intensity of the early dying symptom expression compared with the control in potatoes. Symptoms were greatest when both pathogens were present together. They concluded that combined effect of *P. penetrans* and *V. dahliae* on the symptom expression of early dying and yield of Russet Burbank potato was synergistic.

Fungal spores adhere to *Pratylenchus* spp. In an experiment conducted by Faulkner *et al.* (1970) it appeared that the role of *P. neglectus* in the fungus-nematode complex associated with peppermint wilt was not limited to the opening of infection paths for *V. dahliae.* Even when the nematode and fungus were confined to separate root systems of the same plant incidence and severity of peppermint wilt was increased by the presence of nematodes. Inoculation with *Fusarium solani* and *P. penetrans* together decreased top weight more than did infection by either pathogen alone (Abdel-Hadi and Ghorab, 1987). Taheri *et al.* (1994) showed that when *P. neglectus* was accompanied with fungi including *Bipolaris sorokiniana*, *Pythium irregulare*, *Microdochium bolleyi*, *Fusarium oxysporum*, *Gaeumannomyces graminis* or *Rhizoctonia solani*, a higher number of nematodes per plant was obtained compared to that in the plants not inoculated with fungi. They suggested that these fungi may render the roots more suitable for nematode multiplication.

However, nematodes are not always the primary invaders and may make the plants more attractive to fungi. There are reports that the reproduction rate of *P. neglectus* was increased by the presence of *V. dahliae* on peppermint plants. Faulkner and Skotland (1965) concluded that *V. dahliae* causes changes in host physiology to make the roots more favourable to the nematode.

2.5 CONTROL OF ROOT LESION NEMATODE

2.5.1 Rotation

Crop rotation offers producers control of disease, insects and weeds, allows for increased yields, and helps ensure income stability. Definite crop rotations are not always a part of farming practices in important wheat producing areas such as Australia, due mainly to climatic hazards and lack of alternative crops that can compete economically with wheat. For instance, in parts of Queensland and Western Australia where wheat has been recently introduced, and particularly in drier environments where crop options are limited, wheat is grown almost as a monoculture (A. J. Rathjen, pers. comm.; Clewett *et al.*, 1994). Crop rotations used by farmers to reduce the severity of root lesion nematode problems include sorghum and barley and, in earlier years, linseed and setaria in rotation with wheat (Clewett *et al.*, 1993).

In an experiment conducted by Clewett *et al.* (1993) in Queensland, it was found that some crops including linseed, canary and setaria were non-hosts of *P. thornei*, as the final population of nematodes in these crops was similar to or less than that in unplanted control treatments. For controlling *P. zeae,* the use of sunflower (*Helianthus annuus*) in rotation as a non-host or poor host is effective (Bolton and Waele, 1989). Numbers of *P. thornei* were higher in wheat-fallow-wheat rotations than when *Zea mays*, *Gossypium* spp. or *Glycine max* were used as rotational crops (Van Gundy *et al.*, 1974).

Thorne (1961) stated that rotations including alfalfa and sugar beet for several years reduced the population of *P. thornei.* In investigations conducted by Vanstone *et al.* (1993b), some varieties of legumes such as lupins, peas and faba beans supported much lower populations of *P. neglectus* than did chickpea. Barley varieties in general and some durum wheat lines also supported lower nematode populations than the majority of the bread wheat varieties.

The effect of corn (maize), cowpea (*Vigna unguiculata*), mungbean (*Phaseolus mungo*), rice and sorghum on the population density of *P. zeae* was tested in upland rice field conditions in the Philippines by Aung and Prot (1990). The non- cereal crops were resistant to the parasite, but two successive crops were necessary to reduce the nematode population density to a low level. The yield of rice grown after two crops of corn, cowpea, mungbean or rice was correlated to the population densities of *P. zeae* detected at the end of the preceding crop. The yield of rice grown after cowpea and mungbean was significantly higher than that obtained after cereal crops. The yield of rice after rice was 37% lower than the yield of rice after cowpea. However, surviving *P. zeae* multiplied very rapidly on rice, and only the first rice crop in a rotation benefited.

Of the 45 species of weeds commonly encountered in the tea growing areas of Sri Lanka screened against *P. loosi*, *Radopholus similis* and *Rotylenchulus reniformis* (Nelson *et al.*, 1985), *Oxalis corniculata* was resistant to all three nematodes, but the rest showed varying degrees of susceptibility to at least one or two of the nematodes. This illustrates the problems of developing effective rotations for control of *Pratylenchus* species of nematodes.

2.5.2 Cultivation

Cultivation has mechanical effects on nematodes through abrasion by soil particles, particularly where the soil is more sandy. Best results are obtained when hot, dry conditions follow the cultivation (O'Brien and Stirling, 1991).

Summer cultivation has been practiced for a long time as a method to control weeds, diseases and insects as well as to conserve moisture in the deeper soil layers for the next crop. This exposes the soil to solar heat and desiccation, killing or debilitating a substantial proportion of pest and pathogen populations (Raghaven, 1964). Nematodes are especially vulnerable to rapid desiccation as they can only become anhydrobiotic with slow drying over several days (Charwat, 1994).

Thompson *et al.* (1981) showed that in a top soil with zero tillage there were more nematodes than with normal cultivation. Thompson and Clewett (1984) also found fewer nematodes in the top soil of mechanically tilled plots than in uncultivated plots of the Hermitage long-term fallow experiment for winter cereals after fifteen years of continuous treatment. It was suggested that cultivation kills many of the nematodes but not all. Haak *et al.* (1993) in Queensland, investigating the effects of tillage and stubble retention on populations of *P. thornei* and *P. neglectus* on wheat, also found fewer nematodes in plots with tillage treatments than in plots with zero tillage or reduced tillage. Taylor (1993) showed that conventional cultivation reduced the population of *P. neglectus* by 55% compared with a reduced tillage treatment. She also found a strong negative correlation between the numbers of the nematode and grain yields. She noted that, in addition to the direct effect of cultivation, crops were often sown earlier under reduced tillage. Nematodes are most infective immediately after opening rains, so plants which are sown earlier will suffer from a higher infestation by nematodes.

2.5.3 Solarisation

Recently, the availability of polyethylene and other plastic materials for mulching has opened new ways for controlling nematodes. Polyethylene mulching of moist soil during the summer, raising soil temperature and conserving moisture, is referred to as soil solarisation. It is an alternative to chemical fumigation for control of soil-borne pathogens such as nematodes, fungi, bacteria, insects, mites and weeds (Katan, 1980; Stapleton and DeVay, 1986; Katan *et al.*, 1987). Changes also occur in soil chemistry, with increased levels of nitrogen and exchangeable cations (Chen and Katan, 1980). The combined changes can result in increased plant growth and yield of harvestable products.

In this method of controlling nematodes, the field is tilled and irrigated in mid-summer and, when soil moisture is at about field capacity, a clear polyethylene sheet is spread over the soil and its edges buried. Depending on several factors, including the level of solar radiation, the sheet is left in place for two to nine weeks. At the end of this period the

polyethylene sheet is removed and the field is ready for normal use. The cover material should have high transparency to transmit short-wave solar radiation to the soil, but it should be opaque to long-wave infra-red radiation and to water vapour. Transparent polyethylene has been found more effective than black, since it transmits most of the incident radiation to the soil whereas black polyethylene absorbs and reflects the heat.

In trials in Jordan, Al-Asad and Abu-Gharbieh (1990) recorded temperatures of 50 and 42°C at 10 cm depth under transparent and black sheeting respectively. Raymunda and Alcazar (1986) obtained much higher temperatures in Peru using two layers of polyethylene, one over the soil surface and the other supported at a height of 50 cm. The soil temperature at 10 cm was 60°C under the double layer compared to 47.5°C under the single layer and 32.3°C in the uncovered soil. A sheet about 50-100 µm thick was most practical and could also be reused. Soil solarisation creates accelerated microbial and physico-chemical reactions in the soil resulting in the accumulation of gases, some of which are toxic, and the release of mineral ions which serve as nutrients for, or induce tolerance in, the subsequent crop. Steven *et al.* (1989) found more than a 90% reduction in *M. incognita* populations at a depth of 0-30 cm; the root gall index in the subsequent crop was reduced by 92-98% and yield was increased.

In an experiment conducted by Di Vito *et al.* (1991), soil populations of *P. thornei* were greatly suppressed in plots solarised for six to eight weeks, similar to those treated with aldicarb. As a result of solarisation, root invasion by the nematode was much reduced and fewer nematodes were extracted from the roots of chickpea grown in either the solarised plots or those treated with aldicarb.

In an experiment conducted at several sites in the north-west and south of Victoria, Australia, Porter and Merriman (1985) tested the effect of soil solarisation on controlling diseases. At all sites, treatment reduced the number of viable pathogens propagules to at least a depth of 10 cm. Stapleton and De Vay (1983) used soil solarisation alone or in combination with other treatments for four to six weeks to control nematodes, including

Pratylenchus. Samples taken immediately following treatment demonstrated a reduction in nematodes of 42-100%. Soil solarisation for 40 days during the summer resulted in more than 90% control of plant-parasitic nematodes, including *P. thornei*, to a depth of 15 cm (Sauerborn and Saxena, 1987). The number of *P. thornei* in chickpea roots was greatly reduced and the fresh weight of plants and grain yield of chickpea were significantly increased in plots previously solarised for six or eight weeks (Vito *et al.*, 1991).

Barley plants growing in solarised plots had a significantly higher growth rate and yield and had less lesions compared to those in untreated areas. The yields in solarised plots has been estimated at about 2.5 times those of untreated plots (Vanstone, 1991).

2.5.4 Fertiliser application

Plant parasitic nematodes, by disrupting the normal structure of plant root tissues, hamper water uptake and transport. Altered water transport in turn affects mineral uptake and translocation. Infected plants have fewer (if any) root hairs and consequently a reduced contact surface with the soil surrounding the roots. Fertiliser application, by increasing the availability of nutrients in the root environment, can compensate for reduced access of roots to minerals. Hence, well fertilised crops (with good nutritional status) through a higher availability of nutrients in the soil and higher root growth are subjected less to the effect of nematodes.

Adding mineral fertilisers (N+P) reduced *P. thornei* in wheat roots, and this effect was increased if the crop was grown in rotation with other crops rather than continuously (Tacconi *et al.*, 1988). Nitrogen application of 100 kg N/ha (as urea) reduced numbers of *P. neglectus* per plant by 47-53% when plants were sampled five weeks after sowing. The same trend was observed with experiments conducted under glasshouse conditions, with nitrogen applied to plants two or three weeks after sowing and nematodes extracted after six weeks (Vanstone *et al.* 1993c). They concluded that an early application of a high rate of nitrogenous fertiliser is more effective than a late application. They showed that the

reduction in the nematode population occurred irrespective of the root growth, as the number of nematodes per gram dry root was also reduced. They concluded that an early nitrogen application to nematode infested soil inhibits nematodes prior to their invasion of roots.

It has also been reported that ammonia has nematicidal properties and that even small amounts in soil hinder nematode movement (Kimpiski *et al.*, 1976). In contrast, Orion *et al.* (1984) reported that *P. thornei* was not affected by nitrogenous fertiliser. Good nematode control was obtained when urea was applied at levels in excess of 300 mg N/kg of soil (Rodriguez-Kabana and King, 1980). On the other hand, it has been revealed that urea is phytotoxic at the application rates needed to control nematodes (Rodriguez-Kabana and King, 1980; Huebner *et al.*, 1983). To be nematoxic, the nitrogenous fertiliser should ionise to NH_4^+ which depends on soil characteristics such as pH. The amount of NH_4^+ released is greater above pH 6.

In an experiment carried out in India, the effect of carbonaceous and nitrogenous materials was studied (Gupta, 1990). Chitin flakes, cellulose powder, starch or anhydrous dextrose were added to soil at 0.1, 0.5 and 1.0% w/w and urea, ammonium sulphate or calcium nitrate were mixed with soil at 60, 120 and 240 kg N/ha for control of *Meloidogyne* and *Pratylenchus* spp. in sugarcane on a sandy clay soil. Amendments caused a significant reduction in numbers of both nematode species, with chitin and starch giving the highest level of control of *Meloidogyne* and *Pratylenchus,* respectively. Of the inorganic nitrogenous fertilisers, urea gave the greatest control. The materials added to soil increased its fertility and water holding capacity so that, as a result of improved growth, plants were better able to withstand attack from nematodes. They may also encourage the natural enemies of nematodes. The nitrogen content of amendments can also be toxic to nematodes (O'Brien and Stirling, 1991).

The effects of compost application, alone or in combination with nematicide and mineral fertilisers, for the control of *M. incognita* and *P. zeae* on sugarcane were evaluated (Novaretti *et al.*, 1989). Nematode populations were estimated 4.6 and 8 months after

planting and yield data from the first and second harvests were determined. Application of compost alone at 30 t/ha wet matter showed low efficacy against nematodes, but was beneficial to plant growth; plants became more tolerant to the nematode. At the first harvest, all treated plots including those receiving nematicide (5% carbofuran at 60 kg/ha) had significantly higher yields than untreated plots, the differences ranging from 12 to 22 t/ha. At the second harvest, no significant differences were observed.

2.5.5 Nematicides

Over the years, researchers have attempted to control nematodes with nematicides. In an experiment undertaken by Van Gundy *et al.* (1974), the application of chemical nematicides reduced the number of *P. thornei* in the soil by 70-90% at planting time. Potato seed piece treatment followed by three foliar sprays generally reduced population densities of *P. penetrans* in a sandy loam at mid-season and in the soil and roots at harvest compared to the control (Olthof and Townshend, 1991). However, current concerns about 'clean food', pollution, and de-registration of some nematicides means that fewer are now available for use, and their cost precludes their use on broadacre crops in Australia.

In a greenhouse experiment, fenamiphos 5 GR (40 g/m^2) was incorporated into soil before roses were planted (Amising, 1988). It did not completely prevent invasion by *P. vulnus*, but initially reduced it considerably, so flower production and flower weight were increased by a single treatment. Experiments comparing fenamiphos 40% EC and 10 GR at 5 ml/m^2 and 20 g/m^2 respectively, applied at intervals of nine weeks, with oxamyl or aldicarb, showed that fenamiphos was most effective in reducing *P. vulnus* populations in soil.

Carbofuran was applied as granules at 1.5, 2.5, 3.5 or 4.5 kg /ha a.i. to maize in Nigeria at planting and tasselling to protect against *P. brachyurus* (Atu and Duru, 1990). A significant control of the pests by treatment at 2.5-4.5 kg/ha was obtained, but the lower rate was recommended for cost-effectiveness reasons. More than half the plants in untreated plots began dying before reaching the tasselling stage. Di-Tra Pex® (D-methyl isothiocyanate) at

300 or 500 litres/ha and dazomet (as basimid granules) have been used successfully to control the nematode *P. penetrans* (Rabendel and Szczygiel 1989). Application of Nemacur® 3 (fenamiphos) at 12 lb/acre to *Vaccinum corymbosum* significantly reduced populations of *Pratylenchus* spp., compared to the non-treated control (Schroeder *et al.*, 1988). In the 6 and 12 lb/acre treatments, nematode densities averaged 55% and 5% of those in the nontreated control.

The importance of root knot and root lesion nematodes was assessed on pyrethrum (chrysanthenum) seedlings at two nurseries in Kenya (Anyango, 1988). Nemacur® (5%) granules significantly controlled the population of the two nematodes in the roots and surrounding soil. Plots treated with the nematicide showed faster growth and a higher seedling establishment compared with the untreated control. Large nematode populations resulted in the stunting of seedlings.

Kimpinski *et al.* (1988) showed that the foliage yields of alfalfa in aldicarb treated plots were higher than in untreated check plots in the year after application. However foliage application of aldicarb did not reduce the size of *P. penetrans* populations significantly. Using fenamiphos (Nemacur®) 40% EC at 30 litres/ha increased carrot yields by 38-45% over the untreated control (Orion *et al.*, 1984).

A range of pesticides were tested against *Pratylenchus*. One percent solutions of phosphamidon, dichlorvos, dimethoate, aldrin, endosulfan, malathion and parathion were tested *in vitro*. All possessed nematicidal properties with phosphamidon and dichlorvos being the most effective (Deshmukh *et al.*, 1991).

Experiments were carried out to evaluate the effects of seed treatments with oxamyl and a soil spray treatment with Phenamiphos® 15 G (fenamiphos) for the control of *P. coffeae* on yams. At one location, oxamyl (0.3 and 0.6 ml/plant) was applied alone to soil and in combination with seed immersion in oxamyl for fifteen minutes. Seed immersion was also combined with foliar sprays (4.68 litres/ha every fifteen and 60 days). At another location,

soil sprays (0.6 and 0.9 ml/ha) every fifteen days and soil treatments with fenamiphos (0.64, 0.93 and 1.27 g/plant) were evaluated. Significant yield increases compared to untreated controls were obtained at both locations with all oxamyl treatments (Oramas *et al.*, 1992).

In a field trial in the Philippines, following a five month weed free fallow, control of *P. zeae* using carbofuran increased the yield of rice cv. Upland Ri-5 whilst the yield of cv. Kinandang Patong was unaffected (Plowright *et al.*, 1990). Pre-sowing soil population densities of *P. zeae* were low (0-11 nematodes/100 ml soil) and there were no obvious symptoms of infection during early vegetative growth, although the plant height of Upland Ri-5 was slightly reduced. At harvest, the yield of treated plants was increased by 13-29% of that of untreated plants, the latter having a mean infection of 1350 nematodes/g of root. In the glasshouse, the rate of growth and tillering of cv. IR 36 was significantly reduced, with a high population density of *P. zeae* (630-300 nematodes/100 ml soil). Infected root systems were stunted and the mean root weight was reduced by 40-60%. Although infection reduced the number of spikelets/plant, these plants had a higher harvest index and consequently grain yield was unaffected.

The usefulness of nematicide application depends on the crop, the rate and the time of application. When aldicarb was applied at 10 kg a.i. /ha on spring chickpea, depending on the year, it gave total control of nematodes and increased yields, although this was not statistically significant (Greco *et al.*, 1988). Rodriguez Santana *et al.* (1992), working on the effect of *Pratylenchus* spp. on banana crops, suggested that nematicides should be applied twice or three times annually through drip irrigation to control the nematode efficiently. Extensive testing of various nematicides in 1980 and 1981 indicated that chemical control of *P. thornei* in wheat would be uneconomic (Thompson *et al.*, 1980c), because root lesion nematodes occur deep in the soil (Thompson, 1987c) and the area would be too large to treat.

In recent years, soil nematicides have come under considerable regulatory scrutiny and several have been removed from the market or are subject to restricted use. Thus, control methods must be modified, and one approach is to employ crops in rotations which are poor hosts for nematodes (Townshend, 1989). Nematicides are uneconomic on many lower value crops and when used on high value crops are applied at relatively high rates with consequent risk of toxic residues (Cooke and Evans, 1987).

For these reasons, it is likely that selection for resistance to pests and diseases of economic importance will continue to form an essential part of most breeding programs, probably as part of integrated programs that also exploit other means of disease control.

2.5.6 Biological control

In nature, all organisms are subjected to a series of natural enemies in their environment. Plant parasitic nematodes spend at least some part of their life in the soil, a complex environment. Their activities are controlled by various physical factors and a vast array of living organisms, including other nematodes, bacteria, insects, mites and other soil animals.

The infection of *Pratylenchus* by some bacteria such as *Pasteuria penetrans* has been reported (Davies *et al.*, 1990). The possible use of this genus for biological control of the genera *Pratylenchus* and *Meloidogyne* has been described (Starr and Sayre, 1988). Infection of tall fescue (*Festuca arundinacea*) with the endophytic fungus *Acremonium coenophialum* has been shown to reduce nematode populations in field soils. Reproduction of three plant-parasitic nematodes on endophyte-infected (E^+) and endophyte-free (E^-) tall fescue in greenhouse tests was evaluated. E^+ plants had lower numbers of *P. scribneri*, a migratory endoparasite, than E^- plants, and roots of E^+ plants had fewer egg masses and eggs of *M. marylandi*, a sedentary endoparasite (Kimmons *et al.*, 1990).

In an experiment carried out in India by Jain and Hasan (1986), it was revealed that where roots had more than 50% vesicular-arbuscular mycorrhiza (VAM) infection, nematode

numbers were lower. Presence of nematodes did not adversely affect VAM sporulation. It was concluded that VAM fungi could be used to manage plant parasitic nematode populations.

Despite the fact that various fungi, bacteria, nematodes and other organisms feed on parasitic nematodes, the addition of an exotic organism to the soil will often have only a temporary effect because ecological pressures will restore an equilibrium so that the intended biological control agent assumes a minor role in the microflora or microfauna. On the other hand, an introduced species might become established if its ecological niche was not previously occupied. Little success has been obtained by attempting to supplement predators and parasites in the soil (Jenkins and Taylor, 1976).

In a few cases, plants have been found to be actually antagonistic toward nematodes, and some of them produce materials in their roots that are toxic to nematodes. Such plants have been termed "Feindpflanzen" or enemy plants. Asparagus (*Asparagus officinalis*) and certain marigolds (*Tagetes patula* and *T. erecta)* are the best known examples of these. Fleshy storage roots of asparagus produce a glycoside that is toxic to a large number of plant parasitic nematodes (Jenknis and Taylor, 1976). Certain plants contain toxins effective against endoparasitic species. Foremost are some plants of the genus *Tagetes* that contain terthienyl, a compound toxic to *Pratylenchus* and *Meloidogyne* (Veech, 1981; Bingefors, 1982). *P. neglectus* was susceptible to isothiocyanates extracted from the roots of *Brassica.* When the nematodes were exposed to a concentration of 10^{-2} M of the substance, considerable numbers of the nematode were killed compared to the control (Parker, 1994).

2.5.7 Tolerant varieties

Although using tolerant varieties is not a method of reducing the nematode population, they enable the plant, passively or actively, to counteract the stresses caused by environmental constraints including parasitic nematodes (Wallace, 1987). Tolerance is measured in terms of plant variables, such as yield, which are related to the final outcome of the relationship

between nematode and plant. Thompson and Brennan (1986) have identified some wheat varieties including Gamut, Oxley and Cook tolerant to *P. thornei* in Queensland. In South Australia, Taylor and Evans (1993) have also reported the tolerance of wheat variety Tatiara to *P. neglectus*.

While growing tolerant varieties in nematode infested soils would overcome the yield loss caused by nematodes, they would leave a high population of the nematode to attack the subsequent crop. Tolerance, if combined with resistance, would largely lessen the damage of the nematode to the plant and would lower the population build up of the nematode attacking the next crop.

2.5.8 Resistant varieties

Potentially, the most economical and effective method of controlling nematodes is the use of nematode-resistant plant varieties. These varieties, if available, would allow farmers to produce high-yielding crops in heavily infested soils, would reduce the nematode population, and would lessen the chance of spreading the infestation to unaffected soil.

Resistant cultivars also have several advantages over other methods of reducing nematode populations: their use requires little or no specialised technology and is cost effective; they allow rotation cycles to be shortened allowing the best use to be made of the land; and they do not leave toxic residues (Cooke and Evans, 1987). However, resistant cultivars also need to be tolerant of nematode invasion; those that are intolerant will suffer extensive damage if grown in heavily infested soil and provide low economic returns. Equally, tolerant cultivars that are not resistant lead to increased nematode population densities.

Plant breeders and nematologists have developed cotton, cowpeas, lespedeza, tobacco, lima beans, soybeans, peppers, tomatoes, grape and peach rootstocks resistant to root knot nematode (*D. dipsaci*); potatoes resistant to the golden nematode (*H. rostochiensis*); soybean resistant to the soybean cyst nematode (*H. glycienes*); citrus rootstocks resistant to the citrus

nematode (*Tylenchulus semipenetrans*); corn resistant to stunt nematode (*T. claytoni*); and sugarcane resistant to *P. zeae*. Commercial varieties of cotton, limabeans and soybeans resistant to the stem nematode are grown extensively (Subcommittee on Nematodes, 1968).

In South Australia, cereal cyst nematode (*Heterodera avenae*) has been recognised as a serious pest since 1930 (Davidson and Se, 1930; Fisher, 1987). As a result of the effort devoted to this problem at the Waite Agricultural Research Institute, resistant varieties were identified in the 1960's (O'Brien and Fisher, 1974). Breeding programs in southern Australia have resulted in the release and widespread cultivation of resistant varieties of wheat (Brown and Young, 1982; Rathjen *et al.*, 1989), barley (Sparrow, 1987) and oats (Barr *et al.*, 1988).

Van Gundy *et al.* (1974) tested 51 varieties of wheat to find possible resistance against *P. thornei*. All 51 varieties tested in the field and greenhouse were susceptible to invasion and reproduction of the nematode. In the field, yield reductions ranged from 6 to 32%, and in the greenhouse from 7 to 28%. On the other hand, in an experiment carried out in the greenhouse by Norton (1989), *Zea perennis* and Z. *diploperennis* reduced the number of *Pratylenchus* per gram of dry root by 82 and 98%, respectively, relative to the control.

Five almond (*Prunus amygdalus*) cultivars were evaluated for their reaction to *P. vulnus*, *P. neglectus* and *P. thornei* under greenhouse conditions at 120 days after inoculation with 1000 nematodes per plant. All cultivars were susceptible to *P. vulnus*. This nematode showed high rates of reproduction in all cultivars ranging from 6.8 to 14.8. The number of nematodes per gram of root was significantly higher in relation to other nematode species in all materials tested. *P. neglectus* showed a low population increase, between 1.2 and 1.9, and *P. thornei* also multiplied poorly on almond, indicating that the plant is not a good host of either species (Marull *et al.*, 1990). Variation in the host efficiency for *P. scribneri* in maize has been reported by Edwards and Norton (1988), indicating the possibility of finding varieties supporting few nematodes. *Cajanus cajan* is a resistant crop and *Mucuna pruriens* is a poor host to *P. penetrans* (Haroon and Abadir, 1986). It has been reported that barley

and sorghum crops are not affected by *P. thornei* (Doyle *et al.*, 1987), indicating that these species are at least tolerant to this nematode. Host plant resistance to the root lesion nematode has been reported in tea (*Camellia sinensis* L.), coffee (*Coffea liberica* L.) and tobacco (*Nicotiana tabacum* L.) (Dropkin, 1989). Ferraz *et al.* (1989) have reported that the Barbados cherry (*Malpighia glabra*) is resistant to *P. brachyurus*. The multiplication rate of *P. brachyurus* was studied on local cultivars of 30 vegetable crops in a greenhouse experiment (Khan, 1992). Nine cultivars were resistant, five were poor hosts, twelve were susceptible and six highly susceptible.

Resistance in exotic varieties and related species

Often, due to wheat evolution to the hexaploid level after domestication and the consequent limited genetic variation involved at this level, there is insufficient resistance in adapted wheats and resistance may be sought in varieties that are poorly adapted to the area in which the breeding program is being carried out. However, resistance from within cultivated wheat may be inadequate either in level of expression or because the available resistance is being overcome by the evolution of new races of a pathogen. For both of these reasons, there is an incentive to search for resistance in related species and genera, both cultivated and wild.

One of the potential and most favourable sources of resistance for plant breeding is from the related species and genera to that species which the breeder is trying to improve. This clearly has application to crops such as wheat. Often, it is hoped that such resistance might display greater durability than resistance already available within wheat (Aseidu *et al.*, 1990). However, a wide range of problems arise in interspecific hybridisation and interspecific transfer of genes. In general, progress is often slow and difficult (Knott and Dvorak, 1976).

Inheritance of resistance

The genetics of resistance to nematodes has been reviewed by some authors (Sidhu and Webster; 1981; Fassuliotis, 1987). Almost all the genetically identified resistance used in breeding against endoparasitic *Ditylenchus*, *Meloidogyne*, *Heterodera* or *Globodera* spp. is conferred by dominant major genes. However, resistance in cotton to *Meloidogyne*, in soybean to *H. glycines* and in potato to *G. pallida* appears to be recessive or polygenic (Sidhu and Webster, 1981).

The inheritance of resistance to *P. zeae* and *P. brachyurus* in maize was studied, using resistant and susceptible lines, the F_1 and F_2 generations and backcrosses. The genotypes were sown in a field which was naturally infested with a mixture of *P. zeae* (76%) and *P. brachyurus* (24%). Eighty days after planting the nematode numbers per gram of root were determined. The results indicated that resistance to these pests was due to two dominant genes with an additive effect (Sa$^{\Psi}$azaki *et al.*, 1988). In Queensland, resistance of GS 50A, a selection from the susceptible wheat variety Gatcher, to *P. thornei* was controlled by a single dominant gene (J. P. Thompson, pers. comm.) .

Mechanism of resistance

The mechanisms of resistance to nematodes have been comprehensively reviewed (Kaplan and Davis, 1987). No nematode-derived elicitors of resistance have been identified, but potential sources include the saliva injected via the stylet, excretory products and various surface components. For endoparasites, all three could be involved, but for resistance to migratory ectoparasitic nematodes the elicitor must be in the secretions injected via the stylet. Various carbohydrates have been identified, emanating from cuticular pores, the excretory system, or from the paired amphidial chemoreceptors situated on the nematode head (Robertson *et al.*, 1989) as candidates for the elicitor reactions.

Resistance to nematodes might be caused by the production of toxic root exudates as found in asparagus, the lack of an attractant or a hatching factor in the exudates, a physical barrier

to penetration, or failure of nematodes to develop within plant tissues. The most commonly used form in breeding programs is the failure of nematodes to develop within plant tissues. In this case, the resistant plants produce necrotic areas around the section of the root invaded by the nematodes, so the nematodes are unable to feed and reproduce. This type of resistance is a form of hypersensitivity (Jenkins and Taylor, 1976).

The role of surface glycoproteins in plant-pathogen interactions remains controversial but there is indirect evidence, through blocking by certain lectin treatments, for their involvement in host finding by nematodes (Zuckerman *et al.*, 1984). Treatment of invasive juveniles of a root knot nematode (*M. incognita*, Race 3) with lectins and their complementary sugars increased the tendency for a hypersensitive response to be induced in resistant soybeans and for an incompatible response in a susceptible variety (Davy de Virville *et al.*, 1989). Incubating juveniles in distilled water decreased the proportion of juveniles that initiated a hypersensitive response in the resistant cultivar.

It has been shown that the hairy roots of marigold (*Tagetes patula* L.) induced by infection with *Agrobacterium rhizogenes* produced a terthienyl when grown in darkness and an n-hexane extract of the roots showed nematicidal activity (Kyo *et al.*, 1990). Further analysis indicated that the nematicidal activity against *Caenorhabditis elegans* and *P. penetrans* was due predominantly to a terthienyl. The nematode resistance mechanism in alfalfa appeared to involve low root colonisation and low nematode reproduction in the root (Nelson *et al.*, 1985).

It seems that there is often no difference between resistant and susceptible plants in terms of nematode invasion, as both are attacked by similar numbers of nematodes, ie. resistance to a nematode does not usually protect plants from invasion. Indeed, some resistant genotypes of cereals, potato and soybean are relatively intolerant (ie. sustain more damage than susceptible genotypes) of their respective cyst nematodes (Trudgill and Cotes, 1983).

A range of responses in resistant plants to invasion / feeding by incompatible nematodes has been reported (Kaplan and Keen, 1980). These range from non-specific tissue necrosis, which may not impair nematode development, through delayed localised necrosis around the nematode or its feeding site, which prevents the development of females, but not of males, to a more rapid "hypersensitive" response which prevents development of the nematode and its feeding site. Because of the endoparasitic nature of many nematodes, timing of initiation of the hypersensitive response is uncertain. With *Tylenchulus semipenetrans,* the hypersensitive response in citrus took up to two weeks to develop (Kaplan, 1981). In contrast, a necrotic response to *Meloidogyne* in a resistant tomato was visible within two days of infection and changes could be observed within eight to twelve hours. The reaction was localised around the nematode and its feeding site and initially involved a loss of electron-dense inclusions in the vacuoles, cell membrane disruption, and a rapid increase in the electron density of the cytoplasm. Membrane-bound organelles disappeared but the endoplasmic reticulum became extended, suggesting changes in cell wall and membrane permeability and the synthesis of enzymes leading to general disorganisation (Paulson and Webster, 1972).

Screening methods for resistance to root lesion nematode

A number of different approaches can be adopted in breeding and it is important to screen large numbers of plants rapidly. The ease with which this can be undertaken depends upon the type of pest and the expression of resistance.

Trudgill *et al.* (1975) suggested that counting the number of nematodes per gram of roots six to eight weeks after planting gives the best estimate for screening resistant and susceptible plants against root lesion nematodes. Sorensen *et al.* (1990) used number of nematodes per gram fresh root to screen for resistant varieties of alfalfa.

While most investigators use number of nematodes per gram of dry root to compare the nematode reproduction rate on different plant species or varieties, some workers use number

of nematodes per pot including both the nematodes in roots and the nematodes in soil (Olthof, 1980; Thompson, 1984).

Environmental conditions can affect the efficiency of screening. Dennis *et al.* (1989) showed that *Pestacia atlantica* had a relatively unbranched, stringy root system with very few rootlets when infected with *P. vulnus* in the field, while the root system developed as a mass of rootlets in pots in the greenhouse. They found significantly higher nematode numbers per gram of root for *P. atlantica* in the greenhouse than in the field, while the rate of nematode inoculation and the date of sampling were the same for both experiments. They concluded that these results were due to optimal temperature and moisture conditions for nematode reproduction in the greenhouse and that these results were due primarily to a significant change in the root structure in the greenhouse. They concluded that comparing nematode counts of the genotypes being screened to those of a reference variety would be a more valid method than classifying genotypes based on the actual nematode counts (Dennis *et al.*, 1989).

Measuring resistance

Except on genotypes with qualitative resistance, nematode multiplication rates are density-dependent. Various equations have been produced to model single generation population changes (Seinhorst, 1965; Jones and Perry, 1978). As multiplication rates are density-dependent they vary with inoculum and plant root densities. This basic principle has important implications for the conduct of statutory and breeder pot tests designed to assess levels of quantitative resistance. For the same inoculum density of *G. pallida*, multiplication rates in the field were only one half to one third of those obtained in pots (Phillips and Trudgill, 1986). Qualitatively acting genes can be readily identified in a pot test given a largely virulent nematode population and a susceptible control. Good tests are those where multiplication on the susceptible control is high and there is little or no reproduction on those plants with the resistance gene. Such tests are widespread in breeding for resistance to

potato, soybean and cereal cyst nematodes and several simplified tests have been developed (Philips *et al.*, 1980 and 1979).

To screen large numbers of varieties in a glasshouse for resistance to a nematode, a readily available source of pure inoculum is required as in naturally nematode infected soil other microorganisms may interact with nematode multiplication, thus interfering with the results. Methods of producing a large population of a single aseptic species of *Pratylenchus* for screening experiments have been established, using carrot pieces and chickpea callus cultures at the Waite Agricultural Research Institute (Vanstone and Nicol, 1993) and open pot culture at the Queensland Wheat Research Institute (O'Reilly and Thompson, 1993).

There is usually a continuous range for expression of progeny derived from crosses between resistant and susceptible parents. Rather than recognise this variation in expression, the tendency in nematology has been to fix arbitrary limits for resistance. For potato cyst nematode, plants were classified as resistant if the multiplication rate was less than one in a standard pot test, irrespective of the multiplication rate on the nonresistant controls which may be as low as ten or as great as 50-fold (Trudgill, 1991). In resistance to *Pratylenchus*, plants that do not support substantial nematode reproduction, and have a final population not far from the initial population of the nematode, compared to susceptible check varieties, are considered as resistant (A. J . Rathjen and J. M. Fisher pers. comm.). In Queensland, the wheat variety GS 50A (a selection of Gatcher) which did not allow rapid multiplication of *P. thornei* compared to susceptible check varieties such as Gatcher, was considered as resistant to the nematode (J. P. Thompson, pers. comm.).

A positive, linear relationship has been found between the number of *P. penetrans* invading the roots and inoculum densities below 200, and percentage entering the roots increased as the inoculum level increased (Mauza and Webster, 1982). With high initial soil infestations, low rates of multiplication result, whereas low initial infestations favour higher rates of multiplication (Chitwood and Feldmesser, 1948; Oostenbrink, 1950 (cited by Dropkin, 1955); Fenwick and Reid, 1953).

CHAPTER 3

GENERAL MATERIALS AND METHODS

3.1 Field Experiments

Experimental sites

The location for the field experiment in 1992 was 5 km east of Balaklava, approximately 100 km north of Adelaide, South Australia (Figure 3.1), on the property of Mr. B. Roberts. The soil texture was a sandy loam and this was naturally infested with *P. neglectus* at a density of approximately seven nematodes per gram of moist soil. The site was chosen specifically for the high density of nematodes in the soil.

Field experiments were conducted at two sites (Figure 3.1) in 1993. The first was at Palmer, approximately 65 km east of Adelaide on Mr. J. Eichler's property, located 12 km north east of Palmer. The soil was sandy and naturally infested with *P. neglectus* at an average of 1.5 nematodes per gram of soil. To investigate the interaction between sites and treatments, the Roseworthy Campus of the University of Adelaide, located 6 km west of Roseworthy, was chosen as the second field site in 1993. The experiment was carried out in conjunction with trials conducted by the Waite Agricultural Research Institute wheat and barley breeding programs. The soil was a clay loam, naturally infested with *P. neglectus* at an average density of 0.4 nematodes per gram of moist soil.

Experimental design

The standard field arrangement used by the wheat and barley breeding programs of the Waite Agricultural Research Institute consists of 15 bays of plots, with 6 m between the midpoints of adjacent pathways (Figure 3.2). Between plots, pathways of 1.8 m were cut to allow for spraying operations and the automatic cleaning of harvesters between plots to avoid contamination of grain samples. Each plot consisted of four drill rows, each 15 cm apart with 30 cm between adjacent plots. The total length of each plot, after pathways were

cut, was 4.2 m and the sown area was 4.2 m x 60 cm, or 2.52 m^2. In each plot, 30 g of seed was sown, to give a rate of approximately 60 kg/ha. The plots were sown by a modified fourteen row drill and three plots were sown simultaneously.

Management of field experiments, including cultivation methods and the date of sowing and herbicide application, was in accordance with the local district practices, normally sown after the opening rain with a short period for ground preparation and weed control. The plants were watered naturally by rainfall and no artificial irrigation was used. No pesticide or nematicide was applied except where described in particular experiments. Grain from individual plots was harvested at maturity, using harvesters designed and built at the Waite Agricultural Research Institute.

The experiment conducted in 1992 at Balaklava, and those at Palmer and at Roseworthy in 1993, consisted of six, nine, and twelve bays respectively. The experiment at the Balaklava site was a factorial experiment in a randomised complete block design and those at other sites were split plot designs with a factorial combination of fertiliser regimes and varieties in sub-plots.

Rainfall

Monthly and average rainfalls of locations at which field experiments were conducted in 1992 and 1993 are presented in Table 3.1. These show that 1992 was a year of above average rainfall.

Fertiliser application

Fertilisers used in glasshouse and field experiments included Top Fos® as a source of phosphorus, calcium nitrate as a source of nitrogen, potassium sulphate as a source of potassium and magnesium sulphate as a source of magnesium. The composition of each fertiliser is tabulated in Tables 3.2, 3.3, 3.4 and 3.5.

Table 3.1 Monthly and total rainfall (mm) recorded at Balaklava, Palmer and Roseworthy. Source: Bureau of Meteorology, Kent Town, South Australia.

	Balaklava				Palmer				Roseworthy			
	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
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
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
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
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
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
July	35.0	23.2	44.0	23.6	60.2	38.4	?	15.8	60.2	23.8	36.8	27.4
August	42.8	45.8	19.0	7.4	75.4	118.4	?	11.6	68.4	182.1	35.2	17.6
September	33.2	99.5	50.8	7.8	48.0	101.8	?	34.0	59.6	142.4	77.4	15.2
October	14.8	55.4	43.4	11.0	1.6	71.2	?	27.0	4.0	81.2	93.0	27.8
November	33.8	43.8	12.2	21.6	18.4	77.0	?	15.4	35.0	72.2	21.4	44.6
December	1.2	38.4	?	7.0	4.0	208.8	?	7.4	0.8	75.8	?	8.4
Total	324	547.3	?	202	336.2	819.4	?	268	426	705	?	274.4
Mean annual total	(1880-1993)			**381.8**	(1901-1993)			**421**	(1885-1993)			**446**

Figure 3.1 Location of field sites in the Balaklava, Palmer and Roseworthy regions of South Australia. Latitude, longitude and height above sea level of the locations are as follows:

Location	Latitude	Longitude	Elevation
Balaklava	34° 09' S	138° 25' E	68.0 m
Palmer	34° 51' S	139° 10' E	190.0 m
Roseworthy	34° 32' S	138° 45' E	115.0 m

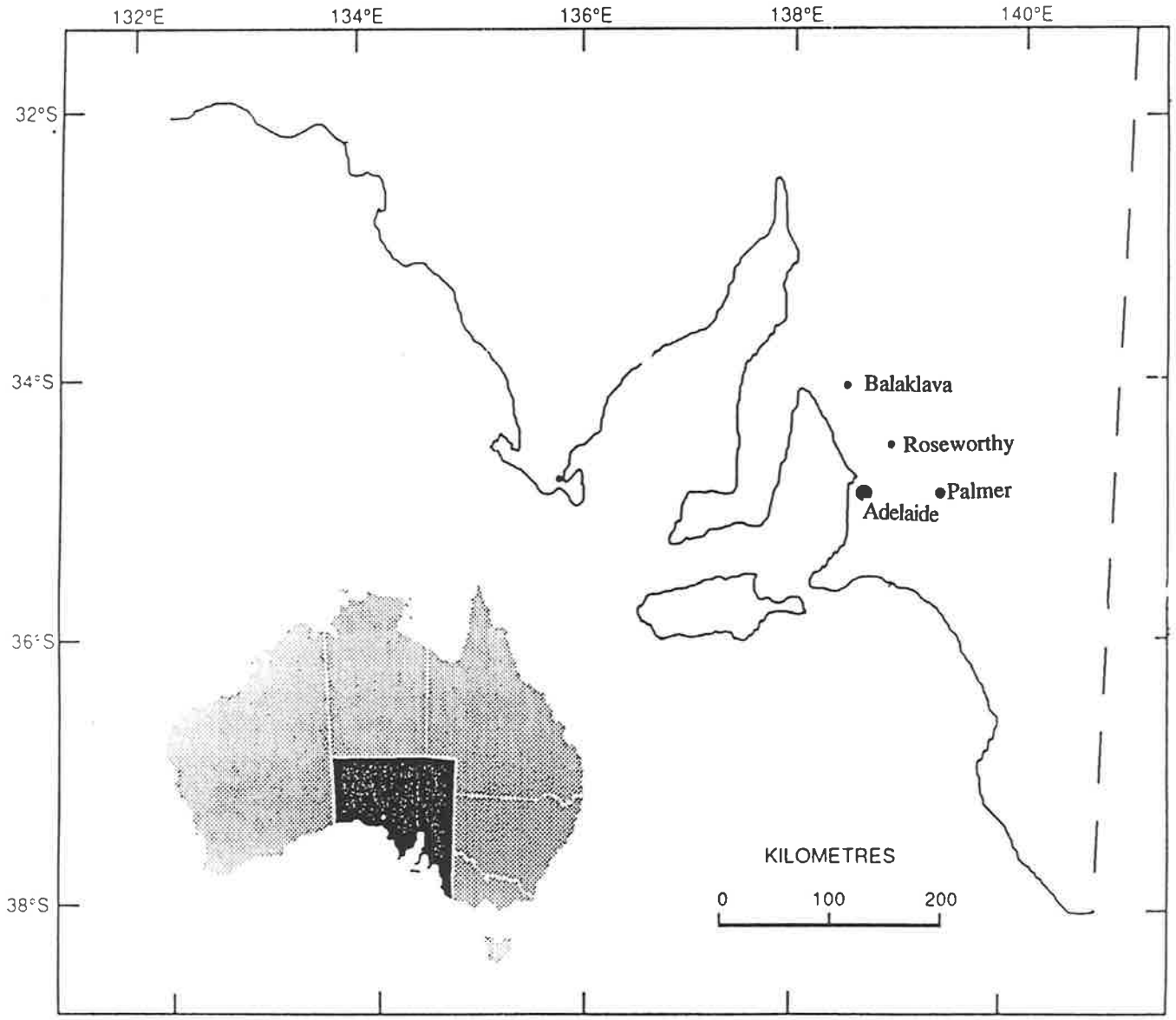
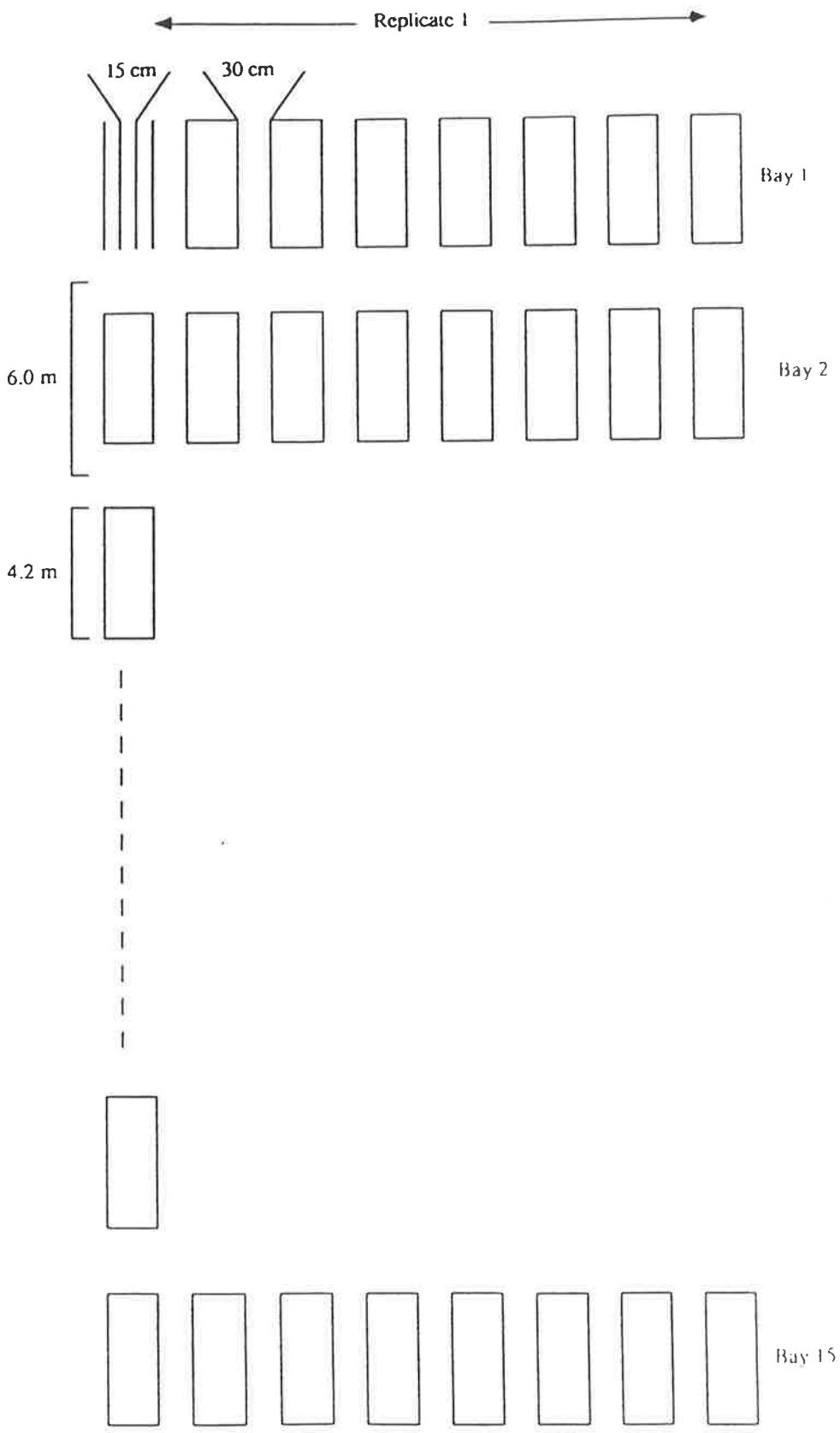

132°E
134°E
136°E
138°E
140°E
32°S
34°S
36°S
38°S
Balaklava
Roseworthy
Palmer
Adelaide
KILOMETRES
0
100
200

Figure 3.2 Experimental field plot layout. The area of each individual plot was 2.52 m^2 (4.2 x 0.6 m). Each plot consisted of four drill rows 15 cm apart, with 30 cm between plots. Number of columns and rows, depending on the experiment was different as explained in text for individual field experiment. A rate of approximately 60 kg ha^{-1} (30 g per plot) of seed was sown in each plot. This diagram was extracted from Paull (1990).


Replicate 1
15 cm
30 cm
Bay 1
6.0 m
Bay 2
4.2 m
Bay 15

3.2 Glasshouse-waterbath experiments

The lack of uniformity of nematode infestation in the soil and seasonal fluctuations are disadvantages for screening lines and varieties under field conditions. Various microorganisms, including bacteria and pathogenic fungi, are also present in field soil and these, by interacting with the nematodes and plants, can interfere with the results. A simple and reliable glasshouse screening procedure permits continuous testing of varieties throughout the year and reduces the reliance on field testing. Soil used for resistance screening experiments, with specific exceptions mentioned in the Materials, was collected from Eichler's property at Palmer, was sandy loam in texture and reddish in colour and generally classified as a solonised brown soil with marked differentiation in texture between the A and B horizons. Nematode reproductive rate was rapid in this soil and the soil was readily separated from roots by washing under tapwater.

For some experiments, naturally nematode-infested soil from cereal farms was used, and because of the non-uniform distribution of nematodes in the field, the soil was mixed to obtain a more uniform level of inoculum for each pot, although a proportion of the nematode population was killed through mechanical movement in the soil. For some other experiments, because of both the non-uniformity of the distribution of the inoculum and contaminating organisms, field soils were pasteurised to kill nematodes and other pathogens and then inoculated with known densities of nematodes.

Soil pasteurisation was accomplished by passing steam through the soil for 30 minutes resulting in a temperature of about 70°C. Pots without drainage holes were filled with 365 or 650 g (depending on the experiment) of pasteurised soil. Fifty millilitres of deionised water was added to each pot to moisten the soil before planting.

Seeds were first surface sterilised by soaking in 2.5% sodium hypochlorite (NaOCl) for 20 minutes and then washed three times in sterile distilled water. Seeds were then transferred to moistened filter papers in Petri dishes and placed at 4°C for about 24 hours and then

transferred to 25°C for about 48 hours. When radicles were 2-5 mm long, one to four germinated seeds were planted at the depth of about 1 cm in each plastic container. In some experiments plants were inoculated with a known population of the nematode. The inoculation techniques have been explained in Section 3.9.

The pots were maintained in 20 ± 2°C waterbaths and hand watered as uniformly as possible with deionised water as required (approximately 20 ml every day or every other day, depending upon the seasonal conditions). For experiments conducted to assess the effect of fertiliser application on nematode infected plants, some fertilisers (mentioned in the related sections) were added. The experiments were usually terminated seven or eight weeks after inoculation. At this temperature, nematodes complete their life-cycle in susceptible plants in about 35 days (Vanstone *et al.*, 1994). Under these conditions the time is enough to discriminate between resistant and susceptible plants.

3.3 Extraction of nematodes from soil

For field experiments, sampling of the nematode population in the soil was performed immediately before sowing by digging the soil with minimal disruption of the structure and bringing the sample in plastic bags to the laboratory. Soils were stored in plastic bags at 4°C until further examination. For the estimation of nematode populations in the soil, the Whitehead Tray Method (Whitehead and Hemming, 1965) was used. A random sub-sample of 200 g was taken from each sample using a small spoon avoiding mechanical damage to the nematodes through mixing.

The soil was placed on two layers of Kleenex® facial tissue supported in a plastic rack, keeping the soil sample slightly above the floor of the tray. Sufficient deionised water was added to the tray to almost cover the soil. Trays were kept at room temperature (15 ± 2°C night and 25 ± 2°C day) for five days (Kerr and Vythilingam, 1966). During this period, water was added whenever necessary, allowing the nematodes to migrate from the soil to the water in the tray (Figure 3.3). After five days, the soil along with tissues and supporting

rack was removed. The suspension containing nematodes was passed through a 10 µm sieve (sintered glass funnel No. 4), using a water tap mounted vacuum pump, to reduce the volume to 10, 20, 30 or 40 ml, depending on the density of nematodes in suspension. After resuspending the nematodes, 1 ml samples were taken from the middle of the suspension column by pipette and transferred to counting dishes. At least three counts were made and averaged for each sample.

Plate 3.1 Extraction of nematodes from soil by the Whitehead Tray method.

3.4 Extraction of nematodes from roots

The number of nematodes inside the roots of plants in field experiments was measured at anthesis. Three sub-samples from each plot (from the middle and nearly each end of each plot) were taken (15 x 15 cm width and 15 cm depth) and combined together. The samples were carried in plastic bags to the laboratory and stored at 4°C until processing.

To remove the soil and debris, roots were carefully washed under running tapwater. Roots were cut into 0.5-1.0 cm segments, and placed on Kleenex® tissue covering a mesh disc with a PVC collar, supported in plastic funnels (Plate 3.2). Nematodes were extracted by

misting (Southey, 1986) with a fine spray of water at 26°C, which is favourable for nematode motility, for ten seconds every ten minutes.

Nematodes were washed from the roots during the five days of misting, and collected in 100 ml test tubes under the funnels. A tubular extension on the funnel stems delivered the nematodes to the bottom of the test tube, allowing excess water to overflow the sides of the tubes. The low rate of flow from the intermittent misting was insufficient to carry away the nematodes, and nematodes settled to the bottom of the tube. An advantage of the mist extraction technique is that it allows dissipation of materials arising from the degradation of roots which may be toxic to nematodes (Lownsbery and Serr, 1963). After extraction, the nematode suspension from each sample was concentrated, depending on the density of nematodes in the suspension, to 20 or 40 ml, using filtration through a sintered glass funnel (No. 4). Samples were stored in screw-top containers at 4°C until counting.

Plate 3.2 Extraction of nematodes from the roots by misting.

The nematode suspension was shaken several times to ensure thoroughly mixing. One millilitre of each sample was taken immediately and the number of nematodes was determined by counting in a modified Doncaster (1962) dish using a low power microscope. At least three counts were made and averaged for each sample.

The washed roots from each field sample were pressed between sheets of paper towel to remove excess moisture, dehydrated at 85°C for 48 hours and weighed.

3.5 Measuring the percentage of nitrogen in plant tissues

For measuring percentage of N in plant materials, a 500 mg plant sample, one Kjeldahl Catalyst Tablet (Low Selenium), and 7.5 ml of concentrated H_2SO_4 were placed in a 50 ml digestion tube. The tube was placed in an aluminium heating block preheated to 380°C and a manifold placed in the mouth of each tube. The sample was digested under high pressure vacuum for ten minutes and at low pressure for another 40 minutes. The tube was removed from the heating block and the sample allowed to cool. After digestion, 25 ml of distilled water was added to the tube and the ammonium released from the digest by steam distillation with 35 ml of alkali (NaOH). Ammonium was collected in a 2% boric acid solution and titrated with 0.1 M HCl. The distilled ammonia was neutralised with a quantity of the standard acid. The boric acid solution contained 1% methyl red/bromocresol green indicator solution (Bremner, 1965) which showed the neutralisation stage by changing the colour of the solution. Ammonium in the digest, corresponding to the amount of nitrogen in the sample, was determined by the relative amount of the titrant consumed, by a KJELTEC Auto 1030 Analyser.

3.6 Measuring the concentration of elements in plant tissues

To measure the plant nutrient concentrations in shoots from field experiments, three quadrats (30 x 50 cm) were randomly placed on plants of the two middle rows of each plot, and the stems of plants inside the quadrat were cut at the soil surface. All three sub-samples from each plot were combined together to give a better representation of plants within each plot.

In glasshouse experiments, the shoots of all the plants in each pot were detached from the roots and analysed as a single sample. Plant shoot and root samples harvested from either the field or pot experiments were washed under tap water and rinsed twice with deionised water in two separate buckets. Shoots were placed in paper bags and dried in a forced draft oven at 85°C for 48 hours. Nematodes were extracted from roots by five to seven days of misting and roots were then dried at 85°C for 48 hours for measuring nutrient concentration.

Digestion tubes were cleaned in 2N HCl overnight, rinsed with deionised water and dried in the oven.

Tissues were ground with a 0.5 ml U.D.Y grinder and kept separately to avoid contamination. Half to one gram of dried sample was used to measure nitrogen percentage by the Kejeldhal method or the other elemental concentrations by ICP-spectrometry.

Immediately prior to digestion, samples were redried for two hours at 65°C. One gram sub-samples were placed in 75 cm^3 Pyrex tubes. Ten millilitres of 70% nitric acid (HNO_3) was added to each tube and the tubes allowed to stand overnight at room temperature under the fume hood. The Tecator Digestion System 40 was heated to 120°C, when the tubes were loaded, and temperature maintained at 120°C for one hour. The temperature was then raised to 140°C and maintained at that level until about 0.5 ml of acid remained in the tubes. After cooling, the samples were diluted to 20 ml with 1% V/V nitric acid.

The digests were filtered through Whatman No. 54 filter papers to remove amorphous siliceous residue and decanted directly into 5 ml plastic tubes. Samples were stored at 1-2°C until analysis. The concentrations of fourteen elements (Al, B, Ca, Co, Cu, Fe, K, Mg, Mn, Mo, Na, P, S and Zn) were simultaneously determined using a Labset V-25 Inductive Coupled Plasma-Optical Emission Spectrometer (Zarcinas and Cartwright, 1987) by Mr. N. H. Robinson of the Department of Plant Science, Waite Agricultural Research Institute, University of Adelaide.

3.7 Multiplication of *P. neglectus* on carrot culture

Cultures of pure *P. neglectus* were provided by Dr. V. A. Vanstone of the Department of Plant Science, Waite Agricultural Research Institute, University of Adelaide. A brief description for culturing *P. thornei* and *P. neglectus* has been published (Nicol and Vanstone, 1993). The following was the general recommended equipment and procedure to culture the nematodes on carrot pieces, modified from the method of Moody *et al.* (1973).

Fresh carrots, preferably regular in shape to facilitate peeling, were washed under tap water gently, removing soil and debris . The carrots were placed on two layers of paper towel in a tray to dry overnight at room temperature.

The surface of the laminar flow was wiped with 70% ethanol, and autoclaved instruments (forceps, knife, peeler and scalpel) placed in 95-98% ethanol. The carrot was held by the narrow end, and 0.5-1.0 cm of the top cut off with the flamed knife and discarded. The carrot was peeled with a flamed peeler using single strokes down the length of the carrot. The peeler was flamed after every other stroke.

The peeled carrots, with the thick end downwards, were transferred into a 2 litre glass beaker, covered for all but 1-2 cm of the thin end with 95-98% ethanol, for ten minutes. Between the steps in the process, the work area was wiped with 70% ethanol and the instruments flamed. Each carrot was held by the narrow end, touched to the flame and dropped onto the pre-sterilised work area for the alcohol to burn. The carrots were picked up, rotated slowly so that all the carrot surface was flamed and placed into a second sterile beaker. While holding the carrot by the narrow end, the flamed area was peeled again, as before. Half a centimetre of the thick end was cut off with the flamed knife and discarded and the sterile section of the carrots cut into about 6 cm lengths. Carrot segments, with the thick end downwards, were placed into sterile plastic tubs (6.7 cm in diameter and 7.5 cm in length). Callus formed on the thinner end which was pointing upwards.

A previously inoculated tub with heavily infected carrots was selected and the carrot removed from the tub with preflamed forceps and placed on its side in a large, sterile glass Petri dish. The callused material and browned areas of the carrot were sliced off with a sterile scalpel and the carrot sliced into a small pieces about 5 mm^3. Infected pieces of carrot were placed on the top of each segment of the new carrots in the tubs. The lids of the plastic tubs were replaced, the tubs labelled and incubated at 20-25°C for about three to four months and then transferred to 5°C until used for inoculum.

3.8 Staining nematodes within roots

Lactic acid fuchsin stain was prepared by mixing 3.5 g acid fuchsin, 250 ml lactic acid and 750 ml distilled water in a beaker and heating the solution to boiling. The solution then was filtered and cooled to room temperature (Byrd *et al.*, 1983).

The method used for staining nematodes was based on that of Byrd *et al.* (1983). Washed roots were cut into 2-3 cm segments, bleached in sodium hypochlorite (NaOCl) for ten minutes, rinsed under running water for about one minute and stained in lactic acid fuchsin (3.5%).

Tubes containing the stain and roots were boiled for 30 seconds, cooled to room temperature and rinsed under running water. The roots were placed in tubes containing glycerin, acidified with a few drops of 5N HCl, heated to remove excess stain, and cooled. The root segments were pressed between glass microscope slides for observation.

3.9 Inoculation

In South Australia, two *Pratylenchus* species commonly occur on cereals in the field (V. A. Vanstone and J. M. Nicol, pers. comm.). Naturally infested soil is also a habitat of many microorganisms including other nematodes, fungi and bacteria, which all interact with each other. For screening varieties for root lesion nematode resistance, the primary requirement

is that only the desired species is present. For these reasons, pure nematodes obtained from carrot culture were used as inoculum in soil pasteurised to kill almost all pathogens.

One week before plants were inoculated, nematodes were collected. Carrots from cultures were cut into thin discs and placed in water in Petri dishes. Over a five day period, the nematodes moved out of the carrot discs and the eggs were released from the thin carrot pieces. Since nematodes are sensitive to the absence of oxygen and cannot tolerate deep water, they were collected every eight hours and the suspension, concentrated to a small volume, was placed in shallow water in a 15 cm Petri dish at 4°C.

One millilitre of the suspension was taken and nematodes counted microscopically. The volume of the suspension was adjusted by concentrating or diluting by adding distilled water to obtain the required number of nematodes per millilitre of suspension. The suspension was shaken thoroughly and the appropriate volume of suspension pipetted close to the plant roots. The inoculum contained all stages of nematodes including eggs, larvae and adults, unless for some specific experiments when eggs alone or a particular stage of the nematodes was prepared.

A positive, linear relationship has been found between the number of *P. penetrans* invading the roots and inoculum densities below 200, and the percentage entering the roots increased as the inoculum level increased (Mauza and Webster, 1982). With high initial soil infestations, low rates of multiplication result, whereas low initial infestations favour higher rates of multiplication (Chitwood and Feldmesser, 1948; Oostenbrink, 1950 (cited Dropkin, 1955); Fenwick and Reid, 1953). To have a high rate of nematode multiplication, and to have the most intolerant varieties undamaged until harvest, a population of around 500 nematodes per pot/plant was usually applied, and the other environmental factors affecting nematode reproduction maintained at an optimum level (eg. temperature at 22 ± 1°C). At 22 ± 1°C, rapid nematode multiplication occurs, shortening the time needed to determine differences between genotypes being examined for nematode multiplication rates.

3.10 Chromosome identification

Since transmission of the additional chromosomes in wheat addition lines to the next generation is about 70%, depending on the genotype of the line (P. A. E. Ellis, pers. comm.), seeds had to be checked for the number of chromosomes before being included as experimental material.

Seeds from each addition line were placed in a tea infuser, soaked in 2-2.5% sodium hypochlorite (NaOCl) for 20 minutes and shaken occasionally, then washed three times in sterile distilled water. Petri dishes and filter papers were sterilised by placing them in boiling water for five minutes. The seeds were placed on two layers of sterile filter paper in sterile Petri dishes, moistened with sterilised distilled water and placed at 4-5°C for 48 hours to imbibe, ensuring rapid and even growth and therefore many dividing cells for examination. The Petri dishes were transferred to an incubator at 25°C for 20-40 hours, depending on the type of seed, until the roots were about 1.5 cm long.

One centimetre long root tips were removed and placed in water in vials which were stored on ice in a refrigerator for 24 hours. The root tips were fixed in 3 absolute ethanol : 1 glacial acetic acid for one to four hours at room temperature or, if it was late afternoon, left in fixative overnight. The roots were washed with distilled water and hydrolysed in 1N HCl at 60°C for fifteen minutes. Roots were washed again with distilled water and stained with Feulgen stain for 1-2 hours at room temperature. After staining, the root tips were transferred to water and placed in a refrigerator at 2-4°C until the chromosomes were counted.

The stained meristematic tip of the root was cut and placed in a drop of 45% acetic acid solution on a slide. Under a dissecting microscope, the root tip was split with a sharp needle and teased apart to free the individual dividing cells.

The bulky root tissue and excess materials were removed from the slide, and a cover-slip gently lowered and tapped to spread the root cells. The slide and cover-slip were placed under a layer of filter paper and the cover-slip pressed firmly onto the slide, ensuring that no lateral movement occurred. The slide was held over a spirit lamp to draw the cover-slip even more closely onto the slide by evaporation of the 45% acetic acid. The slides were examined under the microscope and the number of chromosomes counted.

3.11 Genetic material

The pedigree, Australian Wheat Collection number and geographical origin of all varieties and lines tested for their reaction to the root lesion nematode, *P. neglectus*, are listed in Table 3.6. Some of these were locally cultivated varieties which were generally susceptible to the nematode. Other varieties had shown a lower reproductive rate of the nematode than susceptible check varieties in preliminary experiments.

3.12 Statistical analyses

All presented data are mean values of the number of replicates indicated for each experiment. Data for the number of nematodes were transformed to Ln (number of nematodes + 200) (Proctor and Marks, 1974) prior to analysis of variance. Analysis of variance was carried out on a Macintosh computer using the Super ANOVA program. LSD (least significant difference) method (95% or 99%) was used to detect the differences between means (Gomez and Gomez, 1984).

Table 3.2 The analysis of Top Fos®, a fertiliser used in experiments as a source of phosphorus.

Components	%W/W
Phosphorus (P) as water soluble	13.9
Phosphorus (P) as citrate soluble	2.7
Phosphorus (P) as citrate insoluble	0.2
Phosphorus (P) as total	16.8
Sulphur (S) as sulphate	4.4

Table 3.3 The analytical composition of calcium nitrate ($Ca(NO_3)_2 + 4\ H_2O$), a fertiliser used in experiments as a source of nitrogen.

Components	%W/W
N as Nitrate	14.7
N as NH_3	1.1
Total N	15.8
Calcium	27.5
Fe	0.15

Table 3.4 The analytical composition of the components of potassium sulphate (K_2SO_4), a fertiliser used in experiments as a source of potash (K).

Components	%W/W
Total potassium present as sulphate	41.5
Sulphur present as sulphate	27.5

Table 3.5 The relative amount of components of magnesium sulphate ($Mg(SO_4)$ + 7 H_2O), a fertiliser used in experiments as a source of magnesium (Mg).

Components	%W/W
Magnesium	30.0
Sulphur	33.2
Iron	0.003
Chloride	0.403
Zinc	0.002

Table 3.6 Varieties and lines used in experiments, their pedigrees, Australian Wheat Collection (AUS) accession numbers and origins.

Variety/Line	AUS	Pedigree	Origin
1R (-1A)	Not available	CS/Rye Imperial 405/84	Sears, (USA)
1R (-1B)	Substitution line	CS/Rye Imperial 496/86	Sears, (USA)
1R (-1D)	Substitution line	CS/Rye Imperial 497/86	Sears, (USA)
1R (CS-Imperial rye)	Addition line	365/91 pl 1	Sears, (USA)
2R (CS-Imperial rye)	Addition line	372/91 pl 3	Sears, (USA)
3R (CS-Imperial rye)	Addition line	377/91 pl 1	Sears, (USA)
4R (CS-Imperial rye)	Addition line	363/91 pl 1	Sears (USA)
5R (CS-Imperial rye)	Addition line	26/92 pl 1	Sears, (USA)
6R (-6D)	Addition line	B83-9C-136-3-1	Unknown
6R (CS-Imperial rye)	Addition line	366/91 pl 3	Sears, (USA)
7R (CS-Imperial rye)	Addition line	374/91 pl 2	Sears, (USA)
Wollaroi (Durum)	Durum	TAM1B-17/Kamilaroi Sib// Rokel selection/Kamilaroi Sib	Australia
Abacus	Triticale	Unknown	Mexico
Angas	25418	Schomburgk 3*//Aroona/Moro	Australia
Aroona	20992	(WW-15 x Raven)/24/43.	Australia
AUS 7384	7384	Unknown	China
AUS 16830	16830	Unknown	Turkey
Barunga	25602	((H x Sch #4)x Mx#)8/3;WI034	Australia
Canola (*Brassica napus*, Variety Barossa)	Rape seed	Unknown	Unknown
Currency	Triticale	MIL-BGL "S"	Mexico
Excalibur	25292	RAC177 (Sr26)/UNICULM492 //RAC 311S	Australia
Frame	25601	9/9E;WI-153	Australia
GS 50A	Not available	A selection of Gatcher	Australia
Halberd	11612	(((Scimitar x Kenya C6042) x Bobin) x Insignia 49)	Australia

Table 3.6 (Continued)

Variety/Line	AUS	Pedigree	Origin
Imperial (Rye)	Rye	GH1-1988	USA
Iran 28357	10938	Unknown	Iran
Iraq	7639	Unknown	Iraq
Iraq 48	4930	Unknown	Iraq
Janz	24794	3AG3/4 x Condor//Cook	Australia
King II (Rye)	Not available	403/87 pl 3	Unknown
Local rye	Not available	176/87 pl2	Australia
Machete	23038	((SON 64)//TZPP/Y54)2 x GABD/Maden	Australia
Molineux	24457	((Pitic 62 x Festiguay) x Warigal#)	Australia
Muir	Triticale	Unknown	Mexico
Oxley	16461	Unknown	Australia
Persia 20	5205	Unknown	Iran
Guillemot (Durum)	21142	Unknown	CIMMYT
RAC 589	Not available	MKR//RAC 177/Fleche d'Or//RAC 177 (sr26)	Australia
RAC 613-27	Not available	Madden/Spear (CO2529-417)	Australia
RAC 613-47	Not available	Madden/Spear (CO2529-417)	Australia
Schomburgk	23325	(((W3589 x Oxley) x Warigal #2) x Aroona #2)	Australia
Souri (Durum)	11736	Unknown	Tunisia
Spear	22254	Sabre/MEC3//Insignia	Australia
SUN 146F	25930	4* Cook/VMP	Australia
SUN 277B	Not available	3*Potam//Cook/Sr24	Australia
SUN 289E	Not available	4*Potam//Cook/Sr23	Australia
SUN 290B	Not available	4*Potam/Cook/Sr24	Australia
Surak-I-Bahari	7869	Unknown	Iran

Table 3.6 (Continued)

Variety/Line	AUS	Pedigree	Origin
Tahara	Triticale	Unknown	Mexico
Tatiara	99144	(MKR X MN) W18/18:WT107	Australia
USDA CI 9040	7639	Unknown	Iraq
Virest	11894	Unknown	Italy
Warigal	20593	(WW-15 x Raven)	Australia
Yallaroi (Durum)	23825	Guillemot Sel^{n}-No. 3/Kamilaroi Sib	Australia

CHAPTER 4

EFFECTS OF ROOT LESION NEMATODE (*PRATYLENCHUS* SPP.) ON THE NUTRITION AND GROWTH OF WHEAT

4.1 EXPERIMENT 1

Effects of P. neglectus on wheat growth and nutrition and the assessment of using fertilisers (N, P and Mg) to compensate for the induced nutrient deficiencies

4.1.1 Introduction

In recent years, there has been growing recognition of the role of parasitic nematodes as plant pathogens and of their importance to crop production. Root lesion nematodes attack the roots, damaging the root hairs, cortex and meristems (Melakeberhan *et al.*, 1984, Thompson, 1989, Vanstone, 1991). In the absence of root hairs, there is little direct contact between the root surface and soil surface (Reid and Bowen, 1979). Root systems of infected cereal plants become extensively necrotic. The lesions within the cortex and infected roots have a general light-brown discolouration compared with the white colour of unaffected roots (Thompson, 1989).

The first sign of root lesion nematode damage to wheat plants is the yellowing of the lower (older) leaves, reduced vegetative growth and reduced soil cover, often in patches. Later in the season, poor "spindly" plants with few tillers and little harvestable grain result (Doyle *et al.*, 1986; Pattison and Fisher, 1993).

The main effect of nematodes on plants is in the destruction of the root hairs and reduction in growth of lateral roots as a result of nematodes feeding on the meristems, thereby reducing water absorption (Orion *et al.*, 1984) and nutrient uptake, causing infected plants to be deficient in some elements (Melakeberhan *et al.*, 1984). Reduced yield due to *P. thornei* is a result of relatively poor moisture status of the plants compared to those grown where water

supply has been maintained (Baxter and Blake, 1968). Crops affected by *P. thornei* wilted each day during dry periods (Thompson *et al.*, 1980b).

Yield reduction and many of the symptoms of *Pratylenchus* infection are likely to be the manifestation of the host's ability to compensate physiologically for nematode damage or to limit the damage. When searching for tolerance, understanding the effects of nematodes on nutrient levels and growth of a range of genotypes could be particularly useful. Changes in mineral concentration and total content due to nematode damage have often been noted (Willfarth and Wimmer, 1903, cited by Hunter, 1958): Kruger, 1925; Neuwirth, 1930; Serr and Day, 1949; Oteifa, 1952; Van Gundy *et al.*, 1962; Van Gundy and Stolzy, 1963; Van Gundy *et al.*, 1974; Nasr *et al.*, 1980; Orion *et al.*, 1984; Bhatt, 1986; Thompson, 1987b), but there is no general agreement concerning which elements are most affected. In some cases, fertiliser has been applied on the assumption that the damage caused by nematodes can be compensated for by increasing the availability of specific nutrients.

Wilfarth and Wimmer (1903) (cited by Hunter, 1958), found that nematode infected sugar beets had lower percentage N, K, Na, Ca and Mg, suggesting that the presence of nematodes deprives the plants of these nutritional substances. Application of a large quantity of K fertiliser maintained the sugar content within the beets, but did not prevent a decrease in yield (Kruger, 1925). Neuwirth (1930) confirmed the work of Kruger (1925) and observed that nematode infected beets exhibited signs of K deficiency.

Magistad and Olivera (1934) studied the effect of nematodes on N nutrition of pineapple. Infected plants absorbed 40-50% less N than uninfected plants, and the total dry weight of infected plants was reduced considerably. Nitrogen application resulted in an increased percentage of N in both infected and non-infected treatments, but only increased the growth of infected plants. Otiefa (1952) found that infected lima beans had lower total amounts of N, P, K, Ca and Mg as compared to plants growing in the treatment where there was no control of nematodes. Seeds of chickpea plants infected with *P. thornei* had a lower N content and protein percentage than non-infected plants (Bhatt, 1986). Doyle *et al.* (1987) studied the

effect of fertiliser application including N, P, K, Cu, Mg, B, Mn, Mo and their combinations on yield of wheat infected by *P. thornei* in NSW. The grain yield of plants grown in infected plots was not significantly different from the control, so they concluded that the disorder caused by the nematode was not due to an imbalance or lack of nutrients.

Application of NH_4NO_3 suppressed the reproduction of *P. neglectus* in wheat roots and their mobility in the soil (Kimpinski *et al.*, 1976). It has been suggested that nitrogen acts indirectly on nematodes by increasing the incidence of fungi which attack nematodes (Cook, 1962). On the other hand, Orion *et al.* (1984) showed that nitrogen application (ammonium sulphate) did not have any effect on population levels of *P. thornei.* In the absence of resistant and tolerant varieties, Thompson *et al.* (1981) have recommended the use of high yielding varieties and fertiliser application to reduce the yield loss caused by *P. thornei.* They have also concluded that well fertilised crops will leave more nematodes in the soil for the following year.

During past decades, the low trace element status of many Australian soils for growing agricultural crops and pastures has been demonstrated repeatedly (Hannam and Reuter, 1987; Judson *et al.*, 1987). In South Australia, single and multiple trace element deficiencies, together with macronutrient deficiencies, notably nitrogen, phosphorus, sulphur and potassium (Riceman, 1976; Tiver, 1988), magnesium and zinc (Egan, 1972) have been recognised. Above ground symptoms of nematode infection are usually like those caused by nitrogen, magnesium or phosphorus deficiency. The symptoms of nitrogen deficiency are chlorosis of the whole plant with older leaves more affected. Magnesium deficiency also causes chlorosis on older leaves first, although symptoms alone can often be misleading or non-specific, especially where more than one nutrient is limiting crop production (Reuter and Robinson, 1986).

Although there have been numerous studies on the effect of different species of nematode on the nutritional status of different plants as explained above and in Chapter 3, there are no reports on the effect of *P. neglectus* on the nutrition of wheat. Furthermore, soil physical

factors may be of primary importance in determining migration and penetration of the nematode so the effect of other environmental and biological factors must not be overlooked. As the behaviour of nematodes could be different, depending on site and climate, it was important to investigate how wheat plants were affected by *P. neglectus*, in terms of nutrient absorption and translocation, under South Australian soil and environmental conditions, and whether yield loss caused by the nematode could be compensated for by fertiliser application.

To investigate the effect of nematodes on nutrient uptake, plants should be grown in identical conditions, except for presence or absence of nematodes. One of the most general methods of reducing the nematode population is the use of nematicides. Aldicarb (Temik®) has proved to be an effective nematicide for controlling root lesion nematodes, including *P. thornei* (Thompson *et al.*, 1980c) and *P. neglectus* (Vanstone, 1991). It has been shown that *P. neglectus* can not survive sub-zero temperatures (Townshend and Anderson, 1976). Baxter and Blake (1968) also noted that freezing infested soil inactivated *P. thornei*. It is likely that nematodes are frozen and injured by the formation of internal ice crystals (Sayre, 1964). It is, of course, obvious that the low temperatures could have some influence on other microorganisms living in the soil and involved in soil chemical reactions. Using any means to kill the nematodes in the soil, including Temik®, would have some effects on other soil microorganisms. It is difficult to eradicate nematodes from soil and at the same time maintain the other soil characteristics unchanged.

The availability of equipment such as ICP-spectrometry enables monitoring of the concentration and the whole plant content of many elements. The purpose of experiments reported in this and the next Chapter was to study the problem of *P. neglectus* infestation with respect to nutrient concentrations, including N, P, K, Ca, Mg, Mn, Zn, Na, S and Fe, in plant tissues and to determine which of these elements was more affected by nematode infection and whether there were any differences between South Australian wheat varieties in their tolerance to nutrient/nematode interactions.

4.1.2 Materials and methods

Two varieties of wheat (*Triticum aestivum*), Molineux and Spear, were chosen to examine the effect of root lesion nematodes. Spear was grown only in the comparison between two varieties for symptoms and nutrient contents and concentrations in nematode infested soil supplied with different levels of fertilisers **(Part A).**

Part A	
Fertilisers	**Varieties**
Control	Spear
N	Molineux
P	
N+P	
N+P+Mg	

Part B (Variety Molineux)	
Fertilisers	**Nematode control treatments**
Control	Control
N	Frozen
P	Temik®
N+P	
N+P+Mg	

The effects of nematode control treatments (aldicarb and freezing) and different fertiliser treatments were examined by growing Molineux **(Part B)** only. Both varieties, especially Spear, are widely cultivated in South Australia. Field experience has shown that Spear does not show the yellow leaf symptoms as severely as some other commercial varieties such as Molineux, suggesting that Spear might be more tolerant to the nematode (A. J. Rathjen, pers. comm.). During the testing of a large number of varieties for resistance to root lesion nematode at the Waite Agricultural Research Institute (Vanstone *et al.*, 1993[b]), Spear and Molineux have had a high number of nematodes in their roots, as do most South Australian varieties. Spear has frequently shown a somewhat higher population of the nematodes than Molineux when the varieties have been examined under the same conditions.

Naturally infested soil was collected from Mr. B. Roberts' farm 5 km east of Balaklava, South Australia, on September 16, 1992. The soil was a sandy loam from a paddock in which chickpeas, susceptible to both *P. neglectus* and *P. thornei,* were grown in 1990 and was cropped to cv. Molineux in 1991. The wheat crop showed a noticeable yellowing and death of older leaves. Undisturbed soil cores were extracted under the growing crop at anthesis (September 16), by driving 7.5 cm diameter, 15 cm long PVC tubes into the soil to a

depth of 13 cm and returning them to the laboratory. One third of cores (32) were collected from the plots to which the nematicide Temik® (aldicarb) had been applied at 5 kg per hectare one month earlier in an attempt to control the yellow leaf symptoms. The population density of *P. neglectus* in the soil at the beginning of the trial, determined by the Whitehead tray method (General Materials and Methods), averaged eight nematodes per gram of soil.

Part A was a factorial experiment comparing two varieties in the soil with nematodes. **Part B** consisted of a factorial experiment with three levels of soil treatment including those previously treated with Temik®, frozen (to kill the nematodes) and no control of nematodes. One third of pots (untreated with Temik®) were placed in the cold room at -10°C for a period of 24 hours.

The second factor for both parts of the experiment was five levels of fertiliser as nutrient solution (Nil, N, P, Mg and N+P+Mg). All pots were watered with 50 ml of distilled water containing the appropriate amount of corresponding element or elements. The amount of each nutrient was adjusted to give an equivalent of 110 kg nitrogen, 30 kg phosphorus or 15 kg magnesium and the combination of the three per hectare.

Seed was pre-germinated in Petri dishes on moistened filter paper at 4°C for 24 hours to synchronise germination and transferred to an incubator at 26°C for 36 hours. Four seeds were planted in each pot and each treatment replicated four times.

Plants were grown in the evaporatively cooled glasshouse with a temperature of 15°C during the night and 25°C during the day for seven weeks and were hand-watered with approximately 30 ml of distilled water whenever necessary. Due to space limitations for nematode extraction, half the replicates of each treatment were harvested at seven and the other half at eight weeks after commencing the experiment for **Part B**.

At harvesting, tops were first washed under tap-water and then rinsed twice in separate baths of deionised distilled water and excess moisture removed by blotting between sheets of paper

towelling. The tops were weighed and dried at 80°C for 48 hours and then reweighed. The samples were ground separately and the nitrogen content measured by the Kjeldahl method. The concentration of other elements was measured with an ICP-Spectrometer (Chapter 3). Roots were washed under running tapwater to free them from debris, and nematodes extracted by misting (Chapter 3). Roots were dried at 80°C for 48 hours and the dry weights recorded.

Prior to analysis of variance for number of nematodes in the roots, the data were transformed to Ln (number of nematodes + 200) (Proctor and Marks, 1974) to render the variances independent of the means. All data, means of four plants in each pot, were subjected to analysis of variance and the means compared by LSD values.

4.1.3 Results

Part A

1 Interaction of variety and fertiliser treatments in the presence of nematodes

Shoot and root dry weight

No significant differences were found in top dry weight between the two varieties, Molineux and Spear, although Spear yielded slightly greater top and root dry weights.

The least top growth was obtained where magnesium alone had been applied and the greatest resulted from an application of N+P+Mg (Figure 4.1a). The difference between the control and addition of either magnesium or phosphorus alone was not statistically significant. Plants receiving nitrogen or N+P+Mg produced significantly ($P< 0.01$) more top growth than those with other fertilisers (Figure 4.1a). Although phosphorus had no effect on top dry yield when it was applied alone, it contributed to the highest top growth in combination with nitrogen.

The effect of fertilisers on top growth was the same for both varieties as the interaction effect of variety and fertiliser treatments was not significant.

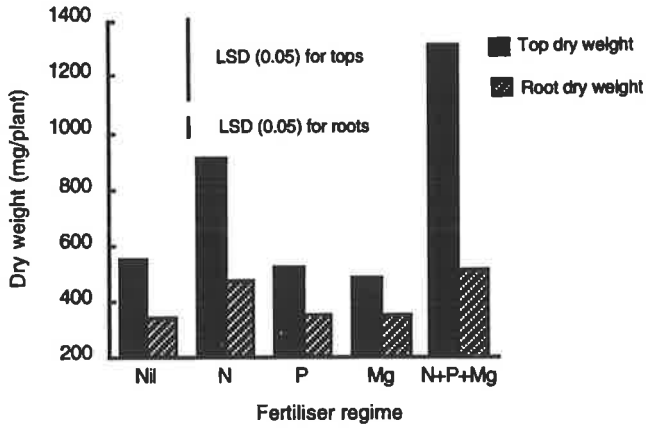
The same results were obtained for root dry weight, and these were highly correlated with the top dry weights ($R^2 = 0.93$) (Figure 4.1a). Root dry weight increased where nitrogen or N+P+Mg were utilised as fertilisers.

Number of nematodes

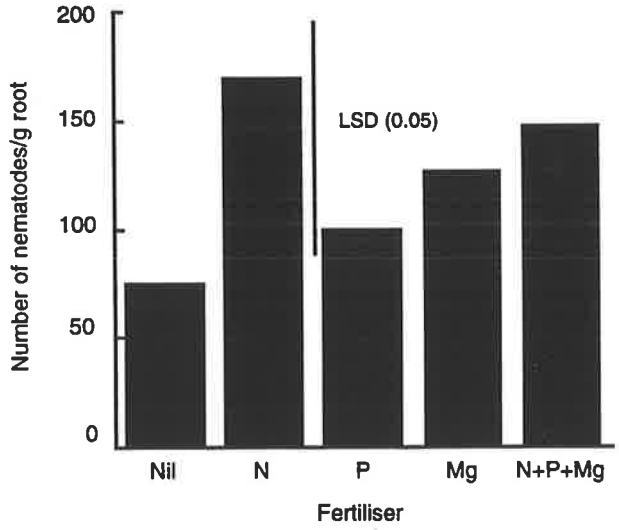
No significant difference was found between varieties or fertiliser treatments for number of nematodes per gram of dry root. Increased root growth increased number of nematodes per plant, so that number of nematodes per gram of root was almost constant.

Figure 4.1 Effect of fertiliser regimes (Nil, N, P, Mg, N+P+Mg) (**a**) on top and root dry weight and (**b**) on nematode multiplication of wheat varieties, Molineux and Spear, grown in 1 kg pots of soil naturally infested with *P. neglectus* (eight nematodes/g of soil) for eight weeks. (Values are the means of six replicates for each treatment).

(a)



(b)



2 Effect of fertiliser on concentration and content of elements in top growth of Spear and Molineux grown in nematode infested soil

Nitrogen

Spear had a higher concentration of N in shoots (Table 4.1) than Molineux, but this was not statistically significant.

The highest concentration of N occurred where nitrogen fertiliser was applied and the lowest concentration where no fertiliser was used (Table 4.1). Plants treated with phosphorus and magnesium fertilisers yielded a slightly higher concentration of N in their tops than the controls. The differences between the control and both the nitrogen and N+P+Mg treatment and that between the nitrogen and N+P+Mg treatments were highly significant ($P>0.01$) (Table 4.1).

In terms of N content in top growth, the difference between the two varieties was not statistically significant. Plants treated with fertilisers containing nitrogen had about four times higher N content than those treated with other fertilisers (Table 4.2).

Phosphorus

Molineux showed a higher concentration of P in its top growth than Spear, but the difference again was not statistically significant.

Fertiliser application had a significant effect on concentration of P in plants (Table 4.1). Highest concentration of P resulted where phosphorus was used alone and the lowest concentration, surprisingly, with N+P+Mg treatment, but the content of the element was significantly higher in plants receiving fertilisers containing nitrogen (Table 4.2). The difference between plants treated with phosphorus fertiliser alone and all other fertiliser regimes, other than magnesium, in terms of P concentration in top tissues was statistically significant (Table 4.1).

The amount of P in top growth of both varieties was the same and the interaction effect of variety and fertiliser application was not statistically significant.

Potassium

Spear had a lower concentration, but a higher amount, of K in its tops than Molineux, but these were not statistically significant.

The highest concentration or amount of K was found with the N+P+Mg treatment, and the lowest concentration and amount in the control and magnesium treated plants (Tables 4.1 and 4.2). A positive correlation ($R^2 = 0.93$) was found between concentration of K and top growth, so that K concentration increased as the top growth increased (Figures 4.2a).

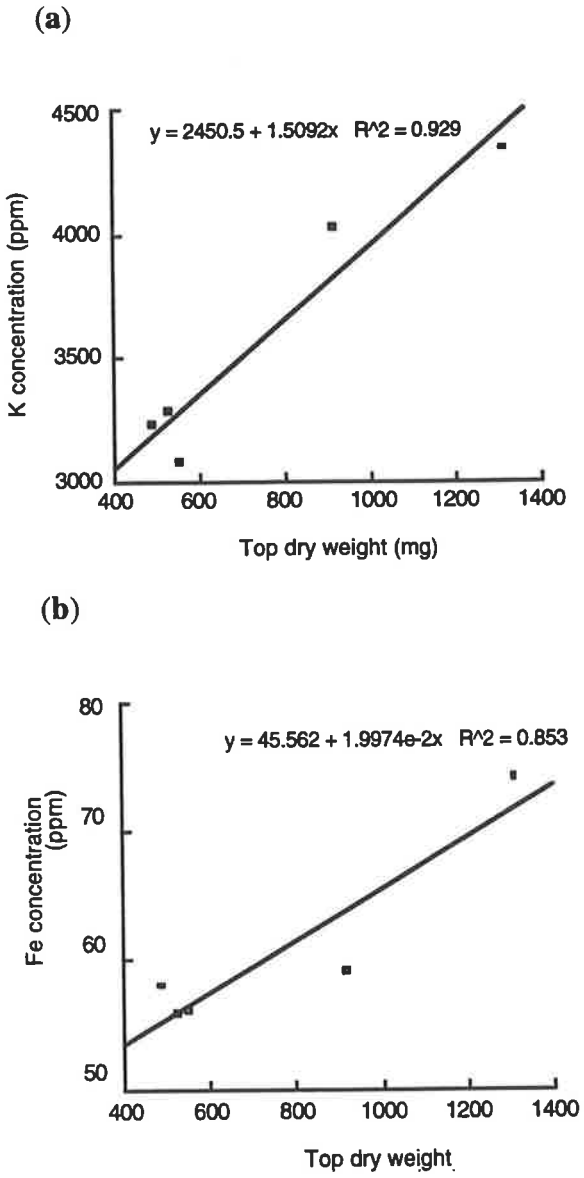
No significant interaction effect was found between varieties and fertilisers for either the content or concentration of this element.

Calcium

The concentration of Ca in Molineux tops was higher than in Spear, but again not statistically significant.

Application of fertilisers had a substantial effect on concentration of Ca in tops, so that the highest concentration of Ca was found with nitrogen addition and the lowest in the control (no added fertiliser) (Table 4.1). A significant difference ($P<0.001$) was found between nitrogen and all other fertiliser regimes in terms of Ca concentration (Table 4.1). Amount of Ca in shoots was significantly higher (about three times) when plants were treated with fertilisers containing nitrogen compared to other fertilisers (Table 4.2). Ca concentration, in contrast with K and Mg, did not follow the top growth pattern.

Figure 4.2 Correlation of (**a**) K and (**b**) Fe concentration (ppm) in vegetative tissues and top dry weight (mg) in wheat varieties Molineux and Spear grown in 1 kg pots of soil naturally infested with *P. neglectus* (eight nematodes/g of soil) at five fertiliser regimes (Nil, N, P, Mg, N+P+Mg) for eight weeks. (Values are the means of six replicates for each treatment).

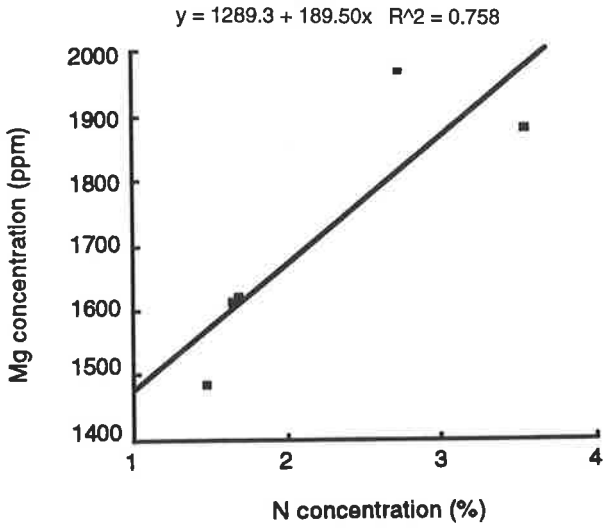


Magnesium

A significant difference was found between varieties for Mg concentration. Irrespective of the type of fertiliser used, the concentration was higher in Molineux than in Spear ($P>0.01$) (Table 4.1). The shoot content of Mg was also higher in Molineux, but was not statistically

significant.

Figure 4.3 Correlation of Mg concentration (ppm) and N concentration (%) in vegetative tissues of wheat varieties Molineux and Spear grown in 1 kg pots of soil naturally infested with *P. neglectus* (eight nematodes/g of soil) at five fertiliser regimes (Nil, N, P, Mg, N+P+Mg) for eight weeks. (Values are the means of six replicates for each treatment).



Fertiliser application also had a significant effect on Mg concentration in plants. The lowest concentration was found in the control treatment and the highest with N+P+Mg (Table 4.1). While the differences between the control and both phosphorus and magnesium treatments for Mg concentration in top growth was not statistically significant, plants growing in pots treated with magnesium and phosphorus had a slightly higher Mg content than the control treatment. Nitrogen application elevated the Mg concentration in tops so that significant differences resulted between plants receiving fertilisers containing nitrogen and other fertiliser treatments (Table 4.1). A positive correlation was observed between plant N concentration and Mg concentration in top tissues ($R^2 = 0.76$) (Figure 4.3). The amount of Mg in plants treated with nitrogenous fertiliser was significantly ($P<0.0001$) higher than with other fertiliser regimes (Table 4.2).

The interaction between varieties and fertiliser application was not statistically significant.

Manganese

Molineux yielded a higher concentration of Mn (Table 4.1) in its top tissues than Spear and the difference was highly ($P<0.01$) significant, but the amount of Mn in shoots was similar (Table 4.2).

The highest concentration of Mn in Molineux was obtained where nitrogen was applied, while for Spear this occurred where no fertiliser was applied to the plants (data not shown). The highest amount of Mn was measured in plants treated by N+P+Mg and followed by those receiving nitrogen alone, and the differences between these two and both with other fertiliser regimes was highly significant ($P<0.0001$) (Table 4.2).

Interaction of fertilisers and varieties was not statistically significant for either Mn concentration or content in top tissues.

Zinc

No significant difference was found between varieties for Zn concentration or content, although Molineux had a higher concentration, but a lower amount of the element than Spear (Tables 4.1 and 4.2).

The difference between fertilisers was highly significant. The highest concentration and amount of Zn was found in plants receiving nitrogen and the lowest concentration and amount in the control and magnesium treatments, respectively (Tables 4.1 and 4.2). While the difference between fertiliser treatments, excluding nitrogen alone, were not significant in terms of concentration of Zn in top growth, the difference between nitrogen alone and all other fertiliser regimes was highly significant ($P<0.01$) (Table 4.1).

Iron

Molineux had a slightly higher Fe concentration in its top growth than Spear (Table 4.1).

The highest concentration of the element was found in plants receiving N+P+Mg and the lowest in those treated with phosphorus fertiliser. Both varieties showed the same pattern for Fe concentration or content at each treatment, so that no interaction was found between varieties and fertiliser regimes. Concentration of Fe followed top growth ($R^2 = 0.85$), so that plants having more top growth demonstrated a higher concentration of Fe in their tissues (Figure 4.2b).

Sodium and *Sulphur*

No significant differences were found between Molineux and Spear for Na and S concentration (Table 4.1).

Fertiliser application had no significant effect on Na and S concentration in top growth of either variety. The interactions of varieties and fertilisers in terms of Na and S concentration or content were not statistically significant.

Table 4.1 Concentration of elements in shoots of varieties Molineux and Spear grown in nematode infested soil and treated with five fertiliser regimes. Plants were grown in an evaporatively cooled glasshouse for eight weeks and watered with distilled water.

	Elements									
Treatments	N (%)	P 10^{-3}	K 10^{-3}	Ca 10^{-3}	Mg 10^{-3}	Mn 10^{-6}	Zn 10^{-6}	Fe 10^{-6}	Na 10^{-6}	S 10^{-3}
Molineux	2.14	5.25	3.63	5.43	1.91	81.0	35.3	61.7	96.3	3.05
Spear	2.30	4.74	3.56	4.94	1.52	63.2	37.7	59.7	97.9	2.29
LSD (0.05)	**0.27**	**0.70**	**0.34**	**0.59**	**0.16**	**10.9**	**4.79**	**4.9**	**24.8**	**1.31**
Nil	1.48	4.87	3.08	4.36	1.49	77.0	34.1	56.2	91.7	2.14
Nitrogen	3.56	4.82	4.02	6.75	1.88	76.2	47.8	59.2	102.5	3.01
Phosphorus	1.68	6.17	3.29	4.62	1.62	68.8	35.8	55.9	86.9	2.34
Magnesium	1.65	5.25	3.23	4.96	1.61	69.7	34.4	58.0	95.3	3.17
N+P+Mg	2.74	3.86	4.35	5.23	1.97	68.7	30.4	74.2	109.2	2.69
LSD (0.05)	**0.43**	**1.10**	**0.54**	**0.94**	**0.25**	**17.30**	**7.57**	**7.8**	**39.2**	**2.08**

Table 4.2 Content (mg/plant) of elements in shoots of varieties Molineux and Spear grown in nematode infested soil and treated with five fertiliser regimes. Plants were grown in an evaporatively cooled glasshouse for eight weeks and watered with distilled water.

	Elements									
Treatments	**N**	**P**	**K**	**Ca**	**Mg**	**Mn**	**Zn**	**Fe**	**Na**	**S**
Molineux	15.87	3.60	2.67	3.89	1.38	0.055	0.025	0.044	0.065	2.06
Spear	21.73	3.66	3.05	4.34	1.33	0.052	0.031	0.051	0.089	1.88
LSD (0.05)	**7.23**	**1.10**	**0.68**	**1.36**	**0.36**	**0.013**	**0.010**	**0.012**	**0.033**	**0.86**
Nil	7.95	2.65	1.70	2.36	0.81	0.040	0.019	0.031	0.051	1.14
Nitrogen	33.98	4.59	3.61	6.45	1.73	0.069	0.046	0.055	0.103	2.28
Phosphorus	8.76	3.19	1.70	2.39	0.84	0.036	0.019	0.029	0.043	1.21
Magnesium	8.06	2.56	1.57	2.41	0.79	0.034	0.017	0.028	0.042	1.55
N+P+Mg	35.29	5.17	5.71	6.96	2.60	0.091	0.041	0.096	0.143	3.67
LSD (0.05)	**11.44**	**1.74**	**1.08**	**2.15**	**0.56**	**0.020**	**0.017**	**0.018**	**0.052**	**1.36**

Part B

1- Effect of fertiliser application on concentration and content of elements in shoots of Molineux grown in soil with or without nematodes

Harvest date

Due to lack of space on the mister, half the replicates of each treatment (no control of nematodes, Temik® and freezing) were harvested after seven weeks. The second harvest was carried out one week later, at eight weeks. As expected, the second harvest yielded significantly ($P<0.05$) more top and root growth than the first one (Table 4.3). Number of nematodes per gram of dry root slightly, but not significantly, decreased. Percentage of N and concentrations of P, K, Mg, Zn and Na significantly decreased from the first to the second harvest. The time of harvest did not have any significant effect on concentrations of Ca, Mn, Fe or S in plant tissues. The amounts of N, P, K, Ca, Mg, Zn, Mn, Fe and S were similar at both harvests, while Na content significantly decreased from the first to the second harvest.

Shoot dry weight

The greatest top growth was recorded for plants supplied with N+P+Mg fertiliser and the lowest where no fertiliser was used (Figure 4.4). While no significant difference was found between no control of nematodes and Temik® treatment with no added fertiliser or fertiliser regimes lacking nitrogen, the difference between the no control of nematodes and Temik® with N and N+P+Mg fertiliser regimes was highly significant ($P<0.01$) (Figure 4.4).

Table 4.3 Effect of harvest time on means of top and root dry weights and element concentration in shoots of plants grown in 1 kg pots of a naturally nematode-infested soil (eight *P. neglectus*/g soil) or soil treated at low temperature (-10°C for 24 h) or nematicide (aldicarb, 5kg a.i. /ha) with five fertiliser regimes (Nil, N, P, Mg or N+P+Mg)

	Top and root dry weight, number of nematodes and concentration of elements												
Treatments	**Top dry weight** (g/plant)	**Root dry weight** (g/plant)	**No. of nematodes** /g root	**N** (%)	**P** 10^{-3}	**K** 10^{-3}	**Ca** 10^{-3}	**Mg** 10^{-3}	**Mn** 10^{-6}	**Zn** 10^{-6}	**Fe** 10^{-6}	**Na** 10^{-6}	**S** 10^{-3}
Seven weeks	3.21	0.402	3200	2.06	4.61	3.98	4.87	1.84	84.0	33.2	59.7	124.1	3.08
Eight weeks	3.60	0.443	2700	1.10	4.07	3.39	4.53	1.68	83.0	29.7	57.5	85.8	2.56
LSD (0.05)	**0.096**	**0.039**	**6700**	**0.20**	**0.443**	**0.223**	**0.352**	**0.119**	**7.93**	**3.06**	**4.180**	**12.90**	**0.885**

Root dry weight

Plants growing in pots without fertiliser application produced the smallest root growth and those in pots treated with N+P+Mg yielded the greatest (Figure 4.5b). No significant differences were found between the control and either the phosphorus or magnesium fertilisers for root dry weight. The difference between the control and both nitrogen and N+P+Mg fertiliser was highly significant, as it was for both Mg and P with N and N+P+Mg (Figure 4.4). The difference between N and N+P+Mg fertiliser was significant, suggesting that in the presence of nitrogen, application of phosphorus was necessary for maximum root growth. Temik® was found to be more effective in the presence of N and N+P fertiliser in terms of root growth (Figure 4.5b).

Figure 4.4 Effect of soil treatments (no control of nematodes, freezing and Temik®) on top and root dry weight of wheat variety Molineux grown in 1 kg pots of soil naturally infested with *P. neglectus* (eight nematodes/g of soil) in the glasshouse.

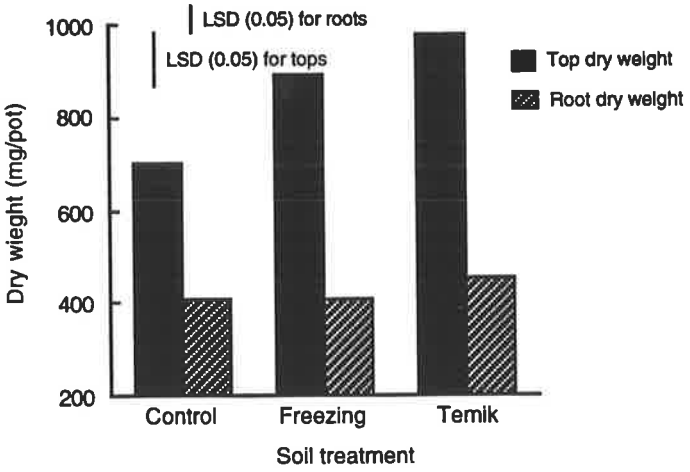
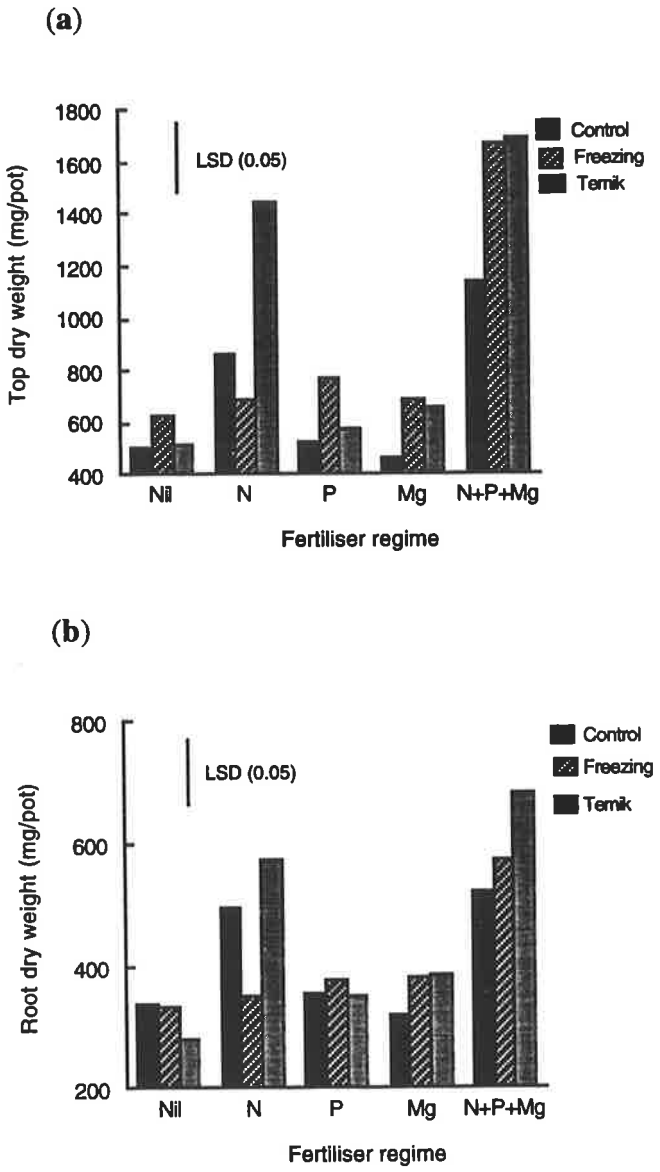


Figure 4.5 Interaction of soil treatment and fertiliser application on (**a**) top dry weight (mg/pot) and (**b**) root dry weight (mg/pot) of wheat variety Molineux grown in 1 kg pots of soil naturally infested with *P. neglectus* (eight nematodes/g of soil) or soil treated by freezing (-10°C for 24 h) or aldicarb (5 kg a.i./ha) at five fertiliser regimes (Nil, N, P, Mg, N+P+Mg) for seven to eight weeks. (Values are the means of six replicates for each treatment).



Number of nematodes

No nematodes were found in roots of plants grown in pots containing soil treated at low temperature. Few nematodes (an average of 35 nematodes per plant) were found in roots of plants growing in soil treated with Temik®. The difference between no control of nematodes and Temik® (with 17,500 and 96 nematodes per gram dry root, respectively) was highly significant. Fertiliser application did not have any significant effect on the density of nematodes in plant roots. As plants were harvested before anthesis, most of the nematode population was expected to have been in the root systems rather than in the soil, so number of nematodes in the soil was not measured. Plants receiving fertiliser containing nitrogen were similar to other fertiliser regimes in terms of number of nematodes per gram of root.

2- Effect of soil treatment and fertiliser application on yellow leaf symptoms and concentration of elements in shoots of wheat variety Molineux

Visual observations showed that yellow lower leaf symptoms were reduced by treating the soil with both Temik® and freezing and the application of nitrogen. Plants growing in Temik® treated soil were greener than those in frozen soil. Nitrogen application restricted the symptoms with a greater effect on plants growing in soil treated with Temik®. Soil treatments alone did not completely remove the symptoms (Plate 4.1).

The effects of the soil treatments and fertiliser applications on concentration and content of each measured element were as follows:

Nitrogen

Application of Temik® reduced the concentration of N in plant top tissues when nitrogen or N+P+Mg fertiliser was applied to plants, but the amount of N was significantly higher (Figure 4.7a). The percentage of N in top growth of the plants grown in no control of nematodes treatment was higher than those grown in both Temik® treated and frozen soil, although this was not statistically significant. Nitrogen application increased both the

percentage and amount of N in top tissues significantly (Tables 4.4 and 4.5). The lowest concentration of N was obtained with the phosphorus fertiliser regime and the highest obtained where nitrogen was applied (Table 4.4). The difference between the control (no fertiliser) and both magnesium and phosphorus in terms of their effects on N concentration was not statistically significant, while the difference between both N and N+P+Mg with other fertiliser regimes was highly significant ($P<0.01$) (Table 4.4).

Plate 4.1 Extent of yellow leaf symptom in plants grown in different soil treatments (control, freezing or Temik®) in wheat variety Molineux with no fertiliser applied.

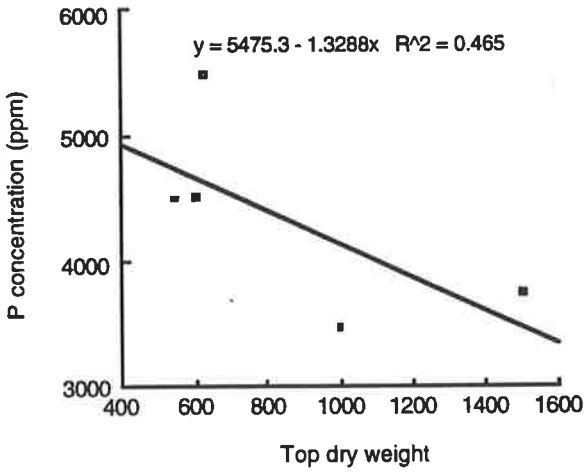
Control **Freezing** **Temik®**

Phosphorus

In terms of concentration of P in plant tissues, Temik® application was similar to the control (Table 4.4). In contrast with other elements (N, K, Fe and Mg), a negative correlation (R^2 = 0.47) was found between P concentration and top growth (Figure 4.6). The highest concentration of P resulted after adding phosphorus to the soil, and the lowest where N alone was applied (Table 4.4).

The amount of P in plants treated with Temik® was higher than in those with no control of nematodes, although the difference was not statistically significant. Both Temik® and no control of nematodes (mean 4.0 mg/plant) had a significantly higher content of P than the freezing treatment (2.4 mg/plant) (Table 4.5).

Figure 4.6 Correlation of P concentration (ppm) in vegetative tissues and top dry weight (mg) in wheat variety Molineux grown in 1 kg pots of soil naturally infested with *P. neglectus* (eight nematodes/g of soil) at five fertiliser regimes (Nil, N, P, Mg, N+P+Mg) for eight weeks. (Values are the means of six replicates for each treatment).



Potassium

Although Temik® application increased the concentration of K in plants, its difference with no nematode control and freezing treatments was not statistically significant. Fertiliser application divided plants into two groups. Those receiving fertilisers containing nitrogen had a significantly ($P<0.01$) higher concentration of K than plants with no fertiliser or fertiliser lacking nitrogen (Table 4.4).

All three soil treatments were significantly different in terms of K content in shoots, with Temik® demonstrating the highest and no control of nematodes the lowest (Table 4.5).

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Interaction of soil treatments and fertiliser application in terms of K concentration in the top growth was not statistically significant, but the highest difference between the soil treatments was observed in the nitrogen fertiliser regime (Figure 4.7b).

Magnesium

The difference between soil treatments for Mg concentration was significant mainly due to reduction of Mg uptake in the freezing treatment (Table 4.4). Although Mg concentration in plants growing in pots receiving Temik® was higher than that of those growing with no control of nematodes, the difference was not statistically significant.

Manganese

Overall the highest concentration of Mn was measured in tissues of plants growing in pots treated with Temik®, and the lowest in those grown with no control of nematodes, but the differences were not statistically significant. But, with application of nitrogen fertiliser alone, freezing yielded a significantly higher concentration of Mn than both the no nematode control and Temik® treatments (Figure 4.7d). Plants receiving only nitrogen as a fertiliser had a higher concentration of Mn than those receiving fertilisers containing phosphorus (Table 4.4). The difference between nitrogen and both phosphorus and N+P+Mg, and that between the control and N+P+Mg, was statistically significant. In contrast, when phosphorus was applied alone, freezing yielded a lower concentration of Mn than did the Temik® treatment (Figure 4.7d).

Zinc

Temik® treated plants yielded a significantly ($P<0.05$) greater amount of Zn (Table 4.5) in top tissues than both freezing and no nematode control treatments, while in terms of concentration (Table 4.4), Temik® was similar to no control of nematodes. The freezing treatment decreased the concentration and its difference with both no control of nematodes and

Temik® was significant (P<0.05) (Table 4.4).

Application of fertilisers containing nitrogen significantly increased the shoot Zn content (Table 4.5), while the difference between the control (without fertiliser) and nitrogenous fertilisers in terms of concentration of the element was not statistically significant (Table 4.4).

Interaction of soil treatments and fertiliser applications for Zn concentration was highly (P<0.001) significant (Figure 4.7e). With no fertiliser treatment, plants grown in soil treated with Temik® demonstrated a significantly higher concentration of the element than those of both the no control of nematodes and freezing treatments. When nitrogen was applied to the plants, the element concentration with no control of nematodes was higher than with both Temik® and freezing treatments (Figure 4.7e).

With phosphorus and magnesium fertiliser regimes, the effect of both Temik® and no control of nematodes was similar for Zn concentration, but both were significantly different from the freezing treatment. No significant difference was found between soil treatments in terms of Zn concentration when all three fertilisers (N+P+Mg) were applied to the plants (Figure 4.7e).

Iron

Temik® application resulted in a significantly higher amount of Fe in plant tissues (P<0.05) than with no control of nematodes or freezing (Table 4.5), but the concentration of the element in plants grown in nematode infested soil and those grown in soil treated with the nematicide was almost the same (Table 4.4).

Plants receiving no fertiliser or fertilisers lacking nitrogen were the lowest in concentration (Table 4.4) or amount (Table 4.5) of Fe and significantly different (P<0.001) from both plants treated with nitrogen alone or N+P+Mg. The combination of all three fertilisers resulted in a greater concentration and amount of the element.

The interaction of fertiliser and soil treatment in terms of Fe concentration was not statistically significant.

Sodium

Soil treatment and fertiliser application did not have any significant effect on concentration of Na in plant top tissues.

In terms of Na content, plants growing in Temik® treated soil were higher than those with no control of nematodes or freezing (Table 4.5). Nitrogen application increased the Na content in top tissues significantly ($P<0.05$) compared to the control or other fertiliser regimes. Where nitrogen was accompanied with phosphorus and magnesium, the effect was greater, so that the difference between nitrogen alone and N+P+Mg in terms of Na content of plants was significant (Table 4.5).

Sulphur

Temik® application elevated the level of S in plant tissues, so that plants grown in pots containing soil treated with Temik® had a higher concentration of the element than those with either no control of nematodes or freezing, although the difference between Temik® and no control of nematodes was not statistically significant (Table 4.4). In contrast, the effect of Temik® was negative in terms of concentration of S with nitrogenous fertiliser regimes compared to other fertilisers, but this interaction was not statistically significant (data not shown).

Table 4.4 Concentration of elements in top tissues of wheat variety Molineux grown for eight weeks in soil with (control) or without (Temik or freezing) nematodes and treated with five fertiliser regimes.

	Element									
	N	**P**	**K**	**Ca**	**Mg**	**Mn**	**Zn**	**Fe**	**Na**	**S**
Treatments	(%)	10^{-3}	10^{-3}	10^{-3}	10^{-3}	10^{-6}	10^{-6}	10^{-6}	10^{-6}	10^{-3}
Control	2.14	5.25	3.63	5.43	1.91	81.0	35.3	61.7	96.3	3.05
Temik	1.97	5.15	3.83	4.87	1.98	86.7	35.1	59.9	110.8	3.34
Freezing	1.94	2.61	3.59	3.80	1.38	82.6	24.0	54.2	107.8	2.06
LSD (0.05)	**0.250**	**0.542**	**0.273**	**0.431**	**0.197**	**9.71**	**3.75**	**5.12**	**15.8**	**1.08**
Nil	1.68	4.49	3.14	4.09	1.46	86.9	32.7	54.6	105.1	2.42
Nitrogen	2.85	3.47	4.49	6.37	1.95	93.7	35.5	64.1	107.2	2.98
Phosphorus	1.48	5.48	3.16	3.96	1.68	77.4	31.3	51.9	101.8	2.64
Magnesium	1.67	4.51	3.26	4.18	1.67	87.4	29.5	50.8	98.9	3.22
N+P+Mg	2.38	3.75	4.37	4.89	2.02	72.3	28.2	71.5	111.7	2.83
LSD (0.05)	**0.323**	**0.700**	**0.352**	**0.556**	**0.254**	**12.54**	**4.84**	**6.61**	**20.4**	**1.40**

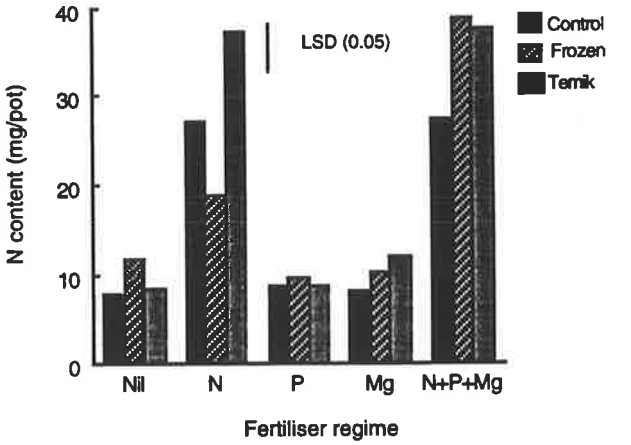
Table 4.5 Content (mg/plant) of elements in top tissues of wheat variety Molineux grown for eight weeks in soil with (control) or without (Temik® or freezing) nematodes and treated with five fertiliser regimes.

	Element									
Treatments	**N**	**P**	**K**	**Ca**	**Mg**	**Mn**	**Zn**	**Fe**	**Na ($x10^{-5}$)**	**S**
Control	15.89	3.60	2.67	3.89	1.38	0.06	0.03	0.04	3.8	2.06
Temik	20.87	4.34	4.06	4.88	2.02	0.08	0.03	0.06	4.9	3.17
Freezing	17.78	2.41	3.29	3.45	1.31	0.07	0.02	0.05	4.3	1.93
LSD (0.05)	**2.33**	**0.79**	**0.48**	**0.74**	**0.28**	**0.01**	**0.01**	**0.01**	**0.74**	**0.72**
Nil	9.25	2.34	1.72	2.17	0.78	0.05	0.02	0.03	3.3	1.29
Nitrogen	27.88	3.48	4.56	6.11	2.02	0.09	0.03	0.07	5.0	2.77
Phosphorus	9.05	3.22	1.95	2.32	1.01	0.05	0.02	0.03	3.6	1.60
Magnesium	10.09	2.64	1.96	2.44	1.00	0.05	0.02	0.03	3.5	1.93
N+P+Mg	34.63	5.57	6.50	7.34	3.04	0.11	0.04	0.11	6.4	4.36
LSD (0.05)	**3.00**	**1.02**	**0.62**	**0.96**	**0.36**	**0.01**	**0.01**	**0.01**	**0.95**	**0.93**

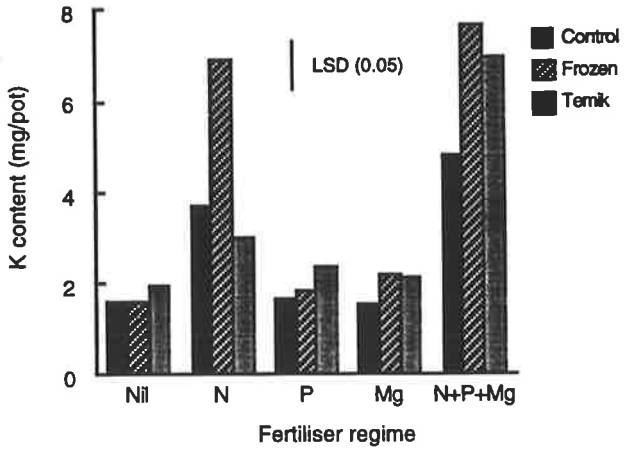
Figure 4.7 Interaction of soil treatments and N (**a**), K (**b**) and Mg content (**c**) and Mn (**d**) and Zn concentration (**e**) in vegetative tissues of wheat variety Molineux grown in 1 kg pots of soil naturally infested with *P. neglectus* (eight nematodes/g of soil) or treated soil by freezing (-10°C for 24 h) or Temik® (5kg a.i./ha) at five fertiliser regimes (Nil, N, P, Mg, N+P+Mg) for seven to eight weeks. (Values are the means of six replicates for each treatment).

Figure 4.7

(a)



(b)



(c)

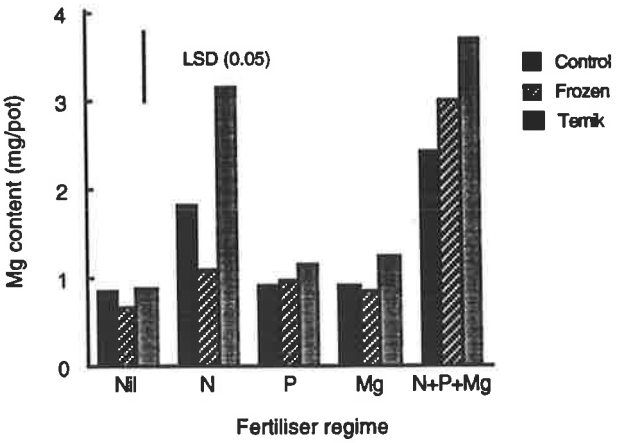
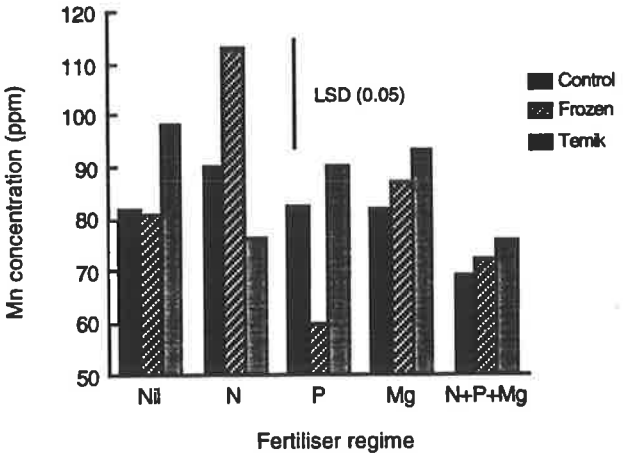
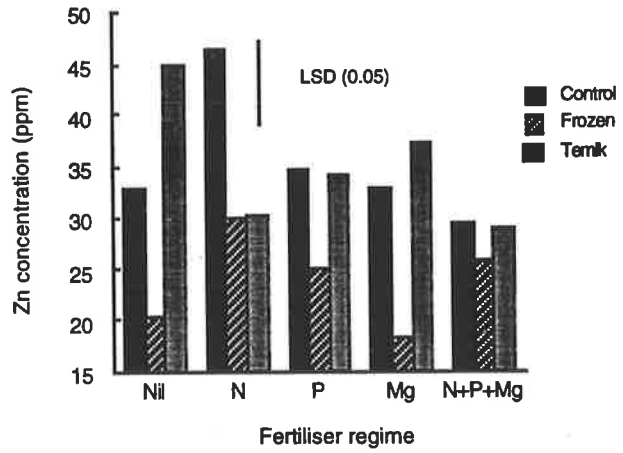


Figure 4.7 continued

(d)



(e)



4.2 EXPERIMENT 2

Further investigation of the effects of nematodes on wheat growth and nutrition by using different methods of controlling the nematode and of different fertilisers to compensate for the damage.

4.2.1 Introduction

Since it has been reported that Temik® has some side effects on plants besides its effect on the nematode population, (J. M. Fisher, pers. comm.), alternative nematode control treatments were used to investigate the effects of the root lesion nematode (*P. neglectus*) on uptake of nutrients and consequently its effects on plant growth. These treatments were short term freezing and rapid drying of the infested soil.

In the previous experiment it was observed that plants growing in pots receiving low temperature treatment (-10°C for 24 hours) had a significantly lower uptake of some elements, including phosphorus. It seems that this temperature regime and its duration had adverse effect on the absorption of these elements and plant growth. Hence a preliminary experiment was conducted to assess the minimum temperature required to kill the nematodes. The results showed that -6°C for a period of ten hours was adequate to kill almost all the nematodes. Periods shorter than ten hours at -6°C were not adequate to eradicate the nematodes, maybe due to the large diameter of cores (about 10 cm), so that with a period of less than ten hours, the centre of these cores did not fall to the temperature required to kill the nematodes.

The aims of this experiment were to assess the effect of *P. neglectus* on elemental concentration by examining different methods of killing the nematodes. Fertilisers including N+P, N+Mg or P+Mg were also applied to partition the effects of the N+P+Mg combination used in the previous experiment and determine the best combination of fertiliser to compensate for nematode infection.

4.2.2 Materials and Methods

The wheat variety used in this experiment was Molineux. This variety has resistance to the cereal cyst nematode which has been a serious pest problem for several decades in southern Australia (Rathjen *et al.*, 1989, 1993).

The treatments investigated in this experiment were presence and absence of the nematode, using a rapid drying treatment at 45°C or low (-6°C) temperature to kill the nematodes, and fertiliser application including control (no fertiliser), N, P, Mg and their combinations (N+P, N+Mg, P+Mg or N+P+Mg).

Soil naturally infested with *P. neglectus* was collected from the property of Mr. B. Roberts (5 km east of Balaklava, South Australia) on February 3, 1992. PVC pipes 7.5 cm in diameter and 15 cm long were driven into the soil to a depth of 13 cm between rows, where the previous crop (wheat cv. Molineux) had been harvested. The lower ends of the cores were covered with Petri dish lids which were taped into position to prevent soil loss. The number of nematodes, determined by the Whitehead tray method (General Materials and Methods), averaged five nematodes per gram of wet soil. Soil texture was determined by the Department of Soil Science at the Waite Agricultural Research Institute as a sandy clay loam soil and its analysis was as follows:

Silt = 12%

Clay = 36%

Sand = 46%

Gravel = 6%

pH = 7.75

To give an even moisture treatment for all pots, 50 ml of distilled water was added to all cores which were then left at room temperature overnight.

Cores treated with low temperature were transferred to a freezer at -10°C for six hours. To

kill the nematodes with minimum damage to other microorganisms, cores with moist soil were placed in a forced air oven at 45°C for a period of 48 hours for rapid desiccation of the nematodes. At this temperature, the drying period was sufficiently intense to kill a high proportion of nematodes (results of preliminary experiments) by preventing them entering the anhydrobiotic state.

Fertilisers used in this experiment were as follows:

As NO_3 is less toxic to nematodes than nitrogenous fertiliser containing NH_4^+, calcium nitrate $Ca(NO_3)_2$ was used as a source of nitrogen, at a rate equivalent to 110 kg nitrogen/ha (0.375 g per pot).

Sodium bi-phosphate ($NaH_2PO_4 + 4\ H_2O$) was applied as a source of phosphorus (equivalent to 25 kg phosphorus/ha or 0.073 gram per pot).

Magnesium sulphate ($MgSO_4$) as a source of magnesium, was applied at a rate equivalent to 14 kg magnesium/ha (0.02 gram per pot).

Appropriate amounts of each fertiliser or their combinations were dissolved in 250 ml of distilled water. Ten millilitres of each solution was added to the corresponding pots. For the pots receiving no fertiliser, the same volume of distilled water was added.

Seeds were surface sterilised with 5% sodium hypochlorite for ten minutes, placed on moistened filter papers in Petri dishes and transferred to a refrigerator at 4°C for a period of 24 hours to give uniform germination. Seeds were then placed in a 25°C incubator to germinate. After about 48 hours, four seedlings were transplanted into each core.

Cores were put in plastic bags, to protect them from seepage, and transferred to controlled temperature waterbaths at 25 ± 2°C in an evaporatively cooled glasshouse.

The experimental design was a completely randomised factorial experiment, with two factors

(soil treatment and fertiliser application) and six replications. The three soil treatments were no control of nematodes, controlling nematodes by rapid drying or by low temperature treatment, and eight fertiliser applications (nil, N, P, Mg, N+P, N+Mg, P+Mg or N+P+Mg).

Plants were grown for seven or eight weeks and watered with distilled water whenever it was necessary. Because of space limitations on the mister, half the replicates (three replicates) were harvested at seven weeks and the remainder at eight weeks after the commencement of the experiment. Nematodes were extracted from the roots for seven days (General Materials and Methods). Roots were then transferred to paper bags and dried. The tops were cut, washed and dried and the element concentrations in roots and tops measured by ICP-spectrometry (General Materials and Methods).

Number of nematodes was transformed to Ln (number of nematodes + 200) (Proctor and Marks, 1974) before analysis. All data were subjected to analysis of variance and the means were compared by LSDs.

4.2.3 Results

About twenty days from commencement of the experiment, there was a significant difference in appearance of plant leaf colour and height between plants with no control of nematodes and those receiving high or low temperature treatment (Plate 4.2a). In the absence of nitrogen, plants growing in pots of rapidly dried soil were darker in colour and appeared to have no nutritional deficiencies. Plants in soil treated at low temperature were slightly darker in colour compared to those grown in rapidly dried soil. Untreated plants, that is grown in soil containing nematodes (other than those receiving nitrogen fertiliser), appeared to have a slightly greater number of yellow lower leaves than those growing in the soil without nematodes (Plate 4.2a).

After four weeks, the yellow leaf symptom was more obvious in plants growing in the nematode-infected soil. All plants receiving nitrogen fertiliser, regardless of soil treatment, had no yellow leaves and appeared to have no nutrient deficiency (Plate 4.2b).

Harvest date

There was no difference in shoot fresh weight between the two harvests, at seven or eight weeks (Figure 4.8). In contrast, plants harvested at eight weeks had a significantly ($P<0.01$) higher shoot dry weight than those harvested after seven weeks (Table 4.6). A significant difference ($P<0.01$) was found between the first and second harvest for root fresh weight (Figure 4.8). Root fresh weight increased by 30% per pot in the period of one week. Harvest time did not have a significant effect on root dry weight.

Nematode number significantly ($P<0.01$) increased from the first to the second harvest (Table 4.6). The first harvest demonstrated a significantly higher percentage of N in shoots than the second harvest. Concentration of P in shoots was reduced from the first to the second harvest. The harvest time had no significant effect on concentration of K, Zn, Fe, Na, S, Ca or Mg in top growth, although at the second harvest the concentration of Ca and Mg was

higher than at the first harvest (Table 4.6).

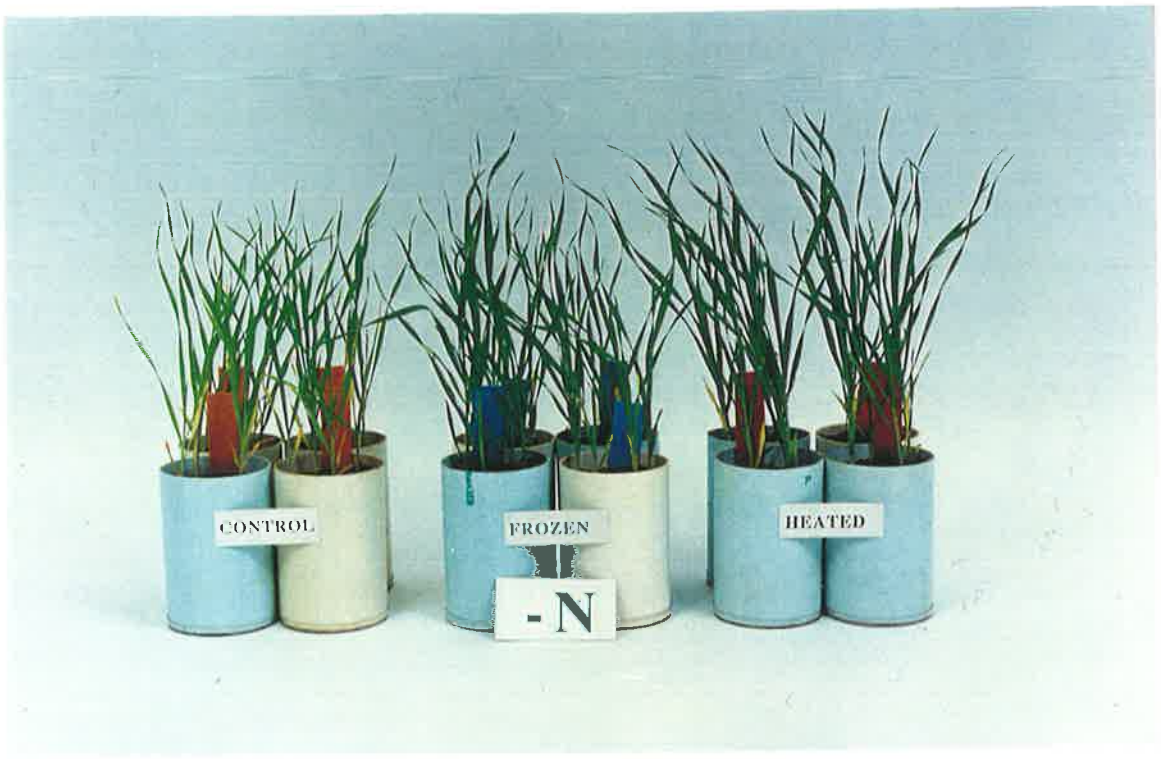
Plate 4.2

(a) The extent of yellow leaf symptom after three weeks in wheat variety Molineux grown in pots with (control) or without (frozen or heated) nematodes without addition of nitrogen fertiliser.

(b) The extent of yellow leaf symptom after three weeks in wheat variety Molineux grown in pots with (control) or without (frozen or heated) nematodes with addition of nitrogen fertiliser.

Plate 4.2

(a)



(b)

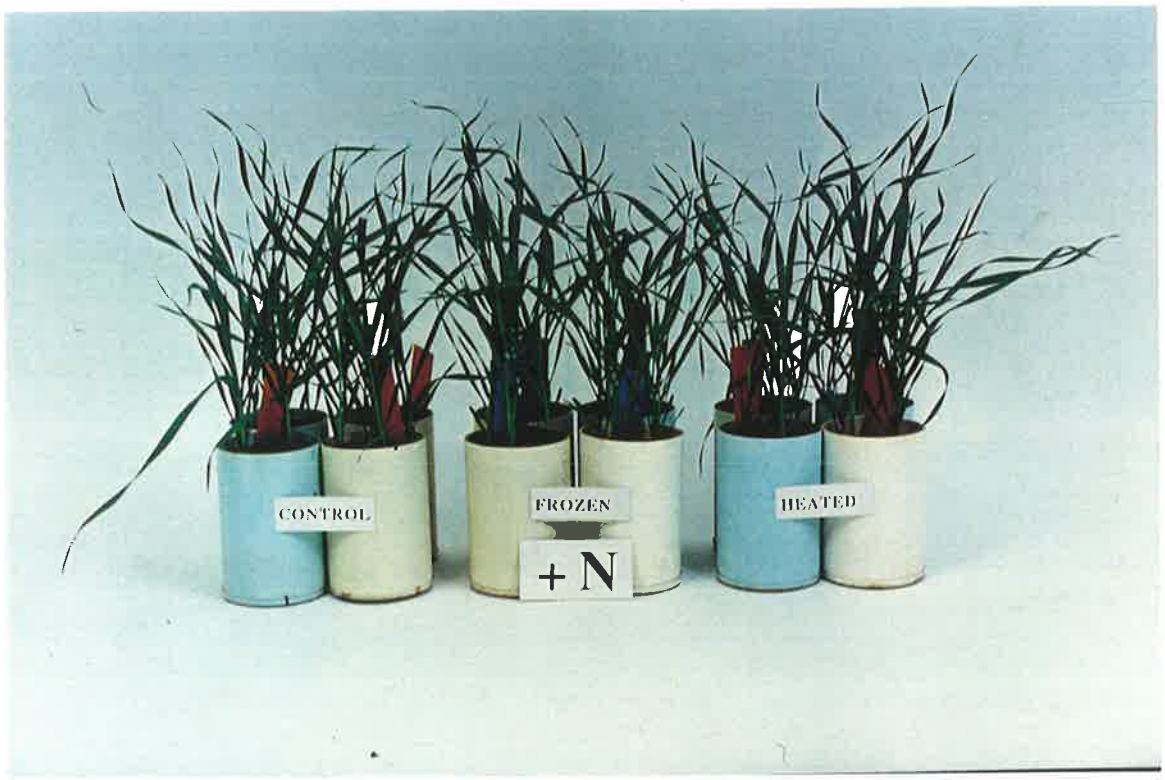
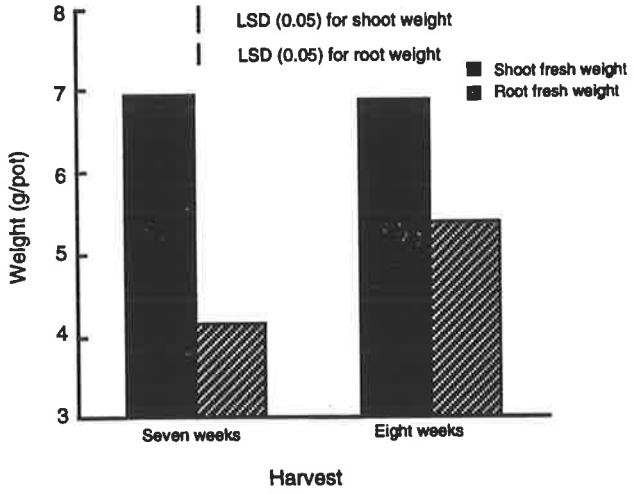


Table 4.6 Effect of harvest time on means of top and root dry weights and element concentration in shoots of plants grown in 1 kg pots of a naturally nematode-infested soil (eight *P. neglectus*/g soil) or treated with rapid drying (+45°C) or low (-10°C for 6 hours) temperature, applied with eight fertiliser regimes (Nil, N, P, Mg, N+P, N+Mg, P+Mg, N+P+Mg) and incubated in a controlled temperature waterbaths for seven or eight weeks. (Values are the means of six replicates).

	Top and root dry weight, number of nematodes and concentration of elements												
Treatments	**Top dry weight** (g/plant)	**Root dry weight** (g/plant)	**No. of nematodes** /g root	**N** (%)	**P** 10^{-3}	**K** 10^{-3}	**Ca** 10^{-3}	**Mg** 10^{-3}	**Mn** 10^{-6}	**Zn** 10^{-6}	**Fe** 10^{-6}	**Na** 10^{-6}	**S** 10^{-3}
Seven weeks	4.20	0.718	50,000	2.05	2.01	22.5	3.67	1.14	66.1	20.5	83.8	0.24	2.07
Eight weeks	4.51	0.729	64,000	1.74	1.98	22.7	3.83	1.16	65.4	20.4	78.7	0.25	2.06
LSD (0.05)	**0.091**	**0.037**	**8200**	**0.120**	**0.136**	**1.32**	**0.208**	**0.048**	**3.07**	**2.11**	**11.02**	**0.044.**	**0.147.**

Figure 4.8 Means of shoot and root fresh weights of wheat variety Molineux at different harvest times (seven or eight weeks). Plants were grown in 1 kg pots of soil naturally infested with *P. neglectus* (six nematodes/g of soil) or soil treated by freezing (-10°C for 6 h) or rapidly drying at five fertiliser regimes (Nil, N, P, Mg, P+Mg, N+Mg, N+P and N+P+Mg) for seven or eight weeks. (Values are the means of six replicates for each treatment).



4.2.3.1 Effects of soil treatments and fertiliser application on plant growth and nematode populations in roots

Shoot fresh weight

Significant differences ($P<0.01$) were found between soil treatments for top growth (Figure 4.9a). Plants growing in rapidly dried soil were the heaviest and those growing in the non-treated soil had the lightest top fresh weight per pot. Plants growing in the freezing treated soil were intermediate and significantly different ($P<0.01$) from both the no control of nematodes and the rapidly dried treatments.

Fertiliser application had significant effects on top weight. Plants receiving nitrogen yielded about twice that of those treated with fertilisers lacking nitrogen (Figure 4.10a). In terms of top weight, treatments could be classified into two groups. One group comprised those

receiving nitrogen and the other included those receiving no fertiliser or fertilisers without nitrogen. The difference between the groups was highly ($P<0.01$) significant, while the differences within groups were not statistically significant.

Shoot dry weight

Soil treatment made a significant difference to shoot dry weight. Plants growing in rapidly dried soil were significantly heavier ($P<0.01$) than both freezing and no control of nematodes treatments in terms of shoot dry weight per pot (Figure 4.9b).

The response of shoot dry weight to fertiliser application was also different. As with shoot fresh weight, treatments could be divided into two groups (Figure 4.10b). Those receiving nitrogen alone or in combination with other fertilisers had significantly higher shoot dry weights than those in the second group which were not treated with nitrogenous fertiliser. Shoot dry weight was the highest where nitrogen alone had been applied to the plants. As shown in Figure 4.10b, phosphorus or magnesium fertiliser application did not have any significant effect on shoot dry weight compared to the control.

Root fresh weight

Plant root fresh weights in pots receiving the rapid drying treatment were significantly ($P<0.01$) heavier than both the freezing and no control of nematodes treatments (Figure 4.9a). Freezing also yielded significantly heavier root fresh weight than the no control of nematodes treatment where the nematodes were present.

The response of root fresh weight to fertiliser application was similar to that observed for the shoot fresh and dry weights. Addition of nitrogen alone or nitrogen accompanied by other fertilisers significantly increased the root fresh weight compared to those without nitrogen. As shown in Figure 4.10a, the heaviest root fresh weight was obtained in plants receiving nitrogen and the lowest in plants receiving phosphorus alone. The differences between the control and both the phosphorus and magnesium treatments for root fresh weight were not

statistically significant.

Root dry weight

The response for root dry weight to the various soil treatments was similar to that obtained for root fresh weight. Application of fertilisers containing nitrogen significantly increased the root dry weight while adding phosphorus or magnesium to the soil did not have any significant effect on this character compared to the plants grown in soils without fertiliser (Figure 4.10b). Nitrogen alone yielded the heaviest roots and plants treated with magnesium the lightest.

Figure 4.9 Effect of soil treatments (no control of nematodes, rapid drying or freezing) on top and root fresh (**a**) and dry (**b**) weights (g/pot) of wheat variety Molineux grown in 1 kg pots of soil naturally infested with *P. neglectus* (six nematodes/g of soil) in the glasshouse for seven or eight weeks and treated with eight fertiliser regimes (Nil, N, P, Mg, P+Mg, N+Mg, N+P, N+P+Mg). (Values are the means of six replicates for each treatment).

(a)

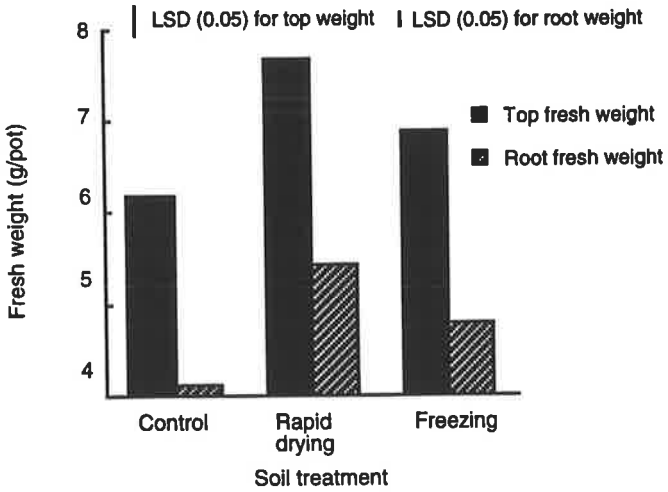


Figure 4.9 continued

(b)

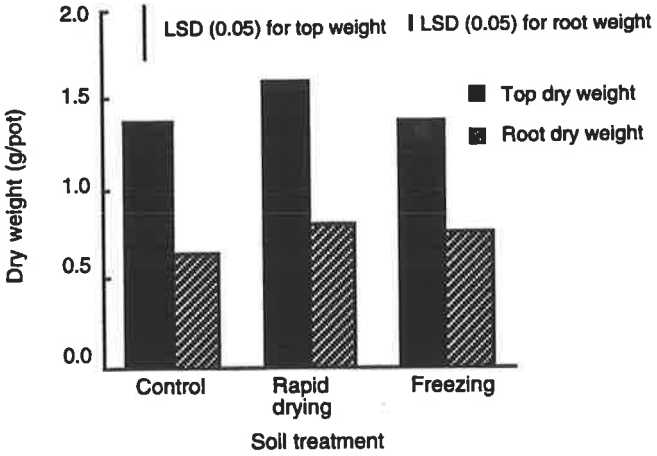


Figure 4.10 Effects of eight different fertiliser regimes (Nil, N, P, Mg, P+Mg, N+Mg, N+P, N+P+Mg) on top (**a**) and root (**b**) fresh weight (g/pot) of wheat variety Molineux grown for seven or eight weeks in 1 kg pots of soil naturally infested with *P. neglectus* (six nematodes/g of soil) or soil treated by freezing or rapidly drying. (Values are the means of six replicates for each treatment).

(a)

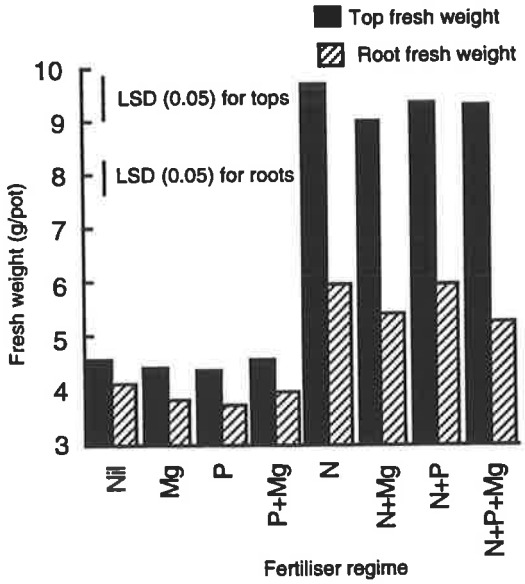


Figure 4.10 continued

(b)

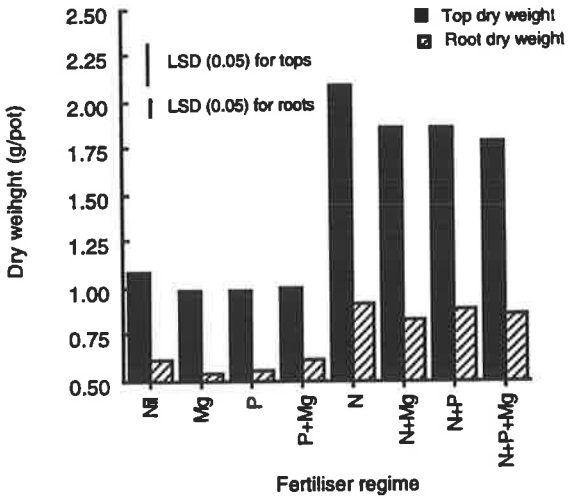
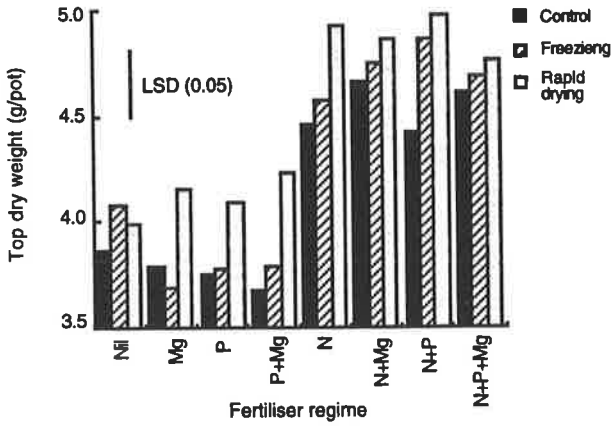


Figure 4.11 Effects of soil treatments (no control of nematodes, freezing and rapidly drying) and eight different fertiliser regimes (Nil, N, P, Mg, P+Mg, N+Mg, N+P, N+P+Mg) on top dry weight (g/pot) of wheat variety Molineux grown for seven or eight weeks in 1 kg pots of soil naturally infested with *P. neglectus* (six nematodes/g of soil). (Values are the means of six replicates for each treatment).



Number of nematodes

The number of nematodes in roots of plants growing in rapidly dried or frozen soil (approximately 18,000 nematodes per pot) was much lower and significantly different from that of the untreated (no control of nematodes) soil (about 100,000 nematodes per pot)

(Figures 4.12a and 4.12b). A higher number of nematodes was found in the roots of plants grown in frozen soil than in the rapidly dried soil.

Fertiliser application had a significant effect on nematode multiplication (Figures 4.12a and 4.12b). All fertilisers containing nitrogen (N+P, N+Mg and N+P+Mg), other than where nitrogen was applied alone, yielded a significantly higher number of nematodes than the control; either per pot or per gram dry root. Plants receiving N+Mg supported the highest and those receiving no fertiliser the lowest multiplication rate of the nematode. Plants receiving magnesium or phosphorus fertiliser were similar to those supplied with no fertiliser in respect of either number of nematodes per pot or per gram dry root.

Figure 4.12 Effects of soil treatments (no control of nematodes, freezing or rapid drying) and eight different fertiliser regimes (Nil, N, P, Mg, P+Mg, N+Mg, N+P, N+P+Mg) on number of nematodes (**a**) per pot and (**b**) per gram of dry root of wheat variety Molineux grown for seven or eight weeks in 1 kg pots of soil naturally infested with *P. neglectus* (six nematodes/g of soil). (Values are the means of six replicates for each treatment).

(a)

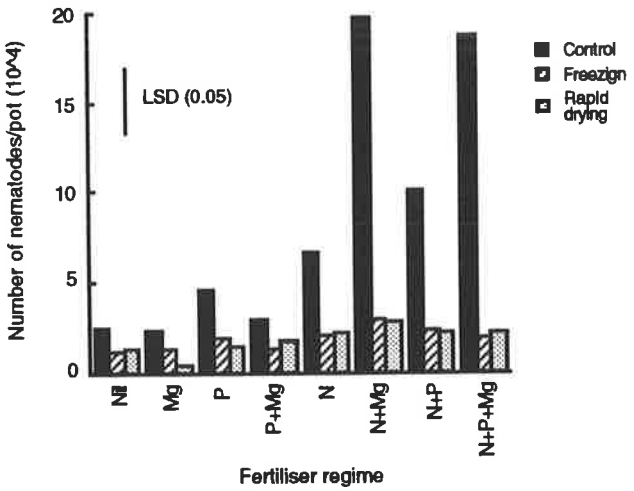
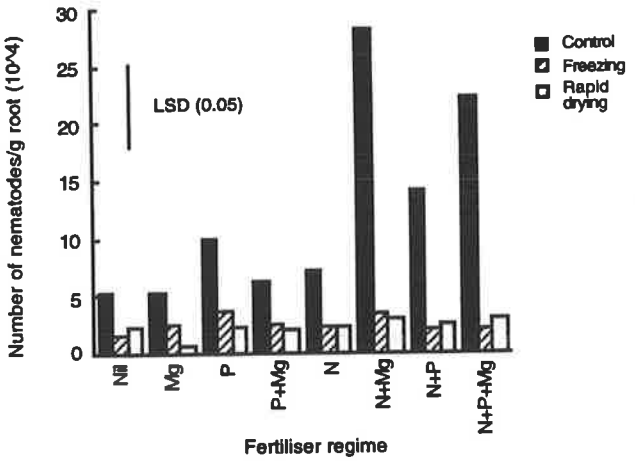


Figure 4.12 continued

(b)



4.2.3.2 Effect of soil treatment (rapid drying or freezing) and fertiliser application on concentration and content of elements in shoots

Nitrogen

Soil treatments did not change the N concentration or content in plants, and both rapidly dried and freezing were similar to the no control of nematodes treatment (Tables 4.7a and 4.7b).

Application of fertilisers containing nitrogen significantly increased the concentration (Table 4.8) and content (Table 4.9) of N in plant shoots compared to the control and other fertiliser regimes. The differences within these two groups were not statistically significant. The highest concentration of N was measured in plants receiving N+Mg and the lowest in plants with no fertiliser.

The interaction of soil treatment and fertiliser application was highly significant ($P<0.01$) (Figure 4.13a). When no fertiliser or fertilisers containing no nitrogen or all three fertilisers together were applied to the soil, all three soil treatments were similar, but with fertiliser regimes containing nitrogen, other than N+P+Mg, the response of N concentration to

fertiliser in different soil treatments was different. When nitrogen alone was applied, freezing resulted in a significantly higher concentration of nitrogen than the no control of nematode treatment, but in the N+P fertiliser regime, the N concentration was lower than that of the control.

Phosphorus

Soil treatment did not have any significant effect on P concentration or content in plants (Tables 4.7a and 4.7b).

Plants receiving fertilisers containing nitrogen showed a significantly lower concentration of P in their top growth. Concentration or content of P was not affected by phosphorus application. The interaction between soil treatments and fertilisers was highly ($P<0.001$) significant (Figure 4.13b). With P+Mg and N+P fertiliser regimes, plants growing in nematode infested soil demonstrated the highest and lowest concentration of P, respectively, and the difference between rapidly dried soil and no control of nematodes was significant for these fertiliser regimes.

Potassium

While no significant difference was found between rapid drying and freezing treatments, both were significantly different ($P<0.01$) from the no control of nematodes treatment in terms of K concentration (Table 4.7a) and content (Table 4.7b).

Fertiliser application had a significant effect on concentration of K in top growth (Figure 4.13c). Plants receiving fertilisers containing nitrogen showed a significantly higher concentration or content of K in the tops (Tables 4.7a and 4.7b). Plants treated with N+Mg showed the highest and those receiving no fertiliser the lowest concentration of K in their shoots ($P<0.05$).

Interaction of soil treatment with fertiliser was significant ($P<0.05$) (Figures 4.13c). Three

soil treatments (no fertiliser, magnesium and P+Mg) were similar in terms of K concentration in top tissues, but with phosphorus or nitrogen regimes the difference between freezing and both rapid drying and no control of nematodes was highly significant ($P<0.001$), with freezing demonstrating the highest and no control of nematodes the lowest concentrations (Figure 4.13c). The rapidly dried demonstrated a higher concentration of K with nitrogen fertiliser than the no control of nematodes treatment. The same result was obtained for amount of K in the top tissues. A positive correlation was observed between top dry weight and concentration of K. Heavier top growth was associated with a greater concentration of the element in the shoots (Figure 4.14).

Calcium

Soil treatment had a significant ($P<0.05$) effect on Ca concentration (Table 4.7a), but not on content (Table 4.7b) in top growth. Plants growing in frozen soil had a significantly higher concentration than those growing in rapid drying soil in terms of the element concentration. With no control of nematodes, Ca concentration was intermediate to that in the rapid drying and freezing treatments and not significantly different from either.

Fertiliser application changed the Ca concentration status in the shoots significantly ($P<0.01$) (Table 4.9). Plants receiving no fertiliser had the highest, and those receiving N+P the lowest. The amount of Ca was not affected significantly by application of any fertiliser regime, compared to the control.

The interaction of soil treatment with fertiliser application was not statistically significant.

Magnesium

Soil treatments were significantly different ($P<0.001$) in their effects on Mg concentration and content (Table 4.7a and 4.6b). Plants growing in soil treated by rapid drying or low temperature demonstrated a significantly higher concentration and amount of Mg in their shoots than those with no control of nematodes. The difference of both high and low

temperature was statistically significant from the no control of nematodes. Rapidly dried soil yielded a significantly greater amount of Mg in shoots than both freezing and no control of nematodes (Table 4.7b).

The effect of fertiliser application on magnesium concentration was significant ($P<0.001$) (Table 4.8). Application of Mg did not have any effect on the concentration or content of the element compared to the control and nitrogen treatments. Nitrogen fertiliser with or without phosphorus or magnesium significantly increased the content of Mg in the shoots (Table 4.9). When nitrogen was accompanied with magnesium, the concentration of magnesium was significantly greater than that of the control.

Interaction of soil treatment and fertiliser application was not statistically significant.

Manganese

Both rapid drying and freezing treatments increased the concentration of Mn in shoots significantly ($P<0.001$) compared to no control of nematodes (Table 4.7a).

Fertiliser application did not have any significant effect on Mn concentration, but Mn content was significantly greater in tissues of plants treated with fertiliser containing nitrogen (Table 4.9).

Interactions of soil treatments and fertiliser applications for both concentration and content of Mn in shoots were significant (Figure 4.13d). In all fertiliser regimes plants grown in soil treated by rapid drying demonstrated a higher concentration or amount of Mn than those with no nematode control.

Zinc

Plants growing in rapidly dried soil were slightly higher in Zn concentration or content than those growing either in the soil with no control of nematodes or soil treated with freezing.

Fertiliser application made no significant difference to the concentration of Zn in plant tissues (Table 4.9).

With the interaction between soil treatments and fertiliser applications, a contrasting effect was observed for plants growing in rapidly dried soil when treated with magnesium or magnesium plus nitrogen (Figure 4.13e). With magnesium fertiliser alone, concentration of Zn in plants grown in rapidly dried soil was significantly higher than with no control of nematodes, but in contrast it was lower when plants were treated with N+Mg.

Iron

Soil treatment also had no significant effect on Fe concentration (Table 4.7a) or content (Table 4.7b).

Fertiliser applications, particularly those containing nitrogen, slightly but not significantly increased the Fe concentration in the top growth (Table 4.9). The highest percentage of Fe was observed in plants receiving N+Mg and the lowest in plants supplied with phosphorus alone.

Sodium

The effect of soil treatment on Na concentration and content was significant ($P<0.05$) (Tables 4.7a and 4.7b). In plants growing in soil with nematodes, Na concentration and content was significantly higher than in those growing in soil treated by low temperature or rapid drying.

Fertiliser application did not have any significant effect on the concentration of Na in top growth, although it was higher in plants receiving no fertiliser or receiving fertiliser containing no nitrogen.

The interaction of fertiliser application and soil treatment was not significant.

Sulphur

Soil treatment had a significant effect on S concentration, so that the highest concentration was obtained in plants growing in frozen soil and the lowest in those in rapidly dried soil (Table 4.7a). Plants growing in the treatment with no control of nematodes (with 2059 ppm of S in the shoots) were between the freezing and rapidly dried treatments.

The effect of fertiliser on S concentration and content of shoots was highly significant ($P<0.001$) (Tables 4.8 and 4.9). The highest concentration and amount of S was obtained under the N+P+Mg treatment and the lowest concentration and content from nitrogen and phosphorus applications, respectively.

Interaction of soil treatment with fertiliser application for both S concentration or content was significant (Figure 4.13f). Plants growing in the rapidly dried soil when treated with phosphorus alone were significantly lower in S concentration than the control, while in other fertiliser regimes the difference between no control of nematodes and rapidly dried soil was not statistically significant.

Table 4.7 Effect of different soil treatments on concentration (**a**) and content (**b**) of elements in top tissues of wheat variety Molineux. Plants were grown in 1 kg pots in a controlled temperature waterbath in an evaporatively cooled glasshouse. Plants were watered with distilled water and harvested after seven or eight weeks. (Values are the means of six replicates).

(a) Concentration of elements in top tissues

	Element									
Treatments	**N** (%)	**P** 10^{-3}	**K** 10^{-3}	**Ca** 10^{-3}	**Mg** 10^{-3}	**Mn** 10^{-6}	**Zn** 10^{-6}	**Fe** 10^{-6}	**Na** 10^{-6}	**S** 10^{-3}
Control	1.89	2.02	21.0	3.75	1.10	59.4	20.6	87.5	277	2.06
Rapid Drying	1.88	1.96	23.2	3.57	1.18	71.7	21.5	79.1	223	1.93
Freezing	1.92	2.01	23.7	3.94	1.17	66.2	19.4	77.0	234	2.20
LSD (0.05)	**0.15**	**0.22**	**1.62**	**0.25**	**0.06**	**3.76**	**2.60**	**13.54**	**40.2**	**0.18**

(b) Content of elements in top tissues (mg/pot)

	Element									
Treatments	**N**	**P** ($\times 10^{-3}$)	**K** ($\times 10^{-3}$)	**Ca** ($\times 10^{-3}$)	**Mg** ($\times 10^{-3}$)	**Mn** ($\times 10^{-6}$)	**Zn** ($\times 10^{-6}$)	**Fe** ($\times 10^{-6}$)	**Na** ($\times 10^{-6}$)	**S** ($\times 10^{-3}$)
Control	82.7	8.5	91.0	16.0	4.7	0.26	0.10	0.39	1.16	8.8
Rapid drying	85.9	8.7	105.5	16.0	5.3	0.32	0.08	0.36	1.00	8.7
Freezing	83.9	8.4	101.8	16.8	5.1	0.28	0.09	0.33	0.99	9.5
LSD (0.05)	**6.6**	**0.81**	**7.84**	**1.15**	**0.28**	**0.02**	**0.01**	**0.08**	**0.16**	**0.86**

Table 4.8 Effect of fertiliser regime on nutrient concentration of vegetative tissues of wheat variety Molineux. Plants were grown in 1 kg pots treated with nine fertiliser regimes and watered with distilled water. Nutrient concentrations were measured seven and eight weeks after sowing. (Values are the means of six replicates).

	Element									
	N	**P**	**K**	**Ca**	**Mg**	**Mn**	**Zn**	**Fe**	**Na**	**S**
Fertiliser	(%)	10^{-3}	10^{-3}	10^{-3}	10^{-3}	10^{-6}	10^{-6}	10^{-6}	10^{-6}	10^{-3}
Nil	1.38	2.20	18.4	4.18	1.10	64.0	20.9	73.2	297	2.31
Mg	1.40	2.25	18.8	3.84	1.05	64.3	20.8	78.5	271	2.05
P	1.40	2.21	20.2	3.74	1.08	64.1	22.1	72.4	257	2.18
N	2.43	1.65	24.4	3.53	1.17	66.6	18.1	87.7	199	1.75
P+Mg	1.42	2.51	18.4	3.95	1.11	63.7	18.7	74.6	239	2.22
N+Mg	2.46	1.66	28.1	3.64	1.25	69.0	22.6	95.2	239	2.20
N+P	2.45	1.60	26.2	3.26	1.19	68.8	21.8	88.4	221	1.79
N+P+Mg	2.27	1.85	26.8	3.87	1.28	65.5	18.6	80.0	235	2.33
LSD (0.05)	**0.24**	**0.271**	**2.65**	**0.416**	**0.095**	**6.14**	**4.30**	**21.9**	**66.7**	**0.292**

Table 4.9 Effect of fertiliser regime on nutrient content of vegetative tissues of wheat variety Molineux. Plants were grown in 1 kg pots treated with nine fertiliser regimes and watered with distilled water. Nutrient concentrations were measured seven or eight weeks after sowing. (Values are the means of six replicates).

	Element									
Fertiliser	**N**	**P**	**K**	**Ca**	**Mg**	**Mn**	**Zn**	**Fe**	**Na**	**S**
Nil	54.6	8.7	73.2	16.6	4.39	0.25	0.09	0.29	1.18	9.23
Mg	54.0	8.7	72.9	14.9	4.05	0.25	0.10	0.30	1.05	7.98
P	53.9	8.6	77.9	14.5	4.20	0.25	0.11	0.28	0.98	7.12
N	120.3	8.4	120.3	17.7	5.79	0.33	0.07	0.47	1.00	8.91
P+Mg	55.3	9.7	71.8	15.3	4.30	0.25	0.09	0.29	0.93	8.64
N+Mg	115.2	7.9	133.6	17.3	5.94	0.33	0.09	0.45	1.12	10.50
N+P	114.3	7.7	124.5	15.6	5.66	0.33	0.08	0.42	1.04	8.52
N+P+Mg	105.5	8.7	121.4	18.3	5.99	0.31	0.08	0.36	1.11	10.97
LSD (0.05)	**10.73**	**1.33**	**12.80**	**1.87**	**0.46**	**0.03**	**0.02**	**0.14**	**0.27**	**1.40**

Figure 4.13 Effects of soil treatments (no control of nematodes, freezing and rapid drying) and eight different fertiliser regimes (Nil, N, P, Mg, P+Mg, N+Mg, N+P, N+P+Mg) on concentration of N (**a**), P (**b**), K (**c**), Mn (**d**) Zn (**e**) and S (**f**) in wheat variety Molineux grown in 1 kg pots of soil naturally infested with *P. neglectus* (six nematodes/g of soil) for seven or eight weeks. (Values are the means of six replicates for each treatment).

Figure 4.13

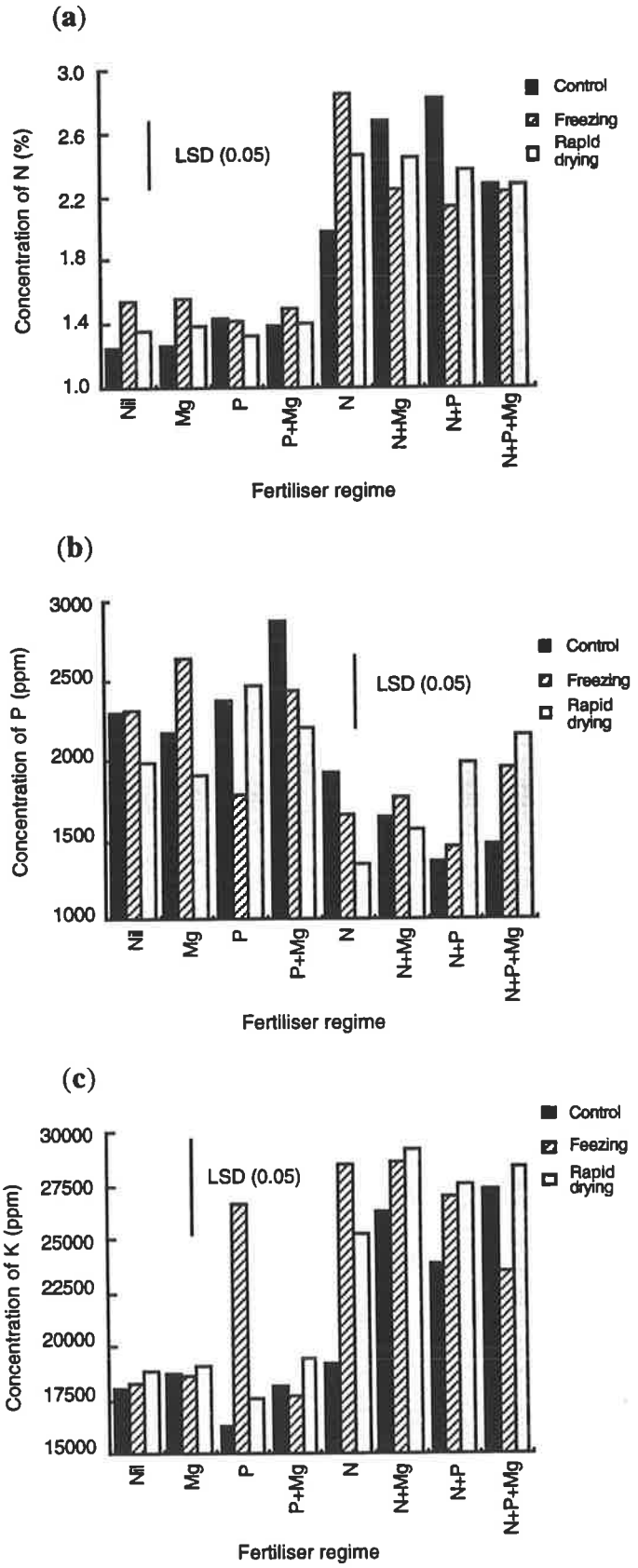

(a)
Concentration of N (%)
3.0
2.6
2.2
1.8
1.4
1.0
LSD (0.05)
Control
Freezing
Rapid drying
Nil
Mg
P
P+Mg
N
N+Mg
N+P
N+P+Mg
Fertiliser regime
(b)
Concentration of P (ppm)
3000
2500
2000
1500
1000
LSD (0.05)
Control
Freezing
Rapid drying
Nil
Mg
P
P+Mg
N
N+Mg
N+P
N+P+Mg
Fertiliser regime
(c)
Concentration of K (ppm)
30000
27500
25000
22500
20000
17500
15000
LSD (0.05)
Control
Feezing
Rapid drying
Nil
Mg
P
P+Mg
N
N+Mg
N+P
N+P+Mg
Fertiliser regime

Figure 4.13 continued

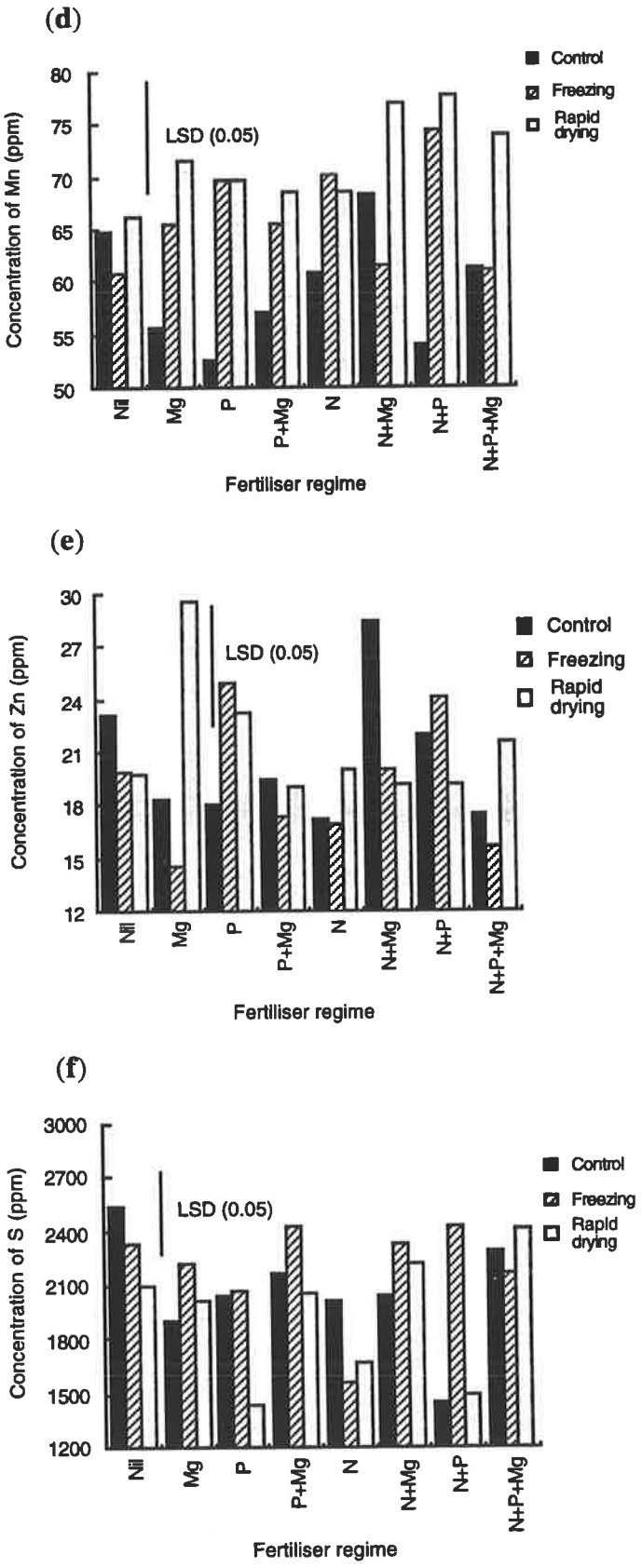
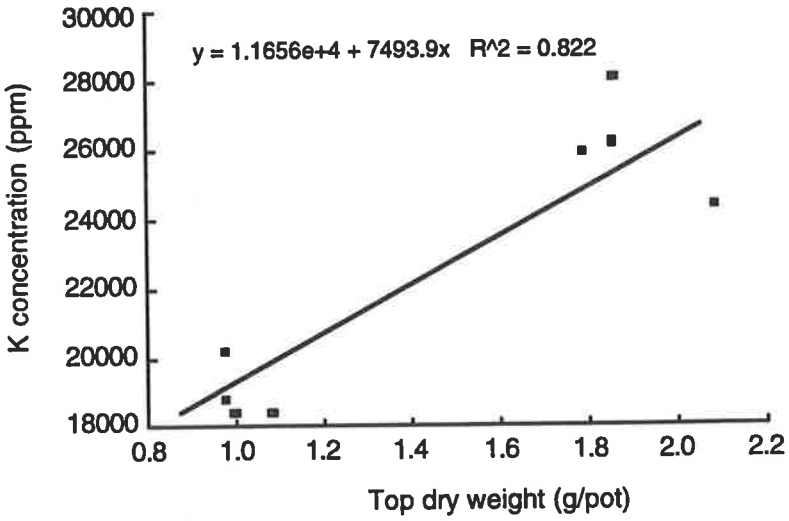


Figure 4.14 Correlation of K concentration in top tissues and top dry weight of wheat variety Molineux grown in 1 kg pots of soil naturally infested with *P. neglectus* (six nematodes/g of soil) or soil treated by freezing or rapid drying. Plants were supplied with eight fertiliser regimes (Nil, N, P, Mg, P+Mg, N+Mg, N+P, N+P+Mg) and grown for seven or eight weeks. (Values are the means of six replicates for each treatment).



4.3 Discussion

The lack of effective sources of cultivar resistance or chemical controls of root lesion nematodes had, at this stage of the investigation, shifted emphasis to the use of tolerant varieties and fertiliser management as a way of reducing crop damage. Tolerant genotypes and well nourished plants suffer less damage from nematodes than intolerant genotypes and/or plants with poor nutrient status. It has been documented that root parasitic nematodes may cause nutrient imbalance and water deficiency as well as disrupting hormone synthesis and other processes (Van Gundy *et al.*, 1974; Bahatt, 1986; Thompson, 1987b). Plants tolerant to nematodes would show less severe symptoms of nutrient deficiency, such as yellow lower leaves, than intolerant plants in the same environment (Brennan and Thompson, 1993).

To relate symptoms of nutrient deficiency to the number of nematodes in the roots, nematodes should be extracted before anthesis, otherwise the nematode population in the roots may decline drastically with the migration of nematodes from the root to the soil (S. P. Taylor, pers. comm., and the results of preliminary experiments). The yellow leaf symptoms of nutrient deficiency due either to nematode attack or other abiotic factors are expressed at about six weeks after seeding. Seven to eight weeks after sowing, therefore, is an appropriate time to extract nematodes under glasshouse conditions and then measure the concentration of elements in top tissues, to examine the role of the nematodes in changing the elemental concentrations in plant tissues thereby helping to define the physiological basis of tolerance and determining the possibility of applying appropriate fertilisers to overcome nutrient deficiencies.

4.3.1 Effects of freezing, nematicide and rapid drying on nutrient availability

Temik® application, through restriction of the nematode population, elevated the content of most nutrients including N, P, K Ca, Mg, Mn, Fe, Na and S (Table 4.5), but did not

significantly effect the concentration of elements in the top tissues. This was mostly because a higher top growth resulted from Temik® application (Figure 4.4b). Nevertheless, it has been suggested that Temik® *per se* has some effect on nutrient uptake, making elements like nitrogen more available in the soil and thereby stimulating plant growth (J. M. Fisher, pers. comm.). However, in some cases the phytotoxicity of Temik® is dominant to its stimulatory effect (Chapter 5).

Lower concentration of phosphorus, potassium, zinc and sulphur in plants growing in the frozen soil appears to be mainly due to the effect of a long duration of freezing rather than the absence of nematodes. As shown in the first experiment, severe cold temperature treatment for 24 h had a significant adverse effect on P, Ca, Mg and Zn availability to plants and reduced their concentration in plants (by 50, 30, 28 and 32%, respectively) compared to those of the no control of nematodes treatments (Table 4.4). When the duration of freezing was shortened in the second experiment, no significant difference was found between freezing and no control of nematode treatments for either concentration or content of these elements (Tables 4.7a and 4.7b). The status of other elements was not affected by the low temperature treatment. Obviously freezing would have some effects on populations of soil microorganisms affecting the availability of nutrients to plants.

Rapid drying through reducing the nematode population (Figure 4.12a and 4.12b) or by affecting other microorganisms in the soil, appeared to have a positive effect on absorption of some nutrients including K, Mg and Mn and a negative effect on that of Na (Table 4.7).

4.3.2 Comparison between Molineux and Spear in terms of tolerance to the nematode

Although the yellow leaf symptoms were not merely confined to nitrogen deficiency, under the conditions of these experiments, nitrogen application alone could mostly remove the symptoms (Plate 4b). When phosphorus was applied to plants in conjunction with nitrogen, it restricted the symptoms to a greater degree. Where N+P was applied to the soil of

nematode infested fields, the symptom of yellow leaves was almost completely removed (Chapter 5).

In nematode infested soils, Molineux shows a greater extent of yellow lower leaves relative to Spear (A. J. Rathjen, pers. comm.). Since, for all the measured elements, Spear was similar to Molineux or even lower (Table 4.1), the yellow lower leaf symptoms are unlikely to be caused by a nutrient deficiency in these experiments. Molineux, despite its higher level of yellow lower leaves, was more efficient in absorption of Mg than Spear, irrespective of fertiliser applied to the plants (Table 4.1), and in all fertiliser regimes exhibited a higher concentration of Mg than Spear. So, this element is unlikely to play a major role in developing yellow leaf symptoms. This conclusion was confirmed in the second experiment where the Mg or Mg+P treatments had no effect on the level of symptoms. Molineux appears inherently more susceptible to expressing this symptom. Other physiological factors such as cytokinin production or the rate of nutrient translocation could also be involved in the lower level of expression of the yellow lower leaf symptoms in Spear than in Molineux.

Numbers of nematodes per gram of root in both varieties were almost the same, indicating that the response of both varieties in terms of resistance was similar and that the yellow lower leaves were not due to the difference in the reproductive rate of the nematode. Nematodes increased the level of yellow leaf symptoms expression as in both Temik® and freezing treated pots plants were greener than in the no control of nematode treatment.

4.3.3 Effects of nematode and fertilisers on plant growth

Greater top dry weight (up to 50%) due to control of the nematode, by all methods adopted in these experiments (Figure 4.5a and 4.11), indicated that infection by *P. neglectus* reduced top growth of infected plants. Results of the first experiments showed that when there was no fertiliser or when the soil was deficient in nutrients, particularly nitrogen, the effects of nematodes on plant growth could be ignored, as the response of infected plants was similar to that of non-infected plants (Figures 4.5a and 4.5b). This might have been due to the fact that

plants were already suffering from some other nutrient deficiency or had a restricted water uptake (due to a small volume of soil in the pots), so the effect of the nematodes on nitrogen absorption was small compared to an over-riding deficiency of other essential elements (Tiver, 1988) or water.

In the first experiment, plants growing in soil treated with Temik® or by freezing, when supplied with N+P+Mg, had a significantly higher top growth than with no nematode control, while in the second experiment these differences were not statistically significant (Figures 4.5a and 4.11). These contrasting results could be due to the different fertiliser status of the soil used in the experiments. Soil of the first experiment was more fertile in terms of nitrogen and particularly phosphorus, but low in potassium and particularly sodium (Tables 4.4 and 4.7). A greater response therefore was achieved by controlling the nematodes through improvement of the absorption of limited elements.

The results of fertiliser application showed that soil used in these experiments was deficient in nitrogen and to some extent in phosphorus as the application of nitrogen increased the concentration of N in plant tissues (70-140%) (Tables 4.1 and 4.4, Figure 4.13a) and plant growth was enhanced (100%) by nitrogen application (Figure 4.11).

Reduction of concentration of N at the second harvest in both the first and second experiments (Tables 4.3 and 4.6) compared to the first harvest undertaken one week earlier was a sign of a lack of sufficient nitrogen in the soil. When nitrogen was available in the soil, it seemed that the amount of phosphorus was not adequate to support maximum growth, so that in the presence of nitrogen, application of phosphorus was necessary (Figure 4.1a). Root dry weight followed the top dry weight and the same discussion also applies to root growth (Figures, 4.1a and 4.10b).

The adverse effect of nematode infection on top fresh weight (Figure 4.9a) could have resulted from water stress imposed by nematode damage to the root system. There are reports that nematodes affect plants through reducing their ability to absorb water (O'Bannon and

Reynolds, 1965; Thompson, 1987c; Riedel, 1988). However when water supply is adequate, even damaged roots can supply sufficient water to the plant to compensate for their reduced activity (Orion *et al.*, 1984). In glasshouse experiments, although the pots were watered regularly, it is possible that serious damage due to lack of water occurred as the restricted soil volume in pots makes the plants very vulnerable to fluctuations in soil water content.

4.3.4 Effects of fertiliser on number of nematodes

The lack of any significant difference between varieties or fertiliser regimes for number of nematodes per gram of dry root in the first experiment, despite differences in volume of root growth, was probably due to a high population density of the nematode (eight nematodes/gram of soil) in the soil, attacking the root system and reaching the equilibrium population level before sampling. It means that the nematode multiplication was so fast which could reach to constant density even in the roots of plants with a high rate of root growth. The lower number of nematodes per gram of root of plants grown in treatments without added nitrogen (Figure 4.12b), was due to their early maturity. At harvesting these had passed the anthesis stage, particularly those in the second experiment. After anthesis, root lesion nematodes leave the root system to find a fresher food source.

In the second experiment, application of calcium nitrate alone while significantly increasing root growth (Figure 4.10b) compared to the control, and increasing potential capacity for nematode reproduction, did not support nematode multiplication to the extent of treatments such as N+P, N+Mg or N+P+Mg (Figures 4.12a and 4.12b). This again suggested that nitrogen, when applied alone to nematode infested soil, could have some detrimental effect on nematode multiplication. This result is in agreement with that of Vanstone *et al.* (1993), who showed calcium nitrate was effective in reducing the nematode population, but not as effective as urea. Vanstone *et al.* (1993) conducted experiments in the field and glasshouse over three years and concluded that application of all forms of nitrogen significantly reduced *P. neglectus* populations (by 32-80%). She suggested that nematodes are inhibited prior to or

during invasion of roots. Tacconi *et al.* (1988) also reported that mineral fertilisers (N+P) reduced *P. thornei* populations in wheat roots. Rodriguez-Kabana and King (1980) stated that good control of nematodes was obtained when urea was applied at levels in excess of 300 mg nitrogen/kg soil. In contrast to the above, Orion *et al.* (1984) reported nitrogen fertiliser had no effect on a population of *P. thornei.*

The higher number of nematodes (2-8 fold) per plant and per gram of dry root in N+Mg and N+P+Mg fertiliser regimes compared to the control (Figures 4.1b, 4.1b, 4.12a and 4.12b), is in agreement with the results of Thompson *et al.* (1981) who concluded that fertiliser stimulates root growth ultimately resulting in a higher population of nematodes. Well fertilised crops will produce greater root growth, providing more food for nematode feeding and reproduction. This will consequently leave a greater population of the nematode to attack the next crop.

4.3.5 Effect of soil treatments and fertilisers on concentration of elements in shoots

Plants must be supplied adequately with nutrients during their entire growth period. For this reason, the concentration of plant nutrients in tissues must be maintained at a satisfactory level for plant growth. Nutrient availability in plant tissues not only depends on nutrient concentration of the soil solution, but also on the capacity of the root to take up nutrients from the soil which depends on the distribution of roots in the soil and the volume of soil which the roots occupy as well as the health of the roots. The ability of plants to exploit the soil for nutrients and water depends on the extent of root branching, the number of root hairs, the number of root tips and other root characteristics. Root hairs are of great importance because of the close contact they maintain between the soil and the root tissues, penetrate moderately impenetrated clays and contributing to nutrient exploitation of less accessible soil regions. Root hairs play a special role for nutrients which are transported towards the roots by diffusion (Itoh and Barber, 1983), notably P and Zn. Besides root length and root density, the number of root tips is also important. Some plant nutrients (such as Ca^{2+}, Mg^{2+} and

Fe^{2+}) are mainly absorbed by young root tissues with unsuberised cell walls (Clarkson and Sanderson, 1978).

Nutrients in the soil can be transported by two different mechanisms: by mass-flow and by diffusion. Mass flow is associated with the amount of water consumption by the plant. Water uptake is also affected by nematode attack through damage to the root system. By feeding on root hairs and on meristems nematodes are likely to reduce the absorption of many elements.

Nitrogen

Nematodes did not have any effect on N uptake by plants (Table 4.5) as the concentration and content of N with no control of nematodes, freezing and rapid drying treatments was similar. These results are in contrast with those of Bhatt (1986), who demonstrated chickpea plants infected with *P. thornei* had a lower nitrogen content than non infected plants. Since chickpea is able to fix atmospheric nitrogen, reduction in number or activity of nodules could be involved in his experiments (Section 2.3.3). A significantly higher content of N (31%) in the plants treated with Temik® (Table 4.5), might be due to the indirect effect of Temik® on nitrogen absorption through other phenomena (J. M. Fisher, pers. comm.), rather than because of the absence of nematodes.

A high response of plants to nitrogen application in terms of top growth (about 100%), and both concentration and content of N in plant top tissues (Tables, 4.2, 4.5 and 4.9), indicated that the soil had insufficient nitrogen to support normal growth of plants.

Phosphorus

Lower concentration of P in the N+P or N+P+Mg fertiliser regimes was due to dilution of the element as a result of greater top growth. Plants with a higher rate of growth demand a higher rate of phosphate uptake, but as the amount of phosphate present in the soil solution is low in

comparison with adsorbed phosphate (Mengel and Kirkby, 1987), the concentration of this element declines in plants treated with nitrogenous fertilisers because of a high demand due to higher growth rate. Phosphate is transported from the soil to the root by both diffusion and mass flow mechanisms, but the latter plays a small role as the phosphate concentration of the soil solution is low (Mengel and Kirkby, 1987).

In both experiments the supply of phosphate to plants appeared to be adequate in all fertiliser regimes, either in the presence or absence of nematodes, as in none of treatments did the level of P fall below the critical level (0.2%) (Reuter and Robinson, 1986). But, since plant growth was increased by phosphorus application in the presence of nitrogen (37% higher compared to nitrogen alone) (Figure 4.1a), its supply would have not been sufficient for optimum growth under the conditions of the first experiment.

Potassium

In all treatments of the first experiment, the concentration of K was below the critical point (1.5%) (Reuter and Robinson, 1986). At the second harvest of the first experiment, concentration of K decreased significantly compared to that measured one week earlier indicating that the soil was deficient in potassium (Table 4.5). Constant concentration of K at both harvests of the second experiment and its higher than critical concentration in top tissues (1.5 times the critical level) indicated that potassium was sufficient in soil of the second experiment.

Concentration of K followed the rate of top growth so that plants with a higher top growth also had a higher concentration of K ($R^2 = 93$) (Figure 4.14). Higher concentration of K in plants treated with nitrogenous fertilisers could be due to the fact that nitrogen causes plants to produce more roots and thereby more root surface is in contact with soil particles. Larger volumes of soil can be exploited by plants as a result of nitrogen application.

Reduction in K concentration in nematode infested soil could result from nematode damage to

the root system. Potassium is taken up via the symplast throughout the length of the root. Reduction in amount of root hairs and destruction of root cortex could have reduced potassium uptake by the plant, and lowered the concentration and content of K in plants in the no control of nematodes treatment. The findings here are in agreement with those of other workers (Lownsbery, 1956; Evans *et al.*, 1975; Trudgill *et al.*, 1975), who noted that efficiency of potassium uptake is decreased by nematode infestation. An increase in the application of potassium fertiliser compensated for the restriction of growth caused by nematodes (Neuwirth, 1930; Otiefa, 1952). As no potassium fertiliser was used in these experiments, further investigation is needed to reveal the response of wheat plants infected with *P. neglectus* to application of potassium fertiliser.

Calcium

Nematodes had no effect on Ca uptake as the concentration of Ca in plants growing with no control of nematodes was higher than in treatments with greater top growth (Table 4.7a). Although Ca concentration increased as a result of fertiliser application, because of the higher root growth, as in the case of potassium, none of the treatments demonstrated a lower concentration than the critical level (0.2%) (Reuter and Robinson, 1986).

Reduced Ca concentration in plants receiving N+Mg (Table 4.8), while there was adequate Ca in the soil and application of $Ca(NO_3)_2$ as nitrogen fertiliser added to the level of Ca, could be explained by Ca uptake being sensitive to antagonism by other cations such as Mg. The reduced concentration of Ca in these fertiliser regimes could be due to a higher concentration of K, as increasing the supply of one cation species results in lowering the concentration of other cation species (Mengel and Kirkby, 1987). It has also been reported that Ca translocation from roots to shoots is depressed by potassium (Ohno and Grunes, 1985). Potassium, which is taken up very quickly either actively or by facilitated diffusion, competes strongly in cation uptake to depress the uptake of other cations (Mengel and Kirkby, 1987).

Magnesium

Soils were not deficient in magnesium as even when no fertiliser was added the concentration of this element was about the critical level. Uptake of Mg can also be seriously affected by excess of other cation species, especially of K^+ and $NH4^+$. Not only the uptake but also the translocation of Mg from the roots to upper plant parts can be restricted by other cations.

Reduced Mg concentration in plants growing with no control of nematodes treatment (Table 4.4) could also be explained on the basis of reduced root hairs and limited root contact with soil particles because of the nematode infection. Lower concentration of Mg in plants receiving no fertiliser or fertilisers containing no nitrogen could be due to the fact that root growth is affected, and in particular, branching is restricted as a result of nitrogen deficiency. These results are in agreement with those obtained by Otiefa (1952) and Viglierchio (1987).

Manganese

All methods of controlling nematodes used in these experiments increased Mn concentration (by 7-21%) (Tables 4.4 and 4.7), so it is suggested that nematodes have a negative effect on Mn absorption or translocation by plants.

It seems that there was sufficient manganese in the soil, as the application of fertilisers containing nitrogen without any source of manganese increased the element concentration particularly when it was accompanied with low or high temperature treatment of the soil. Furthermore, in all treatments, the concentration of Mn was higher than the critical level (30 ppm) (Reuter and Robinson, 1986).

Constant concentration of Mn in plants receiving no fertiliser at all three soil treatments (Figure 4.13d) again confirms that when plants are suffering from a nutrient deficiency, there is no noticeable difference between nematode-infected and non-infected plants. Viglierchio (1987) also found a lower level of Mn in nearly all nematode infested sugar beets and tomatoes.

Zinc

There is no indication here that the soils used in these experiments were deficient in Zn or that nematodes had any noticeable effect on its uptake or translocation. In Queensland, application of zinc to the soil removed the severity of yellow leaf symptoms, but not completely (Thompson *et al.*, 1980b). While the results here are in agreement with that of Nasr *et al.* (1980), who found no changes occurred in Zn concentration of infected almond roots, Sher (1957) showed a higher concentration of the element in uninfected plants.

Iron, Sodium, Sulphur

Higher root growth with nitrogen fertiliser resulted in a higher uptake of Fe, so no deficiency of Fe was found in the soils used in these experiments and it had no contribution to the appearance of yellow lower leaves. Na and S, which demonstrated independence from fertiliser application and thereby from root growth, did not show any connection with the yellow leaf symptoms and number of nematodes.

Percentage of Na was slightly higher (13%) in plants grown with no control of nematodes in soil of the first experiment. Concentration of P and Fe in plants of the first experiment was significantly ($P<0.05$) higher (about 100 and 50%, respectively) and that of K and particularly Na lower (about 20% and 30%, respectively) than those in the second experiment. Concentrations of other measured elements was slightly higher in vegetative tissues of plants grown in soil of the first experiment (Tables 4.4 and 4.7) than the second.

4.3.6 Conclusion and comments

These experiments provided evidences that there are four factors involved in the yellow leaf symptoms;

1 - Root lesion nematode invasion damages the root system and reduces the nutrient absorbing ability of the invaded roots.

2 - Deficiency of nitrogen and to some extent phosphorus in the soil is involved, and becomes more serious in nematode infested soils.

3 - Varietal differences also contribute to the extent of yellow leaf symptoms expression. Some such as Spear are less susceptible for expression of the symptoms, others such as Molineux are more susceptible.

4 - On the basis of observations in other experiments in both the field and glasshouse, water stress induces expression of the symptoms.

The results reported here may not be relevant to field conditions, because in glasshouse conditions water supply to the root environment fluctuates rapidly due to the small soil volume of pots. To make reasonable conclusions on the effect of nematodes on plant growth and nutrient uptake, and to investigate the possibility of reducing nematode damage by application of fertilisers, further experiments were required under field conditions. In field circumstances, natural interactions of other microorganisms with the pest and the host plant are also likely to occur.

CHAPTER 5

EFFECT OF ROOT LESION NEMATODE (*PRATYLENCHUS NEGLECTUS*) ON NUTRIENT CONCENTRATION AND GROWTH OF WHEAT, AND APPLICATION OF FERTILISERS TO REDUCE THE DAMAGE CAUSED BY NEMATODES UNDER FIELD CONDITIONS

5.1 Balaklava 1992

Investigation of the effect of root lesion nematode (Pratylenchus neglectus) *on growth, yield and concentration of elements in roots and shoots and the application of fertilisers to reduce nematode damage*

5.1.1 Introduction

In Chapter 4, the effect of the root lesion nematode, *P.* neglectus, on growth and nutrient concentrations in shoots of wheat plants was investigated in pot experiments under glasshouse conditions. Since the ultimate aim of these experiments was to understand the tolerance mechanism and to develop an economical method for farmers to reduce nematode damage to crop plants, the results obtained under glasshouse conditions needed to be confirmed in the field. Environmental factors such as temperature and moisture availability and soil factors such as texture, fertility and particularly the existence of other microorganisms interacting with nematodes, could be important in affecting the extent of damage to plants caused by nematodes.

The main effect of root lesion nematodes on plant nutrition is by affecting the uptake of water and of nutrients (Sections 2.3.1 and 2.3.2) through damaging root hairs and restricting root growth. However, there are also reports that nematodes affect plant productivity through some physiological changes, inhibiting nutrient translocation from the roots to the shoots (Wilhelm *et al.*, 1985). Comparing the nutrient concentration in shoots of a range of varieties with a series of fertiliser treatments could give an indication of the

physiological basis of tolerance and the key nutrient deficiencies involved in nematode damage.

In 1992, an experiment was conducted in a paddock at Balaklava naturally infested with the nematode. The soil for experiments reported in the previous Chapter had been collected from this site.

5.1.2 Materials and methods

The wheat used in this experiment was Molineux, a variety susceptible to *P. neglectus* (Table 3.6).

The experimental site was on the farm of Mr. B. Roberts at Balaklava, about 100 km north of Adelaide. The soil was a reddish sandy loam naturally infected with *P. neglectus* over a calcareous loam B horizon. The field had been cropped with chickpeas in 1990, and wheat cv. Molineux in 1991. Seed was sown on May 5, 1992, at the rate of 30 g per plot (about 60 kg/ha) (Chapter 3). Cultivation methods were in accordance with the local district practices.

The number of nematodes, determined before seeding, averaged approximately eight nematodes per gram of soil (Chapter 3). The nematode species was identified by Dr. J. M. Fisher as *P. neglectus*.

The experiment was factorial in a randomised complete block design with two factors and six replications:

> The first factor was soil treatment with two levels: one with nematodes (the no control of nematode treatment) and the other with reduced nematodes, using the nematicide Temik® at a rate of 5 kg a.i./ha. Soil samples taken from the cultivated zone two weeks after application of Temik® (May 19, 1992) indicated that there were virtually no plant parasitic nematodes present in treated plots.

The second factor was fertiliser application with eight levels including nil, nitrogen, phosphorus, magnesium, N+P, N+Mg, P+Mg, N+P+Mg. All were applied at sowing. Fertilisers were evenly spread on the soil surface by hand and then mixed with the upper layer of the soil (2-3 cm depth) with a fork at 110, 35 and 15 kg/ha of N, P and Mg respectively. $Ca(NO_3)_2 .4H_2O$ was used as a source of nitrogen, Top Fos® as a source of phosphorus and MgSO4. 7H2O as a source of magnesium (Chapter 3).

About ten weeks after commencing the experiment (August 6, 1992), all plots were scored for plant vigour and the degree of yellow leaf symptoms. Plots with no symptoms of yellow lower leaves were given score 1 and those appearing totally yellow scored 10. Plant vigour was also scored from 10 for the most vigorous plant growth, to 1 for the least vigorous at the time of heading. Three shoot samples (quadrats 30 by 50 cm), were taken at random when plants were at anthesis, from the middle and towards both ends of each plot, and bulked. At the same time, three sets of root samples (15 cm x 15 cm area to a depth of 15 cm) were taken from each plot and bulked.

Shoots were washed twice in deionised water and dried at 85°C for two days. Nematodes were extracted from the roots and from the soil samples and counted (Chapter 3). Roots were washed and the dry weights recorded. Shoots and roots were ground and the concentration of P, K, Ca, Mg, Mn, Fe, Zn, Na and S determined by ICP-spectrometry. The concentration of nitrogen was measured by the Kjeldahl method (Chapter 3).

For number of nematodes a logarithmic transformation based on the recommendation of Proctor and Marks (1974), Ln (number of nematodes + 200), was applied. All data were subjected to analysis of variance and means compared by LSDs.

5.1.3 Results

A- Growth response and nematode population

For some characteristics there was a significant block effect in the analyses of variance.

Plant vigour

Temik® had no significant effect on plant vigour regardless of the fertiliser regime (Table 5.1).

Fertiliser application had a great effect on plant vigour, so that plants receiving N+P or N+P+Mg (with the mean vigour score of 9.0) were significantly (P<.001) different from those which received no fertiliser or magnesium with means of 5.9 and 5.6, respectively (Table 5.3) (Plate 5.1).

Plate 5.1 Effect of fertiliser application on plant vigour and expression of yellow leaf symptoms. A= N+P+Mg, B= N+P, C= Control, D= Mg

The interaction of soil treatments and fertilisers was not statistically significant.

Table 5.1 Effects of soil treatment with nematodes (control) and without nematodes (Temik®) on number of nematodes in roots and soil and growth of wheat at Balaklava.

	Soil treatments		
Characters	**Control**	**Temik®**	**LSD (0.05)**
Vigour score	7.4	7.4	**0.43**
Yellow leaf score	4.5	4.5	**0.43**
Top dry weight (g/quadrat)	236.9	248.5	**19.4**
Number of nematodes/200 g of soil	603.1	488.0	**161.9**
Number of nematodes/ g of dry root	22316.5	4833.7	**866.5**

Yellow lower leaves

Temik® application did not change the status of plants in terms of the yellow leaf symptoms compared to plots with no control of nematodes (Plate 5.1).

Plants with fewer yellow lower leaves were more vigorous (Figure 5.1). Fertiliser regimes were significantly different in their effects on the yellow leaf symptoms (Table 5.3). Plants receiving N+P+Mg fertiliser demonstrated the lowest and those that received magnesium the highest score for yellow leaf symptoms. Application of nitrogen compared to other fertilisers was more effective in reducing the symptoms, but the best result was obtained where both N and P fertilisers were applied together so that the difference between both N+P+Mg and N+P with N+Mg was significant ($P<0.01$). Application of phosphorus and magnesium alone or both together (P+Mg) did not have any positive effect in removing the symptoms compared to the nil fertiliser treatment (Figure 5.2).

The interactions of soil treatments and fertiliser applications were not statistically significant.

Figure 5.1 Correlation of yellow lower leaf score with plant vigour score for the wheat variety Molineux grown in soil naturally infested with eight nematodes/g at Balaklava.

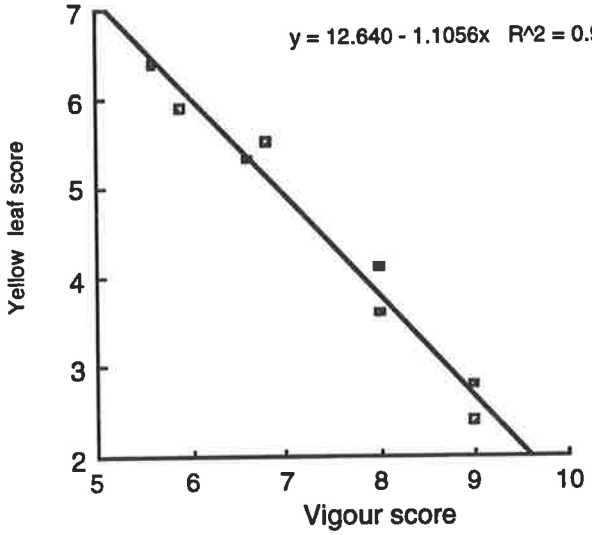
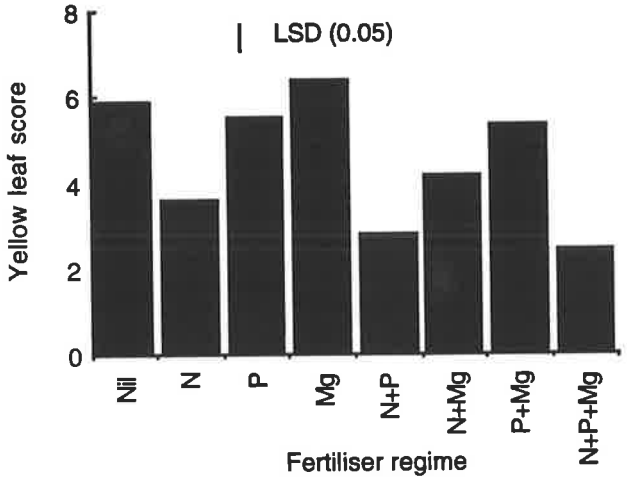


Figure 5.2 Effect of different fertiliser regimes on yellow leaf symptoms of the wheat variety Molineux grown in soil naturally infested with eight nematodes/g at Balaklava.



Top dry weight

Plots treated with Temik® had greater shoot dry weight (249 g per quadrat) than those receiving no nematicide (237 g per quadrat), but this was not statistically significant (Table 5.1).

Fertiliser application had a significant effect on top growth. Plots supplied with N+P or with N+P+Mg were significantly heavier than those from the other fertiliser regimes. Plots

treated with magnesium alone, with 62% the top growth of the N+P treatment, were the lightest. Plants receiving nitrogen alone were not as heavy as those treated with N+P or N+P+Mg (Table 5.3).

The interactions of fertilisers and soil treatments were not statistically significant.

Grain yield

As a result of abnormally high rainfall in the early summer of 1992 (Table 3 1, Chapter 3), leaf rust was severe and the plants had lodged and stems broken before harvest. Harvesting was difficult and the grain yield results inaccurate so no significant differences were recorded.

Number of nematodes

As the density of plants varied within plots, and sampling the root system of individual plants was not possible, calculation of number of nematodes per plant was not appropriate. If the multiplication rate of the nematode was unaffected by treatments, those treatments with a higher root growth would have a higher number of nematodes per plant, but the number of nematodes per gram dry root would be unaffected. Results were therefore presented as number of nematodes per gram of root and number of nematodes per 200 g of soil without root material.

Although Temik® application significantly reduced the number of nematodes per gram of root ($P<0.001$) compared to the no control of nematode treatment (Table 5.1), no significant difference was observed in terms of number of nematodes in the soil. Since Temik® is effective to the depth of the cultivated zone, the depth at which it is placed, the nematodes in the lower layers migrate to the cultivated zone and invade the root system. Before anthesis most nematodes inhabit the root system and a small proportion of their population exists in the soil. This could be an explanation for the non-significant

difference between Temik® treated plots and no control of nematodes treatment in terms of number of nematodes per 200 g of soil.

The differences between fertiliser regimes in terms of number of nematodes per gram dry root, except the difference between the control and N+P, were not statistically significant (Table 5.3). Plants receiving no fertiliser had about 56% the number of nematodes found in plants receiving N+P fertiliser. Plants treated with nitrogen alone had a lower number of nematodes per gram than those supplied with nitrogen and phosphorus. Nitrogen plus magnesium and the control had the lowest number of nematodes per gram dry root (Table 5.3). No significant difference was found between fertiliser treatments in terms of number of nematodes per 200 g of soil without root material sampled at anthesis (Table 5.3).

B- Effect of Temik® and fertiliser application on concentration of elements in shoots

Plants growing in plots treated with Temik® had significantly higher concentrations of Mn ($P<0.0001$) and S ($P<0.05$) in top tissues than those grown in nematode infested soil (Table 5.2). Temik® application also slightly increased concentration of K, Ca, Fe and Na in shoots compared to the no control of nematodes treatment, but the differences were not statistically significant. Temik® application had no effect on the concentrations of N, P, Mg and Zn in plant tops.

Nitrogen application alone or in combination with phosphorus and magnesium significantly increased the concentration of N, Ca, Mg, Mn, Na and S in top tissues (Table 5.3). The treatments could be classified into two completely separate groups in terms of concentration of the above elements, one included plants receiving fertilisers containing nitrogen and the other included those receiving no fertiliser or fertilisers containing no nitrogen (Table 5.3).

Fertiliser application, particularly nitrogen, increased Fe concentration, but did not have any major effect on the concentration of Zn. In contrast, nitrogen application slightly, but

not significantly reduced the concentration of K . Nitrogen application caused a reduction in P concentration in plant top tissues. Plants supplied with phosphorus fertiliser had a significantly higher concentration of P in their shoots (Table 5.3).

None of the interactions of soil treatments and fertiliser applications for nutrient concentrations were significant.

Table 5.2 Effect of soil treatments with nematodes (control) and without nematodes (Temik®) on concentration of elements in roots and shoots of Molineux wheat at Balaklava.

Element	**Nutrient concentration**					
	Roots			Shoots		
	Control	**Temik®**	**LSD (0.05)**	**Control**	**Temik®**	**LSD (0.05)**
N (%)	1.3	1.3	**0.06**	1.8	1.8	**0.13**
P (10^{-3})	1.2	1.2	**0.07**	1.9	2.0	**0.11**
K (10^{-3})	3.8	4.0	**0.38**	21.1	21.4	**1.23**
Mg (10^{-3})	1.6	1.7	**0.06**	1.3	1.3	**0.08**
Ca (10^{-3})	3.9	4.2	**0.29**	2.4	2.4	**0.17**
Mn (10^{-5})	4.0	4.7	**0.32**	3.7	4.4	**2.40**
Na (10^{-3})	1.4	1.5	**0.07**	0.72	0.84	**0.23**
Fe (10^{-3})	1.8	1.9	**0.16**	0.07	0.07	**0.01**
S (10^{-3})	1.74	1.72	**0.13**	1.78	2.02	**0.14**
Zn (10^{-4})	1.29	1.34	**0.20**	0.18	0.19	**0.04**

Table 5.3 Mean vigour and yellow leaf scores, top dry weights, nematode populations and nutrient concentrations in tops of plants grown at Balaklava in field under eight different fertiliser regimes. Plants were sampled at anthesis, twelve weeks after sowing.

	Fertiliser regime								
Characters	**Nil**	**N**	**P**	**Mg**	**N+P**	**N+Mg**	**P+Mg**	**N+P+Mg**	**LSD (0.05)**
Vigour score	5.9	8.0	6.8	5.6	9.0	8.0	6.6	9.0	0.60
Yellow leaf score	5.9	3.6	5.5	6.4	2.8	4.1	5.3	2.4	0.57
Top dry weight (g/quadrat)	197.1	252.5	226.2	189.6	308.4	257.0	214.9	295.1	24.1
No. nematodes /g root	10987.0	15709.0	12926.0	15015.0	18817.0	10795.0	11054.0	13297.0	5818
No. nematodes /200 g of soil	560.0	412.0	535.0	760.0	523.0	514.0	515.0	542.0	358
N (%)	1.6	2.1	1.6	1.6	2.1	2.0	1.6	1.9	0.18
P (10^{-3})	1.9	1.8	2.2	1.9	2.0	1.8	2.1	2.0	0.18
K (10^{-3})	21.3	21.1	21.5	21.5	20.8	21.8	20.9	20.8	1.69
Mg (10^{-3})	1.1	1.4	1.1	1.1	1.6	1.4	1.2	1.4	0.12
Ca (10^{-3})	2.1	2.7	2.2	2.0	2.7	2.6	2.2	2.7	0.27
Na (10^{-4})	5.5	13.4	4.9	4.8	10.6	9.0	5.2	9.0	3.92
Fe(10^{-5})	6.4	6.8	6.6	6.3	6.9	6.9	6.8	6.8	0.09
S (10^{-3})	1.6	2.3	1.7	1.6	2.1	2.1	1.7	2.1	0.04
Zn (10^{-5})	1.8	1.9	2.2	1.7	1.9	1.9	1.7	1.9	0.45
Mn (10^{-5})	3.6	4.3	3.7	3.6	4.6	4.3	3.7	4.5	0.39

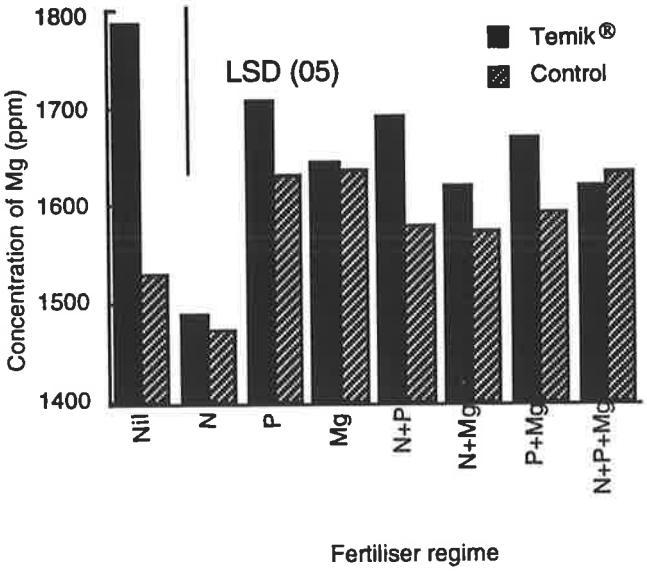
* 10^{-3}, 10^{-4} and 10^{-5} stand for par per thousand, part per ten thousands and part per hundred thousands respectively.

C- Effects of soil treatment and fertiliser application on element concentration in roots

Soil treatment

Temik® application significantly ($P<0.05$) increased the concentration of Mn and Mg compared to no control of nematodes treatment, and slightly, but not significantly, elevated the concentration of N, P, K, Ca, Fe, Zn and Na and decreased that of S in the roots (Table 5.2). Application of Temik® increased the level of Mg in the roots more effectively in the absence of any additional fertiliser (Figure 5.3).

Figure 5.3 Interactions of soil treatments (control and Temik®) and eight different fertiliser regimes on concentration of Mg in roots of the wheat variety Molineux grown in soil naturally infested with eight nematodes/g at Balaklava.



Fertiliser application

Plants receiving fertilisers containing nitrogen were significantly ($P<0.001$) higher in N concentration than those receiving fertilisers lacking nitrogen. Nitrogen application significantly decreased the concentration of K and Mg in root tissues (Table 5.4).

Plants receiving fertilisers containing phosphorus were higher in concentration of P compared to those receiving no fertiliser or fertilisers containing nitrogen without phosphorus (Table 5.4).

Fertiliser application was effective in terms of Zn concentration in plant roots. The lowest level of this element was measured in roots of plants receiving no fertiliser and the highest level in those treated with P+Mg. The difference in Zn between of P+Mg compared with N+P+Mg was highly ($P<0.001$) significant (Table 5.4).

Application of fertilisers, particularly phosphorus, elevated the concentration of Fe in plant roots. In terms of Fe concentration in the roots, plants could be classified into two separate groups: one including those receiving fertilisers containing phosphorus, with high concentration of the element (1.94×10^{-3}), and the other including those receiving no phosphorus fertiliser with low concentration (1.74×10^{-3}). Plants receiving nitrogen or phosphorus fertiliser alone had the lowest and highest concentration of Fe, respectively (Table 5.4).

Plants growing in plots receiving N+P had the highest and those in plots receiving magnesium showed the lowest concentration of Na in their root tissues and the difference between them was highly significant ($P<0.001$) (Table 5.4).

Fertiliser application did not have any significant effect on the status of Ca, Mn or S concentration in roots, but plants receiving P+Mg had the highest and those receiving no fertiliser the lowest concentration of Mn (Table 5.4).

Interactions of soil treatments and fertiliser regimes were not significant for any nutrient concentrations in the roots.

Table 5.4 Means of concentration of elements in the roots of plants grown at Balaklava under eight different fertiliser regimes. Plants were sampled at anthesis, twelve weeks after sowing.

	Fertiliser regime								
Nutrient concentration	**Nil**	**N**	**P**	**Mg**	**N+P**	**N+Mg**	**P+Mg**	**N+P+Mg**	**LSD (0.05)**
N (%)	1.20	1.4	1.2	1.2	1.5	1.5	1.2	1.4	0.11
P (10^{-3})	1.19	1.03	1.33	1.22	1.28	1.15	1.20	1.25	0.14
K (10^{-3})	4.93	3.11	4.14	4.86	3.48	3.06	3.97	3.47	0.76
Mg (10^{-3})	1.66	1.48	1.67	1.64	1.64	1.60	1.63	1.63	0.13
Ca (10^{-3})	4.15	3.78	4.04	4.05	3.98	4.40	4.09	4.03	0.57
Na (10^{-3})	1.39	1.36	1.34	1.29	1.74	1.45	1.39	1.51	0.15
Fe (10^{-3})	1.86	1.64	2.02	1.79	1.83	1.68	1.99	1.93	0.32
S (10^{-3})	1.84	1.55	1.80	1.64	1.67	1.76	1.80	1.76	0.31
Zn (10^{-4})	0.99	1.54	1.21	1.25	1.48	1.25	1.56	1.22	0.04
Mn (10^{-5})	3.85	4.13	4.46	4.50	4.32	4.42	4.72	4.16	0.89

* 10^{-3}, 10^{-4} and 10^{-5} stand for par per thousand, part per ten thousands and part per hundred thousands respectively.

5.2 Palmer and Roseworthy 1993

The effects of nematode control and fertiliser application on wheat growth and nutrient concentration of different genotype in two environments in 1993

5.2.1 Introduction

In previous experiments, conducted in the glasshouse and in the field at Balaklava, it was shown that nitrogen and phosphorus fertilisers were required to achieve optimum growth in nematode infested soil. The extent of damage and alteration of nutrition due to nematode attack depends on the host genotype and the growing conditions. Some genotypes are more tolerant to the nematode, being less affected by nutritional changes brought about by nematode invasion. Hence, genetic differences between varieties in terms of efficiency in nutrient uptake might alter their responses in nematode infested soils.

The aim of these experiments was to assess the reaction of different genotypes to root lesion nematode invasion under a range of fertiliser applications.

5.2.2 Materials and methods

Three varieties, Janz, Molineux and Spear (Table 3.6), important commercial cultivars grown in southern Australia, were included in these experiments to investigate the interactions between fertiliser applications and genotypes. Janz and Spear, often with greener lower leaves and suspected to be tolerant to the nematode, and Molineux, often with a high level of yellow lower leaves thought to be indicative of intolerance to the nematode, were grown.

Two sites in the cereal growing region (see details below) with different characteristics were used. One was at Palmer, on the property of Mr. J. Eichler, and the other at Roseworthy on the Roseworthy Campus of the University of Adelaide. The number of

nematodes per gram of soil was determined immediately after sowing. Three samples were taken at random from the cultivated layer of each plot (depth 0.0 to 15.0 cm) and bulked. Nematodes were extracted and counted (Chapter 3). The Palmer site, with pasture in the previous year, had an average of 1.4 nematodes per g of soil. Roseworthy, with an annual medic as the previous crop, had an average of 0.4 nematodes per g of soil.

Both trials were designed as split plot experiments with six replications. The main plots had two levels, of nematode population: presence (control) or absence of nematodes (application of Temik®). Sub-plots included 18 treatments, being combinations of three varieties and six fertiliser regimes. Fertiliser applications were control (nil), nitrogen, phosphorus, potassium and two combinations of the three elements, N+P and N+P+K. Calcium nitrate was used as a source of nitrogen, Top Fos® as a source of phosphorus and potassium sulphate as a source of potassium. The rate of each fertiliser used in the experiments was suggested by Dr. A. J. Rathjen, on the basis of local experimental results and commercial practice, as follows:

Nitrogen: 100 kg N/ha

Phosphorus: 25 kg P/ha

Potassium: 60 kg K/ha

The properties of each fertiliser have been given in Tables 3.2-3.5.

Temik® was used at the rate of 5 kg a.i./ha. All fertiliser treatments (N, P, K, N+P and N+P+K) and Temik® were applied at the time of sowing (Chapter 3).

Plots were 0.75 m wide and 6.00 m long with four rows of plants, each row being 15 cm apart from neighbouring rows with 30 cm between plots. Seeds were sown at Palmer on May 10 and at Roseworthy on July 15, 1993. Properties of the surface soils, determined by the Department of Soil Science at the Waite Agricultural Research Institute, were as follows:

Palmer

pH $_{1:5}$ = 8.0 (soil:water ratio 1:5)

EC $_{1:5}$ = 0.12 dS m $^{-1}$ (mmhos/cm) (soil:water ratio 1:5) = 116 μ s cm $^{-1}$

Total C = 0.83% (determined by dry combustion in an induction furnace, Leco CR - 12 Automatic Carbon Analyser)

Texture: Sand (sand = 86.1%, silt = 6.3% and clay = 7.6%), determined by the Hydrometer method.

Roseworthy:

pH 1:5 = 8.1 (soil:water ratio 1:5)

EC 1:5 = 0.18 dS m $^{-1}$ (mmhos/cm (soil: water ratio 1:5) = 182 μS cm $^{-1}$

Total C = 1.35% (determined by dry combustion in an induction furnace, Leco CR - 12 Automatic carbon Analyser)

Texture: Sandy Loam (sand = 57.7%, silt = 32% and clay = 10.3%), determined by the Hydrometer method.

Sampling was carried out on September 22 at Palmer and on October 4 at Roseworthy, when the plants were at anthesis. Three small areas (25 x 25 cm) containing two or three plants were chosen at random in each plot. The tops for each sample were cut at the soil surface and bulked. Corresponding roots of the samples were dug out (from an area of 20 x 20 cm to 15 cm depth) and bulked.

Tops were washed twice in deionised water, dried and ground for analysis by ICP - spectrometry. Roots were washed in tapwater, and nematodes extracted for five days under the mister and counted. Roots were dried, ground, and nutrient concentration in tissues was determined by ICP - spectrometry (Chapter 3). Grain was harvested on December 6, 1993 and grain yield was determined.

5.2.3 Results

A- Top and root dry weights

Due to enormous variability of plant density in each sampling area as a result of low rainfall in 1993 (Table 3.1), top and root dry weights were not measured. A larger sampling area was not chosen because the aim of the experiment was to determine grain yields.

B- Number of nematodes

Number of nematodes per gram dry root remained the same for all three varieties with an average of about 2500 and 8000 nematodes per gram of dry root at Roseworthy and Palmer respectively.

Fertiliser application had a significant ($P<0.05$) effect on the number of nematodes per gram dry root at Palmer, so that the lowest number was obtained after phosphorus alone had been applied and the highest resulted from the application of N+P and N+P+K (Figure 5.4). No significant difference was found between fertilisers in terms of number of nematodes per gram of root at Roseworthy.

None of the interaction effects were statistically significant.

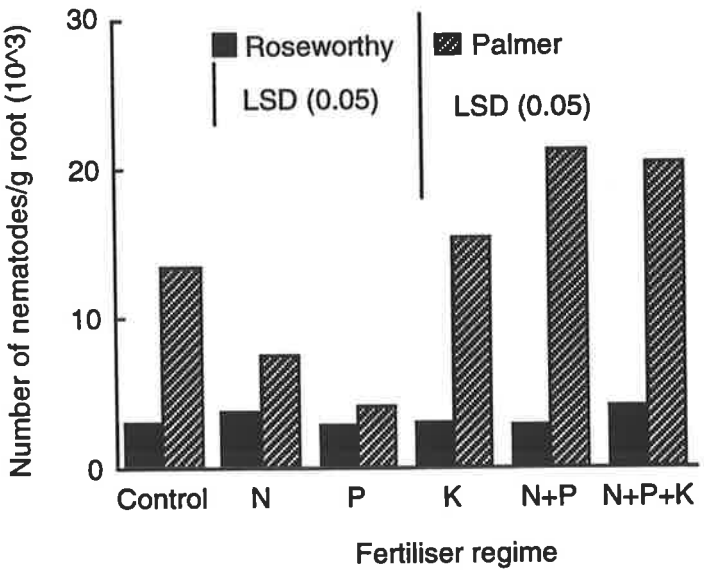
C- Effect of Temik® application on nutrient concentration in shoots

Temik® application increased the level of N slightly and that of S significantly at Palmer. In contrast, at Roseworthy, a significant reduction of N concentration resulted from Temik® application (Table 5.6). Concentration of Zn in plants growing in soil treated with Temik® was similar to those growing with no control of nematodes at Palmer, but at Roseworthy Temik® application increased the concentration of Zn in top tissues significantly ($P<0.05$).

Depending on the nutrient, the sites used for this experiment performed differently. Temik® application increased Na concentration in top tissues at both locations and the difference between Temik® and no control of nematodes treatment at Roseworthy was statistically significant ($P<0.05$) (Tables 5.5 and 5.6). While application of Temik® at Palmer significantly ($P<0.01$) increased concentration of Mn in the shoots, a reduction was observed for its concentration at the Roseworthy site (Tables 5.5 and 5.6).

Application of Temik® slightly decreased the concentration of Ca and Fe in plant tissues at both locations, but the difference was not statistically significant. No significant difference was found between Temik® and the no control of nematodes treatment for P, K, Mg concentration in plant top tissues at both locations (Tables 5.5 and 5.6).

Figure 5.4 Number of nematodes per gram dry root in wheat variety Molineux at anthesis. Plants were grown in soil naturally infested with an average of 1.4 or 0.4 nematodes per g at Palmer and Roseworthy, respectively.



D- Effects of fertilisers on nutrient concentration in shoots of different genotypes

Nitrogen

Concentration of N in the shoots of variety Janz was significantly ($P<0.05$) higher than for both Molineux and Spear at Palmer, while at Roseworthy both Janz and Spear demonstrated a significantly ($P<0.05$) higher concentration of N in the shoots than Molineux (Tables 5.5 and 5.6).

Plants receiving fertilisers containing nitrogen were significantly higher in terms of N concentration in shoots than those receiving no fertiliser or fertilisers lacking nitrogen.

The interactions of both soil treatments and fertiliser regimes with varieties were not statistically significant.

Phosphorus

While all three varieties were similar in terms of P concentration at Palmer, Molineux demonstrated a significantly lower concentration of this element in top tissues than both Spear and Janz at Roseworthy (Table 5.6).

Phosphorus application significantly ($P<0.001$) increased P concentration in top tissues. The highest concentration was measured where phosphorus alone was added and the lowest occurred with nitrogen or potassium fertiliser alone at Palmer and Roseworthy, respectively (Table 5.6).

Interactions of the soil treatments with both varieties and fertiliser regimes were not significant.

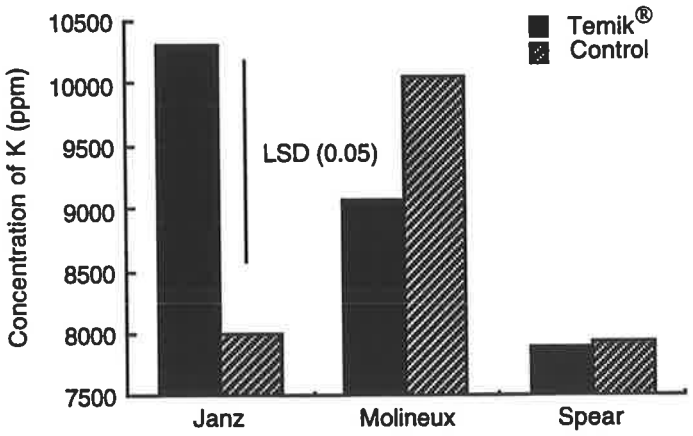
Potassium

While at Palmer Spear demonstrated a significantly lower concentration of K in top tissues than Molineux, no significant difference was found between varieties at Roseworthy (Tables 5.5 and 5.6).

Potassium application did not increase the level of K concentration in shoots at either location. In contrast, application of nitrogen statistically significantly ($P< 0.01$) elevated the concentration, more than twice that of the control or the potassium treatment ($P<0.01$) (Tables 5.5 and 5.6).

Interactions of the soil treatments and varieties in terms of K concentration at Palmer were significant (Figure 5.5), so that Temik® application did not have any effect on K concentration of Spear, but increased the level in Janz significantly ($P<0.05$). At Roseworthy none of the interactions were statistically significant.

Figure 5.5 Interactions of soil treatments (control and Temik®) and different genotypes on concentration of K in shoots of wheat plants grown in soil naturally infested with nematodes at Palmer.



Calcium

While at Palmer Molineux demonstrated a non-significantly lower concentration of Ca in top tissues than Spear and Janz (Figure 5.6a), Janz had a significantly higher concentration of Ca in its top tissues at Roseworthy (Table 5.6).

Treatments in terms of Ca concentration in response to fertiliser applications, could be classified into two groups at both locations. One group included those supplied with no fertiliser, phosphorus or potassium and the other group included those supplied with fertilisers containing nitrogen which had a significantly ($P<0.05$) higher concentration of Ca in their tops (Table 5.6).

Interactions of fertiliser application with both variety and soil treatment were not statistically significant.

Figure 5.6 Interactions of soil treatments (control and Temik®) and different genotypes on concentration of (**a**) Ca and (**b**) Mg in shoots of wheat plants grown in soil naturally infested with nematodes at Palmer.

(**a**) Concentration of Ca in top tissues

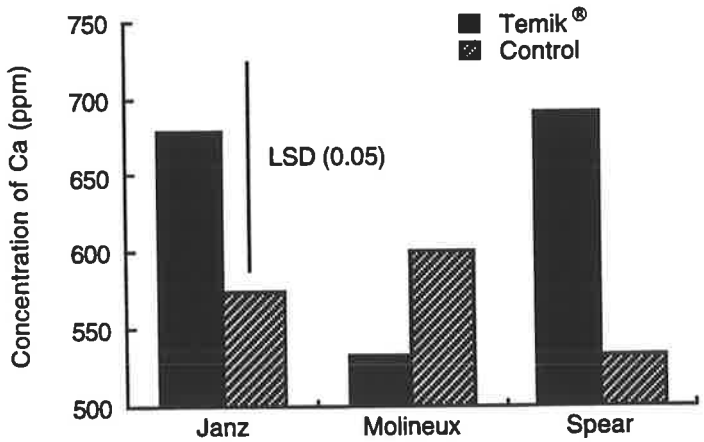
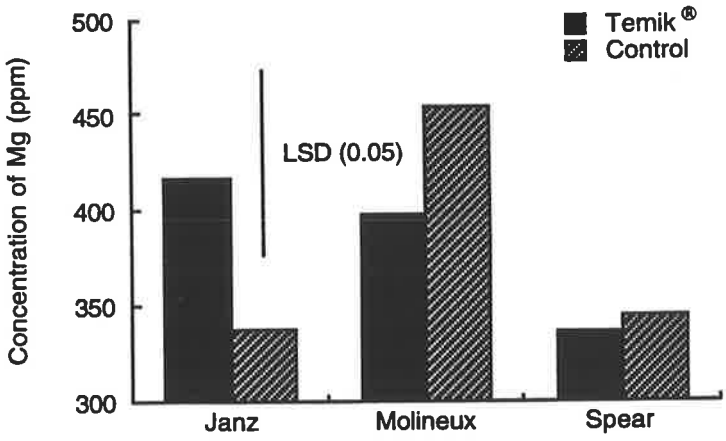


Figure 5.6 continued

(b) Concentration of Mg in top tissues



Magnesium

At Palmer Molineux showed a significantly higher concentration of Mg in top tissues than Spear, but at Roseworthy no significant difference was found between varieties (Tables 5.5 and 5.6).

Fertilisers containing nitrogen significantly ($P<0.001$) increased Mg concentration in top tissues at both locations (Tables 5.5 and 5.6). Interaction of soil treatment with variety at Palmer was statistically significant (Figure 5.6b). While Temik® application increased concentration of Mg in Janz, it had the reverse effect on the concentration in Spear and Molineux.

Manganese

Spear demonstrated a significantly ($P<0.01$) higher concentration of Mn than both Janz and Molineux at the Palmer site, whereas at Roseworthy all three varieties were similar (Tables 5.5 and 5.6).

Fertiliser application grouped plants in three classes. Plants receiving no fertiliser or only potassium or phosphorus had the lowest and those treated with nitrogen plus phosphorus,

with or without potassium, demonstrated the highest concentration of Mn. Those supplied with nitrogen fertiliser alone had intermediate concentrations. The difference between all three groups was statistically significant ($P<0.05$).

Interactions of soil treatments with both varieties and fertilisers were not statistically significant.

Iron

Molineux demonstrated a significantly ($P<0.05$) higher concentration of Fe in top tissues than both Janz and Spear at Palmer, while at Roseworthy, Janz had a lower concentration of Fe than both Molineux and Spear, but this not was statistically significantly (Tables 5.5 and 5.6).

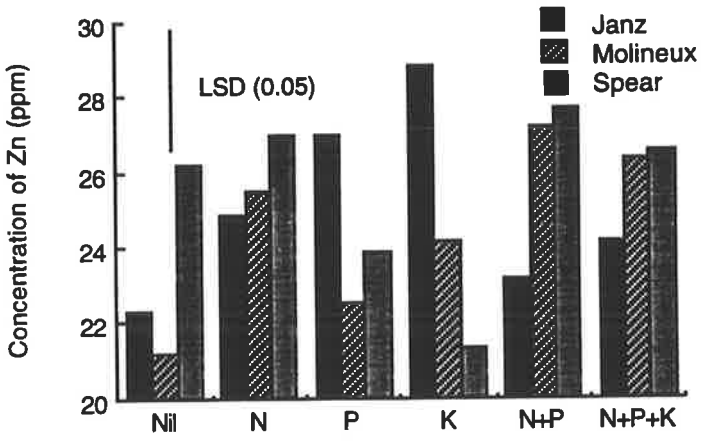
Zinc

All three varieties were similar in terms of concentration of Zn in top tissues at both locations.

Application of nitrogen fertiliser resulted in an increase in the Zn concentration so that the differences between plants receiving fertilisers containing nitrogen and other fertiliser regimes were statistically significant at Palmer (Table 5.5), but at Roseworthy, while the same trend was observed, the differences were not significant (Table 5.6).

Interaction of varieties with fertilisers in terms of Zn concentration, was statistically significant at Roseworthy (Figure 5.7). The responses of all varieties for Zn concentration in all fertiliser regimes (except potassium) was similar, but with the application of potassium, Janz demonstrated a significantly higher Zn concentration than the other two varieties.

Figure 5.7 Interaction effects of different genotypes and fertiliser regimes on concentration of Zn in shoots of plants grown in soil naturally infested with nematodes at Roseworthy.



Sodium

Janz had a significantly lower concentration of Na than both Molineux and Spear at Roseworthy. Molineux demonstrated a higher concentration of the element than both Spear and Janz at Palmer, but the difference was not statistically significant.

Fertilisers containing nitrogen elevated the level of Na and this effect was more obvious at the Palmer site (Table 5.5).

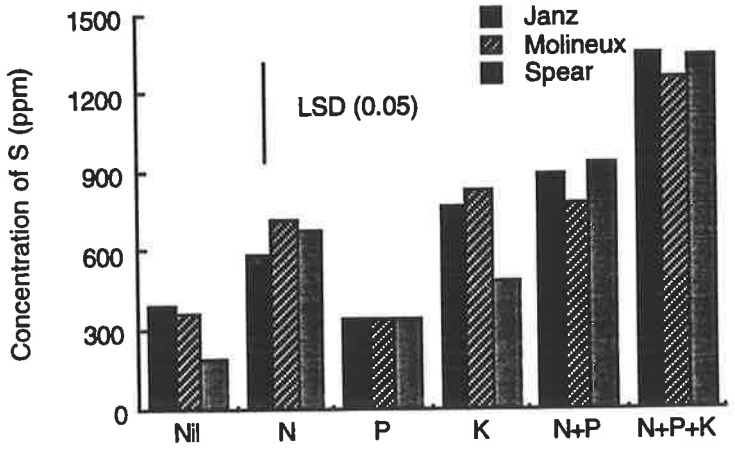
None of the interaction effects of soil treatments, varieties and fertilisers were statistically significant.

Sulphur

No significant difference was found between varieties for concentration of S at Palmer, but at Roseworthy Spear demonstrated a significantly higher concentration than Molineux (Tables 5.5 and 5.6).

Fertilisers containing nitrogen caused an increase in the S concentration in top tissues at both sites. Interaction effects of varieties with fertilisers at Palmer were significant (Figure 5.8).

Figure 5.8 Interaction effects of different genotypes and fertiliser regimes on concentration of S in shoots of plants grown in soil naturally infested with nematodes at Palmer.



E- Effect of soil treatment and fertiliser application on grain yield

Application of Temik® to control nematodes caused a significant reduction in grain yield compared to the no control of nematode treatment at both Palmer and Roseworthy sites (Tables 5.5 and 5.6). Plants treated with Temik® at the Palmer site were highly infected with *Gaeumannomyces graminis* var. *tritici* (take-all disease).

No significant difference was found between varieties in terms of grain yield at Palmer, while at Roseworthy, Janz had a significantly higher grain yield ($P<0.05$) than Spear (Table 5.6).

At both sites, plants receiving fertilisers containing nitrogen had a significantly higher (44% and 11% at Palmer and Roseworthy, respectively) grain yield than those receiving other fertilisers lacking nitrogen (Tables 5.5 and 5.6). Phosphorus and potassium

fertiliser, where applied alone did not have any significant effect on grain yield compared to those receiving no fertiliser.

At Palmer, the adverse effect of Temik® on grain yield was greater when plants were supplied with N+P or N+P+K fertilisers (Figure 5.9). No significant difference was found between Temik® and no control of nematodes treatments for other fertiliser regimes.

Figure 5.9 Interactions of soil treatments (control and Temik®) and different fertiliser regimes on grain yield of plants grown in soil naturally infested with nematodes at Palmer.

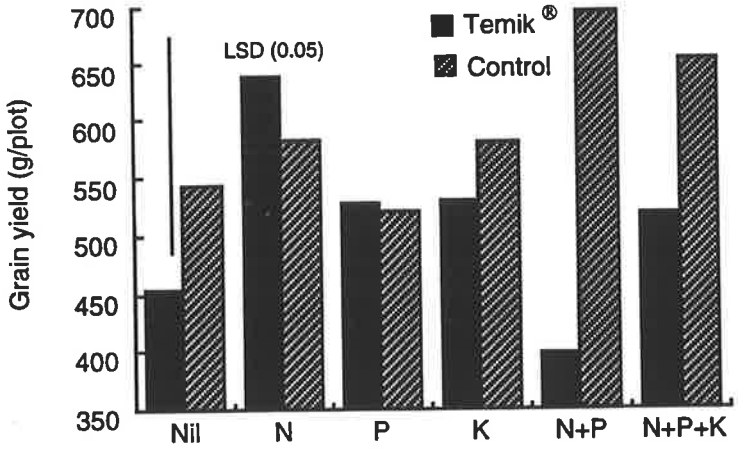


Table 5.5 Mean grain yield and nutrient concentrations in shoots of three wheat varieties grown in soil with nematodes (control) and without nematodes (Temik®) and six fertiliser regimes at Palmer.

Treatments	Grain yield and concentration of nutrients in shoots										
	Grain yield	**N**	**P**	**K**	**Ca**	**Mg**	**Mn**	**Zn**	**Fe**	**Na**	**S**
	g/plot	(%)	(10^{-4})	(10^{-3})	(10^{-4})	(10^{-4})	(10^{-5})	(10^{-5})	(10^{-5})	(10^{-4})	(10^{-4})
Control	505.7	3.06	6.63	8.66	5.68	3.78	4.80	1.76	10.03	2.22	5.82
Temik®	396.3	3.19	6.61	9.10	6.34	3.82	6.61	1.84	9.01	1.84	8.15
LSD (0.05)	**41.6**	**0.27**	**0.22**	**0.10**	**3.33**	**1.34**	**0.75**	**0.38**	**1.29**	**0.44**	**0.59**
Janz	450.5	3.19	6.81	9.16	6.26	3.76	5.53	1.74	9.06	1.86	7.24
Molineux	447.7	3.08	6.88	9.55	5.66	4.25	5.59	1.84	10.19	2.37	7.13
Spear	454.9	3.11	6.17	7.91	6.12	3.39	6.00	1.82	9.31	1.86	6.59
LSD (0.05)	**21.8**	**0.08**	**0.94**	**1.33**	**0.90**	**0.54**	**0.36**	**0.13**	**0.77**	**0.61**	**0.84**
Nil	356.0	2.51	5.43	4.49	3.99	2.11	4.83	1.86	9.52	1.49	3.17
N	495.7	3.55	3.67	11.91	7.41	4.40	5.74	1.96	9.68	2.37	6.61
P	390.6	2.46	10.19	3.97	3.98	2.20	5.09	1.72	9.36	2.13	3.41
K	370.9	2.55	5.90	5.56	4.13	2.37	5.01	1.76	9.49	1.33	6.97
N+P	531.8	3.83	7.13	12.44	8.55	6.11	6.66	1.62	9.57	2.80	8.67
N+P+K	581.1	3.84	7.41	14.88	8.00	5.62	6.90	1.88	9.51	2.05	13.08
LSD (0.05)	**30.8**	**0.151**	**1.34**	**1.89**	**1.27**	**0.77**	**0.51**	**0.19**	**1.09**	**0.86**	**1.19**

* 10^{-3}, 10^{-4} and 10^{-5} stand for par per thousand, part per ten thosands and part per hundred thosands respectively.

Table 5.6 Mean grain yield and nutrient concentrations in shoots of three varieties grown in soil with nematodes (control) and without nematodes, (Temik®) at six fertiliser regimes at Roseworthy.

	Grain yield and concentration of nutrients in shoots										
	Grain yield	**N**	**P**	**K**	**Ca**	**Mg**	**Mn**	**Zn**	**Fe**	**Na**	**S**
	g/plot	(%)	(10^{-3})	(10^{-3})	(10^{-3})	(10^{-3})	(10^{-5})	(10^{-5})	(10^{-5})	(10^{-4})	(10^{-3})
Control	595.8	3.33	3.07	39.2	2.86	1.58	5.86	2.19	10.74	2.91	2.56
Temik®	511.3	3.09	2.85	37.2	2.39	1.45	5.22	2.81	9.81	3.86	2.44
LSD (0.05)	**16.4**	**0.13**	**0.36**	**2.7**	**0.81**	**0.22**	**1.24**	**0.13**	**1.9.5**	**0.15**	**0.13**
Janz	608.7	3.24	3.04	37.90	2.76	1.55	5.60	2.51	9.88	2.93	2.49
Molineux	555.1	3.07	2.87	38.02	2.44	1.48	5.33	2.45	10.31	3.42	2.43
Spear	496.9	3.32	2.98	38.73	2.67	1.52	5.69	2.54	10.64	3.81	2.57
LSD (0.05)	**76.38**	**0.16**	**0.115**	**1.84**	**0.29**	**0.09**	**0.49**	**0.20**	**0.84**	**0.48**	**0.13**
Nil	499.2	2.82	2.95	34.71	2.16	1.34	5.31	2.32	10.43	2.91	2.20
N	611.3	3.67	2.92	41.50	3.29	1.71	5.91	2.58	10.40	3.64	2.46
P	524.4	2.80	3.15	35.19	2.06	1.33	5.14	2.44	9.58	2.98	2.29
K	555.4	2.44	2.66	32.07	1.93	1.20	5.07	2.48	9.91	2.77	2.19
N+P	545.9	3.74	3.16	42.53	3.06	1.74	5.94	2.60	10.42	3.95	2.71
N+P+K	585.1	3.79	2.95	43.29	3.26	1.78	5.87	2.57	10.91	4.05	3.14
LSD (0.05)	**108.02**	**0.23**	**0.16**	**2.61**	**0.40**	**0.12**	**0.70**	**0.28**	**1.19**	**0.68**	**0.19**

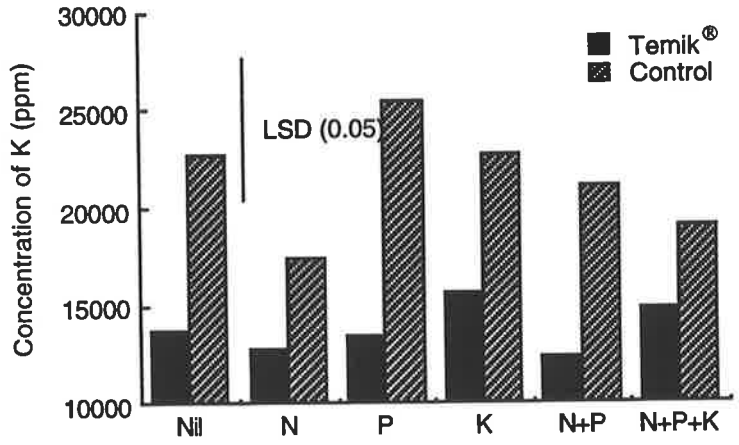
* 10^{-3}, 10^{-4} and 10^{-5} stand for par per thousand, part per ten thosands and part per hundred thosands respectively.

F- Effects of soil treatment and fertiliser application on concentration of elements in roots

Effect of Temik® application on concentration of nutrients

Application of Temik® increased the concentration of N, Zn and S in root tissues of plants growing at both sites, but only at Palmer was the difference significant ($P<0.05$) (Tables 5.7 and 5.8). In contrast, Temik® application caused a significant reduction in the concentration of Na at both sites, but that of K was significantly lower only at Palmer (Figure 5.10). No significant difference was found between Temik® application and the no control of nematodes treatment in terms of P, Ca and Mg concentration in plant roots either at Palmer or at Roseworthy (Tables 5.7 and 5.8).

Figure 5.10 Interactions of soil treatments (control and Temik®) and different fertiliser regimes on concentrations of K in roots of plants grown in soil naturally infested with nematodes at Palmer.



Difference between genotypes for concentration of elements in roots

No significant difference was found between varieties for concentration of N in roots at either site. Janz had a significantly ($P<0.01$) lower concentration of P than both Molineux and Spear at the Palmer site (Table 5.7). In contrast, while at Palmer all three varieties behaved similarly with regard to concentrations of K, Ca, Mg, Na and S in the roots, at

Roseworthy, Janz was significantly lower in concentrations of Na, Ca and S than the other two varieties and Molineux had a significantly ($P<0.01$) lower concentrations of K and Mg than the other two varieties. All three varieties were similar in terms of concentration of Mn, Fe and Zn at both sites (Tables 5.7 and 5.8).

G- Effects of fertilisers on nutrient concentrations in roots

Nitrogen

Application of nitrogen increased the absorption of N by the plant roots so that the difference between plants grown with fertilisers containing nitrogen and those with other fertiliser treatments was highly significant ($P<0.001$) at both sites (Tables 5.7 and 5.8)

No significant interaction was found between all three factors (soil treatment, variety and fertiliser regime).

Phosphorus

Fertiliser application had a significant effect ($P<0.01$) on P concentration. The highest concentrations were obtained where N+P or N were applied to the soil at Palmer and Roseworthy, respectively (Tables 5.7 and 5.8). Application of phosphorus at Palmer increased the concentration of the element by about 16% compared to the control, but at Roseworthy the concentration increased about 7% over the control treatment.

Potassium

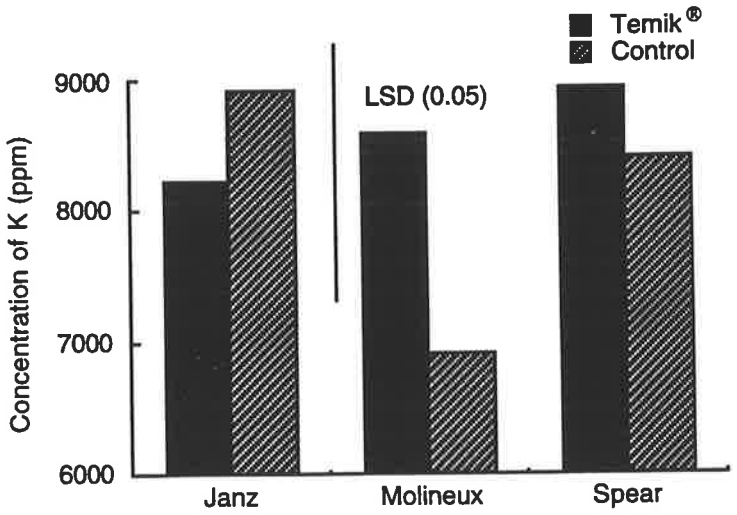
Fertilisers containing nitrogen lowered the concentration of K significantly ($P<0.01$) at both sites (Tables 5.7 and 5.8). Application of potassium had no significant effect on its concentration compared to the control treatment.

Interaction effects of soil treatments with varieties (Figure 5.11a) and fertiliser applications (Figure 5.11b) and that of the varieties with fertilisers (Figure 5.11c) at Roseworthy were

significant. While Temik® application increased concentration of K in Molineux, its effect was similar to that with no control of nematodes in Spear and Janz. The effect of Temik® was greater in terms of concentration of K in roots which received nitrogen and phosphorus fertilisers (Figure 5.11b).

Figure 5.11 Interactions of soil treatments (control and Temik®) with (**a**) varieties and (**b**) fertiliser regimes and (**c**) varieties with fertiliser regimes on concentrations of K in roots of plants grown in soil naturally infested with nematodes at Roseworthy.

(**a**) Interactions of varieties with soil treatments



(**b**) Interactions of soil treatments and fertiliser regimes

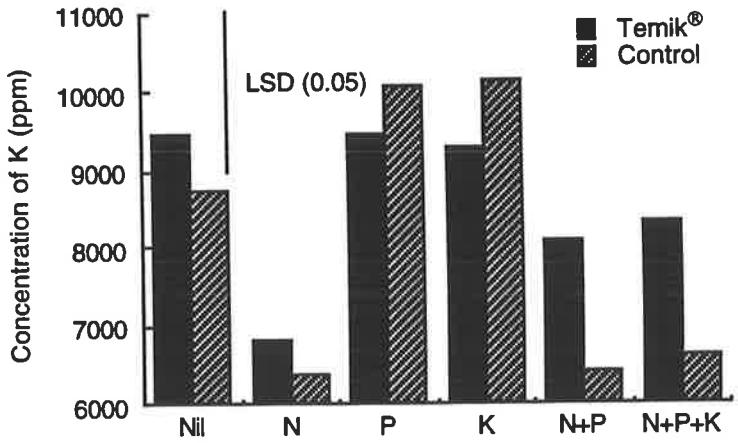
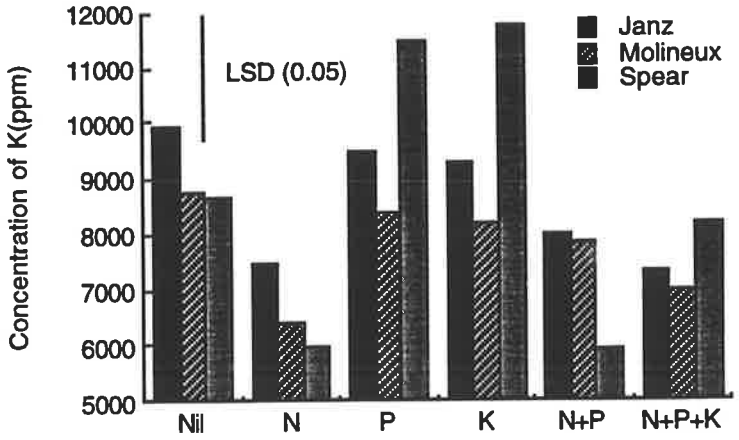


Figure 5.11 continued

(c) Interactions of varieties with fertiliser regimes

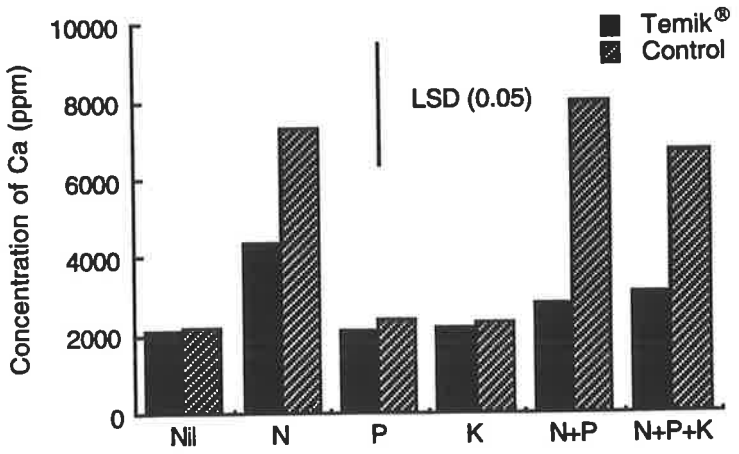


Calcium

The effects of fertiliser regimes on Ca concentration in roots were not significant at Palmer, but at Roseworthy using fertilisers containing nitrogen increased the concentration significantly (Table 5.8).

Interactions of soil treatments with fertiliser regimes were highly significant at Roseworthy ($P<0.001$) (Figure 5.12). The difference between Temik® and no control of nematode with fertiliser regimes containing nitrogen was significant ($P<0.05$), while with other fertiliser regimes both Temik® and no control of nematodes were similar.

Figure 5.12 Interactions of soil treatments (control and Temik®) and different fertiliser regimes on concentrations of Ca in roots of plants grown in soil naturally infested with nematodes at Roseworthy.



Magnesium

Fertiliser application did not have any effect on Mg concentration at Palmer, but treatment with fertilisers containing nitrogen increased its concentration at Roseworthy (Table 5.8).

Zinc, Iron and manganese

No meaningful trends were observed for the effects of fertilisers on the Zn, Fe and Mn concentrations in the roots (Tables 5.7 and 5.8).

Sodium

Fertilisers containing nitrogen increased the concentration of Na in plants growing at the Palmer site, but not at Roseworthy, compared to the control. At Roseworthy application of phosphorus and potassium resulted in the highest and lowest concentration of Na, respectively.

Sulphur

Fertiliser application had a significant effect on S concentration at both sites. All fertilisers somewhat elevated the level of this element in roots, but the highest concentration was obtained where all three fertilisers (N+P+K) were applied.

Interaction effect of soil treatment and variety was significant at Roseworthy in terms of the S concentration in roots. While Temik® application reduced concentration of S in Janz, Molineux had a higher concentration of the element in the presence of Temik® (Figure 5.13).

Figure 5.13 Interactions of soil treatments (control and Temik®) and different genotypes on concentration of S in roots of wheat plants grown in soil naturally infested with nematodes at Roseworthy.

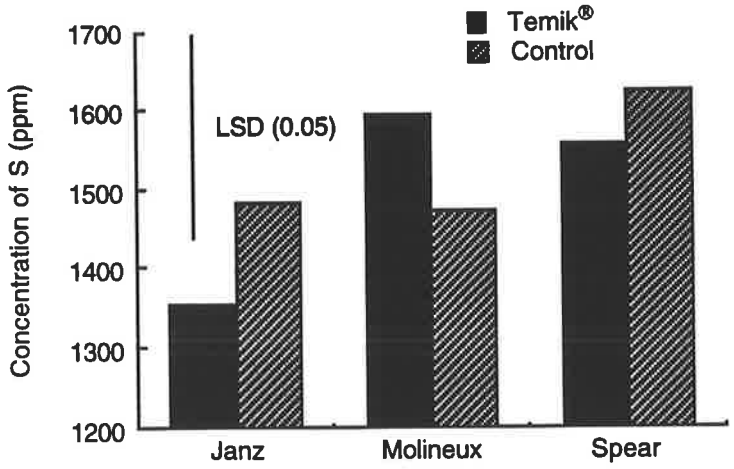


Table 5.7 Mean of nutrient concentrations in roots of three varieties grown in soil with nematodes (control) and without nematodes, (Temik®) at six fertiliser regimes at Palmer.

Treatments	Nutrient concentrations									
	N (%)	P (10^{-3})	K (10^{-3})	Ca (10^{-3})	Mg (10^{-3})	Mn (10^{-5})	Zn (10^{-4})	Fe (10^{-3})	Na (10^{-3})	S (10^{-3})
Control	1.43	2.58	21.43	4.95	2.68	7.68	2.95	2.87	2.83	3.21
Temik®	1.58	2.73	13.75	5.45	2.51	9.30	3.54	2.98	2.49	3.42
LSD (0.05)	**0.10**	**0.18**	**1.64**	**0.43**	**0.22**	**2.28**	**0.50**	**0.99**	**0.22**	**0.15**
Janz	1.49	2.59	17.61	5.08	2.66	8.45	3.62	2.92	2.67	3.32
Molineux	1.48	2.76	16.78	5.46	2.52	8.61	3.34	2.98	2.62	3.27
Spear	1.54	2.62	18.42	5.06	2.61	8.42	2.78	2.87	2.69	3.37
LSD (0.05)	**0.083**	**0.19**	**1.60**	**0.42**	**0.14**	**0.72**	**0.82**	**0.34**	**0.19**	**0.18**
Nil	1.31	2.36	18.21	4.82	2.50	8.36	2.76	3.02	2.57	3.02
N	1.80	2.60	15.07	5.11	2.60	8.60	3.40	2.71	2.64	3.27
P	1.26	2.80	19.43	5.25	2.56	8.63	3.02	3.20	2.45	3.05
K	1.27	2.51	19.24	5.06	2.56	8.49	3.75	3.05	2.58	3.52
N+P	1.67	2.89	16.75	5.66	2.76	8.41	3.25	2.97	2.97	3.35
N+P+K	1.73	2.79	16.91	5.30	2.58	8.48	3.29	2.61	2.74	3.69
LSD (0.05)	**0.117**	**0.27**	**2.27**	**0.59**	**0.20**	**1.02**	**1.16**	**0.49**	**0.26**	**0.25**

* 10^{-3}, 10^{-4} and 10^{-5} stand for par per thousand, part per ten thousands and part per hundred thousands respectively.

Table 5.8 Mean of nutrient concentrations in roots of three varieties grown in soil with nematodes (control) and without nematodes, (Temik®) at six fertiliser regimes at Roseworthy.

		Nutrient concentrations									
	Grain yield	**N**	**P**	**K**	**Ca**	**Mg**	**Mn**	**Zn**	**Fe**	**Na**	**S**
	g/plot	(%)	(10^{-3})	(10^{-3})	(10^{-3})	(10^{-3})	(10^{-5})	(10^{-4})	(10^{-3})	(10^{-3})	(10^{-3})
Control	595.8	1.28	1.41	8.07	4.81	1.49	6.06	1.27	1.85	2.07	1.53
Temik®	511.3	1.36	1.19	8.58	2.79	1.31	5.01	1.18	1.49	1.90	1.50
LSD (0.05)	**16.44**	**0.13**	**0.43**	**1.94**	**2.80**	**0.27**	**1.18**	**0.11**	**0.48**	**0.16**	**1.06**
Janz	608.7	1.30	1.20	8.56	3.09	1.42	5.08	1.15	1.62	1.88	1.42
Molineux	555.1	1.31	1.33	7.74	4.19	1.33	5.70	1.28	1.63	2.08	1.53
Spear	496.9	1.35	1.37	8.66	4.11	1.44	5.83	1.24	1.76	2.01	1.59
LSD (0.05)	**76.38**	**0.07**	**0.09**	**0.67**	**0.80**	**0.07**	**0.39**	**0.20**	**0.18**	**0.13**	**0.07**
Nil	499.2	1.09	1.14	9.10	2.14	1.27	5.41	1.03	1.70	2.03	1.35
N	611.3	1.55	1.51	6.62	5.84	1.56	5.76	1.52	1.69	1.93	1.49
P	524.4	1.12	1.24	9.77	2.28	1.28	5.26	1.09	1.62	2.17	1.52
K	555.4	1.05	1.07	9.73	2.24	1.26	5.41	1.03	1.59	1.84	1.53
N+P	545.9	1.53	1.47	7.25	5.42	1.50	5.63	1.44	1.73	2.01	1.50
N+P+K	585.1	1.57	1.36	7.47	4.87	1.52	5.74	1.21	1.71	1.95	1.70
LSD (0.05)	**108.02**	**0.10**	**0.12**	**0.95**	**1.13**	**0.10**	**0.55**	**0.28**	**0.25**	**0.19**	**0.10**

* 10^{-3}, 10^{-4} and 10^{-5} stand for par per thousand, part per ten thousands and part per hundred thousands respectively.

5.3 Discussion

Nematodes attack the roots of crops and reduce absorption ability so that plants require a greater supply of nutrients. To reduce nematode effects on crop productivity adequate fertilisation must be applied to plant to keep it in a vigorous healthy condition.

The results of experiments carried out in the glasshouse and reported in Chapter 4 demonstrated that the root lesion nematode reduced top growth of infected plants (Section 4.3). In contrast, the results of the field experiment at Balaklava reported in this Chapter showed that Temik® application had no effect either on plant vigour nor on the severity of yellow lower leaves.

At Balaklava, as expected, plants treated with Temik® yielded a higher top growth in 1992, although this was not statistically significant. Surprisingly, Temik® application in 1993 at Palmer and Roseworthy, not only did not increase the grain yield, but caused a significant reduction at both sites. When sampling the roots of plants at the Palmer site, it was recognised that roots of plants treated with Temik® were highly infected with *G. graminis*, a pathogenic fungus widespread in southern Australia. While the Temik® treated plots had significantly lower numbers of nematodes compared to the control treatment, the damage caused by *G. graminis* to roots of plants grown in these plots was more serious than that of those grown with no control of nematodes. V. A. Vanstone (pers. comm.) also obtained similar results when using the nematicide Furadan® to control the nematode in adjacent plots. Taheri *et al.* (1995) confirmed these results in both glasshouse and field conditions. Temik®, apparently by reducing the natural enemies of *G. graminis* in the soil, appeared to enhance the growth of the fungus, consequently damaging the plants more severely.

The adverse effect of Temik® on grain yield at Roseworthy (Table 5.8) could be explained on the basis that the benefits from Temik® application depend on the potential for damage to the plants by the nematodes. The latter depends on nematode density at the time of

sowing, soil fertility and other environmental factors. Roseworthy soil was more fertile (higher organic carbon) than Palmer. It had a lower density of nematodes and a higher proportion of clay, restricting nematode movement, and provided conditions where nematode invasion was reduced. Not only would the nematodes have been less destructive to plant roots at Roseworthy than Palmer, but the plants would have been likely to be damaged by the application of Temik® because of its phytotoxicity effects. Under these conditions, application of Temik® could be expected to reduce plant growth. The significant reduction of N concentration in plants treated with Temik® at Roseworthy (Table 5.6), associated with a low population of nematodes, could be an effect of phytotoxicity. In contrast, when the soil was more heavily infected and the texture more favourable for nematode movement, as at Palmer, nematode damage to plant would be more serious, so any method of reducing the nematode population would have a positive effect on plant growth (Chapter 4).

The soil at Balaklava, which was used in the glasshouse experiments reported in Sections 4.1.3 and 4.2.3.1 and for the field experiment in 1992, was high in magnesium. Application of magnesium alone to this soil reduced top and root growth (Table 5.3). This might be because the high concentration of the element in the soil environment could influence the absorption of other elements such as Ca and Fe (Table 5.3) (Dr. R. D. Graham, pers. comm.). When a plant is suffering from the deficiency of other essential elements, its growth could be further retarded by high levels of a nutrient which is already in liberal supply. As discussed in Chapter 4, in these experiments it has been demonstrated that magnesium deficiency was not responsible for the symptoms of yellow lower leaves. It is also unlikely that the symptoms are caused by Mn deficiency as this is a relatively immobile element and deficiency symptoms usually show up first in the younger leaves (Tisdale *et al.* 1993). Mn deficiency is normally expressed as pale green floppy leaves, dissimilar to the yellow leaf symptoms.

Varietal differences

Janz and Spear were more efficient in absorbing nitrogen compared to Molineux as they showed a higher concentration of N in their shoots than Molineux (Tables 5.5 and 5.6). Molineux also showed less efficiency in absorbing phosphorus from the soil (Table 5.6). Plants with greater efficiency in nitrogen and phosphorus absorption may be less sensitive to reduction of the root's ability to uptake nutrients resulting from nematode infection. It seems that the symptom of yellow lower leaves is highly related to nitrogen deficiency and to a less extent to phosphorus (Figure 5.2 and Table 5.3). This confirms the results of Chapter 4 which revealed that N+P fertiliser could restrict the symptoms of yellow leaves of plants growing in nematode infested soil (Section 4.4).

Effect of Temik® application on nutrient concentration

The effect of Temik® application on nutrient concentration depends on the site and the nutrients. While results from the Palmer site agreed with the suggestion that Temik® improves the ability of plants to absorb nitrogen (J. M. Fisher, pers. comm.), a contrasting reduction of nitrogen concentration was recorded at Roseworthy (Table 5.6).

Increased Na concentration in plants growing in Temik® treated plots at Roseworthy (Table 5.9) was in agreement with the results obtained under glasshouse conditions. At Roseworthy, plants treated with Temik® had a lower concentration of K in their shoots (Table 5.6). In contrast, plants growing in Temik® treated plots at Palmer had a significantly ($P<0.01$) higher concentration of K (Table 5.5) than those in untreated plots, but were lower in Na. These contrasting effects may be explained by the fact that potassium and sodium can substitute for each other and affect the absorption of each other negatively (Tisdale *et al.*, 1993).

Both K and P absorption was highly reduced by nematode infection (Table 5.5), as Temik® treatment yielded a significantly higher concentration of these two nutrients in roots than the no control of nematodes treatment. Temik® application at Palmer increased

K concentration in roots by about 60%. Significantly lower concentrations of these nutrients in shoots of plants growing at the Palmer site (with low fertility and soil favourable for nematode multiplication) compared to that of their corresponding concentrations in the roots (Tables 5.5 and 5.7) together with a high level of *G. graminis* infection and the contrasting results at the Roseworthy site (Table 5.9) with a higher fertility status and soil less favourable for nematode reproduction (Table 5.6 and 5.8), indicated that plants infected with a high population of nematodes or *G. graminis* were not able to translocate nutrients from the roots to shoots efficiently.

Table 5.9 Percentage increase in nutrient concentrations in vegetative tissues of wheat due to Temik® application compared to the control treatment at three locations.

	% increase Temik® vs no control of nematodes		
	Site		
Nutrient	Balaklava	Palmer	Roseworthy
N	2.0	4.3	-7.2
P	1.6	-0.4	-7.1
K	1.4	5.1	-5.1
Ca	3.6	11.5	16.5
Mg	3.2	1.2	-8.6
Mn	18.8	37.5	-11.0
Na	17.0	-20.6	32.5
Fe	6.1	-10.0	-8.6
S	13.0	40.1	-4.7
Zn	5.3	4.1	28.5

Higher concentration of Ca in top tissues of plants treated with nitrogen fertiliser compared to those receiving no fertiliser, could have been due to application of calcium in the calcium nitrate and a higher root growth resulting from nitrogen application as demonstrated in Chapter 4 (Figure 4.1). Nitrogen application, also through improving root

growth, caused an increase in Mg uptake. A good supply of nitrogen is associated with increased root proliferation in the area of applied nitrogen (Mengel and Kirkby, 1987).

A lower concentration of K (Tables 5.7 and 5.8) in roots of plants treated with calcium nitrate might be due to this fact the Ca competes with K for entry into plants. Soils high in this cation may require high levels of K for satisfactory nutrition of crops. K uptake would be reduced as Ca is increased and *vice versa* (Tisdale *et al.*, 1993). As the concentrations of P, K, Ca, Mg, Mn, Na and S were significantly higher in shoots of plants treated with nitrogenous fertilisers than those supplied with fertilisers lacking nitrogen, it could be concluded that uptake of these elements was facilitated by nitrogen application.

Effects of fertilisers on nematode population and nutrient concentration

Application of nitrogen to nematode infested soil almost removed the symptoms of yellow lower leaves in all experiments, confirming that it is the most critical element in respect of these symptoms for plants in nematode infested soil. Nitrogen fertiliser, through enhancing root growth (Section 4.2.3.1), stimulates the root system to proliferate extensively through the soil environment, giving greater access of roots to the nutrients. Nitrogen application also has a nematicidal effect. It was documented that the number of nematodes was reduced by application of calcium nitrate (Section 4.2.3.1). As shown in Figure 5.4, number of nematodes per gram dry root in nitrogen treated plots was not as high as the number obtained in N+P and N+P+Mg treatments, while they had a similar root growth (Chapter 4) and consequently equal potential for nematode multiplication. Through these mechanisms, nitrogen application could remove the symptoms of yellow leaves of plants growing in nematode infested soil. The best results for reducing the yellow lower leaf symptoms were obtained when nitrogen and phosphorus were both applied (Chapter 4 and Figure 5.2).

However, since the number of nematodes per gram dry root of plants treated with nitrogen and phosphorus was higher than those of the control treatment (Table 5.3 and Figure 5.4),

and as these plants produced more root growth (Chapter 4), they would leave a higher population of nematodes in the soil to invade the next crop.

In contrast to the results of the glasshouse experiments (Section 4.3.5), plants supplied with nitrogenous fertiliser under field conditions were not lower in concentration of P compared to the other treatments. In field conditions, plants were apparently able to explore a higher volume of soil and consequently had access to larger quantities of the element than they did in the limited soil volume in a pot.

Both in the glasshouse experiments (Chapter 4) and in the field experiments at Balaklava and Palmer, the Mn concentration was highly elevated by a reduction in the nematode population (Table 5.9). Nematodes, through wounding the roots, increase the opportunity for infection by fungi and bacteria. They may reduce the ability of plants to absorb Mn. Pedler (1994) noted that fungal and bacterial infestation promote the oxidation of Mn in the rhizosphere and make it unavailable for uptake by plants. However, the higher concentration of Mn in plants grown in Temik® treated plots at Palmer, while heavily infected with *G. graminis*, was in contrast to Pedler's hypothesis.

Deficiency of Zn is usually associated with concentrations of less than 20 ppm (Tisdale *et al.*, 1993). Since in almost all fertiliser regimes in the experiment conducted at the Palmer site the concentration of Zn was less than 20 ppm in plant top tissues (Table 5.5), the soil was probably deficient in the element.

Effect of Temik® on other soil microorganisms needs to be investigated. It may have negative effect on growth of plants by controlling the natural enemies of other pathogens. Temik® application to reduce nematode population is effective up to the cultivated layer. Unaffected nematodes will migrate from the lower layers to soil surface and invade plant root systems, so that at harvesting both treated and untreated plots might have equal density of nematodes to attack the next crop.

In the absence of resistant varieties, nitrogen application to nematode infested paddocks will improve the root growth of infected plants and overcome the damage caused by nematodes. However, to reduce the nematode population satisfactory, a high rate of nitrogen is required.

These both methods might be uneconomic to control nematodes for low value crops such as wheat. Screening resistant varieties , while would give a satisfactory yield in nematode infested soils, would also reduce the nematode population and minimise the nematode damage to the next crop.

CHAPTER 6

SCREENING WHEAT VARIETIES FOR RESISTANCE TO ROOT LESION NEMATODE (*P RATYLENCHUS NEGLECTUS*)

6.1 Introduction

Genetic variation among the members of any species is the key to adaptation to various environmental stresses and ultimately survival. The continued survival of any species, in spite of various natural enemies including diseases and pests, is that some members have genetically controlled mechanisms to enable them to tolerate or resist invaders. Root lesion nematode attack the roots of cereals and legumes and damage the cortex. It is to be expected that some wheat varieties would be less favourable for nematode reproduction due to the factors disscussed in Section 2.5.8.

After finding a suitable host, root lesion nematodes enter the roots where they develop and reproduce. Mature larvae, after recovery from penetrating, start to lay eggs. As the time required for an egg to develop and hatch is seven to ten days in optimal conditions, counting the number of nematodes in the roots during this stage after initial inoculation will measure the rate of penetration. Nematode reproduction will continue until confronted with a lack of food or other factors limiting nematode multiplication. If sampling is performed at this stage, nematode reproduction rate can be determined. The length of this period varies, depending on the rate of root growth and the time to maturity of the host. Apart from the inherent rate of multiplication of the nematode, the reproduction rate will depend upon the suitability of the host to the nematode, the level of tolerance and the rate of root growth of the host. Hence, sequential sampling should be undertaken at appropriate times to estimate the relative rates of penetration and reproduction for different varieties.

To estimate differences in susceptibility of a range of varieties, some investigators have suggested growing plants in a nematode-infested soil for a duration of six to eight weeks

and counting nematodes per gram of dry root (Trudgill *et al.*, 1975). Others have recommended sampling plants after maturity and comparing final to initial populations (Thompson, 1984). The problem with both these methods is that the time of maturity and the growth rate of plants vary between varieties and could affect the results. Given equal reproduction rates, varieties with early maturity will have a smaller number of nematodes per plant than late maturing varieties. In contrast, varieties with a higher root growth and late maturity will have a lower number of nematodes per gram of dry root. To measure the nematode reproduction rate in order to identify resistant varieties to the root lesion nematode, the time of sampling is therefore very important.

This experiment was undertaken to understand the effect of sampling time on nematode multiplication in genetically different varieties and to assess how nematode multiplication is influenced by time of maturity of the host, rate of root growth and the time of sampling.

6.2 Materials and methods

In preliminary experiments, P. F. Lonergan and V. A Vanstone (unpublished data) tested many varieties and found that two lines, RAC 589 and AUS 16830 (for pedigree and origin refer to Table 3.6), showed a relatively low number of nematodes per plant and Machete (a variety cultivated in South Australia) and AUS 7384 showed a high number of nematodes in the roots among a large number of varieties tested.

A reddish sandy loam soil was collected from the farming property of Mr. J. W. Eichler at Palmer, 65 km east of Adelaide (Chapter 3). As movement of soil particles can kill nematodes and reduce total numbers (J. M. Fisher, pers. comm.), and nematodes tend to be unevenly distributed throughout a field, the infested soil was mixed gently to provide more even distribution of nematodes in the soil. Nematode density in the soil was determined using the Whitehead Tray method (Chapter 3) in a 200 g soil sample accumulated by taking approximately 100 random samples with a spatula. After one

week, the extracted nematodes were counted. An average of seven nematodes per gram of soil was recorded.

Plastic pots filled with 650 g of infected soil were arranged in a completely randomised design in a temperature controlled waterbath (20 ± 2°C) in an evaporatively cooled glasshouse. The experiment was a split plot design with the times of sampling (4, 7 and 10 weeks) as the sub plots and the varieties the main plots. Each variety was replicated six times.

Seeds were surface sterilised and germinated (Chapter 3). When radicles were about 1-3 cm long, four seedlings from each variety were planted per pot. One third of pots were sampled after four weeks, one third after seven weeks and the remainder after ten weeks.

The growth of plants in terms of number of tillers, time of first appearance of the yellow leaf symptom and time of heading were recorded. At each sampling, tops were cut from the crowns at ground level and fresh weights measured. Tops were dried at 85°C for 24 hours and dry weights recorded. Nematodes were extracted from the roots by misting for five days and counted under a microscope (Chapter 3).

At each sampling, the number of nematodes in the soil was also measured. The soil from each pot was gently mixed after removal of the roots, and a sample of 100 g was accumulated by taking random spoonfuls. Soils were placed in WhiteheadTrays (Chpter 3) and after one week at a temperature of 22 ± 3°C the number of nematodes per pot was counted.

A logarithmic transformation (Ln number of nematodes + 200) based on the recommendations of Proctor and Marks (1974) was used for analysis of the nematode data. Data were analysed using Super ANOVA (v1.11) program on a Macintosh computer and the means were compared by LSDs.

6. 3 Results

Six weeks after commencing the experiment, RAC 589 started heading, the plants at that stage had one or only a few tillers. Machete headed 47 days after seeding, having several tillers per plant. The other two varieties, AUS 16830 and AUS 7384, remained vegetative seven weeks after seeding and had a large number (4-5) of tillers per plant. After seven weeks, RAC 589 had completed heading and was at the grain filling stage.

RAC 589 was the most vigorous variety followed in order by Machete, AUS 16830 and AUS 7384. After four weeks (Table 6.1), the differences between top fresh weights for all varieties, other than AUS 16830 and AUS 7384, were significant ($P<0.05$) with RAC 589 having the highest weight. None of the varieties were significantly different for root fresh weight or dry weight at the first sampling (four weeks), although Machete and AUS 16830 showed the lowest and highest root fresh weights, respectively (Table 6.1). The roots of AUS 7384 and AUS 16830 were brighter and apparently more healthy.

No significant difference was found between the varieties for number of nematodes either per gram of dry root or per plant four weeks after commencing the experiment (Figures 6.2 and 6.3). Numbers of nematodes in the soil surrounding the roots of the four varieties were also similar (Table 6.1).

After seven weeks (Table 6.1), AUS 16830 had the lowest top fresh weight and was significantly different from AUS 7384 and Machete, but not from RAC 589. During the three weeks, from the first sampling to the second, the top fresh weight of all varieties increased about three times (Table 6.1). The differences between all varieties were significant for top dry weight ($P<0.05$), except for AUS 16830 and AUS 7384 (Table 6.1). AUS 7384 and AUS 16830 had significantly ($P<0.01$) higher root fresh weights than the other two varieties, with twice the weight of RAC 589 and about 35% more than Machete (Table 6.1).

In contrast to samples at four weeks, after seven weeks varieties were significantly different ($P<0.05$) for the number of nematodes per gram of root, with RAC 589 showing the highest numbers of nematodes and AUS 16830 the lowest (Figure 6.2). When varieties were compared in terms of numbers of nematodes per plant, only the difference between AUS 16830 and Machete was statistically significant and, in contrast to numbers of nematodes per gram, AUS 16830 had the highest number per plant (Figure 6.3). No significant difference was observed for number of nematodes in soil for the four varieties at this stage, and the number was negligible compared to that in the roots (Table 6.1).

After ten weeks, AUS 7384 (with 18.32 g of top fresh weight) was significantly heavier ($P<0.05$) than the other varieties (Table 6.1), whereas RAC 589 had the highest dry weight. There was a large difference for root fresh weight between AUS 16830 and AUS 7384 and both RAC 589 and Machete (Table 6.1).

In terms of numbers of nematodes per gram of dry root, RAC 589 had the highest number and was significantly different from both AUS 7384 and AUS 16830 (Figure 6.2). There were no significant differences between varieties in terms of numbers of nematode per plant (excluding numbers of nematodes in the soil) (Figure 6.3). The numbers of nematodes per pot (including both those in the roots and in the soil), differed significantly for Machete and RAC 589 from AUS 7384 and AUS 16830 (Figure 6.4b).

As shown in Figure 6.4, ten weeks after commencing the experiment, the numbers of nematodes extracted from the soil from Machete and RAC 589 were higher than AUS 7384 and AUS 16830 ($P<0.01$).

From the second to the third sampling, no growth of roots took place for RAC 589 and Machete whereas that of AUS 16830 and AUS 7384 increased from 1.32 to 2.48 and from 1.49 to 2.63 g per pot, respectively

Table 6.1 Top weight (fresh and dry) and root fresh weight of four varieties sampled after 4, 7 and 10 weeks. Plants were grown in soil naturally infested with *P. neglectus* (7 nematodes /g of soil) at 22± 2°C.

		Varieties				
Sampling	**Tissue**	**Machete**	**RAC 589**	**AUS 7384**	**AUS 16830**	**LSD (0.05)**
Four weeks	Top fresh weight (g/pot)	5.52	5.98	4.88	4.86	0.40
	Top dry weight (g/pot)	0.85	1.20	0.75	0.84	0.12
	Root fresh weight (g/pot)	5.25	5.47	5.79	5.99	1.06
	No. of nematodes per pot in the soil	350	437	375	287	223
Seven weeks	Top fresh weight (g/pot)	15.68	13.32	14.93	12.6	1.20
	Top dry weight (g/pot)	2.91	3.29	2.41	2.29	0.29
	Root fresh weight (g/pot)	9.82	5.97	13.67	14.00	1.40
	No. of nematodes per pot in the soil	854	761	361	546	506
Ten weeks	Top fresh weight (g/pot)	16.61	14.12	18.32	14.96	1.67
	Top dry weight (g/pot)	5.32	5.63	4.76	4.01	0.31
	Root fresh weight (g/pot)	6.93	4.84	16.23	17.7	2.05

Figure 6.1 Root dry weight of four varieties sampled after 4, 7 and 10 weeks. Plants were grown in soil naturally infested with *P. neglectus* (7 nematodes /g of soil) at 22± 2°C.

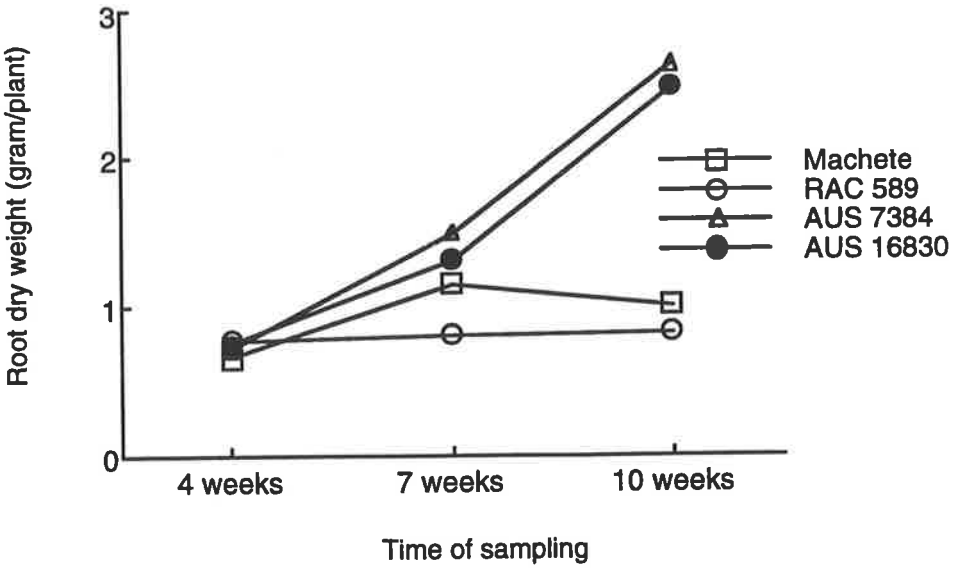
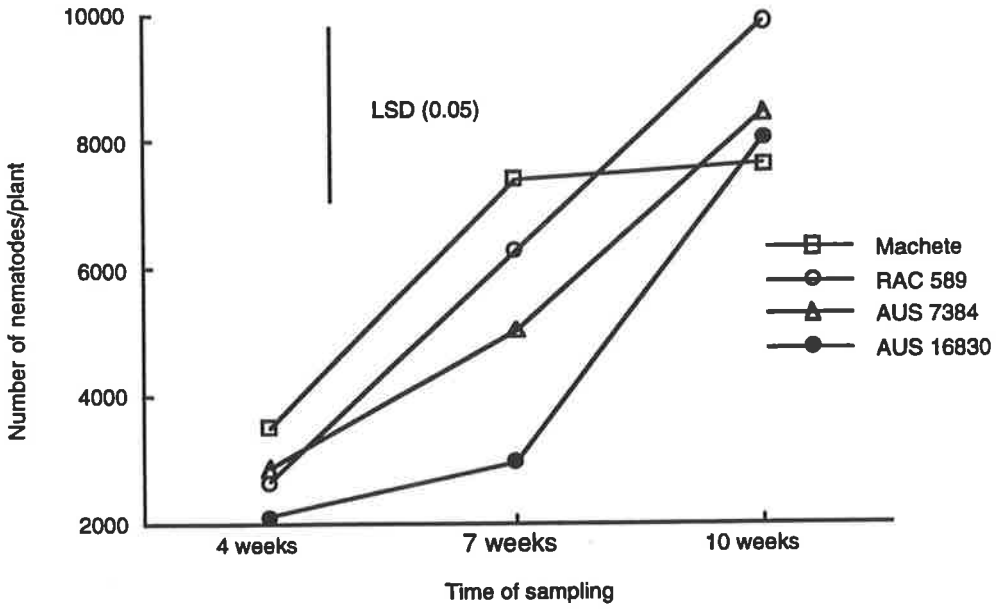


Figure 6.2 Numbers of nematodes (**a**) per plant and (**b**) per gram of dry root of four varieties sampled after 4, 7 and 10 weeks. Plants were grown in soil naturally infested with *P. neglectus* (7 nematodes /g of soil) at (22± 2°C).

(**a**) Number of nematodes per plant



(**b**) Number of nematodes per g of dry root

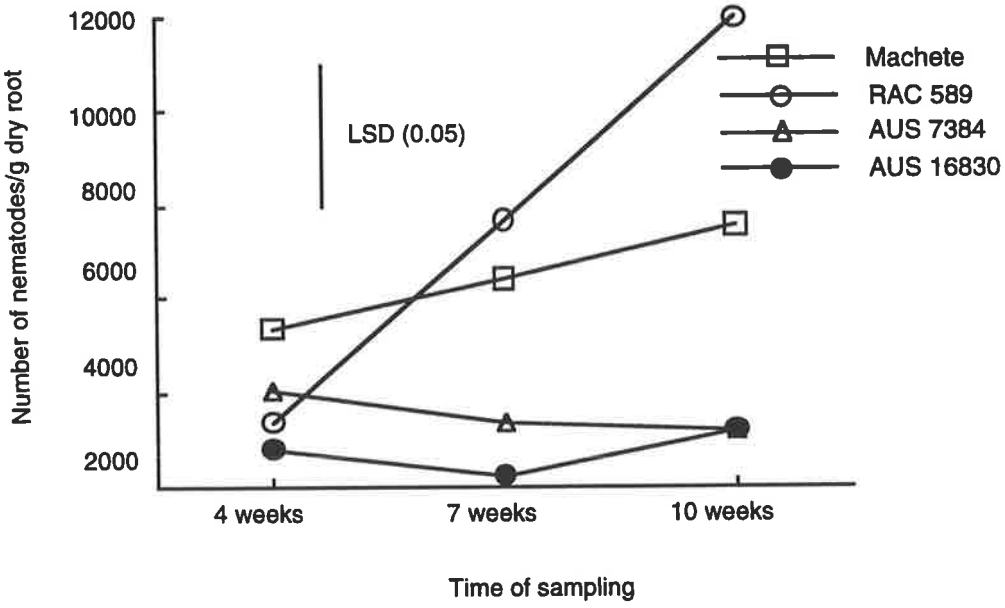
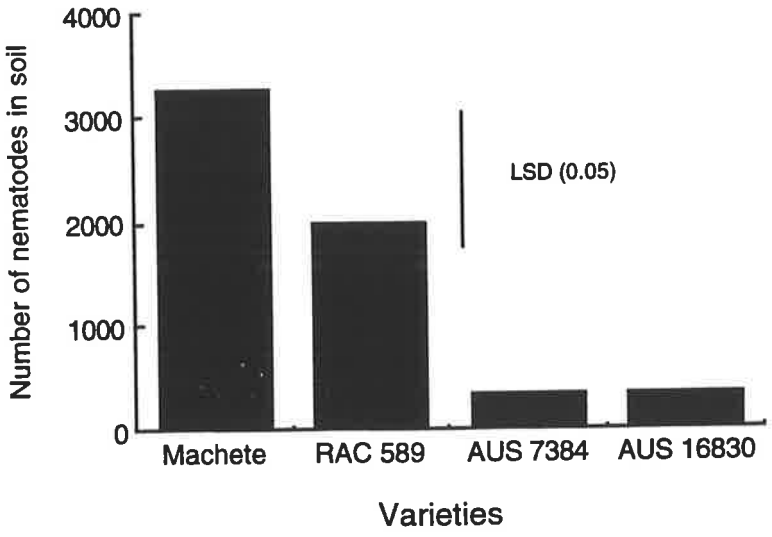
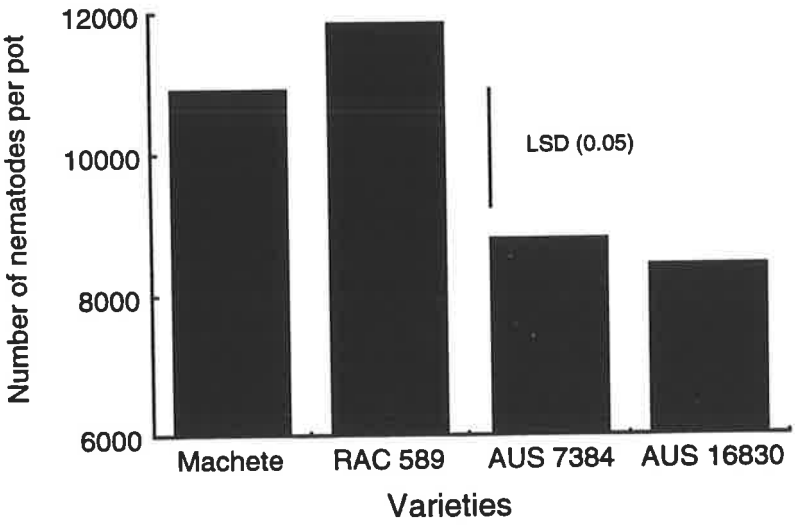


Figure 6.4 Numbers of nematodes (**a**) per pot in the soil and (**b**) in the soil and root for four varieties sampled after 10 weeks. Plants were grown in soil naturally infested with *P. neglectus* (7 nematodes /g of soil) at 22± 2°C.

(**a**) Number of nematodes per pot in the soil



(**b**) Total number of nematodes per pot (in the soil and roots)



Discussion

Factors affecting nematode multiplication will determine the appropriate sampling time for screening varietal resistance. Varietal characteristics including the rate of growth, time of maturity and extent of tolerance are likely to be involved. Plants with low root growth will reach the equilibrium level of nematode density earlier than those with a high rate of root growth. Likewise, intolerant plants that are suscptible to the nematode, are likely to show a lower number of nematodes than tolerant varieties at late sampling times because extensive damage from nematode attack leaves the host unsuitable for nematode reproduction. Duration of the nematode reproduction phase will be shortened in early maturing varieties compared to those with a longer growing season. The time of sampling, therefore, is very important in screening for varieties resistant to the nematode.

In healthy roots, exchange of nematodes between the soil and roots occurs (Pitcher, 1965), but after anthesis it seems that the root becomes less attractive to the nematodes and they prefer to leave the root habitat and migrate to the soil. Nematodes leave the roots and enter the soil to find new sources of food or enter the anhydrobiotic stage. Machete, with no further root growth from seven to ten weeks, had lost about 30% root moisture compared to that lost by RAC 589 with about 19%. With both of these varieties, migration of nematodes from the root to the soil might be caused by reduction of root moisture or by root senescence.

It is therefore suggested that plants should be sampled before the earliest maturing variety goes to anthesis. Depending on daylength, temperature and varietal characteristics the time of maturity varies. Seven to eight weeks is an optimal period for pot experiments. If sampled later, the nematodes would have left the roots of early maturing varieties and migrated to the soil so that the number of nematodes in both the roots and soil must be determined. Thompson *et al.* (1993) and Vanstone and Nicol (1993) have also suggested in the case of late sampling, that the number of nematodes

per pot (including roots and soil) should be examined. Nevertheless, the number of nematodes which are extracted from the roots or from the soil after anthesis is reduced compared to the population extracted before anthesis (S. P. Taylor and J. P. Thompson, pers. comm.).

As shown in Figure 6.1, at the first sampling there were no significant differences between varieties for number of nematodes either per plant or per gram dry root. This is to be expected if resistance does not affect the penetration rate or the establishment of feeding in the roots. If resistance reduces multiplication rate of the nematode, at this period nematodes have yet to complete one generation, as the life cycle would take about 30 days at this temperature (20 ± 2°C) (Vanstone and Nicol, 1993). This confirms the suggestion of Vanstone and Nicol (1993) and indicated that sampling of screening experiments for root lesion nematode resistance should not be undertaken until at least four weeks after germination. During this period, penetration may still be taking place, as at earlier stages some nematodes do not have access to the roots due to low volume of root growth.

After seven weeks, root growth rate of RAC 589 and Machete decreased because of their early maturity. The high number of nematodes per gram dry root for RAC 589 at the second sampling was more likely due to a lower root dry weight compared to root growth of other entries (Figure 6.1). At this stage, both AUS 7384 and AUS 16830 showed significantly lower number of nematodes per gram dry root than Machete and RAC 589. In terms of number of nematodes per plant, only AUS 16830 was significantly lower than the other varieties.

In terms of number of nematodes per plant at ten weeks, all four varieties were similar, but a large number of nematodes had migrated from the roots of Machete and RAC 589 into the soil (Figure 6.4a). For Machete, the constant number of nematodes in the root system after seven weeks could be explained by two phenomena. Firstly, nematode reproduction rate has been slowed down through a lack of sufficient food, and secondly,

as shown in Figure 6.4a, after a reduction in root moisture content, nematodes had started to migrate to the soil. Nematodes had also migrated from the roots of RAC 589, following its early maturity, but this line still could have accommodated a large number of nematodes (Figure 6.2a).

In contrast to earlier results of P. F. Lonergan and V. A. Vanstone (unpublished data) (Machete, AUS 7384, AUS 16830 and RAC 589 with 627, 752, 469 and 240 nematodes per plant respectively), RAC 589 must be considered as susceptible (Figure 6.2a and 6.2b). AUS 7384 also showed no significant difference from Machete or RAC 589, in term of nematode multiplication. AUS 16830, despite a high root dry weight which was favourable for nematode reproduction, had a significantly lower number of nematodes per pot and was relatively resistant to the nematode compared to RAC 589 and Machete. This is in agreement with the results of A. J. Rathjen and P. F. Lonergan (unpublished data).

As ranking of varieties did not change from seven to ten weeks for number of nematodes per pot (at the seven week sampling no significant differences were found between varieties for number of nematodes outside the roots in the soil), it is recommended that screening experiments be terminated at eight weeks and nematodes extracted from the roots only.

CHAPTER 7

EXAMINATION OF POSSIBLE VARIATION IN POUALATIONS OF *PRATYLENCHUS NEGLECTUS* IN DIFFERENT SOILS

7.1 Introduction

In screening experiments, it is important to use a single genotype of nematode, as the response of a given variety or line of the host may vary from biotype to biotype of the pest within the same species. In the case of different biotypes of nematode species existing in different soil types, or under different crop rotations, the investigator should be concerned about mixing different soils or using soils from different fields in glasshouse pot screening programs.

Different biotypes of nematodes can be distinguished through the different reaction of individual host varieties to different populations of the nematode. If two varieties of host react differently to two nematode populations of the same species, it is concluded that the two pest populations are different in their pathogenicity and belong to two different biotypes.

The objective of this experiment, therefore, was to investigate the response of ten of the most widely cultivated wheat varieties in Southern Australia to two populations of the root lesion nematode (*P. neglectus*) from different soil types and rotational systems.

7.2 Materials and methods

Varieties included in this experiment are local bread wheats. Machete and Molineux are varieties susceptible to *P. neglectus* that have already been checked in South Australia. Machete and Molineux are good hosts of the nematode. Spear, supporting a high number of nematodes, usually has shown comparatively greener leaves in nematode-infested soils (Chapter 4 and 5). The reaction of Janz to the nematode (in terms of tolerance) has been

found to be similar to Spear (Chapter 5), but supporting a lower number of nematodes. Angas, Aroona, Halberd, Warigal and Oxley are intermediate hosts of *P. neglectus*. Excalibur has shown lower numbers of nematodes in field trials over three years (Vanstone *et al.*, 1993b). Pedigrees of all these varieties are given in Table 3.6 in Chapter 3.

Numbers of nematodes per gram of soil were determined by taking soil samples at random from fields at a depth of 0-15 cm. Samples were taken from the unsown area between plots at Smythe's property at cooke plains, where the field experiment of S. P. Taylor was established, and from the farming areas of the property at Palmer.

The soil from Palmer (Schimer's property) was a solonized brown soil, duplex, reddish in colour and sandy in texture with a calcareous clay loam subsoil. The field was under pasture (naturally regenerating) in 1993 and left as fallow in 1992. The soil from Cooke Plains (Smythe's property) was characterised as solonized solonetz, sandy loam in texture, grey in colour and highly impermeable with a solodic clay subsoil. The field was under lupins in 1991 and cultivated with wheat in 1990.

Six sub-samples each containing 100 g of soil, were taken at random from each soil (Chapter 3) and the numbers averaged. The nematode population from the Palmer site, with an average of 263 nematodes per 200 g of soil, was higher than that from Cooke Plains with an average of 188 nematodes per 200 g of soil.

Individual plastic containers were filled with 650 g of soil and transferred to waterbaths at 22 ± 2°C in an evaporatively cooled glasshouse. Pots were arranged in a completely randomised design.

Seeds were first surface-sterilised and then germinated (Capter 3). When the radicles were 1-2 cm long, one seedling was sown in each pot.

Because of space limitation on the mister for nematode extraction, half of the replicates of each entry were sown one week earlier than the second half. The first three replicates were sown on August 13, 1992 and the second three replicates sown one week later. Pots remained in the waterbath for seven weeks and plants were then harvested, nematodes extracted and counted and root and shoot dry weights recorded (Chapter 3).

Numbers of nematodes were transformed to Ln (number of nematodes + 200) (Proctor and Mark, 1975) and data were analysed using the Super ANOVA program.

7.3 Results

Plants in pots containing grey soil from Cooke Plains showed significantly ($P<0.0001$) higher shoot and root growth than those in Palmer soil, with shoot dry weight slightly more than twice that of plants in Palmer soil (Figure 7.1a). Root dry weight of plants growing in Cooke Plains soil was also significantly higher than that from Palmer (Figure 7.1).

Aroona had the highest shoot dry weight and it was significantly different ($P<0.05$) from other varieties, except for Excalibur and Angas. Molineux, one of the susceptible check varieties, had the lowest shoot dry weight and was followed by Oxley, Spear and Warigal, respectively. Machete, Janz and Halberd were intermediate in terms of shoot dry weight compared to Molineux and Aroona (Figure 7.3a).

In terms of root dry weight the ranking changed somewhat. Spear had the lowest and Oxley the highest root dry weight. The difference between Oxley and other varieties other than Excalibur and Warigal, in terms of root dry weight was statistically significant ($P<0.05$). Oxley (which had the second lowest shoot growth) demonstrated the highest root growth among all varieties tested. Machete, one of the susceptible check varieties, was intermediate for root growth as well as for shoot growth (Figure 7.4b).

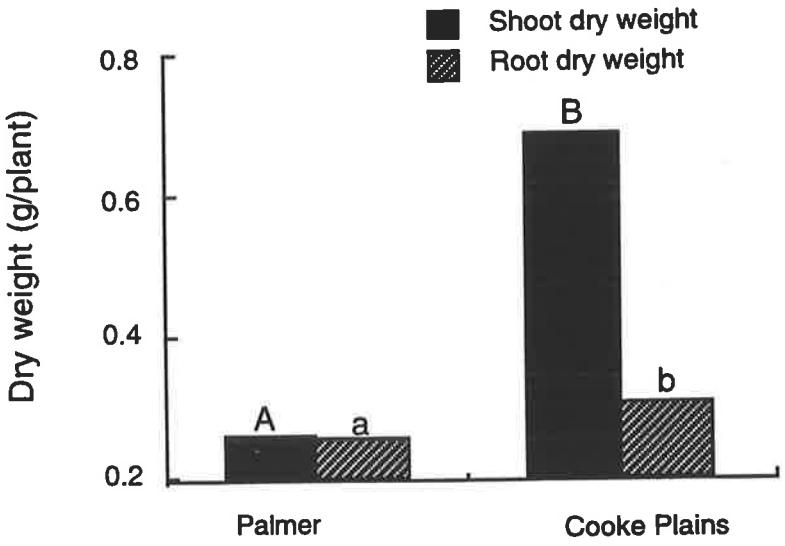
The differences between the two soils in terms of numbers of nematodes per plant and per gram dry root were statistically significant ($P<0.0001$) (Figure 7.1b). Number of nematodes per plant in sandy soil (Palmer) was about eight times that in the grey sandy loam soil (Cooke Plains), and the same trend was observed for the number of nematodes per gram of dry root (Figure 7.1b).

In terms of number of nematodes per plant, the reaction of varieties in the two soils was different. On the Cooke Plains soil, varieties were all similar and there were no significant differences between them. At the Palmer site, Machete had the highest number of nematodes, which was significantly different from all other varieties ($P<0.05$) (Figures 7.4a and 7.4b). Angas and Janz, while similar to Molineux, had a lower number of nematodes per plant than Spear, Halberd and Oxley.

Interaction of soil type and variety was highly significant ($P<0.001$). No correlation was found between the two soils for either number of nematodes per plant nor for number of nematodes per gram dry root (Figures 7.2a and 7.2b). Both numbers of nematodes per plant and per gram of soil in Angas, Janz, Aroona, Excalibur and Molineux varieties were similar, but those of other varieties were significantly higher in Palmer soil. While both number of nematodes per plant and per gram dry root of Machete was similar to Angas and Janz when growing in the Cooke Plains soil, they both were significantly higher in the Palmer soil.

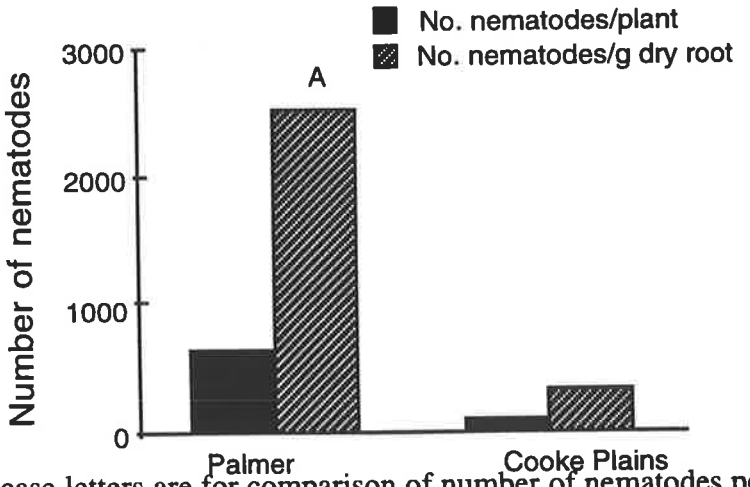
Figure 7.1 Average shoot and root dry weights (**a**) and number of nematodes per plant and per gram dry root (**b**) of ten wheat varieties grown in Cooke Plains or Palmer soil. Cooke Plains soil was clay loam in texture and grey in colour with an average initial population of 188 nematodes per 200 g of soil and Palmer soil was loam in texture and yellow in colour with an average of 263 nematodes per 200 g of soil. Plants were grown in infected soil for seven weeks in a waterbath (22 ± 2°C).

(a)



Upper and lower case letters are for comparison of shoot and root weights, respectively. Data with the same letter are not significantly different (P<0.05).

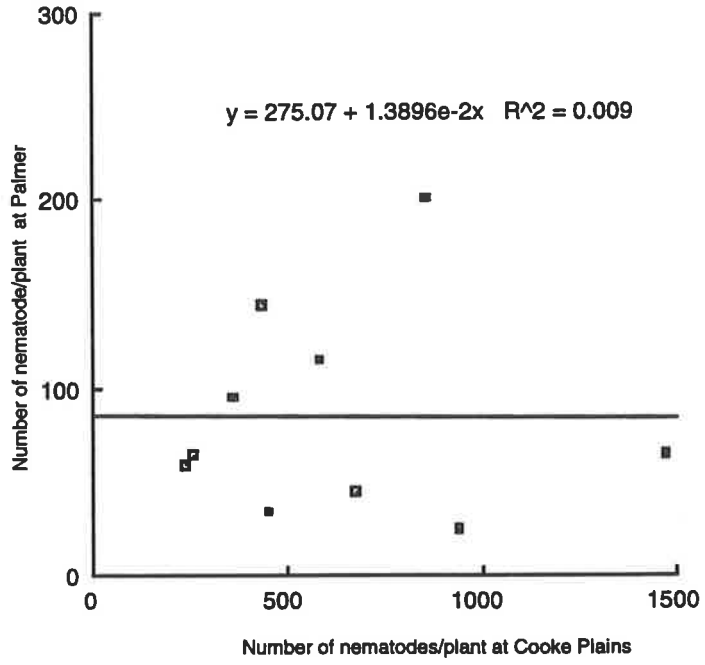
(b)



Upper and lower case letters are for comparison of number of nematodes per gram of root and per plant respectively. Data with the same letter are not significantly different (P<0.05).

Figure 7.2 Correlation between the two soils used in the experiment for number of nematodes per plant (**a**) and per gram of dry root (**b**).

(a)



(b)

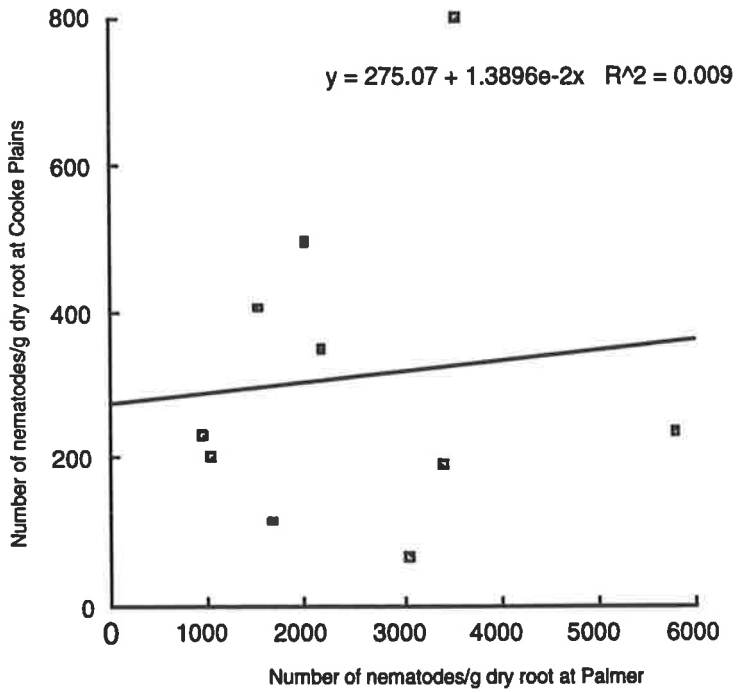
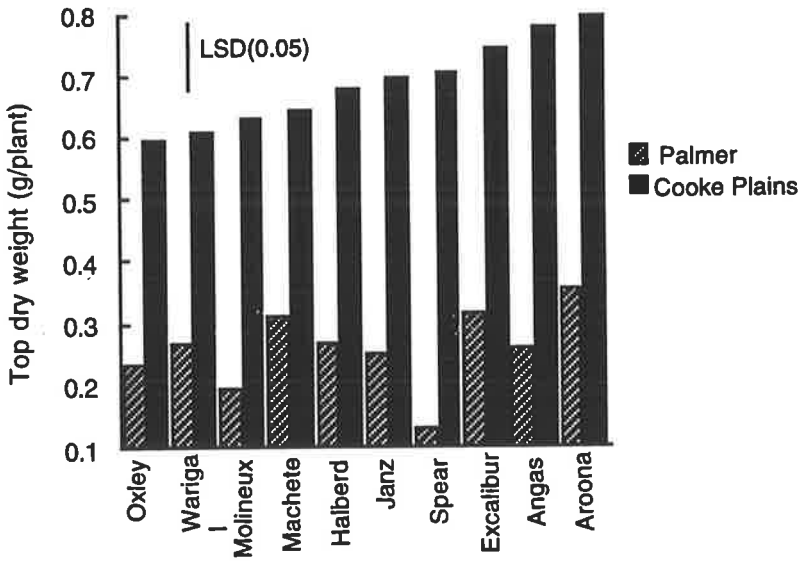


Figure 7.3 Top dry weight (**a**) and root dry weight (**b**) of ten wheat varieties grown in different soil types infected with *P. neglectus* for seven weeks in a waterbath (22 ± 2°C). Cooke Plains soil was clay loam in texture and grey in colour with an initial average nematode population of 188 per 200 g of soil and Palmer soil was loam in texture and yellow in colour with an average of 263 nematodes per 200 g of soil.

(a)



(b)

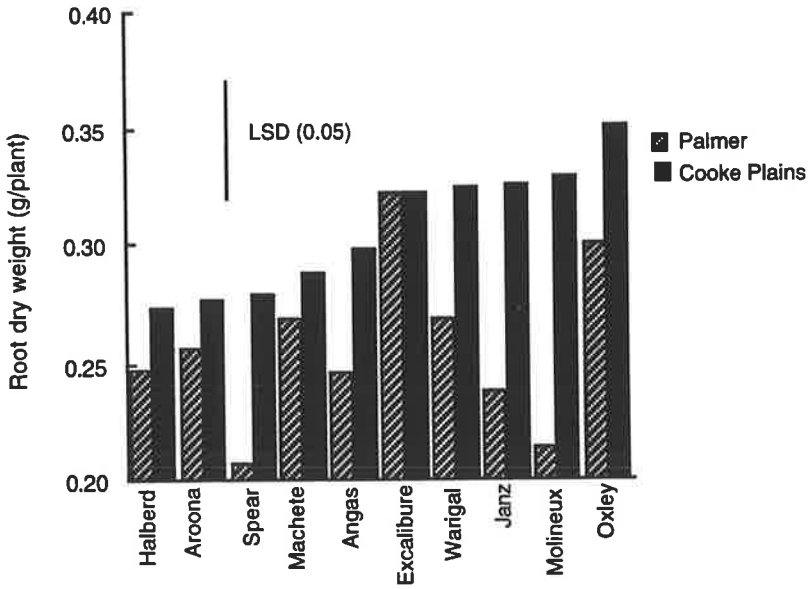
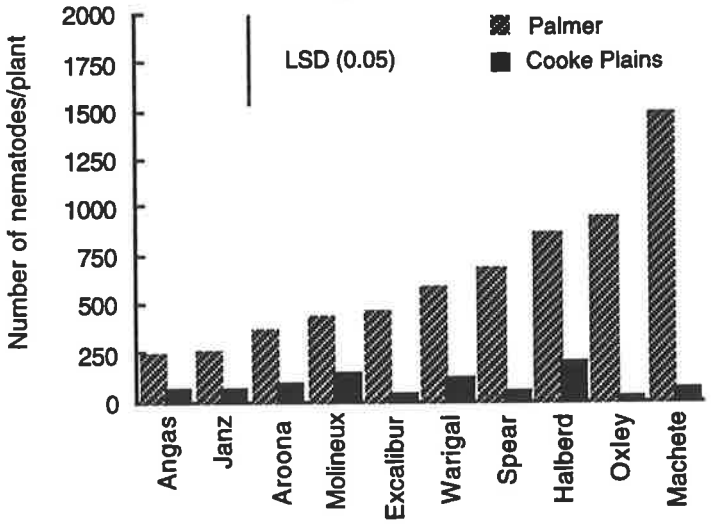
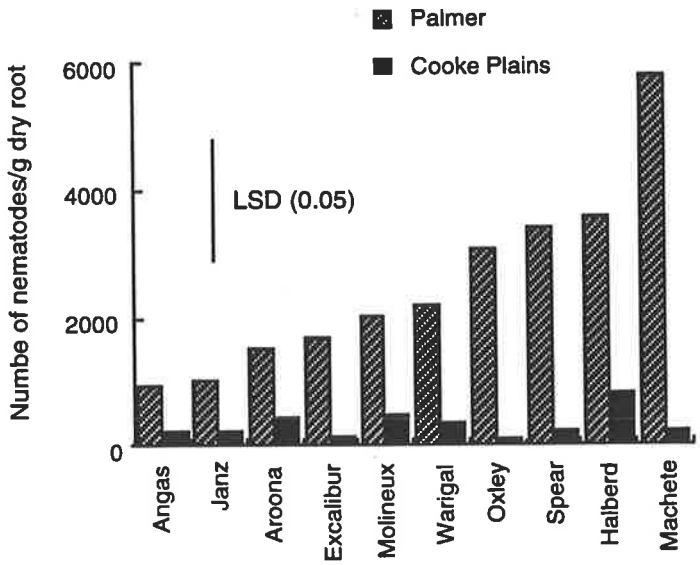


Figure 7.4 Number of nematodes (*P. neglectus)* per plant (**a**) and per gram dry root (**b**) of ten wheat varieties grown in Cooke Plains or Palmer soil. Cooke Plains soil was clay loam in texture and grey in colour with an average initial nematode population of 188 nematodes per 200 g of soil and Palmer soil was loam in texture and yellow in colour with an average of 263 nematodes per 200 g of soil. Plants were grown in infected soil for seven weeks in a waterbath (22 ± 2°C).

(a)



(b)



7.4 Discussion

The significantly higher shoot and comparatively higher root growth obtained in the Cooke Plains soil was probably due to a higher level of soil fertility and lower number of nematodes in the soil, affecting the rate of plant photosynthesis and nematode numbers invading roots.

A higher number of nematodes in plants growing in Palmer soil which had a lighter texture, could be due to a higher initial number of nematodes per gram of soil, as initial population density influence final nematode density. As the results of experiments in Chapter 4 showed, nematodes could cause reduction of shoot and root growth of infected plants. Number of nematodes per gram dry root of some varieties in Palmer soil reached to about 6,000, which is high enough to cause reduction in shoot growth (Griffin and Gray, 1990).

Water deficiency caused by root lesion nematodes to the infected plants is one of the major effects on yield reduction. The nematodes destroy the lateral roots and thereby reduce the rate of water absorption (Thompson, 1987c). Nutrient and water availability are also affected by soil type, influencing both nematode reproduction and host response. Water is more easily evaporated from the lighter soils than the heavy soils and the light soil needs more frequent to be irrigated. Water stress can in turn exacerbate nutrient deficiency and thereby reduce productivity. Vanstone and Nicol (1993) also found a higher number of *P. neglectus* in roots of wheat (Machete and Yallaroi) grown at a low water regime.

Soil physical factors may be of importance in determining migration and penetration of nematodes (Van Gundy and Stolzy, 1963). Cooke Plains soil moisture with a heavier texture could be maintained around field capacity all the time. This may have an adverse effect on nematode reproduction. In contrast , the ligther soil from Palmer could allow water to evaporate more freely and make conditions more favourable for nematode reproduction. In light textured soils nematodes can move more easily and may have

greater access to the roots than in heavy textured soils, so that root penetration is greater in sandy soils (Vanstone and Nicol, 1993).

Number of nematodes per plant as (expected) was the highest for Machete the susceptible check variety, but Molineux (the other susceptible check variety) was intermediate here and significantly different from Janz and Machete, demonstrating the lowest number of nematodes and the highest one respectively. There was no significant difference between Machete, Molineux and Janz for root dry weight, so the difference for number of nematodes per plant could not have been affected by root growth. Janz had lower numbers of nematodes per plant and had greener leaves when growing in nematode infested soil compared to Machete or Molineux. This could be an indication that yellow leaf symptoms are caused by nematode infection. Alternatively, Janz may be relatively resistant to the nematode but also relatively tolerant.

In terms of number of nematodes per gram dry root, again Janz had the lowest and Machete the highest numbers of nematodes per gram dry root. This result also suggests that Janz is comparatively resistant to *P. neglectus* under the conditions of this test. Spear, Molineux and Machete behaved similarly for number of nematodes per gram dry root and all are considered susceptible to the nematode.

As the behaviour of varieties tested here was not the same and interaction of soil and variety for both number of nematodes per plant and per gram of soil was highly significant (Figures 7.5 and 7.6), it is possible that the biotype of the nematode in the soil of Cooke Plains is different from that at Palmer. However, no difference between varieties was found for nematode numbers in Cooke Plains soil and the only observed difference was in Palmer soil. Further investigation is needed to make a precise conclusion. The screening of the ten varieties could be repeated, using a common soil and nematodes from cultures established from soil collections made at different sites. Care should be taken to ensure that initial densities were the same in all treatments.

As explained in the previous experiment (Chapter 6), a single genotype or biotype of the pest should be used as inoculum in screening experiments to detect any resistant varieties. A single genotype population of the nematode is vital as the initial population, otherwise the mixture of different species or different biotypes of the nematode in the soil may result in misleading conclusions.

CHAPTER 8

THE CAUSE AND GENETIC BASIS OF YELLOW LOWER LEAF SYMPTOMS OF WHEAT IN *PRATYLENCHUS* INFESTED SOIL

8.1 Experiment 1

The effect of soil fertility and wheat variety on the extent of yellow lower leaf symptoms

8.1.1 Introduction

Symptoms of *Pratylenchus* infection on wheat usually appear after six weeks as patchy growth with a high frequency of yellow lower leaves. Plant symptoms consist of reduced tillering and chlorosis of lower leaves and some necrosis of leaf tips (Thompson, 1985). Thompson (1985) reported variation in tolerance of wheat varieties to the root lesion nematode *P. thornei*. Depending on extent of the yellowing of the lower leaves and yield reduction in nematode infested soil, he classified varieties ranging from tolerant (GS 28, a selection of Gatcher), to moderately tolerant (Cook) and intolerant (Gatcher).

A. J. Rathjen (pers. comm.) observed that some of the derivatives of Molineux and Spear, when growing in the field in soil naturally infested with *P. neglectus,* contrasted in the extent of yellow leaf symptoms, resulting in lines with distinguishable differences for relatively green or yellow leaves. Whether this genetically controlled propensity to develop symptoms was caused solely by root lesion nematode invasion or whether it reflected a reaction to some other environmental factor was not apparent. Brennan and Thompson (1993) concluded that yellow lower leaf symptoms were controlled by a single gene, although the segregation ratio of the F_2 population fitted neither the ratio of 1: 2: 1 for a single gene nor that for two or more genes. A. J. Rathjen (pers. comm.), on the basis of the performance of closely related lines, suggested that the yellow leaf symptoms were also probably controlled by one or two major genes in South Australian varieties. Apart from differential tolerance to the nematode, reaction of varieties in different soil types may vary depending on soil nutritional status

(Chapters 4 and 5), soil texture (Chapter 7) and other environmental factors such as water availability and temperature.

The objective of this experiment was to investigate the relative effects of *P. neglectus* and soil fertility on the causation of yellow leaf symptoms by comparing varieties varying in their propensity to develop the yellow lower leaf symptoms.

8.1.2 Materials and methods

Genetic material in this experiment included some of those varieties which in the field experiment behaved differently in terms of the yellow leaf symptoms and also susceptible varieties to *P. neglectus* as checks. Pertinent characters of these varieties are presented in Table 8.1 and their pedigrees are shown in Table 3.6.

A factorial experiment was carried out in a completely randomised design with four replicates. The first factor was soil layer with two levels, and the second factor was variety with twelve levels.

Machete and Molineux were included as check varieties as both develop a high level of yellow lower leaves when grown in nematode infested soil. Spear and Janz were included as varieties showing a low frequency of yellow lower leaves in nematode infested soil. Corrigin and Tincurrin (closely related varieties) and RAC 589 were included because they also demonstrated greener leaves than Machete. RAC 613/47 (g) and RAC 613/27 (y) were two backcross derivatives of Spear with the former showing greener and the latter more yellow leaves. Similarly (Mx x Sch # 3)/ A19/8/1(g) and (Mx x Sch # 3)/ A18/13/6 (y) were two closely related derivatives of Schomburgk and Molineux with contrasting performance in nematode infested soil with the former showing greener and the latter a similar level of yellow leaves to the donor parent, Molineux.

Sandy loam soil was collected from Mr. John Krause's farming property at Palmer from two depths in the A horizon, the cultivated layer (0-10 cm depth) and the layer below that (10-20 cm). The upper layer was higher in organic matter and phosphorus than the lower layer. Pots (30 cm diameter) were filled with 5 kg of either soil.

Table 8.1 Genetic materials included in the experiment to test yellow leaf symptoms in cultivated soil layer or the layer below cultivated depth.

Line or Variety	Propensity to develop yellow lower leaves	Putative susceptibility to *P. neglectus*
Machete	Moderately high	High
Janz	Very low	High
Corrigin	Low	Unknown
Tincurrin	Low	Unknown
Spear	Moderately low	Very high
RAC 589	Low	Moderate
RAC 613/27 (y)[a]	High	Unknown
RAC 613/47 (g)[a]	Moderately low	Unknown
(H x Sch # 4)/ 2/5[b]	Very high	High
Molineux	Very high	High
(Mx x Sch # 3)/ A19/8/1 (g)[b]	Moderately low	Unknown
(Mx x Sch # 3)/ A18/13/6 (y)[b]	Very high	Unknown

a: Backcrosses derivatives of Spear

b: Backcrosses derivatives of Schomburgk

Seeds were first surface-sterilised (Chapter 3), placed on moistened filter paper in Petri dishes and transferred to 4°C for 24 hours to imbibe synchronous. Seeds then were transferred to 25°C to germinate.

On September 9, 1992, six germinated seeds of each variety were planted in each pot. Pots were placed on benches in an evaporatively cooled glasshouse. They were watered with distilled water whenever necessary until harvest. After six weeks, about the tillering stage, plants were scored for appearance of yellow leaf symptoms ranging from a score of 1, with no yellow leaves, to a score of 10 with the whole plant yellow. Seven weeks after commencing the experiment, plants were harvested, nematodes extracted and counted and root dry weights recorded (Chapter 3).

The number of nematodes were transformed to Ln (number of nematodes + 200) (Proctor and Marks, 1974) and all data were analysed with Super ANOVA (v1.11) on a Macintosh computer and means were compared by LSDs.

8.1.3 Results

Maturity and extent of vegetative growth of varieties grown in the upper layer were recorded after six weeks (Table 8.2). In terms of yellow leaf symptoms, plants growing in upper layer soil with a mean score of 3.4 were significantly greener ($P<0.05$) than those growing in the lower layer with a mean score of 3.7 (Plate 8.1). Some varieties, including RAC 589, RAC 613/47 (g), Machete, Corrigin and (H x Sch # 4)/2/5, were similar in both soil layers in terms of yellow leaf symptoms, while others, including RAC 613/27 (y), Spear, (Mx x Sch # 3)/A19/8/1 (g), Janz and Tincurrin had a greater proportion of yellow lower leaves when grown in the lower layer of the soil than in the upper layer (Figure 8.1). Surprisingly, Molineux and one of its derivatives, (Mx x Sch # 3)/A18/13/6 (y), when grown in upper layer soil showed a significantly greater proportion of yellow leaves than when grown in the lower layer. In contrast, the other derivative of Molineux and Schomburgk, (Mx x Sch # 3)/A19/8/1 (g), was significantly greener in the upper layer soil compared to its appearance in the lower layer (Figure 8.1).

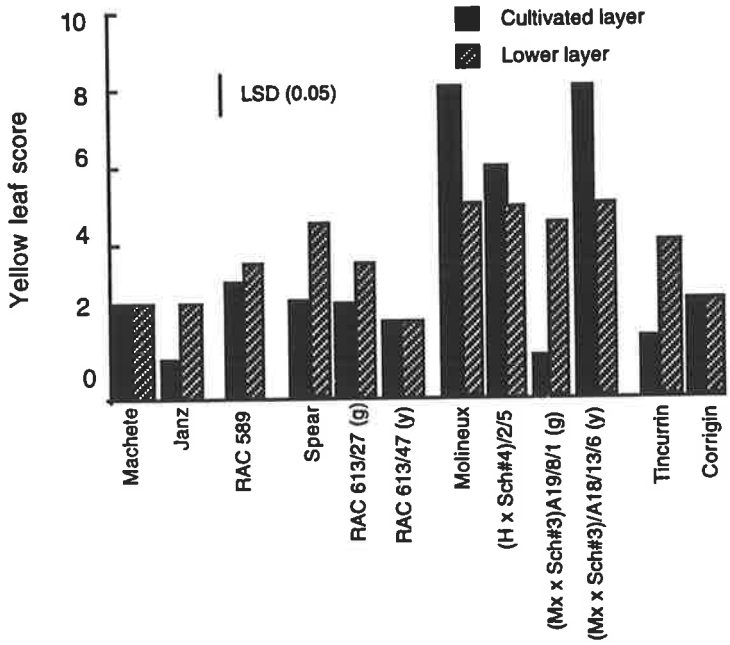
Plate 8.1 Extent of yellow leaf symptom on plants growing in the upper cultivated soil layer (right) or in the layer below (left).

In the cultivated layer, Janz was the greenest variety. Spear and its derivatives, RAC 613/27 (y), RAC 613/47 (g) and RAC 589 were ($P<0.01$) significantly greener than Molineux and its derivative (Mx x Sch # 3)/A18/13/6 (y), but had a higher proportion of yellow leaves than Janz. Machete, Tincurrin and Corrigin were similar to Spear.

In the soil from below the cultivated layer, Janz was again the greenest variety and Machete was greener than Spear. RAC 613/27 (y) was significantly more yellow than its sister line

RAC 613/47 (g) in the lower layer. Molineux and the Schomburgk derivatives were the most yellow in this layer (Figure 8.1).

Figure 8.1 Interactions of varieties and soil profiles for yellow leaf score. Plants were grown in the glasshouse in 5 kg pots in soil naturally infested with *P. neglectus*.



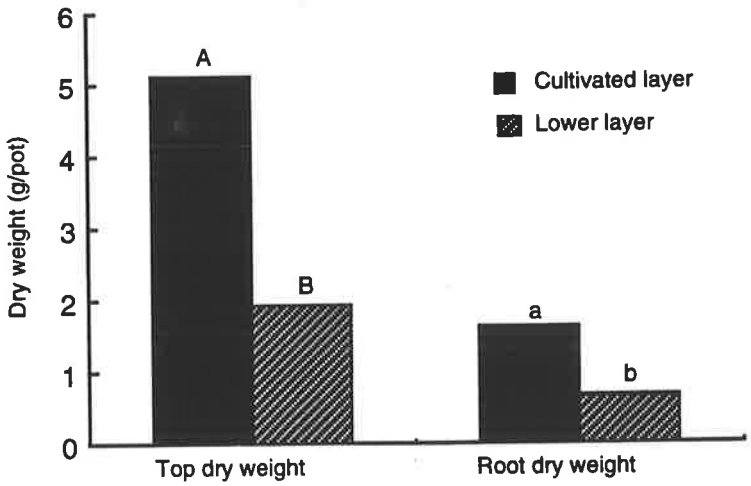
The roots of all varieties were infected by *Gaeumannomyces graminis* var. *tritici* (the take-all fungus) causing a root rot with typical black lesions, but (Mx x Sch # 3)/A18/13/6 (y) was more severely damaged by the fungus compared to the other varieties. No measurement of the degree of rotting was taken.

Both shoot and root dry weights of varieties grown in the upper layer were significantly heavier than those in the lower layer (Figure 8.2). No significant difference was found between genotypes either for shoot or root dry weight (Figure 8.3a and 8.3b). Tincurrin had the highest shoot and root weight. Molineux and RAC 589 had the lowest shoot and root dry weight, respectively.

Table 8.2 Characteristics of plants grown in the cultivated layer of soil naturally infested with *P. neglectus*.

Variety / Line	Tillering and stage of maturity at seven weeks
Machete	Mostly three tillers with the first at anthesis.
Janz	One or two tillers at about the flowering stage.
Corrigin	One strong and one weak tiller at about anthesis.
Tincurrin	Two tillers one strong and one weak. Plants were at anthesis.
Spear	One to two tillers, some at anthesis.
RAC 589	One strong and one weak tiller. All were at anthesis.
RAC 613/47 (g)	Three to four tillers, vegetative.
RAC 613/27 (y)	Two to three tillers nearing anthesis.
Molineux	One to two tillers, vegetative.
(H x Sch #4)/2/5	Mostly three tillers with the first at anthesis.
(Mx x Sch # 3)/A19/8/1 (g)	Three tillers, vegetative.
(Mx x Sch #3)/A18/13/6 (y)	Strong main stem with one or seldom two small tillers. All at heading.

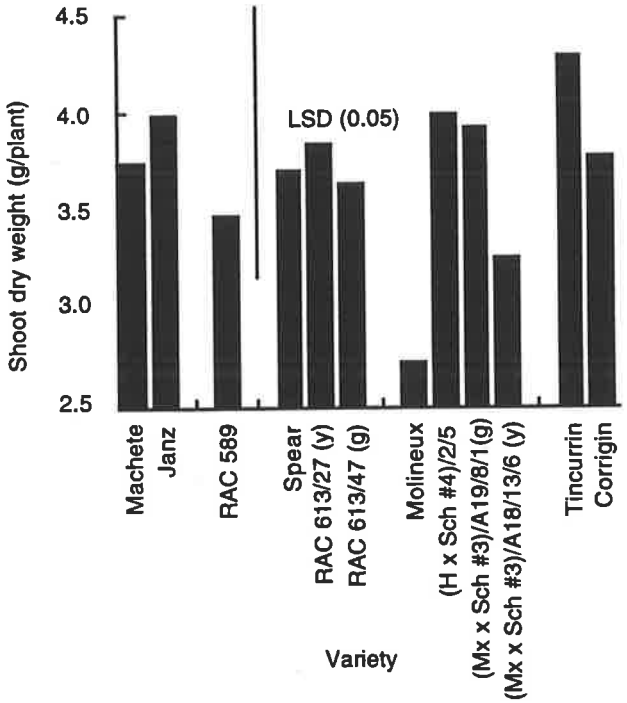
Figure 8.2 Mean shoot and root dry weights of all ten varieties grown in two different soil layers (cultivated or the layer below) at seven weeks after seeding. Plants were grown in the glasshouse in 5 kg pots of soil naturally infested with *P. neglectus*.



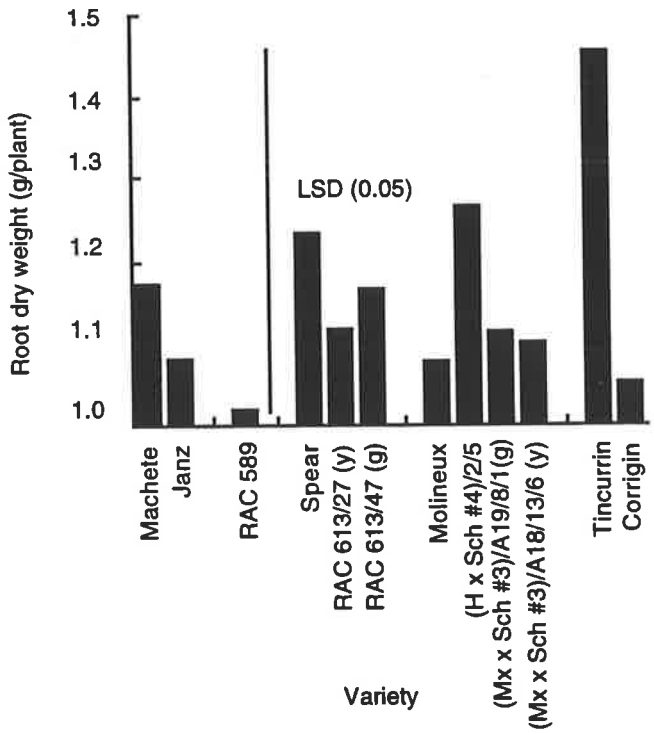
Upper and lower case letters are for comparison of soil layers for plant top and root dry weights, respectively. Values followed by the same letter are not significantly different ($P<0.05$)

Figure 8.3 Means of shoot (**a**) and root (**b**) dry weights of ten varieties grown in two layers of soil. Plants were grown in the glasshouse in 5 kg pots of soil naturally infested with *P. neglectus*.

(**a**) Shoot dry weight



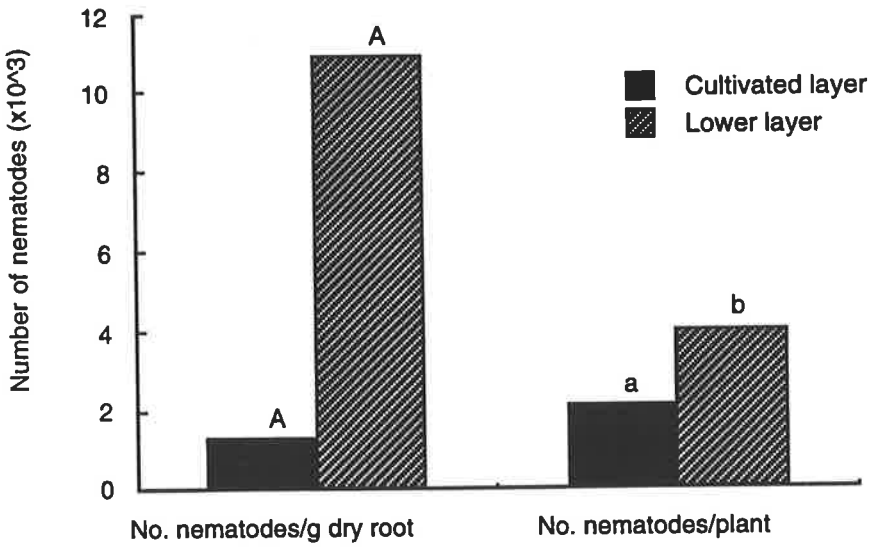
(**b**) Root dry weight



The number of nematodes per plant in the lower layer was significantly higher than that in the cultivated layer (Figure 8.4). The number of nematodes per gram of dry root of plants grown in the lower soil layer was about six times that observed in the cultivated layer (Figure 8.4). The differences between varieties for number of nematodes either per plant or per gram of dry root were not statistically significant (Figures 8.5a and 8.5b). Machete and Corrigin had the highest number of nematodes per plant and RAC 589 had the lowest for both number of nematodes per plant and per gram of dry root.

None of the interactions of variety and soil profile for the characters measured, other than that for yellow leaf symptoms (Figure 8.1), were statistically significant.

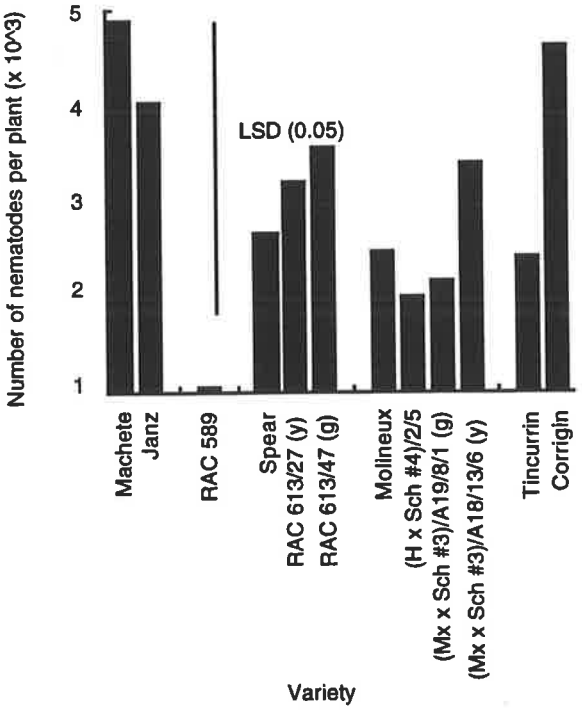
Figure 8.4 Mean number of nematodes per gram dry root and per plant of all ten varieties grown in two different soil layers, cultivated or the layer below, seven weeks after seeding. Plants were grown in 5 kg pots of soil naturally infested with *P. neglectus* in the glasshouse.



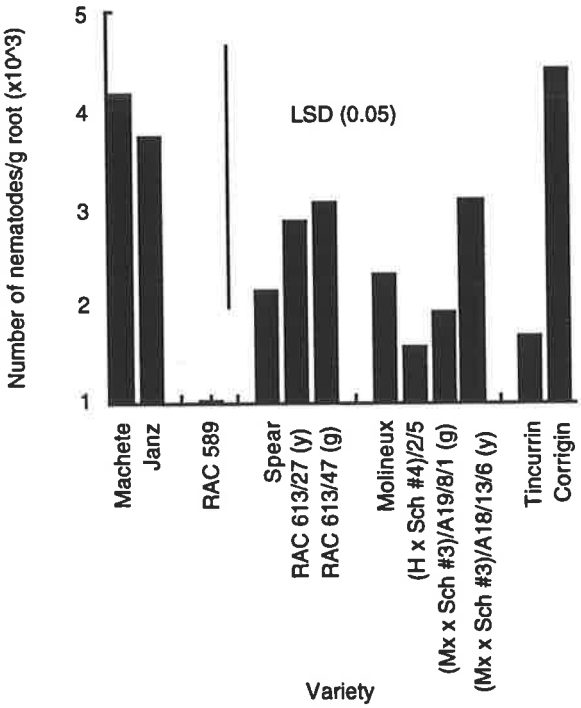
Upper and lower case letters are for comparison of soil layers for number of nematodes per gram of dry root and per plant, respectively. Values followed by the same letter are not significantly different ($P<0.05$).

Figure 8.5 Means of number of nematodes per plant (**a**) and number of nematodes per g root (**b**) of ten varieties grown in two layers of soil. Plants were grown in the glasshouse in 5 kg pots of soil naturally infested with *P. neglectus*.

(**a**) Number of nematodes per plant



(**b**) Number of nematodes per g root



8.1.4 Discussion

Yellow leaf symptoms, which are characteristic of root lesion nematode attack, are caused by a deficiency in some nutrients: perhaps nitrogen (Van Gundy *et al.*, 1974; Thompson, 1987b, Chapter 4), or phosphorus and zinc (Thompson, 1987c). In previous experiments (Chapters 4 and 5), it was demonstrated that the symptom of yellow leaves could be mostly removed by nitrogen application, and when phosphorus accompanied the nitrogen, the symptom was totally removed.

The significant differences in appearance between varieties in terms of the yellow leaf symptoms, particularly in genetically closely related ones, such as sister lines (Mx x Sch #3)/A19/8/1 (g) and (Mx x Sch #3)/A18/13/6 (y) or of RAC 613-27 (y) and RAC 613-47 (g), could be an indication of the existence of a simple genetic basis for the yellow lower leaf symptoms (Table 3.6).

Varieties tolerant to root lesion nematodes grow well in spite of nematode infection. The growth of these varieties compared to intolerant lines is likely to be less affected by nutrient deficiency caused by root lesion nematode. Spear and Janz, as demonstrated in field experiments (Chapter 5, Experiment 2), are less likely than Molineux to develop yellow leaf symptoms in nematode infested soils. These two varieties were more efficient in nitrogen uptake than Molineux and this may enable them to tolerate the nutrient deficiency caused by the nematode. One of the derivatives of Molineux and Schomburgk, (Mx x Sch #3)/A19/8/1(g) was one of the greenest varieties when grow in the cultivated layer, suggesting that (Mx x Sch #3)/A19/8/1 (g) is more efficient in absorbing or translocating the elements responsible for minimising the yellow leaf symptoms. The remaining healthy roots of these varieties can compensate for the reduction in nutrient uptake caused by the nematode.

Some varieties, such as Spear, Janz and (Mx x Sch #3)A19/8/1(g), behaved differently in terms of the extent of yellow leaf symptoms when growing in the lower layer of the soil

compared to the cultivated layer, suggesting that poor nutrient fertility of the horizon below the cultivated layer in essential elements particularly nitrogen and phosphorus, could accounted for their higher yellow lower leaf symptoms. Alternatively, a higher number of nematodes per gram of root in plants growing in the soil below the cultivated layer may have caused more damage to the cortex and reduced root hairs, thereby affecting water and nutrient uptake.

The contrasting behaviour of Molineux and, particularly, one of its derivatives (Mx x Sch #3)/A18/13/6 (y) compared to other varieties (when grown in the cultivated layer) could have been partly the result of infection by *G. graminis* as (Mx x Sch #3)/A18/13/6 (y) seemed more severely infected than other varieties. The fungus enters the root cortex and breaches the endodermis and blocks the xylem, thereby effectively cutting off supplies of carbohydrates to the root tip and supplies of water and minerals to the shoot (Graham and Webb, 1991).

8.2 Experiment 2

Assessing the cause of yellow lower leaf symptoms under aseptic conditions

8.2.1 Introduction

In the previous experiment it was shown that in some varieties the yellow leaf symptoms were greater in plants grown in the cultivated layer. One of the Molineux derivatives, (Mx x Sch #3) A19/8/1 (g), was significantly greener than its parent Molineux and its sister line (Mx x Sch #3)/A18/13/6 (y) when grown in the cultivated layer. Differences were less apparent for the lower layer.

To investigate the cause of yellow leaf symptoms in the absence of other soil pathogens and pests, other than the one under investigation, the soil must be sterilised and followed by introduction of an aseptic single genotype of the nematode. Comparing the infected with the non-infected controls could lead us to an accurate indication of the role of the root lesion nematode in the induction of the symptoms.

The aim of this experiment was to examine the effect of the nematode on the extent of yellow lower leaf symptoms, in the absence of other microorganisms, and to assess the effect of nitrogen and phosphorus fertilisers on mitigation of the symptoms.

8.2.2 Material and methods

Varieties included in this experiment were Molineux and Molineux derivatives, (Mx x Sch # 3)/A19/8/1 (g) and (Mx x Sch #3)/A18/13/6 (y) which behaved differently for yellow leaf symptoms in the previous experiment.

The trial was designed as a factorial experiment; soil with and without nematodes, fertiliser at four levels (control, nitrogen, phosphorus, and N+P), and three different wheat varieties in a completely randomised block design with four replications.

Soil was collected from the cultivated layer from the same location as the previous experiment. The soil was pasteurised by heating to 70°C (Chapter 3). Plastic pots, 20 cm in diameter, were filled with 3 kg of sterilised soil. Pots were placed in an evaporatively cooled glasshouse with the temperature at 22 ± 5°C.

Seeds were surface sterilised (Chapter 3), transferred to 4°C for a period of 24 hours and then transferred to 25°C in an incubator. When the radicles were 1-2 cm long, four seedlings were planted into the 3 kg pots.

Three days after planting, seedlings were inoculated with an aseptic single genotype of *P. neglectus* extracted from carrot cultures. Nematodes were separated from the eggs by passing the suspension of larvae and eggs through a 30µm sieve about 30 times and collecting the remaining nematodes. The volume of the suspension was adjusted to give a density of 3000 nematodes per ml. One millilitre of nematode suspension was inoculated adjacent to each of the four seedlings in the pots, giving 12,000 nematodes per pot.

One week after planting, fertilisers were added to the pots. Fertilisers were calcium nitrate ($Ca(NO_3)_2$) as a source of nitrogen, and Top Fos® as the phosphorus source (Tables 3.2 -3.5). Calcium nitrate was chosen to minimise the adverse effect of N fertiliser on nematodes. Fertiliser was added at the rate of 2.2 g of calcium nitrate (110 kg N/ha) and 0.44 g of Top Fos® (35 kg P/ha).

Plants were grown for seven weeks and watered when required with distilled water. To control aphids a non-systemic pesticide (Pyrethrum®) was sprayed over the leaves weekly after the third week.

About six weeks (45 days) after commencing the experiment, plants were scored for the extent of yellow leaf symptoms (Section 8.1.2) and one week later plants were harvested,

nematodes extracted and shoot and root dry weights recorded (Chapter 3). Data for number of nematodes were first transformed to Ln (number of nematodes + 200) (Proctor and Marks, 1974) to render the variances independent from the means. All means were compared with LSDs.

8.2.3 Results

Plants of Molineux reached anthesis five weeks after the commencement of the experiment. At 45 days after sowing, the lower leaves of Molineux were completely yellow. (Mx x Sch #3)/A19/8/1 (g) was completely green and the other derivative, (Mx x Sch #3)/A18/13/6 (y), was intermediate between its parent and its sister line for the yellow leaf symptoms (Figure 8.6). This line was earlier in maturity than the other two genotypes.

With both Molineux and (Mx x Sch #3)/A18/13/6 (y) the rate of yellow leaf symptom was non significantly higher, but in general the effect of nematode infection on the extent of the symptom development was not obvious in this experiment. Both infected and non-infected plants appeared similar (Plate 8.2). Interaction of nematodes with genotypes was not significant (Figure 8.6). Nematode infection did not have any significant effect either on top fresh or dry weight or on the root dry weight, except for (Mx x Sch #3)/A19/8/1 (g) which, in the presence of nematodes, had a significantly decreased root weight (Figure 8.7b).

Figure 8.6 Yellow lower leaf score of Molineux and its two derivatives. Plants were grown in pasteurised soil and inoculated with 12,000 aseptic *P. neglectus* per pot.

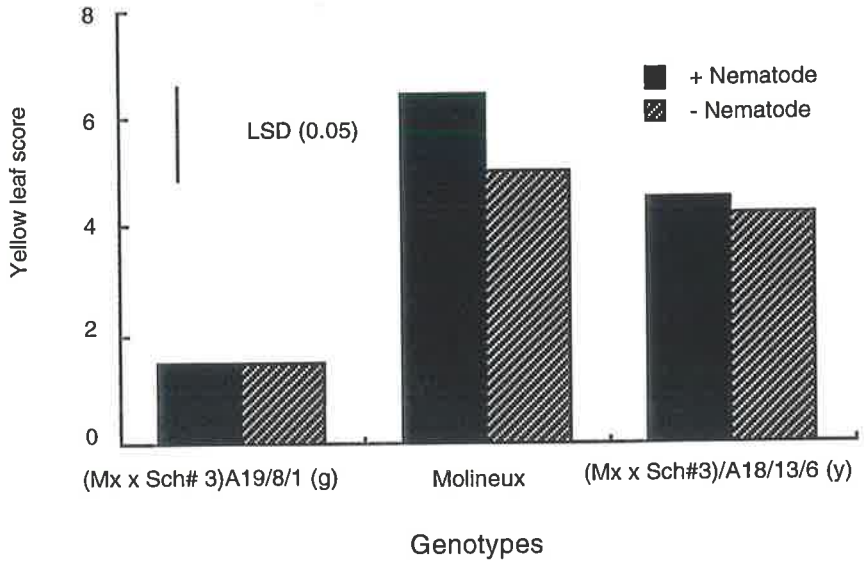
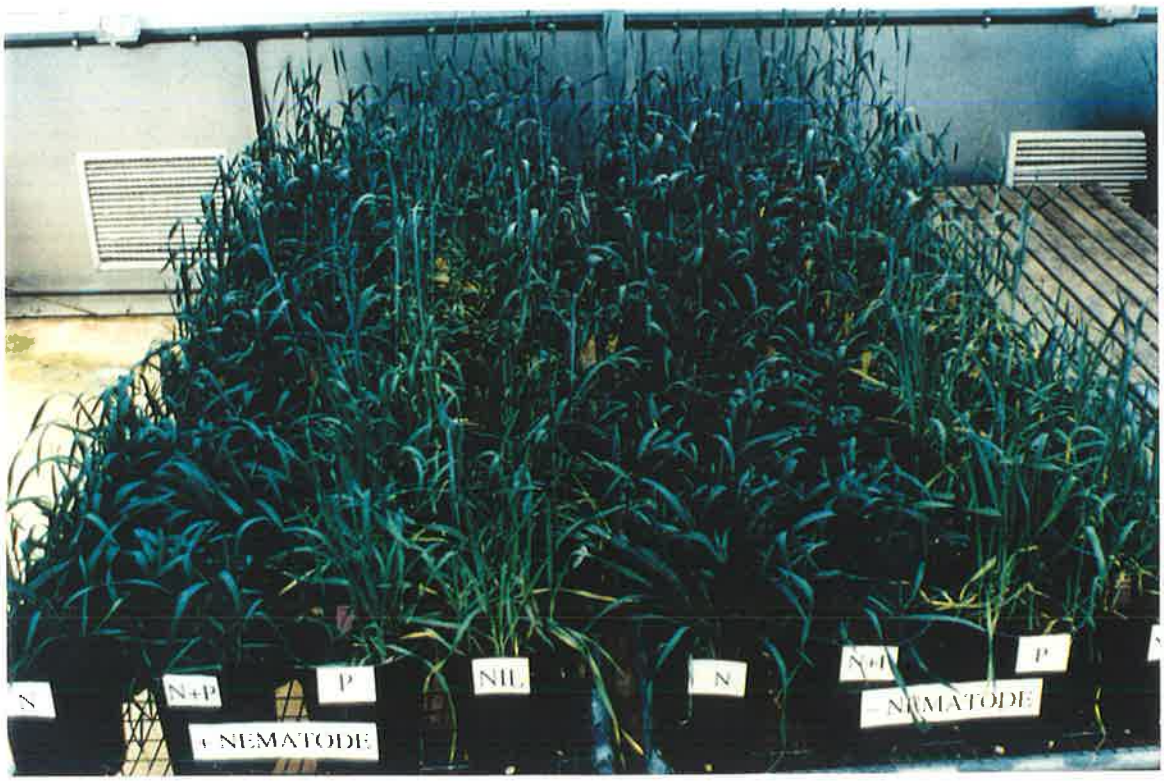


Plate 8.2 Effect of nematodes and fertiliser application on the extent of yellow leaf symptom in wheat variety Molineux. Aseptic nematodes were added to pasteurised soil.



Significant differences were found between varieties for shoot fresh weights (Figure 8.7a). (Mx x Sch #3)/A8/19/8/1 (g), the greenest genotype in the previous experiment, had the highest shoot fresh weight and it was significantly different from Molineux with a high level of yellow leaves (Figure 8.7). The intermediate line for yellow leaf ranking (Mx x Sch #3)/A18/13/6 (y), was the lowest for shoot fresh weight. Shoot fresh weight was negatively correlated to the yellow leaf score (Table 8.3). No significant difference was found between varieties in terms of shoot dry weight, although the ranking was almost similar to shoot fresh weight. The difference between genotypes for root dry weight was greater than that of the shoot dry weight, and the three genotypes were significantly different ($P<0.05$) (Figure 8.7b). (Mx x Sch #3)/A8/19/8/1 (g) with 1.4 gram root dry weight per pot was the highest and line (Mx x Sch #3)/A18/13/6 (y) with 0.5 gram the lowest and Molineux was intermediate.

Figure 8.7 Means of top fresh weight (g/pot) (**a**) and root dry weight (g/pot) (**b**) of Molineux and its two derivatives with or without nematodes. Plants were grown in pasteurised soil with four fertiliser treatments.

(**a**) Top fresh weight

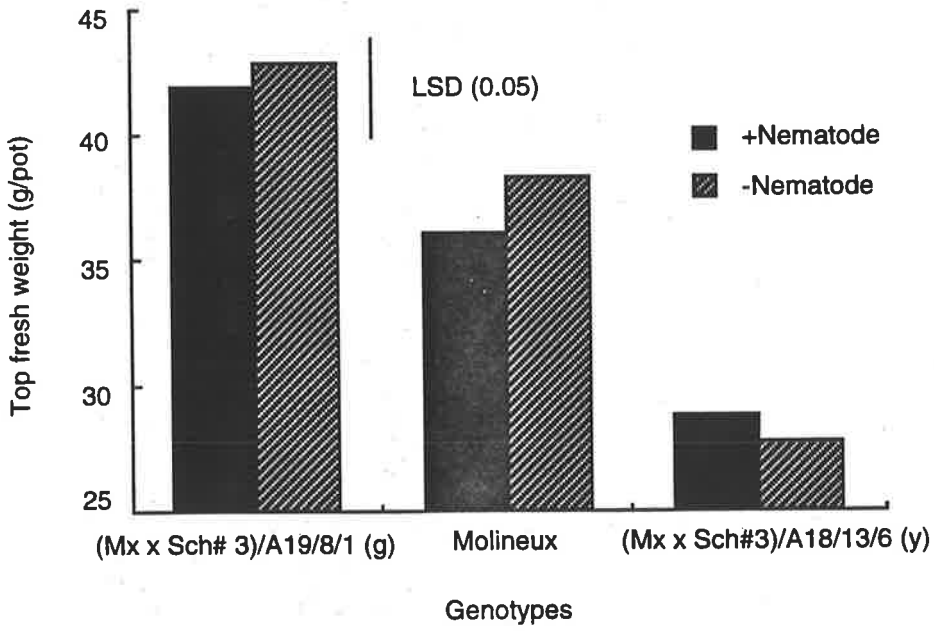
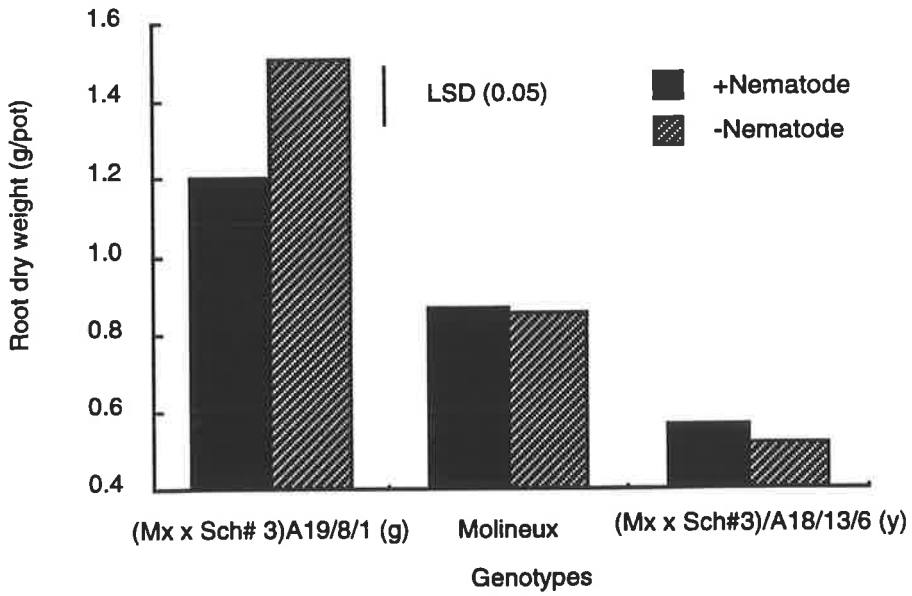


Figure 8.7 continued

(b) Root dry weight



Yellow leaf symptoms were significantly reduced by application of phosphorus compared to the control. Nitrogen almost completely removed the symptom of yellow lower leaves (Table 8.3). Application of phosphorus slightly increased the means of shoot fresh and dry weights compared to the control (no fertiliser). With no added fertiliser, the line (Mx x Sch #3)/A19/8/1 (g) was significantly greener than Molineux and its sister line (Mx x Sch #3)A18/13/6 (y). When plants were supplied with nitrogen or N+P, all three genotypes were the same in terms of yellow lower leaf symptoms (Figure 8.9).

Nitrogen treatment increased the shoot fresh and dry weight significantly ($P<0.001$) (Table 8.3). Nitrogen application to the soil also significantly ($P<0.05$) increased root dry weight, while the differences between the control and both the N+P and phosphorus treatments were not statistically significant (Table 8.3).

In terms of numbers of nematodes per pot, (Mx x Sch #3)/A18/13/6 (y) with 6800 had the highest and (Mx x Sch #3)/A19/8/1 (g) with 3728 the lowest (Figure 8.8). While the differences between both derivatives with Molineux were not significant, they were significantly ($P<0.05$) different from each other. In terms of number of nematodes per gram

dry root, the line (Mx x Sch #3)/A18/13/6 (y) was significantly (P<0.001) higher than Molineux and its sister line (Mx x Sch #3)/A19/8/1 (g) (Figure 8.8).

Table 8.3 Mean effects of fertiliser treatments (control, N, P, N + P) on the growth and nematode reproduction of the three genotypes. Plants were grown in pots in a controlled temperature glasshouse (22 ± 5°C) for seven weeks.

Fertiliser	Measured characters					
	Yellow leaf score	Top fresh weight (g/pot)	Top dry weight (g/pot)	Root dry weight (g/pot)	Number of nematodes per pot	Number of nematodes per g dry root
Nitrogen	1.5	42.6	5.98	1.03	3329	4626
N+P	1.3	44.6	6.08	0.87	5028	6681
Phosphorus	4.0	29.1	5.31	0.94	6399	7980
Control	6.2	27.5	5.10	0.84	4463	8293
LSD (0.05)	**1.3**	**5.2**	**0.37**	**0.11**	**2357**	**3564**

Plants receiving phosphorus or no fertiliser had a higher number of nematodes per gram of dry root than those receiving nitrogen alone or nitrogen plus phosphorus together, with the differences being statistically significant (P<0.05) (Table 8.3). The P and N + P treatments had the highest number of nematodes per pot.

Figure 8.8 Means of number of nematodes per gram dry root and per pot for Molineux and its two derivatives. Plants were grown in pasteurised soil with four fertiliser treatments and with or without nematodes.

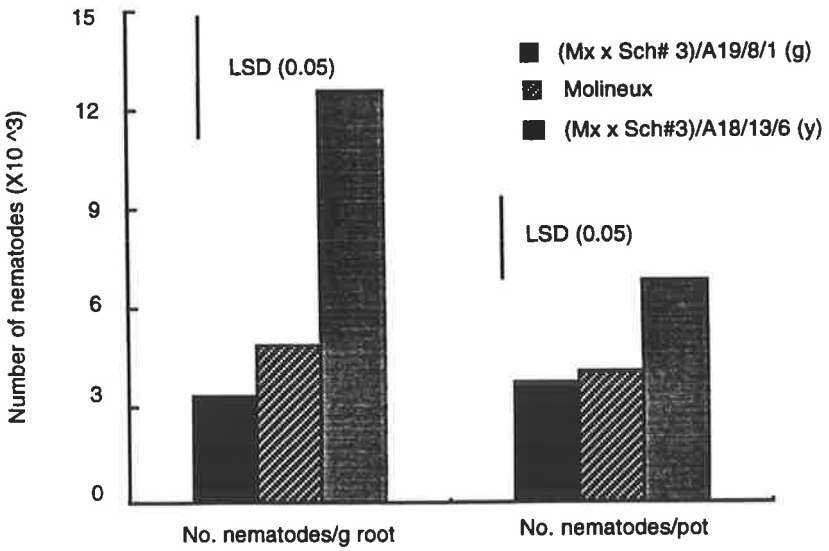
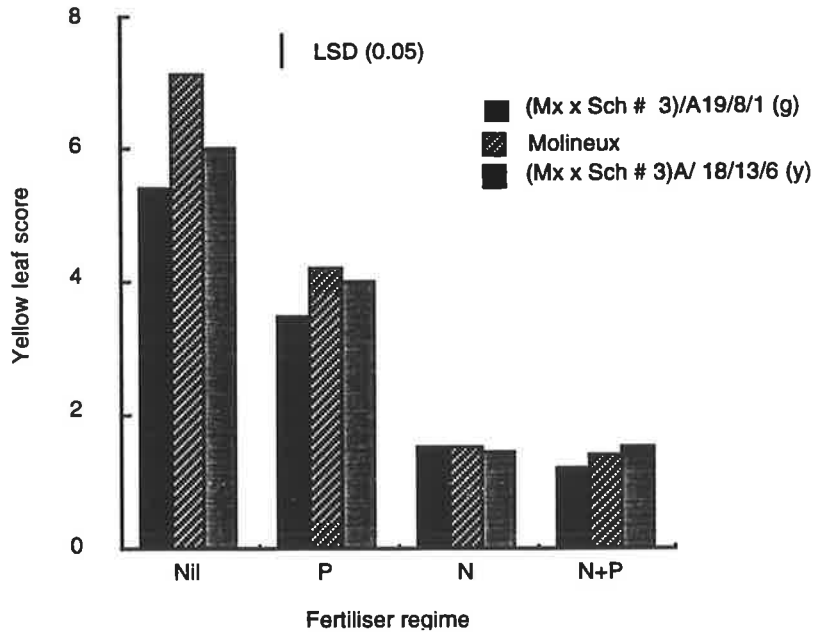
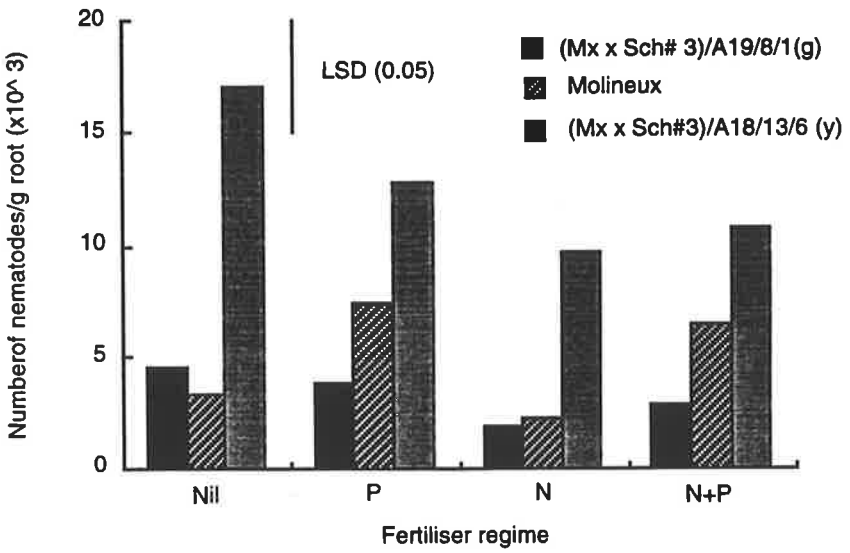


Figure 8.9 Interactions of yellow leaf score (**a**) and number of nematodes per gram dry root (**b**) of Molineux and its two derivatives with four fertiliser treatments, with or without nematodes.

(**a**) Yellow leaf score



(**b**) Number of nematodes per gram of dry root



8.3.4 Discussion

More yellow leaves on (Mx x Sch #3)/A18/13/6 (y) could be due to at least two factors: firstly the low root growth (Figure 8.7b), and secondly the damage by nematodes to the plant as it developed a nematode population twice as large as the other genotypes (Figure

8.8), although the comparatively low number of nematodes in Molineux (Figure 8.8) tend to argue against the second of these. The line with the higher root growth rate, (Mx x Sch #3)/A19/8/1(g), was greener than Molineux and had a significantly greater shoot fresh weight (Figure 8.7b). This line, by more thoroughly exploring the soil volume and having a lower nematode density in its roots, probably had a higher portion of healthy roots compared to the heavily infected plants, and would be likely to be more efficient in taking up nutrients from the soil.

The lack of any significant difference between all the three genotypes when supplied with nitrogen or N+P (Figure 8.9a) again indicated that the yellow lower leaf symptom is mostly caused by lack of nitrogen and, to some extent, phosphorus. Plants receiving nitrogen or nitrogen plus phosphorus were quite healthy with little yellowing of lower leaves symptoms. Application of nitrogen resulted in almost complete disappearance of the yellow leaf symptoms even in line (Mx x Sch #3)/A18/13/6 (y) (Figure 8.9a). Nitrogen application to the root zone appeared to promote root growth, thereby increasing nutrient uptake and compensating for the nutrient deficiency in this case probably a deficiency of P.

Application of phosphorus slightly reduced the extent of yellow leaf symptoms (Figure 8.9a) indicating that plants were suffering from phosphorus deficiency. However, with addition of phosphorus to the soil, (Mx x Sch #3)A19/8/1 (g) was still significantly different from Molineux, indicating that the yellow leaf symptom was not created by P deficiency alone.

The extent of yellow leaf symptoms, although lower in plants grown in pots without nematodes than in those infected with nematodes (Figure 8.6), was not significantly different in the two treatments. However, it is likely that the yellow leaf symptoms partly result from nematode invasion reducing the area of root surface in contact with soil particles. In this experiment, nematodes were applied to plants in positions adjacent to the seedling roots. Plants, therefore, are not infected as evenly as in naturally nematode infested soil where root hairs could be attacked almost immediately. Since the root lesion nematode

impairs the plants' ability to take up water and nutrients from the soil by damaging the root system (Vanstone, 1991) and disrupting the normal structure of plant tissues or changing physiological mechanisms (Krusberg, 1963), affected crops become inefficient in uptake of nutrients like nitrogen and phosphorus, accounting for the chlorotic appearance (Thompson, 1987c). Other investigators such as Mountain (1954) and Thompson (1985), have also suggested that the yellow leaf symptoms are caused by root lesion nematodes (*Pratylenchus* spp.).

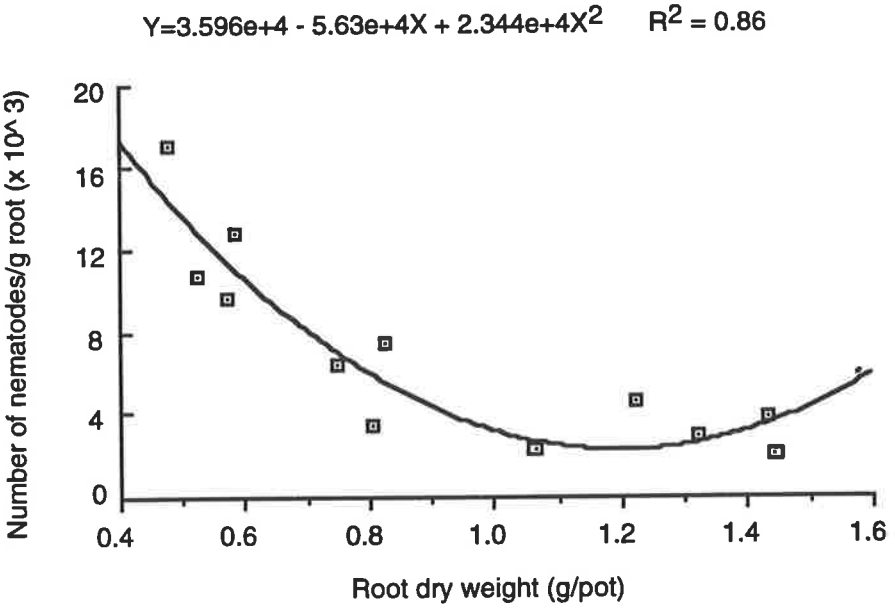
The difference between line (Mx x Sch #3)/A18/13/6 (y), Molineux (the susceptible check variety) and its derivative line (Mx x Sch #3)/A19/8/1 (g) in respect of yellow leaf symptoms could be also explained on the basis of their genetic differences in reaction to the nematode (Figure 8.8), or to a difference in tolerance related to the extent of root growth or a difference in their production of cytokinin.

Nitrogen application significantly ($P<0.05$) reduced number of nematodes both per gram of dry root and per pot (Table 8.3). Lower numbers of nematodes per gram of root in plants receiving nitrogen could be accounted for by significantly higher root growth stimulated by the nitrogen fertiliser. The reason for significantly lower numbers of nematodes per pot in the plants treated with additional nitrogen (Table 8.3) can be explained by the nematicidal effects of nitrogen, again supporting the results of Kimpinski *et al.* (1976) and Vanstone *et al.* (1993c).

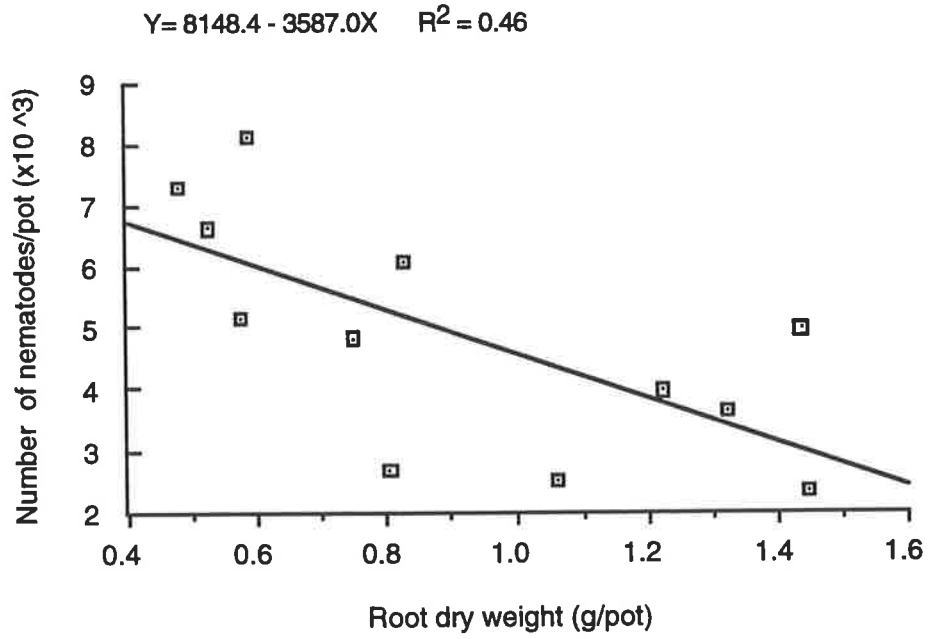
When plotting the number of nematodes per gram of root against the amount of root, a highly significant negative correlation ($R^2 = -0.86$) was found between these two variates (Figure 8.10a). Plants with higher root growth therefore show a lower number of nematodes per gram of root. On the other hand when fitting the number of nematodes per pot against root weight the correlation was not significant (Figure 8.10b). In the absence of resistance, plants with a low root growth would suffer more severely from a high density of nematodes than those with a high root growth.

Figure 8.10 Relationship between root dry weight and (**a**) number of nematodes per g root and (**b**) number of nematodes per plant.

(**a**) Relationship between root dry weight and number of nematodes per g root



(**b**) Relationship between root dry weight and number of nematodes per pot



CHAPTER 9

RESISTANCE TO *PRATYLENCHUS NEGLECTUS* IN DURUM WHEAT (AABB), RYE (RR) AND TRITICALE (AABBRR).

9.1 Introduction

Resistance (reduced multiplication of the nematode imposed by the plant) offers the most economical and environmentally acceptable method of control. Resistance is being used increasingly for the control of plant parasitic nematodes, either through the incorporation of resistance genes or by use of resistant root stocks. In South Australia, cereal cyst nematode (*H. avenae*) has been recognised as a serious problem since 1930 (Davidson and Se, 1930) and resistance was identified in the 1960's (O'Brien and Fisher, 1974). Breeding in southern Australia has resulted in the release and widespread cultivation of resistant varieties of wheat (Brown and Young, 1982; Rathjen *et al.,* 1989), barley (Sparrow 1987) and oats (Barr *et al.,* 1988).

While resistance to many root lesion nematodes including *P. neglectus* (Marull *et al.,* 1990 in almonds; Townshend, 1989 in oats) and *P. thornei* in wheat (Thompson and Clewett, 1989) has been recorded, there have been few attempts to breed for resistance. One of the problems has been the availability of a suitable assay technique, but the recent development of a practical assay, coupled with the aseptic culture of the nematodes on carrots (Nicol and Vanstone, 1993) has made this possible. No commercial wheat varieties resistant to *P. neglectus* are available in Australia and no resistance has been reported in *T. aestivum.*

Plant breeding programs were initiated using local or introduced landraces which have been selected by farmers over a long period for desirable traits. But land races contain a small sample of the total germplasm base of a plant species. In most self pollinated crops including wheat, breeders follow a program of hybridisation and subsequent selection of

uniform lines from segregating populations, with some selection for disease resistance taking place during the early phases of the breeding program. Homozygous genotypes are then obtained and selected for disease resistance, yield, and other features in the final stages of breeding programs. Relatively few varieties are released each year from a breeding program and only a few of these become widely grown by farmers. Therefore, the genetic base of our cultivated crops, particularly self fertilised species such as bread wheat, has often become very narrow. Furthermore, because of high selection pressure imposed to landraces in the absence of some pathogens and the limited genetic base, advanced current varieties often lack genes required by plant breeders (Hooker, 1977).

In many genera, and particularly in those that have experienced polyploidy phenomena such as wheat, having three ancestral species, undomesticated species related to the cultivated species provide an important reservoir of genes for use by breeders. Related wild species, particularly in the *Triticeae*, sometimes demonstrate greater genetic diversity, especially in respect of resistance genes, than their cultivated relatives. Tetraploid *Triticum* spp., for example, have a greater number of genes for resistance to rusts and other diseases than the hexaploid bread wheats (Knott and Dvorak, 1976).

Bread wheat is a hexaploid species having 42 chromosomes. These 42 chromosomes belong to three genomes, A, B and D. It is generally believed that these three genomes have been derived from a single ancestral species having seven pairs of chromosomes, so that the genetic content of these three genomes is similar (Hart, 1987). A number of studies have shown that the chromosomes within these three genomes in wheat not only are related to each other, but also are similar to particular chromosomes in other species within the *Triticinae* (eg. *Secale cereale*, *Aegilops comosa*, *Ae. umbellulata* and *Agropyron* spp. (Sears, 1958; Athwal and Kimber, 1972; Shepherd, 1973).

Wheat breeders now use genera related to wheat (eg. *Secale*, *Agropyron*, *Aegilops*) as the source for transferring desirable genes from these wild relatives into wheat (Knott, 1987; Gale and Miller, 1987). Wheat-rye translocation lines carrying rye arm 1RS have been

used extensively in wheat breeding programs around the world, and now many widely-grown and high-yielding wheat cultivars carry one or other of these translocations. Lines carrying the 1RS(-1BL) translocations have been found to have several agronomically desirable characters including increased disease resistance, broad adaptation, tolerance to stress and potentially high yield (Rajaram *et al.*, 1984).

There are, however, several reports that a wide range of problems arise in interspecific hybridization and interspecific transfer of genes. Progress is often slow and difficult. For instance, many of the cultivars carrying a 1RS translocation produce grain with serious quality defects (Pena *et al.*, 1990). The alien chromosome segment therefore should be as small as possible, otherwise the introduction of the segment may result in undesirable duplication and linkage of genes. Techniques have been developed for the removal of deleterious genes linked to a desired gene through homology of donor and recipient genomes. Nevertheless, outstanding examples of successful exploitation of alien genetic material, especially for stem rust resistance derived from species related to bread wheat, have been achieved (Roelfs, 1988). McIntosh (1991) has published a comprehensive review of the alien genetic material transferred into wheat from different species of tetraploid wheat and related diploids including *Agropyron* sp. and *S. cereale*.

Hence the search here for sources of resistance to *P. neglectus* also included species related to *T. aestivum* in anticipation that it would be possible to introgress the resistance genes from these related species into wheat. In particular, rye (*S. cereale*) has been a source of many valuable characters for wheat, including resistance to rusts on chromosome 1R (Zeller, 1973). Therefore rye, triticale, durum and some of the wheat-rye substitution and addition lines were included in experiments reported in this and following chapters.

In the current wheat breeding program carried out at the Waite Institute, screening lines and cultivated bread wheat (*T. aestivum*) varieties for resistance to root lesion nematode (*P. neglectus*) has been of a high priority. At the time this experiment was undertaken, no

such resistant line or variety had been found in the material tested for resistance to the nematode.

In preliminary experiments carried out by V. A. Vanstone and P. F. Lonergan and A. J. Rathjen (unpublished data), some rye varieties and durum (*T. turgidum*) had a lower number of nematodes in their roots. To further the investigation of these alien materials and to confirm the results of the preliminary tests, various rye, durum and triticale varieties, along with two cultivated susceptible check varieties, were included in this experiment.

9.2 Materials and methods

The genetic materials included in this experiment are listed in Table 9.1 and their pedigrees given in Table 3.6. All the rye and durum varieties included in this experiment had previously shown a lower number of nematodes than susceptible check varieties. The check varieties, Molineux and Machete, are bread wheats widely cultivated in South Australia.

To determine the initial number of nematodes in the soil, five sub samples each containing 100 g were randomly collected from the field samples from Palmer, with a small spatula. Nematodes were extracted for five days at room temperature by the Whitehead Tray method (Chapter 3). The average number of nematodes was 0.35 per gram of soil or 235 nematodes per pot. Since the number of nematodes was relatively low, presumably because of high temperatures and lack of fresh root material in the field, another 2400 nematodes obtained from carrot culture were added to each pot five days after commencing the experiment, so the total number of nematodes was increased to about four nematodes per gram of soil or about 2600 nematodes per pot.

Small plastic pots were filled with 650 g of nematode infested soil from the Palmer site (Chapter 3) where *P. neglectus* is a major pest and the soil texture, as demonstrated in

previous experiments, is favourable for nematode invasion and reproduction. On 11 December, 1993, seeds were surface sterilised and germinated (Chapter 3). When radicles were 3-5 cm long, two seeds were sown in each pot. Pots were incubated in a temperature controlled waterbath for seven weeks, arranged in a completely randomised design with six replications. Nematodes were extracted and the weight of roots measured (Chapter 3).

Data were transformed to Ln (number of nematodes + 200) (Proctor and Marks, 1974) and subjected to analysis of variance. Means were compared using LSDs.

Table 9.1 Genetic materials used in this experiment to measure the multiplication rate of the nematode (*P. neglectus*) in roots.

Genotypes	Reason for inclusion	Genomic constitution
Machete	Susceptible wheat check variety	(ABD)
Molineux	Susceptible wheat check variety	(ABD)
Guillemot	Durum	(AB)
Souri	Durum	(AB)
Yallaroi	Durum	(AB)
Wollaroi	Durum	(AB)
Imperial	Rye	(R)
King II	Rye	(R)
Local rye	Rye	(R)
Abacus	Triticale	(ABR)
Currency	Triticale	(ABR)
Muir	Triticale	(ABR)
Tahara	Triticale	(ABR)

9.3 Results

In terms of number of nematodes per plant, triticale and durum varieties performed similarly and both were significantly ($P<0.05$) different from the wheat and rye groups (Table 9.2). In terms of number of nematodes per plant, triticales overall demonstrated the lowest number of nematodes per plant and were followed by the durums. Susceptible wheat checks showed the highest number of nematodes.

In terms of number of nematodes per gram dry root the triticales, with the lowest number, were also significantly different from the other three groups (Table 9.2). Among the other three groups, rye had the highest number of nematodes per gram and durum the lowest. The difference between these three groups was not statistically significant.

Root dry weight for triticales, in contrast to number of nematodes per gram of dry root, was the highest among these four groups and significantly ($P<0.05$) different from all other groups (Table 9.2). The difference between bread wheats, durums and ryes for root dry weight was not statistically significant.

The two susceptible wheat check varieties, Molineux and Machete, were not significantly different from each other both in terms of number of nematodes per plant or per gram dry root (Figures 9.1a and 9.1b). Root dry weight of Machete was significantly lower than that of Molineux (Figure 9.1c).

All durum varieties were similar in terms of number of nematodes per plant, but Wollaroi had a significantly higher number of nematodes per gram dry root than the other durums (Figure 9.1b). Wollaroi had the lowest root dry weight and its difference from the other three durums was significant (Figure 9.1c).

Among the triticales, Tahara (with the lowest number of nematodes per plant) was only significantly different from Currency (with the highest number of nematodes per plant).

Interestingly, the order of triticales for number of nematodes per gram dry root was similar to their order for number of nematodes per plant and the difference between Tahara and Currency was again statistically significant. Currency demonstrated the lowest root dry weight and its difference with Muir was highly significant ($P<0.01$), as was the difference between the other two triticales, Tahara and Abacus ($P<0.05$).

No significant difference was found between the ryes in terms of numbers of nematodes per plant, while the difference between South Australian rye and King II and Imperial in terms of numbers of nematode per gram dry root was statistically significant ($P<0.01$). The root dry weight of South Australian rye was significantly lower than King II and Imperial.

Overall, the triticale Tahara had the lowest number of nematodes both per plant and per gram dry root and its difference from both wheat varieties, Molineux and Machete, in terms of number of nematodes per plant, was statistically significant. In terms of number of nematodes per gram dry root, only Tahara was significantly different from Machete (Figure 9.1b).

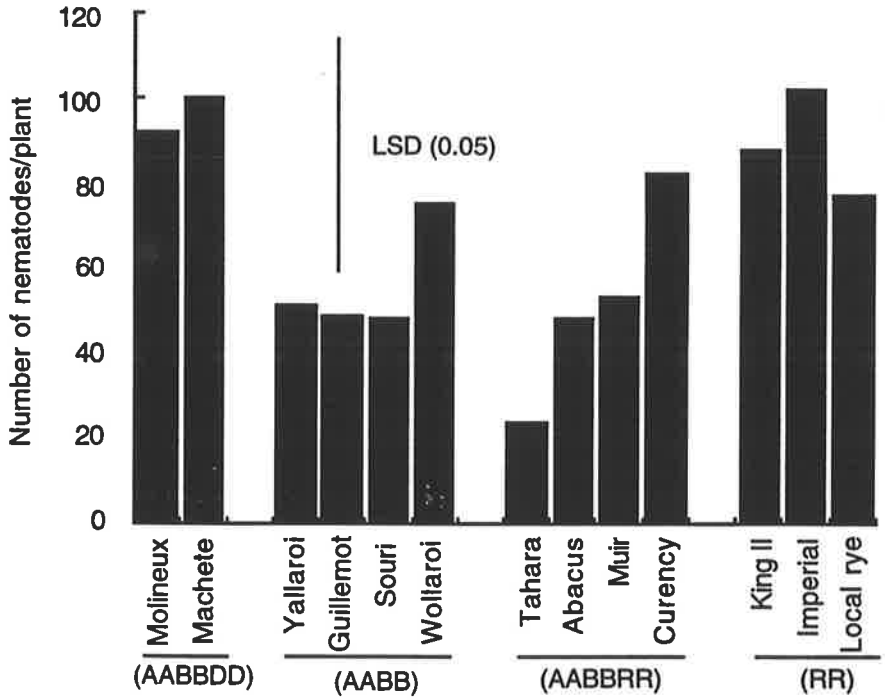
Table 9.2 Number of nematodes per plant and per gram dry root and root dry weight of four groups of genotypes tested for their reaction to nematode reproduction in their roots. Plants were grown for seven weeks in pots each containing about 2600 of *P. neglectus*.

Species	**Nematodes**		**Root dry weight**
	/plant	**/gram of dry root**	**(g/pl)**
Wheat	95.8	527.3	0.178
Durum	55.5	469.7	0.148
Triticale	51.7	242.4	0.231
Rye	88.9	610.2	0.174
LSD (0.05)	**26.6**	**227.1**	**0.042**

Figure 9.1 Number of nematodes per plant (**a**) and per g dry root (**b**) and root dry weight (**c**) of thirteen genotypes from different species, tested for their reaction to nematode reproduction. Plants were grown for seven weeks in pots containing 2600 *P. neglectus* .

Figure 9.1

(a) Number of nematodes per plant



(b) Number of nematodes per gram dry root

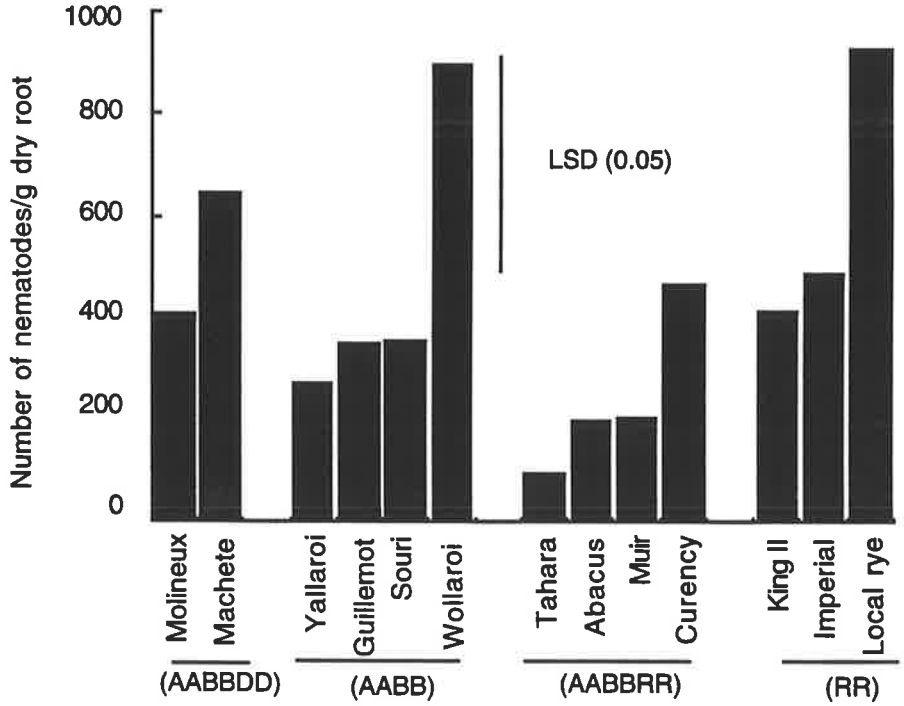
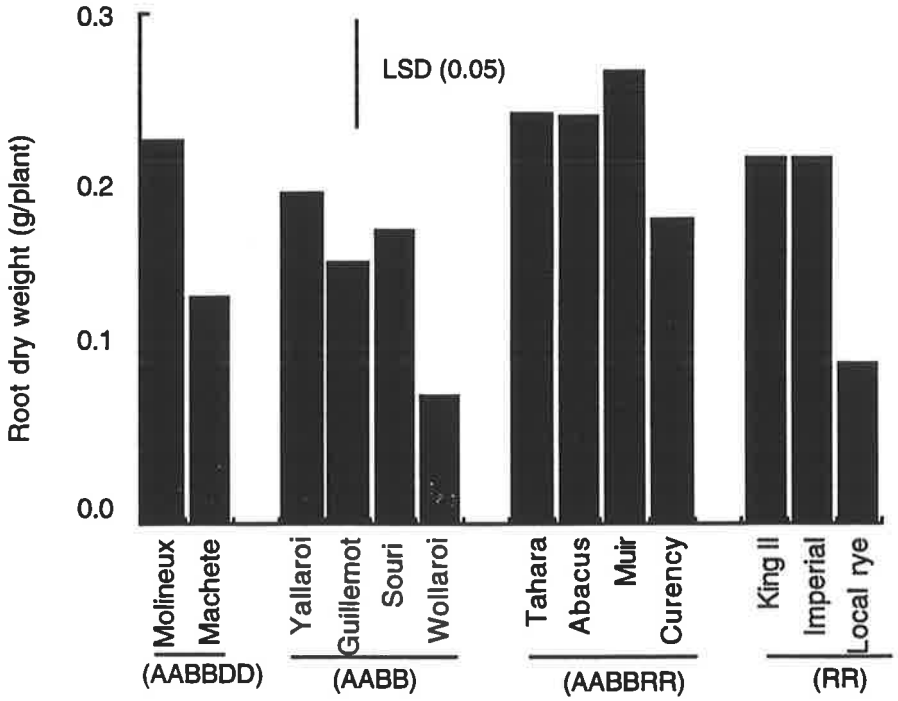


Figure 9.1 Continued

(c) Root dry weight (g/plant)



9.4 Discussion

For screening to detect resistant varieties, the number of nematodes per gram of root dry weight (Dennis *et al.* 1989; Marull *et al.*, 1990) and occasionally, both number of nematodes per plant and per gram dry root (Townshend, 1989), have been used. The number of nematodes per gram of root depends on variation for two characters: nematode reproductive rate per gram of root and root growth rate, either of which could influence the results. Faster growing roots will always appear more resistant when judged on the number of nematodes per gram, even though this character may have no influence on multiplication of the nematode and hence on the number of nematodes present in the soil to invade a subsequent crop. So, screening for resistance on the basis of the number of nematodes per gram of root could result merely in selecting plants with a higher root growth rate. As the economic damage to a subsequent crop is predominantly related to the size of the nematode population invading the seedlings, the number of nematodes per plant

is a more appropriate estimate of resistance for an agricultural crop than the number per gram of root.

Nematode multiplication rate is a density dependent phenomenon affected by both root growth and the initial numbers of nematodes invading the plant (Dennis *et al.*, 1989). The latter has an important implication for the conduct of screening experiments. In assessing varietal resistance, it is important that the number of nematodes in the inoculum be at a level such that even the most intolerant varieties with the least root growth are not substantially damaged before the nematode reproductive rate can be measured. A large number of nematodes introduced to plants growing in small pots, conditions favourable to maximise nematode invasion, could result in intolerant varieties reaching threshold damage before harvest, thus reducing the apparent reproductive rate (J. M. Fisher, pers. comm.; Krusberg, 1963). Under the conditions of this experiment, nematode reproduction was not affected by root growth as the number of nematodes per plant in Wollaroi and South Australian rye, with the lowest root dry weights, was the same as those with a high root growth such as Yallaroi and King II.

The significantly higher number of nematodes per gram dry root of Wollaroi compared to the other durums was merely due to significantly lower root dry weight and its equal number of nematodes per plant. The same conclusion could be applied to South Australian rye.

The lower number of nematodes in durums and triticales was the result of their being less favourable for nematode reproduction as the root dry weights of both species, particularly the triticales, was higher than the susceptible check varieties. These result confirmed the results of the previous work carried out by V. A. Vanstone, P. F. Lonergan and A. J. Rathjen (unpublished data). Durum wheats (AABB) and triticales have two genomes (A and B) in common and both lack the D genome. This suggests that some of the genes responsible for susceptibility to the root lesion nematode are located on the D genome (A. J. Rathjen, pers. comm.). The lower number of nematodes per plant of Tahara could be

also due to the presence of the rye genome (R). The rye genome has many valuable characters for wheat, including resistance to rusts on chromosome 1R (Zeler, 1973) and resistance to *Puccinia graminis tritici* (Sr27) (Luig and Watson, 1976).

In South Australia, a gene found in triticale T 701-4-6 (Asiedu *et al.*, 1990), has been found to confer a higher level of resistance to cereal cyst nematode than that of the gene found in the wheat AUS 10894, originating from Afghanistan (O'Brien and Fisher, 1974). There have been attempts to transfer the gene from triticale T 701-4-6 into commercial wheat varieties in South Australia, but the linkage of undesirable genes with the resistance gene has made this difficult to achieve (Dundas *et al.*, 1987).

As the soil used in this experiment was naturally infested with nematodes, there was a possibility that interacting root rotting fungi and bacteria and different biotypes of the same nematode species could have been present. To confirm these results, further experiments were needed with these genotypes using sterilised soil and a pure aseptic population of *P. neglectus*.

CHAPTER 10

RESISTANCE TO *PRATYLENCHUS NEGLECTUS* IN WHEAT-RYE SUBSTITUTION LINES AND WHEAT VARIETIES RESISTANT OR TOLERANT TO *PRATYLENCHUS THORNEI*

10.1 Introduction

In the previous experiment (Chapter 9), it was found that triticales, particularly Tahara did not support such rapid nematode multiplication as did susceptible wheat varieties when inoculated with a mixture of nematodes in naturally infested soil and aseptic nematodes from carrot cultures. The soil used in the previous experiment was not sterilised and in addition to the nematodes contained other microorganisms including root invading fungi and bacteria. When searching for resistance, as explained in Section 3.7, using sterilised soil and inoculating the plants with a pure population of aseptic nematodes is of importance, as the reaction of any given plant genotype to the nematode may differ in the presence of other microorganisms.

The aims of conducting this experiment were:

(1) To examine the effect of the absence of (1A), (1B) or (1D) chromosomes on nematode reproduction by examining 1R substitution lines. From the results of the previous experiment, it was concluded that the presence of the D genome may facilitate nematode reproduction in plant roots as most durum varieties with the AB genome and most triticales with the ABR genome demonstrated a significantly lower number of nematodes in their roots compared to the susceptible wheat varieties.

(2) To test the resistance reaction of some wheat varieties varying in their expression of the yellow lower leaf symptom (putatively tolerant to the nematode) (Chapter 8).

(3) To investigate the reaction to *P. neglectus* of some wheat varieties demonstrated by Queensland workers to be resistant or tolerant to *P. thornei*.

(4) To confirm the nematode reproductive rate in the triticales tested in the previous experiment, as these are cultivated in South Australia as rotational crops and could be useful sources of resistance genes for bread wheat.

10.2 Materials and methods

The genetic material examined included two susceptible local commercial wheat varieties, Spear and Molineux, and two derivatives of Spear, RAC 613-27 and RAC 613-47, the former often demonstrating yellow lower leaves under field conditions (the symptom of intolerance to *Pratylenchus* spp. recognised by Queensland workers) (Table 10.3). Wheat varieties varying in resistance and tolerance to *P. thornei*, rye and rye derivatives, triticales and 1R substitution lines in Chinese Spring, were also screened (Table 10.3).

A reddish grey, sandy loam soil from a cereal farming property at Palmer (65 km east of Adelaide) was steam pasteurised for 30 minutes at 70°C. Sterilised plastic pots without drainage holes were filled with 650 g of soil.

Twenty seeds from each entry were soaked in 2.5% sodium hypochlorite (NaOCl) for 20 minutes and washed three times in sterile distilled water. Petri dishes and filter papers were sterilised in boiling water for five minutes. The seeds were germinated on moistened filter paper at 25°C for 48 hours and transplanted, with one germinated seed in each pot. After five days, each plant was inoculated with approximately 250 larvae (mixed stages) and 150 eggs of aseptic *P. neglectus* prepared from carrot cultures (Nicol and Vanstone, 1993). The pots were transferred to a waterbath at 22 ± 1°C in an evaporatively cooled glasshouse and watered with distilled water whenever necessary.

Table 10.3 Lines included in the screening experiment and reasons for their inclusion. Pedigrees have been presented in Table 3.6.

Name	Reason for inclusion
Molineux	Susceptible wheat check variety
Spear	Susceptible wheat check variety
RAC 613-27	More yellow leaves in *P. neglectus* infested soil
RAC 613-47	Greener leaves in *P. neglectus* infested soil
RAC 589	Lower number of *P. neglectus* in previous experiments
SUN 277B	Tolerant to *P. thornei* (QWRI)[a]
SUN 289E	Tolerant to *P. thornei* (QWRI)[a]
SUN 146F	Tolerant to *P. thornei* (QWRI)[a]
GS 50A	Resistant to *P. thornei* (QWRI)[a]
King II	Rye genome
Tahara	Triticale, AB and R genomes
Abacus	Triticale, AB and R genomes
Currency	Triticale, AB and R genomes
Muir	Triticale, AB and R genomes
1R (-1A)	1A replaced by 1R in *T. aestivum*
1R (-1B)	1B replaced by 1R in *T. aestivum*
1R (-1D)	1D replaced by 1R in *T. aestivum*
6R (-6D)	6D replaced by 6R in *T. aestivum*

(a) - Identified by Dr. J. P. Thompson and co-workers, Queensland Wheat Research Institute.

After seven weeks, soil was washed from the roots. Roots were chopped into 1 cm lengths and misted for five days to extract nematodes (Chapter 3). The nematodes were counted and the roots were dried and weighed. The number of nematodes per gram of dry root and per plant was calculated.

The experiment was a completely randomised design with eight replications. Where necessary, the data were transformed prior to analyses of variance to Ln (number of nematodes + 200) (Proctor and Marks, 1974) to render variances independent of the means. Means were compared with LSDs.

10.3 Results

In this experiment, there were no significant differences between the bread wheats in either number of nematodes per plant (Figure 10.1a) or in the number of nematodes per gram dry root (Figure 10.1b). In contrast to earlier trials (V, A. Vanstone, P. F. Lonergan and A. J. Rathjen, unpublished data), RAC 589 was indistinguishable from Spear and Molineux and there is no indication here that RAC 589 supports a lower nematode reproductive rate. Nor is there any indication that the Spear derivative (RAC 613-27) (which has a greater propensity to produce yellow lower leaves) is associated with any difference in nematode reproductive rate from its green leafed sister selection (RAC 613-47). The varieties selected on the basis of their resistance or tolerance to *P. thornei* did not differ from the susceptible local checks in their reaction to *P. neglectus*.

In terms of numbers of nematodes both per plant and per gram of dry root, substitution lines were intermediate between bread wheats and triticales. Among the substitution lines, 1R(-1B) had the lowest and 6R(-6D) the highest number of nematodes, but the difference between them was not statistically significant.

There were no significant differences between the entries containing a whole rye genome, other than Currency which had a slightly, but significantly, higher number of nematodes than Muir. The triticales Abacus and Muir, as expected, showed the lowest number of nematodes both per gram of root and per plant (Figures 10.1a and 10.1b) and can be regarded as being resistant to *P. neglectus*.

When the entries were analysed in three groups: wheat varieties, substitution lines and whole genome of rye including triticales, a significant difference was found between the three means for numbers of nematodes per plant, but for number of nematodes per gram of dry root the difference between the means for the substitution lines and that for the whole genome of rye was not significant (Table 10.2).

Abacus had the highest root growth which was significantly different from all other entries except those which had chromosome 1R from Imperial rye substituted for another group 1 chromosome. Substitution lines produced a higher root dry weight than the other two groups (Table 10.2) and the difference between 6R(-6D) and both 1R(-1B) and 1R(-1D) was statistically significant ($P<0.05$). Triticales, other than Abacus, and the rye King II had root dry weights similar to those of the wheat varieties (Figure 10.1c).

Table 10.2 Root dry matter and number of nematodes per plant and per gram of root dry weight for three genetic groups: the wheats, the substitution lines and lines with the whole rye genome.

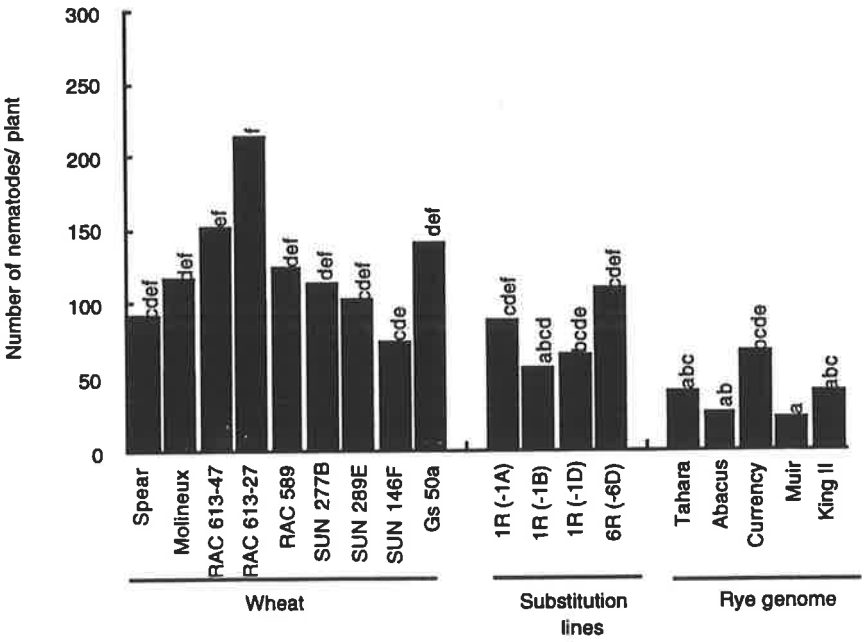
Genetic groups	Root weight	Nematodes	
	(g) /plant	/plant	/g dry root
Wheat	0.61 ^{c}	138.4 ^{a}	277.7 ^{a}
Substitution lines	0.74 ^{a}	86.5 ^{b}	160.0 ^{b}
Whole rye genome	0.64 ^{ab}	40.0 ^{c}	108.6 ^{c}

Means followed by the same letter are not significantly different ($P<0.05$).

Figure 10.1 Mean number of nematodes per g dry root (**a**), number of nematodes per plant (**b**) and root dry weight (**c**) of eighteen entries grown in a waterbath at 22 ± 1°C for 7 weeks and inoculated with *P. neglectus*, 250 (mixed stages) larvae + 150 eggs. Treatments with common letter(s) are not significantly different at ($P<0.05$).

Figure 10.1

(a) Number of nematodes per plant



(b) Number of nematodes per gram dry root

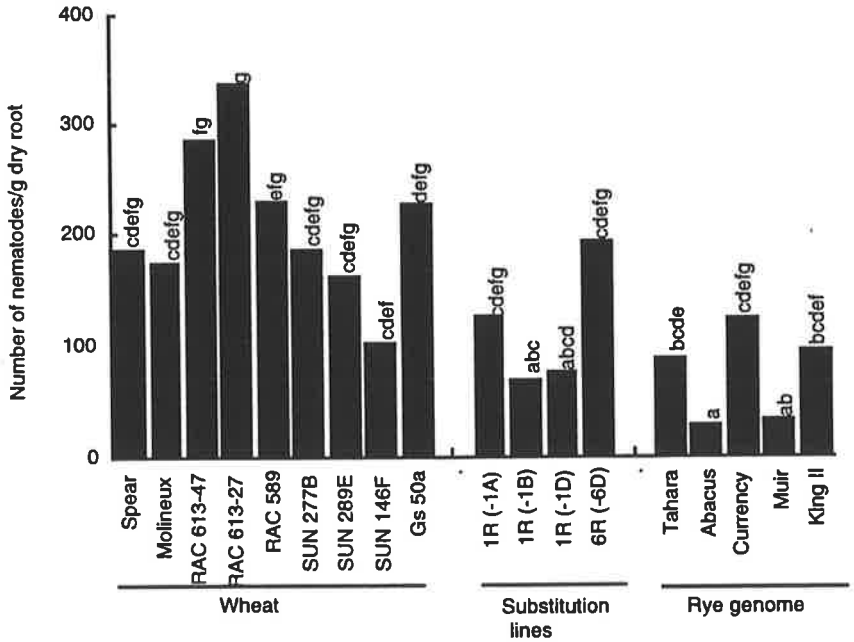
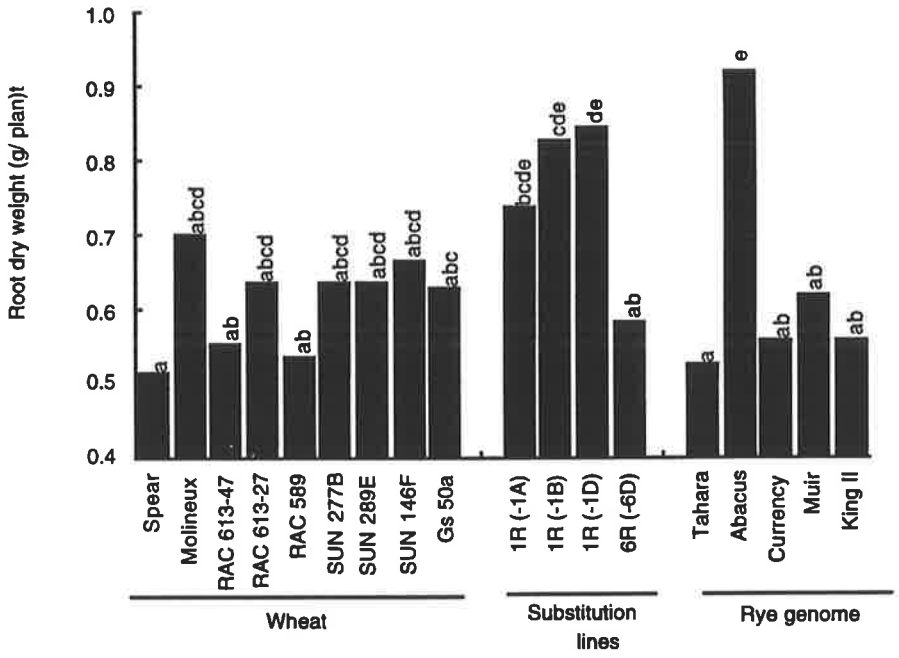


Figure 10.1 Continued

(c) Root dry weight (g/plant)



10.4 Discussion

In this experiment, GS 50A (resistant to *P. thornei* in Queensland (Thompson and Clewett, 1989)), was not significantly different from the susceptible check varieties, Molineux and Spear, so it is susceptible to *P. neglectus*. All lines reported to be tolerant to *P. thornei* were susceptible to *P. neglectus* and even SUN 146F, which had the lowest number of nematodes in this group, was not statistically different from the susceptible checks. The genetic mechanisms conferring resistance or tolerance to *P. thornei* do not affect the reproductive rate of *P. neglectus*.

RAC 589, found to have a low number of nematodes per plant in earlier experiments (V. A. Vanstone, P. F. Lonergan and A. J. Rathjen, unpublished data), showed a comparatively high number of nematodes per plant in this experiment. The low number in previous experiments could have been due to its slow root growth (Figure. 10.1c).

All lines with rye chromosomal material demonstrated a comparatively low number of nematodes compared to the wheats (Figures 10.1a and 10.1b). The triticales, with the exception of Currency, had fewer nematodes than the substitution lines with the 1R(-1B) line lower (but not significantly) than the other substitution lines. More detailed examination of the substitution lines is warranted to partition the effects of the background genotype, Chinese Spring, from that of the rye chromosomal material. The 1R(-1B) effect is particularly interesting, as this substitution has been widely successful in commercial wheats including the Bluebird series from CIMMYT and Aurora from Germany (A. J. Rathjen pers. comm.). Similarly, the contribution of the whole rye genome to the reduced multiplication rate needs to be compared to that from the tetraploid wheat present in the triticales. In the previous experiment (Chapter 9), some of the durums showed relatively fewer *P. neglectus* in their roots compared to bread wheat varieties. Since triticales have both the durum and rye genomes, these genomes may have a complementary effect in increasing resistance to the nematode.

CHAPTER 11

RESISTANCE TO *PRATYLENCHUS NEGLECTUS* IN WHEAT-RYE ADDITION LINES AND SOME EXOTIC WHEAT VARIETIES

11.1 Introduction

As no Australian wheat variety resistant to *P. neglectus* had been found, a search for sources of resistance to *P. neglectus* in related species and imported material was undertaken. In preliminary screening experiments (A. J. Rathjen and M. C. Kroehn, unpublished data), two wheat varieties, Persia 20 (from Iran) and Virest (from Italy), showed lower numbers of nematodes per plant. The experiment reported here was conducted to confirm the resistance of these sources, to test the resistance of other overseas material identified as being tolerant to *P. thornei*, and to investigate the possibility of locating the gene or genes for resistance on rye chromosomes by using wheat-rye (Imperial) addition lines.

11.2 Materials and methods

The genetic material examined included two susceptible local commercial wheat varieties (Spear and Molineux), lines reported by Dr. J. P. Thompson of the Queensland Wheat Research Institute, to be tolerant to *P. thornei* some overseas wheat varieties (Virest, Persia 20, Surak-I-Bahari and Iraq 48), canola as a less susceptible species (Vanstone *et al.*, 1993a), a 1R(-1B) substitution line and seven Chinese Spring wheat-Imperial rye addition lines (Table 11.5).

Since the transmission of addition chromosomes to the next generation is about 70% depending on the type of addition line (P. A. E. Ellis, pers. comm.), root tips were first checked for the presence of the rye chromosome by staining root tip cells by the Feulgen method and examining the chromosomes under a light microscope (Chapter 3) and only those with correct chromosome constitution were used.

Table 11.1 Genetic material investigated for resistance to *P. neglectus*. Pedigrees and Australian accession numbers are given in Table 3.6.

Name of Line/ Variety	Reason for inclusion
Spear	Susceptible local wheat check variety
Molineux	Susceptible local wheat check variety
Virest	Resistant to *P. neglectus* in previous experiment
Persia 20	Resistant to *P. neglectus* in previous experiment
SUN 290 B	Tolerant to *P. thornei* [a]
Iran 28357	Tolerant to *P. thornei* [a]
Surak -I- Bahari	Tolerant to *P. thornei* [a]
USDA CI 9040	Tolerant to *P. thornei* [a]
Iraq 48	Tolerant to *P. thornei* [a]
Abacus (triticale)	Confirming previous results (resistant to *P. neglectus*)
1B/1R (Cs [b]-Imperial rye substitution line)	Locating resistance genes
1R (Cs-Imperial rye) [c]	Locating resistance genes
2R (Cs-Imperial rye) [c]	Locating resistance genes
3R (Cs-Imperial rye) [c]	Locating resistance genes
4R (Cs-Imperial rye) [c]	Locating resistance genes
5R (Cs-Imperial rye) [c]	Locating resistance genes
6R (Cs-Imperial rye) [c]	Locating resistance genes
7R (Cs-Imperial rye) [c]	Locating resistance genes
Canola (*Brassica napus*, variety Barossa)	Less susceptible to *P. neglectus* and *P. thornei*

a - Detected by Dr. J. P. Thompson, Queensland Wheat Research Institute (QWRI).

b - Chinese Spring, hexaploid wheat variety.

c - Addition line

A reddish gray sandy loam soil collected from a farming property at Palmer (65 km east of Adelaide) (Chapter 3) was steam pasteurised for 30 minutes at 70°C. Sterilised plastic pots without any drainage holes were filled with 650 g of soil. Seeds were surface sterilised, pre-germinated and sown one to a pot in a completely randomised design of 19 entries with eight replications.

After five days, each pot was inoculated with about 350 larvae (mixed stages) and 200 eggs of aseptic *P. neglectus* obtained from carrot cultures (V. A. Vanstone, pers. comm.). Pots were cultured in a waterbath at 22 ± 1°C in an evaporatively cooled glasshouse, and watered with distilled water whenever necessary

Due to space limitation in the mister, four replicates were harvested after seven and the remaining four after eight weeks, when soil was washed from the roots under running tapwater. Roots were chopped into 1 cm lengths and misted for five days to extract nematodes (Southey, 1986). The nematodes were counted and the roots were dried and weighed (Chapter 3).

Prior to analyses of variance, the data were transformed to Ln (number of nematodes + 200) (Proctor and Marks, 1974) to render variances independent of the means. Means were compared using LSDs.

11.3 Results

The average number of nematodes both per plant and per gram of dry root significantly ($P<0.01$) increased between the seven and eight week harvests, from 1129 to 3272 nematodes per plant and from 1617 to 3939 nematodes per gram dry root.

The differences between entries both in number of nematodes per plant and in number of nematodes per gram of dry root were statistically significant ($P<0.01$). The entries could be classified into two groups for the number of nematodes per plant or per gram of dry root (Figures 11.1a and 11.1b). The first group, including all addition lines, the substitution line 1R(-1B), Abacus, Persia 20 and Virest, was significantly different ($P<0.01$) from the second group including checks and other wheat varieties.

The differences between entries for root dry matter were also statistically significant ($P<0.01$) (Figure 11.1c). Canola, which was included as a less susceptible species, had the

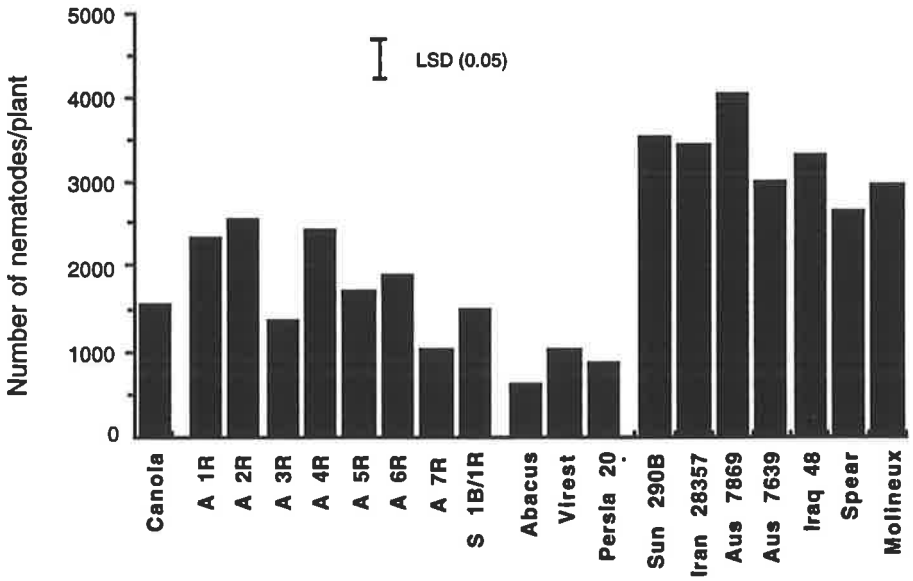
lowest root dry matter (Figure 11.1c) and thus was associated with a high number of nematodes per gram, whereas, with 1481 nematodes per plant, canola was as low as the addition lines and significantly different from Molineux the wheat check (Figure 11.1a). Persia 20 had the highest amount of root dry matter, while its number of nematodes per gram of dry root was the lowest of all the genotypes.

Among wheat varieties other than Persia 20 and Virest, only Spear was significantly different from Surak in terms of the number of nematodes per plant (Figure 11.1a). Abacus (triticale), with 594 nematodes per plant, showed the lowest and Surak (AUS 7869), with 4050 nematodes per plant (about five times that of Abacus), the highest number of nematodes per plant (Figure 11.1a). Persia 20 and Virest occupied the second and fourth positions, respectively, following Abacus, but the difference between them was not significant. Among addition lines, 7R showed the lowest number of nematodes per plant and was significantly different ($P<0.05$) from the 1R, 2R and 4R addition lines.

Figure 11.1 Number of nematodes per gram dry root (**a**), number of nematodes per plant (**b**) and root dry weight (g/plant) in 19 entries grown for seven or eight weeks in a waterbath at 22±1°C and inoculated with 550 *P. neglectus* (350 larvae + 200 eggs). Each value is the mean of eight replicates.

Figure 11.1

(a) Number of nematodes per plant



(b) Number of nematodes per gram dry root

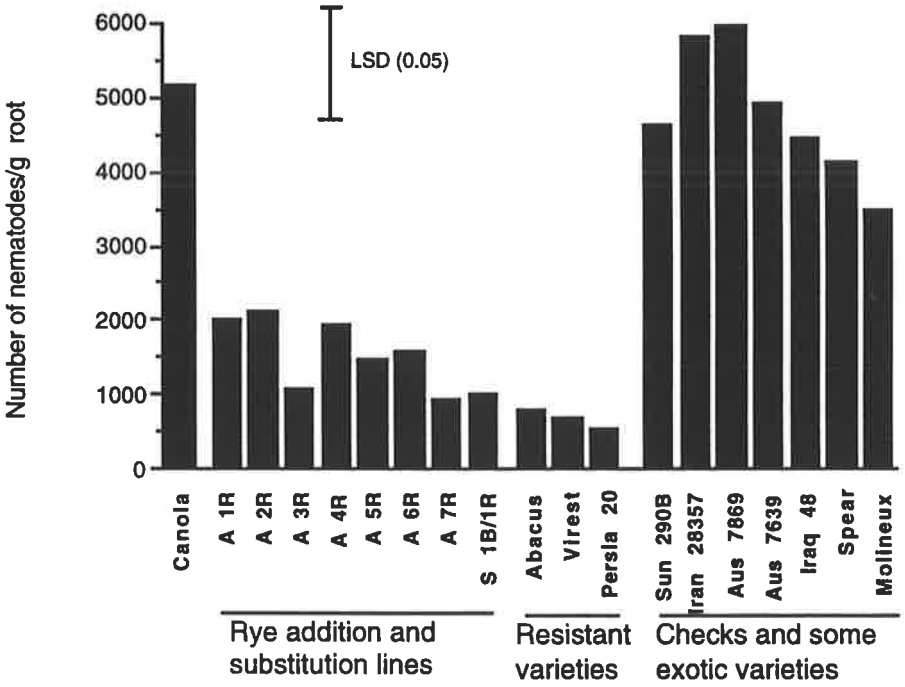
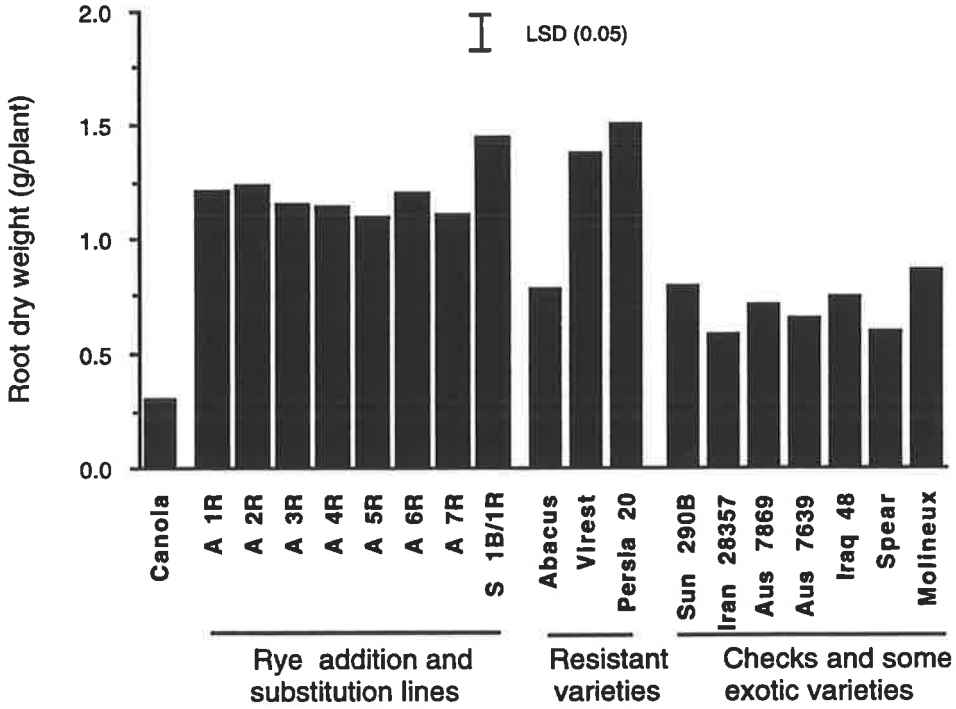


Figure 11.1 continued

(c) Root dry weight (g/plant)



11.4 Discussion

Although there are various methods of controlling root lesion nematodes, including rotation with non-host crops (Bolton and Waele, 1989; Vanstone, 1993a), cultivation (Thompson *et al.*, 1981), solarisation (Vito *et al.*, 1991), fertiliser application (Vanstone *et al.*, 1993b) and nematicide application (Farsi *et al.*, 1993b), the most economical method of nematode control is the use of resistant varieties. These varieties, if available, would allow farmers to produce high-yielding crops without a substantial increase in nematode numbers which could damage subsequent crops.

In terms of the number of nematodes per plant, canola was as resistant as the addition lines but, in the number of nematodes per gram because of low root dry weight, it would be regarded as being susceptible. As the economic damage by nematodes is predominantly related to the size of the population invading the seedlings of a subsequent crop, the

number of nematodes per plant is a more appropriate estimate of resistance for an agricultural crop than the number per gram of root (J. M. Fisher, pers. comm.).

A low number of nematodes per plant was counted for Abacus, Persia 20 and Virest, as in previous experiments (Chapter 10; A. J. Rathjen and M. C. Kroehn, unpublished data), and this suggests the existence of a resistance gene or genes in Persia 20 and Virest (from Iran and Italy, respectively). To confirm this result, further experiments were conducted to study the feeding, moulting and reproduction of the nematode inside the plant roots (Chapter 12).

Although the 7R addition line had the lowest number of nematodes per plant among the addition lines, its difference with 3R was not significant, and since all addition lines were significantly different from the second group of wheats (Figure 11.1a), it is not possible to locate the resistance gene or genes on a specific chromosome of rye.

Abacus, consisting of rye and tetraploid wheat (durum) genomes, could be used either as a rotational crop or as a donor parent to incorporate the *P. neglectus* resistance gene (or genes) into commercial local wheat varieties. Fortunately, there appears to be sufficient resistance to *P. neglectus* in wheats such as Persia 20 and Virest, so such translocative will not be necessary.

At the time of sampling, the number of nematodes extracted from susceptible plants was about seven times the population initially introduced, while in the roots of Abacus and resistant wheat varieties, Virest and Persia 20, the numbers were almost equal or about twice the numbers initially inoculated to the plants, respectively (Figure 11.1). So it is concluded that in resistant plants nematode reproduction rate is reduced compared to that in susceptible plants.

CHAPTER 12

EXAMINING *PRATYLENCHUS NEGLECTUS* LIFE CYCLE IN ROOTS OF RESISTANT AND SUSCEPTIBLE PLANTS

12.1 Introduction

In the previous experiments (Chapters 9, 10 and 11), it was found that the triticale Tahara and two wheat varieties (Virest and Persia 20) supported substantially lower numbers of *P. neglectus* than the susceptible commercial wheat variety Spear. In resistant plants some mechanism has been developed that does not allow nematodes to multiply as rapidly as they do in susceptible plants. The resistance mechanism may vary depending on the plant and species of the nematode.

Hatching factors for cyst nematodes do not play a part in resistance mechanisms, and there is no evidence that resistant plants are less attractive to the nematodes (O'Brien and Fisher, 1977). There are cases where larvae fail to penetrate resistant plants or where secretions from roots of some plants are toxic to nematodes (Southey, 1978), or resistant and susceptible plants are equally invaded by the nematode, but fewer larvae develop within resistant plants (Cooke, 1974). Mechanisms of resistance to *P. neglectus* in wheat varieties have not been investigated, nor is there any report on the mechanisms of resistance to *Pratylenchus* in any host species. What has been found already in studies undertaken for this thesis is that when plants were inoculated with a predetermined number of nematodes and grown under controlled and optimum conditions for nematode reproduction, the number of nematodes extracted after seven or eight weeks from resistant varieties was significantly less than from susceptible ones, and usually less than the initial population introduced into the soil (Chapter 11).

Resistance is measured by the ability of the nematode to penetrate, feed, develop and reproduce on its host and could operate at any time during the nematode's life cycle. Once inside the host, nematode growth and reproduction may continue at varying rates depending

on the suitability of the environment offered to the parasite by the host. This experiment was conducted to investigate differences in penetration rates, growth, development and life cycle (the time necessary for a newly hatched larva to grow to adult and commence egg laying) of the nematode *P. neglectus* in the roots of resistant and susceptible wheat and triticale varieties (Farsi *et al.*, 1994b).

12.2 Materials and methods

Genetic material used in this experiment included Tahara triticale, and Virest and Persia 20 as resistant wheat varieties. Tatiara was included as a moderately susceptible wheat (Vanstone *et al.*, 1994), and Spear as a susceptible commercial variety.

Aseptic eggs were collected from carrot cultures (Nicol and Vanstone, 1993) and purified by passing the suspension of eggs and mixed stages of nematodes through a 30 µm sieve about 30 times. Eggs passed through the sieve and the nematodes remaining on the sieve were washed off. Eggs were then placed in a Petri dish in a 25°C incubator to hatch. Every three hours over a twelve hour period, freshly hatched larvae were separated from eggs using a 30 µm sieve and stored at 4°C. Thus, juveniles, of the same stage and freshly hatched (synchronised), were collected to use as inoculum. Nematodes inside the roots were observed at each sampling by first bleaching roots in 5% NaOCl and then staining with lactoglycerol acid fuchsin (Chapter 3).

Small Eppendorf tubes (2.0 ml) were filled with pasteurised fine sandy soil to maximise nematode penetration (Vanstone and Nicol, 1993; Chapter 7), and were sown with one surface sterilised pre-germinated seed. Three day old seedlings were inoculated with 1000 juveniles (early second stage larvae) and incubated in a temperature controlled waterbath at 25°C for 36 hours. The seedlings were then removed from the tubes and the roots washed to remove all debris, soil particles and any nematodes that had not penetrated the root cortex, but were adhering to root surfaces. Three seedlings from each entry were stained to determine nematode penetration rate. Remaining seedlings were transplanted to 360 g pots

containing pasteurised reddish grey sandy loam from Palmer (Chapter 3). The pots were transferred to a waterbath at 25°C until sampling.

Pots were arranged in a completely randomised design and harvested at five day intervals (5, 10, 15, 20, 25, 29 days) and then after 37, 45 and 51 days. Three pots, each containing one plant, were harvested at each sample time and their roots stained.

Stained roots were placed between two glass microscope slides and gently pressed. The shape of at least 20 nematodes, where possible, were first drawn on paper, using microscope with drawing tube attached, and then the length of the nematodes was measured by using a flexible wire and ruler.

Since finding nematodes in large amounts of root from 30 day old plants was too difficult, the roots from the last two harvests were misted to extract nematodes (Southey, 1986), which were then counted microscopically.

The data were analysed as a completely randomised design with sampling within each treatment. For analysis of variance the data were transformed to Ln (number of nematodes + 200) (Proctor and Marks, 1974) to render means independent from variances. Means were compared by LSDs.

12.3 Results

Penetration

In general, the nematodes penetrated roots as a group and formed a cluster within the cortex. Thus, some sections of root were invaded by many synchronised nematodes (Plate 12.1), while other sections had none. No statistically significant difference was found between entries for nematode penetration (Table 12.1), although Abacus, the resistant triticale with an average of 81 nematodes per plant, showed the lowest and Spear, with 239 nematodes per plant, the highest rate of penetration. The two resistant wheat varieties, Persia 20 and Virest with 108 and 189 nematodes per plant, respectively, showed lower penetration rates than Spear.

Table 12.1 Mean number of nematodes penetrating roots of resistant wheat varieties (Virest and Persia 20), Abacus (triticale) and susceptible check wheat variety Spear over a period of 36 hours at 25°C in 2.0 ml Eppendorf tubes.

Genotypes	Number of nematodes penetrating the roots
Spear	239.0 [a]
Virest	189.0 [a]
Persia 20	107.7 [a]
Tatiara	131.7 [a]
Abacus	80.7 [a]

Values followed by the same letter are not significantly different ($P<0.05$).

Nematode development

No significant differences were found between entries for nematode lengths measured at five day intervals, other than for five and fifteen days after inoculation (Table 12.2). At five days, nematodes in Tatiara (0.180 mm long) were the shortest and those in Abacus (0.195 mm) the longest. At fifteen days after inoculation, Tatiara again had the shortest (0.193 mm)

and Spear (0.228 mm) the longest nematodes.

Table 12.2 Mean nematode lengths (mm) at 5, 10, 15, 20, 25 and 29 days after inoculation with second stage juveniles obtained from freshly hatched eggs.

	Days after inoculation					
Genotypes	**5 days**	**10 days**	**15 days**	**20 days**	**25 days**	**29 days**
Spear	0.19 ab	0.20 a	0.23 b	0.20 a	0.25 a	0.36 a
Virest	0.19 ab	0.20 a	0.20 ab	0.21 a	0.22 a	0.37 a
Persia 20	0.19 ab	0.18 a	0.20 ab	0.21 a	0.29 a	0.25 a
Tatiara	0.180 a	0.18 a	0.19 a	0.23 a	0.25 a	0.27 a
Abacus	0.20 b	0.19 a	0.20 ab	0.22 a	0.17 a	0.27 a

Letters following values are for comparison between entries on a given date. Values with common letters are not significantly different ($P<0.05$).

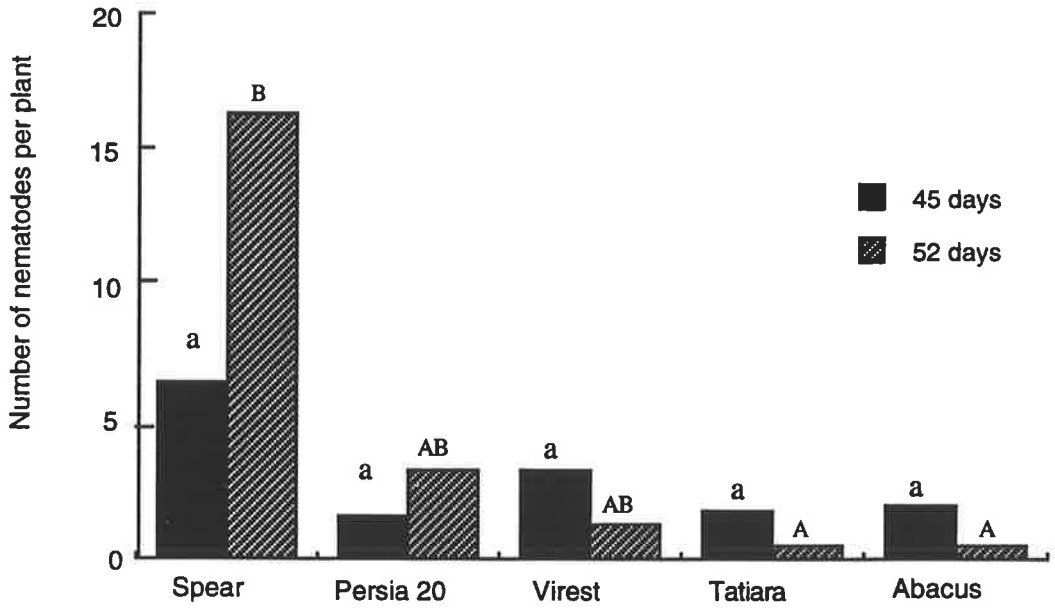
Moulting

While the entire root system for each plant was checked under the microscope some replicates at later samplings (particularly Abacus, Tatiara and Virest), contained no nematodes and others only a few. Twenty days after inoculation, the number of nematodes inside the roots had decreased, and after 25 days the majority of eggs found in Spear were among root hairs instead of being in the cortex, where the nematodes usually feed.

In roots of Spear, the susceptible check variety, a large number of nematodes (about 30%) were moulting (Plate 12.2) ten days after inoculation, while in Persia 20 only one nematode out of the 60 examined was moulting. In Tatiara and Virest, some moulting was observed at fifteen days after inoculation. In Abacus, the first moulting was observed 29 days after egg hatching.

At 52 days after inoculation, Spear (with an average of 16 nematodes per plant) was significantly ($P<0.05$) different from both Abacus and Tatiara which had an average less than one nematode per plant (Figure 12.1).

Figure 12.1 Number of nematodes per plant extracted from the roots of five cereal varieties 45 and 52 days after inoculation.



Values followed by the same letter are not significantly different ($P<0.05$). Lower and upper cases are for comparison of number of nematodes extracted at 45 and 52 days, respectively.

Egg laying

The first egg laying occurred 25 days after inoculation in the roots of Spear and Tatiara. After 29 days, a few eggs were observed in Persia 20. No eggs were observed in Virest and Abacus, even though the whole root system was examined.

Hypersensitivity of host tissue

When examining nematodes under the microscope, some in Abacus and Persia 20 appeared to be trapped inside cells with thickened walls. Appearance of some vacuoles in the bodies of nematodes trapped in the necrotic cells showed that nematodes in the dead cells were decomposed (Plate 12.3), indicating they had died before being fixed and stained. In the resistant plants, most necrotic cells were those which had surrounded the dead nematodes and were confined to the surrounding cells, while in the susceptible plants the necrotic areas

were larger and more expanded with no nematode inside.

Plate 12.1 Nematodes had penetrated roots in a cluster when plants were examined 36 h after inoculation with second stage juveniles.

Plate 12.2 Nematodes first started moulting in the susceptible wheat variety, Spear, but in resistant genotypes moulting occurred later.

Plate 12.1

Plate 12.2

Plate 12.3 Hypersensitive reaction in resistant cereal genotypes (Abacus triticale and Virest wheat) to nematode invasion. Nematodes were trapped by thickened cell walls, and vacuoles within nematodes indicated death had occurred prior to staining.

12.4 Discussion

In this experiment, resistant varieties (Persia 20 and Virest wheat and Abacus triticale) showed lower nematode penetration than Spear, the susceptible check variety, although the difference was not statistically significant. The absence of a significant difference between Abacus, with a mean of 81 and Spear with 239 nematodes per plant, was due to large variation between replicates. Although temperature and nematode density were controlled in this experiment, root growth within the same variety was not consistent. Variation in soil structure and distribution of nematodes around the roots resulted in diverse numbers of nematodes penetrating the cortex. To differentiate the varieties in terms of nematode penetration rate, further investigation with a greater number of replicates is needed.

In general, resistance in plants is expressed after nematode invasion, resulting in juveniles being unable to complete development and reproduce. When Barrons (1939) counted the number of root-knot nematodes in resistant and susceptible plants, he found no differences in the rate of larval entry into either the seedling or adult stage of the plant. Some barley varieties resistant to cereal cyst nematode also do not show any difference between susceptible and resistant plants in terms of nematode penetration, but nematode development within resistant plants is slower (Cotten, 1970). However, Georgaras (1990) showed that resistant oat varieties were less attracted to the nematode.

The first significant result obtained in this experiment was the difference between resistant and susceptible varieties for the time of moulting and commencement of egg laying. In the susceptible check variety (Spear) and in Tatiara, a moderately susceptible variety, the first egg laying occurred 25 days after inoculation. In Persia 20, the first egg was observed after 29 days. For Virest and Abacus, no eggs were observed even after 35 days. It could be that resistant plants lack substances necessary for the development and reproduction of the nematodes, or contain them in insufficient amounts which results in the failure of nematodes to reach maturity (Giebel, 1982).

The second significant result, illustrated in Plate 12.3, was the phenomenon of dead cells surrounding nematodes in the resistant plants. This may be based on plant tissue hypersensitivity to nematode infection. Hypersensitive reaction is a rapid response of plant to the nematode that prevents development of the nematode and/or its feeding site. The host-parasite interaction can stimulate biochemical reactions in the host that cause histological changes, such as host cell necrosis. Necrosis can occur around the nematode, walling it off and either delaying development or causing the nematode to die. In resistant plants the necrotic area, therefore, is confined to the cells surrounding the nematode (Plate 12.3), while in the susceptible plants its occurrence is slow, but expanded to neighbouring cells (Trudgill, 1991; Cramer *et al.*, 1993).

Pratylenchus spp. move between soil and roots, and can feed from root hairs while remaining outside the roots. This ectoparasitical behaviour has been observed for *P. penetrans* (Townshend, 1987: Zunke, 1990b). The eggs can be deposited either within the roots or in the soil particularly in the region where large numbers of root hairs are available. *Pratylenchus* as enters roots primarily in the region of root hair development (Dropkin, 1989). The difficulty of finding nematodes in the roots twenty days after of commencement of the experiment might be due to nematodes leaving roots. Nematode may have left because roots of resistant varieties lacked or had low concentration of substances which keep them in roots of susceptible plants.

Although penetration rate of nematodes to roots of the triticale Tahara was one third of that in Spear, it seemed that this was not the main mechanism responsible for the significantly lower number of nematodes per plant in resistant varieties. In resistant plants, a higher proportion of those nematodes entered the roots, failed to establish feeding sites and therefore left the invaded region to find another source of food. This fact caused difficulties in finding nematodes in the roots of resistant plants at later stages. To investigate the life cycle of the nematode in resistant varieties, it is suggested that plants should be inoculated with a higher initial nematode population.

Since it appeared that nematode development and reproduction are delayed in resistant plants, it is suggested that in screening experiments, plants should be inoculated with a synchronised population of juveniles, so that relative number of nematodes extracted from the roots after seven to eight weeks would reflect nematode development and reproduction rather than differences in penetration rates.

In future works it might be better to extract all nematodes from roots at all sampling times by mister, count, fix and stain for later examination of gonad primordium development. This is more reliable way of assessing nematode development than size (Fisher and Triantaphyllou, 1964).

CHAPTER 13

GENETICS OF RESISTANCE TO *PRATYLENCHUS NEGLECTUS* IN WHEAT

13.1 Introduction

The genetics of resistance to nematodes has been reviewed by Sidhu and Webster (1981) and Fassuliotis (1987). Almost all the genetically identified resistances used in breeding programs are controlled by single, dominant major genes (O'Brien *et al.*, 1979; O'Brien *et al.*, 1980; Sidhu and Webster, 1981). Resistance of maize to *P. zeae* and *P. brachyurus* is due to two dominant genes with an additive effect (Savazaki *et al.*, 1988). In Queensland, J. P. Thompson (pers. comm.) reported that resistance of the Gatcher selection Gs 50A to *P. thornei* is controlled by a single, dominant gene.

The probability of success in a breeding program aimed at incorporating a specific character is inversely correlated to the number of genes by which the character is controlled. So, questions of great importance to a breeder are how many genes are involved and whether the gene effects can be recognised unambiguously. A small environmental component compared to the genetic effect enhances the accuracy of identification of the individual genotypes and thereby increases the likelihood of selecting superior segregates.

An understanding of the genetic control of resistance in wheat to *P. neglectus* would facilitate the breeding of resistant varieties. As the response of genotypes to *P. neglectus* can be highly affected by environmental factors such as temperature and contaminating microorganisms such as fungi, which can apparently break the resistance of some resistant varieties (A. Taheri, pers. comm.; Section 2.4), this investigation was conducted under controlled environmental conditions in pasteurised soil with aseptic nematodes.

This experiment was conducted to determine the number of genes involved in controlling the resistance of wheat variety Virest to *P. neglectus.*

13.2 Materials and methods

The reaction of 21 plants of each of three susceptible parents, Barunga, Frame and Tatiara and the resistant parent Virest (Table 3.6) and 84 plants of progeny from the F_2 generation of three crosses, (Virest x Barunga), (Virest x Frame) and (Virest x Tatiara), were assessed for their response to *P. neglectus*. Frame and Barunga are resistant to cereal cyst nematode, but support rapid multiplication of *P. neglectus*, and Tatiara is moderately susceptible to *P. neglectus* (A. J. Rathjen and V. A. Vanstone, pers. comm.). The crosses were made in the glasshouse, and the F_1 plants grown for seed propagation. F_1 plants were allowed to self-fertilise and F_2 seeds were collected.

Nematodes were collected from carrot cultures (Chapter 3) and the suspension containing eggs and all stages was passed through a 40 µm sieve about 30 times to separate adults from eggs and other larval stages. The adults were stored at 4°C until being used (Section 12.3). The reason for using adults instead of second stage juveniles was to increase the rate of multiplication and hence reduce the time to final assessment.

Seeds were surface-sterilised and incubated in Petri dishes in a refrigerator at 4°C for 24 hours and then at 25°C for germination. A reddish grey sandy surface soil from Palmer was pasteurised at 70°C (Chapter 3). Plastic pots (Chapter 3) were filled with 650 g of the soil. When radicles were 2-3 cm long, one seedling was transplanted into each pot. Three days later, seedlings were inoculated with about 500 adult nematodes (after counting the number of nematodes per millilitre and adjusting the solution to 500 nematodes per millilitre) by pipetting the nematode suspension into a small hole (3-4 cm deep) adjacent to each seedling.

Plants were grown in a controlled waterbath at 25 ± 1°C in an evaporatively cooled glasshouse for eight weeks and watered with distilled water whenever required. Plants were harvested after eight weeks, and nematodes extracted and counted (Chapter 3).

The data were transformed to Ln (number of nematodes + 200), (Proctor and Marks, 1974) and then subjected to analysis of variance. As it was not possible to classify the F_2 plants into discrete categories due to the continuous distribution of the data, comparisons between the observed and expected variances of each F_2 population were used for the estimation of the number of genes controlling resistance to the nematode. The expected variances were calculated from the following equations on the assumption of no epistasis, no linkage and no dominance (Mather and Jinks, 1971).

(a) One gene:

$$V_{F2} = 1/2\ d^2 + E$$

(b) Two genes:

$$V_{F2} = 1/4 d^2 + E$$

Where d is the departure from the mid-point m of each homozygous genotype and E is the environmental variance ($E = 1/4\ V_{p1} + 1/4\ V_{p2} + 1/2\ V_{F1}$; V_{p1} and V_{p2} are the variances of the parents, V_{F1} is variance of the F_1 between P_1 and P_2). Since the F_1 generation of the populations examined in this experiment was not tested, the environmental variance was estimated from the average variance of the two parents ($E = (V_{p1} + V_{p2})/2$).

Confidence limits were calculated based on the following formula suggested by Dr. David Peterson (pers. comm.).

$$(S^2 \times \text{d.f})/\chi^2$$

Where S^2 is the observed variance, d.f is the degrees of freedom for number of plants in each population and χ^2 is the corresponding figure for the degree of freedom and the level of significance (eg. for α=0.05 and d.f = 80, the $\chi 2$ of the lower limit is 57.15 and that of the upper limit is 106.63) (Table of χ^2).

13.3 Results

The differences between root dry weights of all the varieties and the F_2 populations were statistically significant (Figure 13.1). Root weights of all the susceptible parents were significantly lower than that of Virest, the resistant parent. Surprisingly, the root dry weights of two of the F_2 progenies, (Virest x Tatiara) and (Virest x Barunga), were significantly higher than both parents indicating a high level of heterosis (Figure 13.1).

Number of nematodes per plant

As in previous experiments (Chapters 11 and 12), Virest had a lower number of nematodes per plant compared to the other parents and the F_2 populations, and was significantly different from them (Figure 13.2). Number of nematodes per plant (Figure 13.2) for F_2 progenies of (Virest x Barunga) (1429) was slightly higher than for Barunga (1172), the susceptible parent. The mean number of nematodes per plant of the F_2 population of (Virest x Frame) (1085) was intermediate to the two parents (Frame 1458, Virest 461), and that of the F_2 populations of (Virest x Tatiara) (2117) was significantly higher than the susceptible parent, Tatiara (1294) (Figure 13.2). Variances of number of nematodes per plant for the four parents and three F_2 populations were highly correlated ($R^2 = 0.77$) to the mean numbers (Figure 13.4a).

No significant correlation was found between number of nematodes per plant and root growth either for parents (Figure 13.6a), means of parental and F_2 populations (Figure 13.3a), or the F_2 populations (Figures 13.7a-13.7c).

In terms of number of nematodes per plant, none of the F_2 populations (Figure 13.11a-13.11c) could be interpreted genetically as all the expected variances for one gene, which has the highest variance of all the simple models (as the number of genes increase, the F_2 variance decreases), were lower than the lowest limit of corresponding observed variance (Table 13.1a). The F_2 population with variance (705×10^3) closest to the expected variance (437×10^3) for a single gene was the progeny of (Virest x Frame).

Number of nematodes per gram dry root

In terms of number of nematodes per gram dry root, Virest (568) was the lowest of the parents and Frame (3691) the highest (Table 13.1b and Figure 13.2). The number of nematodes per gram dry root of all the F_2 populations (Figure 13.2) was between that of their corresponding parents, and the mean of their distributions was significantly higher than Virest and lower than the susceptible parent, except for the mean of the F_2 of (Virest x Tatiara) (2259) which was not significantly different from that of Tatiara (2788) (Table 13.1b). When F_2 populations were considered individually, the number of nematodes per gram of dry root of F_2 progenies of (Virest x Frame) and (Virest x Barunga) was independent of root growth (Figures 13.9a and 13.9b). That of (Virest x Tatiara) was slightly, but not significantly, inversely related to the amount of root growth (Figure 13.9c). When the means of parents and F_2 populations were considered, the number of nematodes per gram of dry root was inversely related to root growth (Figure 13.3b). In contrast, the variances of number of nematodes per gram of dry root was positively correlated to root growth (Figure 13.4b).

In terms of number of nematodes per gram dry root, the upper 95% confidence limit of expected variance for F_2 progenies of (Virest x Barunga) was lower than that of the expected variance for two genes (Table 13.1b). A greater number of genes (more than two) could be an interpretation for the genetic basis of this distribution. The distribution of the F_2 population of (Virest x Tatiara) (Figure 13.7c) could not be distinguished between being due to the segregation at one or two genes, as the expected variances for both fall between the confidence limits of the corresponding observed variance (Table 13.1b). The segregation of (Virest x Frame), could be the result of two genes as the expected variance for two genes was closer to the observed variance than that for one gene (Table 13.1b).

Transformed data

The transformation to Ln (number of nematodes per plant + 200), recommended by Proctor and Marks (1974), rendered the variances independent of means (Figure 13.4c), so the results contrast to those above. With this transformation, the Ln (number of nematodes per plant + 200) was also independent of root growth (Figure 13.3c).

The results of F_2 distribution of (Virest x Frame) could be interpreted as being due to separation at a single gene as the expected variance for one gene was slightly lower than the confidence lower limit of the observed variance (Table 13.2). In contrast, the expected variance of the F_2 population of (Virest x Tatiara) for two genes was slightly higher than the upper limit of observed variance and it could be interpreted as segregating at two genes. That of (Virest x Barunga) (Table 13.1c) could be attributed to either one or two genes, but it was closer to that of the expected values for two genes.

The variance of both the number of nematodes per plant and per gram of dry root for the resistant variety Virest, was less than the variances for the susceptible parents and F_2 populations which were from three to ten and from four to fifteen times that of the resistant parent, respectively (Table 13.1). As the distribution of F_2 populations was continuous (Figures 13.11a-13.11c), objective classification of F_2 progenies into different groups was not possible. If an arbitrary demarcation of resistance (those with a mean ≤ the mean of resistant parent plus two standard errors are considered as resistant, i.e. $461+(2 \times 71) = 603$ and $568 +(2 \times 95) = 758$, for number of nematodes per plant and per gram of dry root, respectively), is imposed on progeny in the F_2 populations, then the distributions of some F_2 populations could reflect an underlying 1:3 ratio of resistant:susceptible based on the one gene hypothesis (Table 13.2).

Table 13.1 Observed means and variances of (**a**) number of nematodes per plant, (**b**) number of nematodes per gram dry root and (**c**) Ln (number of nematodes per plant + 200) of F_2 populations compared to the expected variances based on the one or two genes models.

(a) Number of nematodes per plant

Parent and F_2 population	Mean	d[a]	E[b] ($x10^3$)	Expected variance[c] ($x10^3$) 1 gene	2 genes	Observed variance ($x10^3$)	95% Confidence limits for observed variance ($x10^3$)
Barunga	1175					352	
Frame	1458					525	
Tatiara	1294					582	
Virest	461					100	
(Virest x Barunga)	1429	357	226	290	258	822	633<x<1176
(Virest x Frame)	1085	498.5	312	437	374	705	543<x<1009
(Virest x Tatiara)	2117	416.5	341	427	384	1034	796<x<11480

(b) Number of nematodes per gram dry root

Parent and F_2 population	Mean	d[a]	E[b] ($x10^3$)	Expected variance[c] ($x10^3$) 1 gene	2 genes	Observed variance (x103)	95% Confidence limits for observed variance ($x10^3$)
Barunga	2384					1848	
Frame	3691					2992	
Tatiara	2788					2165	
Virest	568					183	
(Virest x Barunga)	1350	908	1016	1428	1222	731	563<x<1049
(Virest x Frame)	1509	1561	1588	2807	2197	1616	1244<x<2313
(Virest x Tatiara)	2259	1110	1174	1790	1482	1399	1077<x<2003

Table 13.1 continued

(c) Ln (number of nematodes per plant + 200)

Parent and F_2 population	Mean	d[a]	E[b]	Expected variance [c] 1 gene	2 genes	Observed variance	95% Confidence limits for observed variance
Barunga	7.12					0.24	
Frame	7.31					0.25	
Tatiara	7.17					0.35	
Virest	6.38					0.25	
(Virest x Barunga)	7.24	0.370	0.245	0.31	0.28	0.34	0.27<x<0.49
(Virest x Frame)	6.94	0.465	0.250	0.36	0.30	0.48	0.37<x<0.70
(Virest x Tatiara)	7.64	0.395	0.300	0.38	0.34	0.23	0.18<x<0.33

a - The departure of one pair of corresponding homozygotes from their mid-point *(P1 + P2)/2 - P1 or (P1 + P2)/2 - P2.*

b - Environmental variance = $1/2V_{P1} + 1/2\ V_{P2}$

c- Expected variance for 1 gene ($1/2\ d^2 + E$) and 2 genes ($1/4\ d^2 + E$).

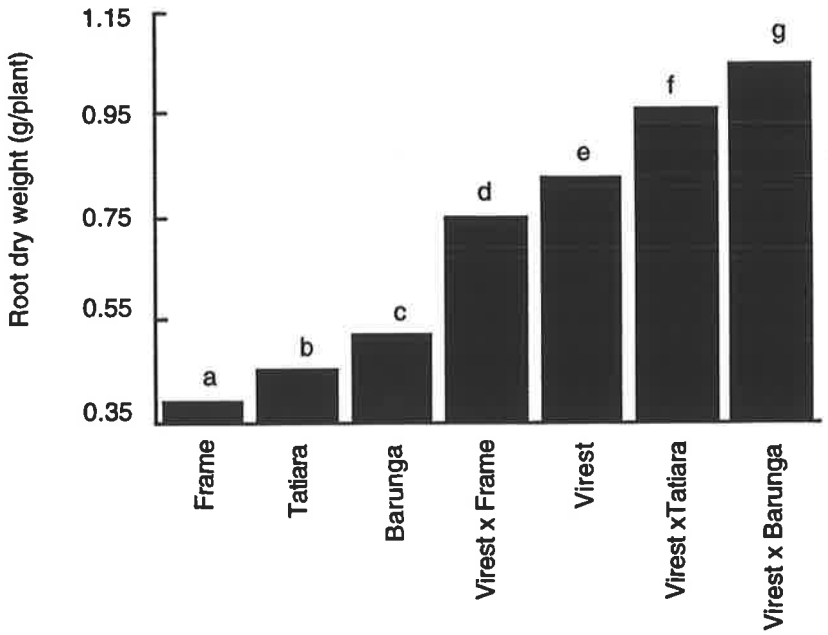
Table 13.2 Chi-square analysis of the observed and expected segregation ratios of F_2 populations, based on defining those segregants within the range of the mean of Virest plus two standard deviations as being resistant, derived from crosses of the resistant variety (Virest) with susceptible parents (Frame, Barunga and Tatiara).

Population	Number of nematodes per plant					χ^2
	Observed values			Expected values		
	Resistant	Susceptible	Ratio	Resistant	Susceptible	
(Virest x Frame)	28	56	1:3	21	63	3.10
(Virest x Barunga)	16	68	1:3	21	63	1.59
(Virest x Tatiara)	5	79	1:3	21	63	16.20
Population	**Number of nematodes per gram of dry root**					χ^2
	Observed values			Expected values		
	Resistant	Susceptible	Ratio	Resistant	Susceptible	
(Virest x Frame)	21	63	1:3	21	63	1.00
(Virest x Barunga)	16	68	1:3	21	63	1.59
(Virest x Tatiara)	3	81	1:3	21	63	20.50

Figure 13.1 Mean root dry weight (g/plant) of resistant and susceptible parents and their F_2 progenies. Plants were grown for seven weeks in 650 g pots containing pasteurised soil and inoculated with 500 *P. neglectus*.

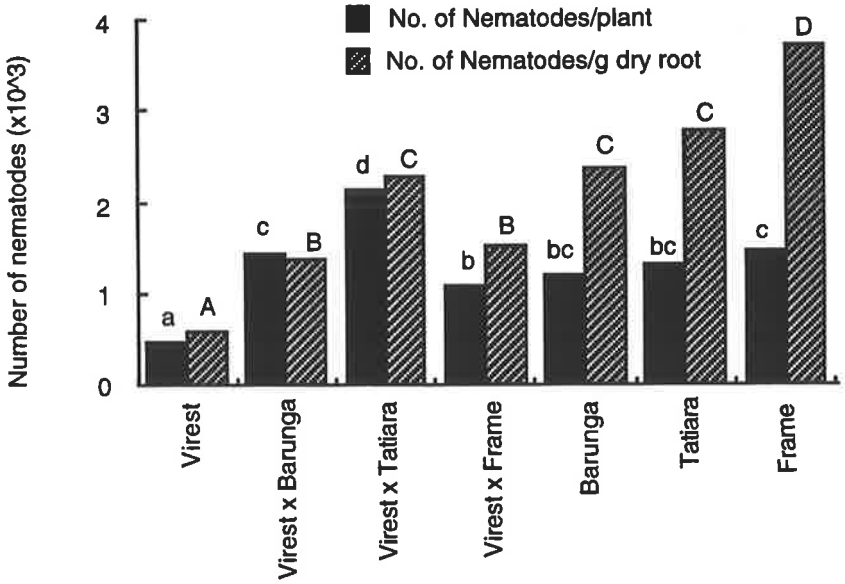
Figure 13.2 Mean number of nematodes per plant and per gram dry root of resistant and susceptible parents and their F_2 progenies. Plants were grown for seven weeks in 650 g pots containing pasteurised soil and inoculated with 500 *P. neglectus*.

Figure 13.1



Values followed by the same letter are not significantly different (P<0.05).

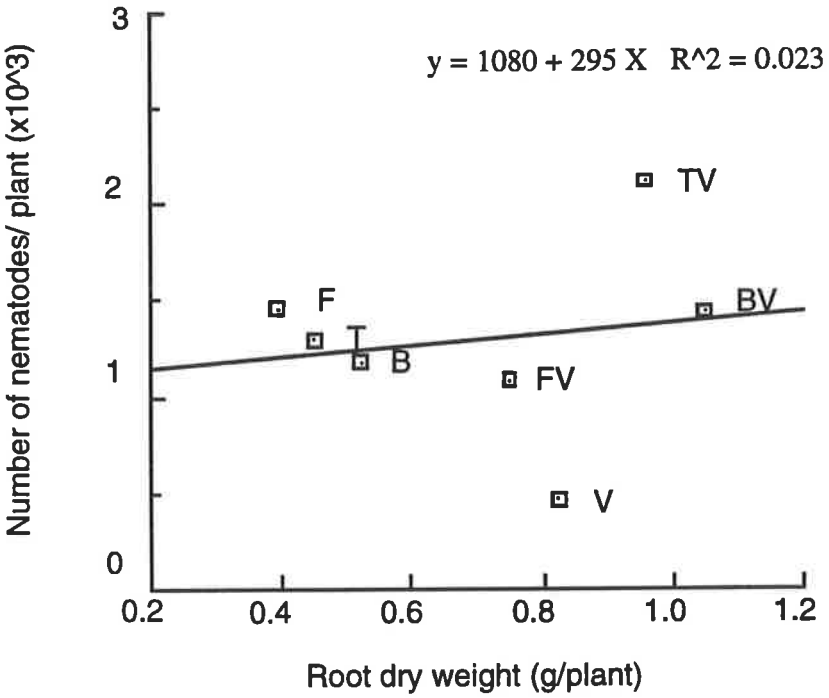
Figure 13.2



Values followed by the same letter are not significantly different (P<0.05). Lower and upper case letters are for the comparison of number of nematodes per plant and number of nematodes per gram of dry root, respectively.

Figure 13.3 Relationship between the means of (**a**) number of nematodes per plant and (**b**) number of nematodes per gram of dry root and root dry weight. Plants were grown for seven weeks in 650 g pots containing pasteurised soil and inoculated with 500 *P. neglectus*.

(**a**) Number of nematodes per plant



(**b**) Number of nematodes per gram of root

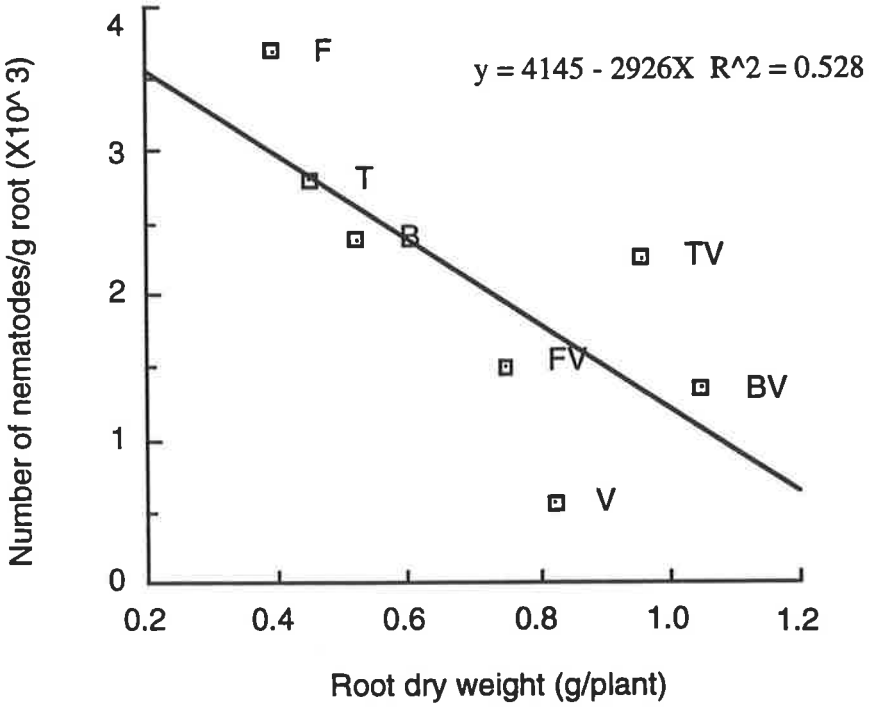
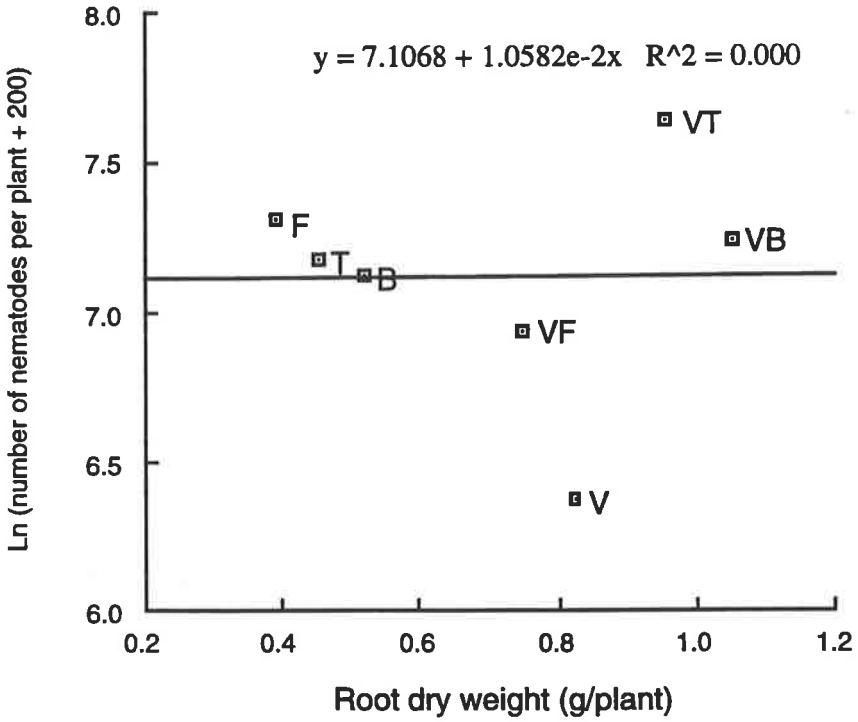


Figure 13.3 continued

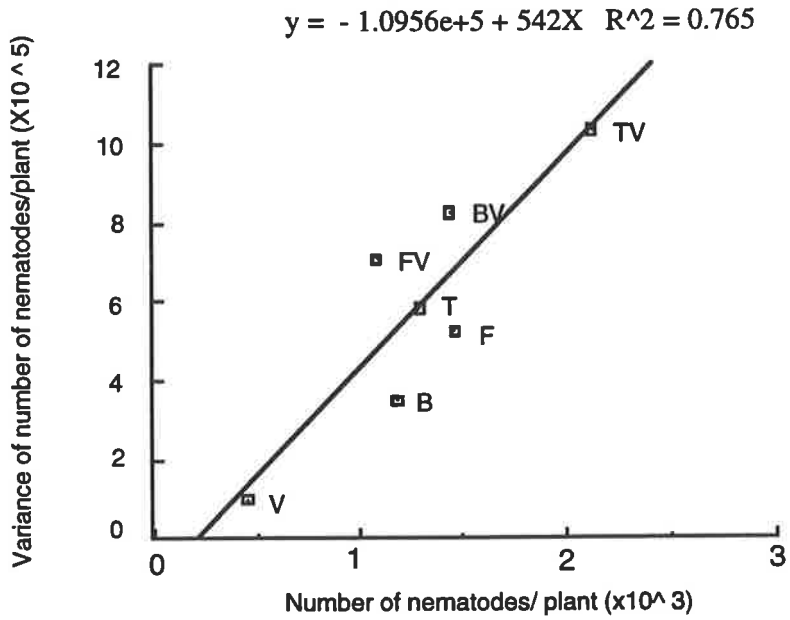
(c) Ln (number of nematodes per plant + 200)



B= Barunga, F = Frame, T= Tatiara, V = Virest; VB, VF and VT=F_2 populations of (Virest x Barunga), (Virest x Frame) and (Virest x Tatiara), respectively.

Figure 13.4 Relationship between the variances and (**a**) number of nematodes per plant, (**b**) number of nematodes per gram of dry root and (**c**) Ln (number of nematodes + 200) of parents and F_2 populations. Plants were grown for seven weeks in 650 g pots containing pasteurised soil and inoculated with 500 *P. neglectus*.

(**a**) Number of nematodes per plant



(**b**) Number of nematodes per gram of root

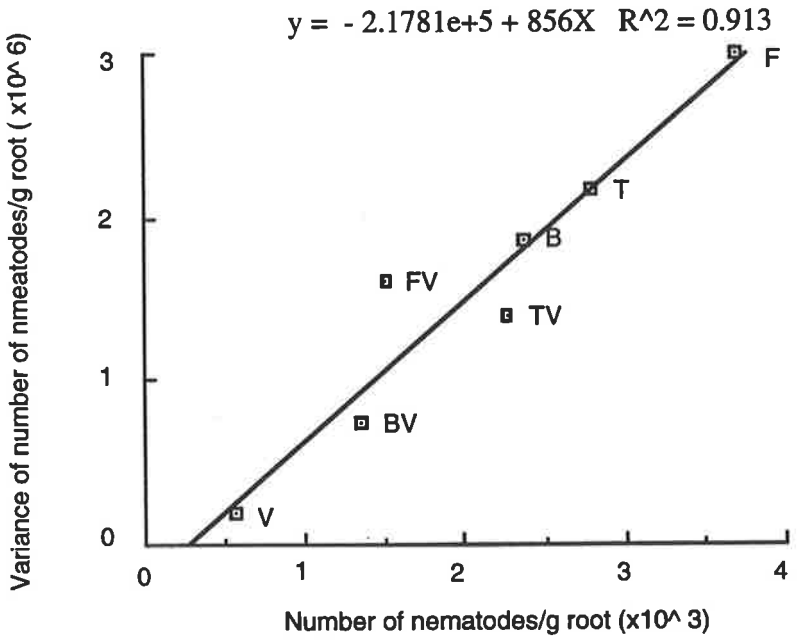
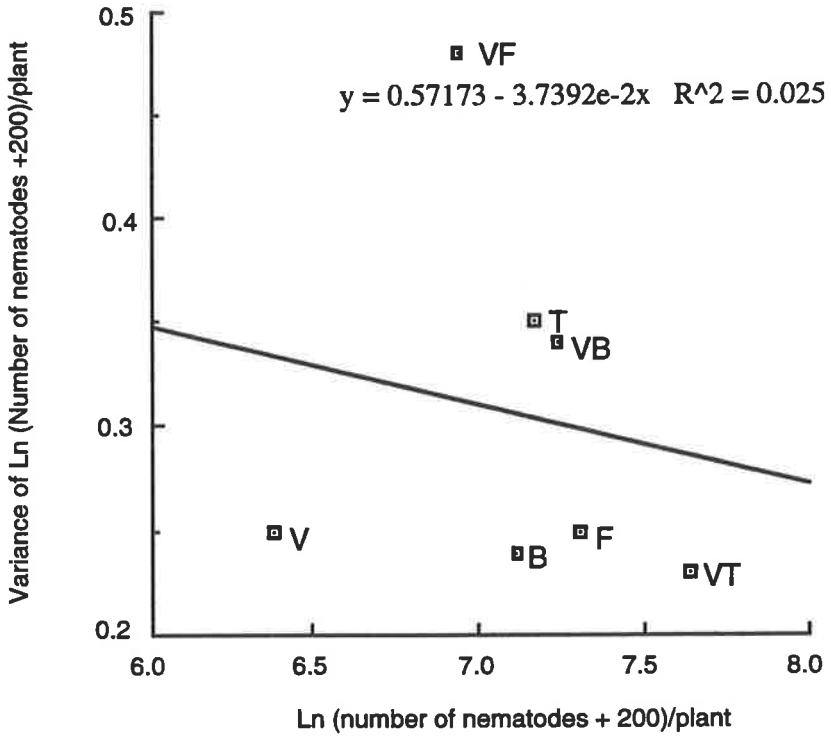


Figure 13.4 continued

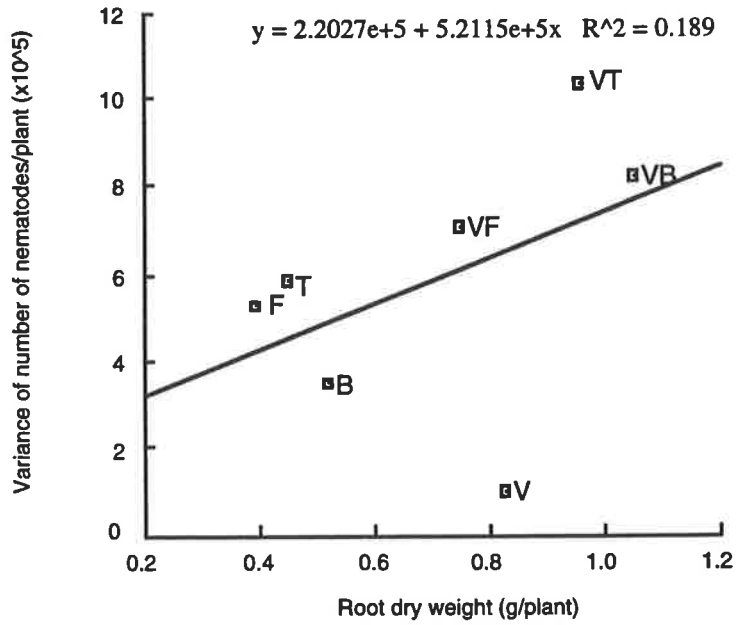
(c) Ln (number of nematodes per plant + 200)



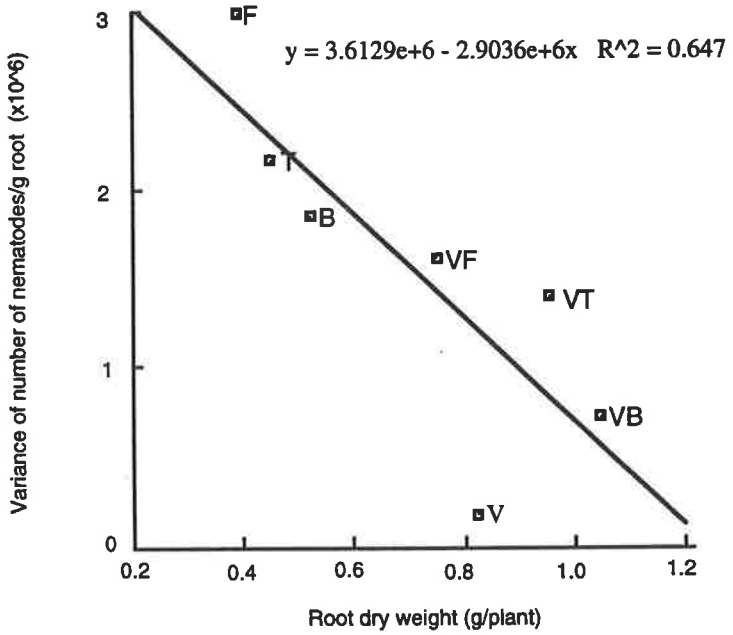
B= Barunga, F = Frame, T= Tatiara, V = Virest; VB, VF and VT = F_2 populations of (Virest x Barunga), (Virest x Frame) and (Virest x Tatiara) respectively.

Figure 13.5 Relationship between the variances of (**a**) number of nematodes per plant and (**b**) number of nematodes per gram of dry root and root dry weights of parents and F_2 populations. Plants were grown for seven weeks in 650 g pots containing pasteurised soil and inoculated with 500 *P. neglectus*.

(**a**) Number of nematodes per plant



(**b**) Number of nematodes per gram of root

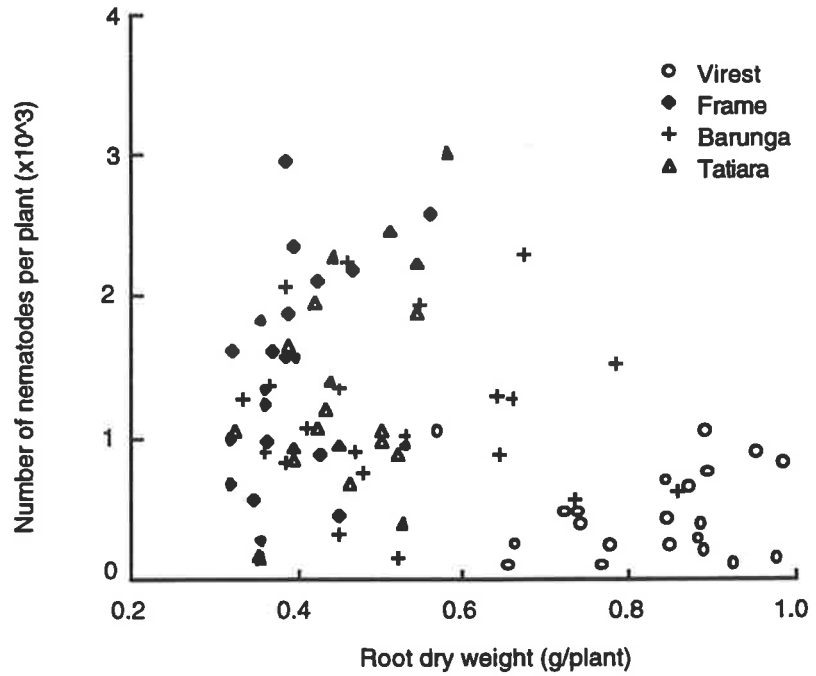


B= Barunga, F = Frame, T= Tatiara, V = Virest; VB, VF and VT = F_2 populations of (Virest x Barunga), (Virest x Frame) and (Virest x Tatiara) respectively.

Figure 13.6 Relationship of (**a**) number of nematodes per plant and (**b**) number of nematodes per gram of dry root with root dry weight. Plants were grown for seven weeks in 650 gram pots containing pasteurised soil and inoculated with 500 *P. neglectus*.

Figure 13.6

(a) Number of nematodes per plant



(b) Number of nematodes per gram of root

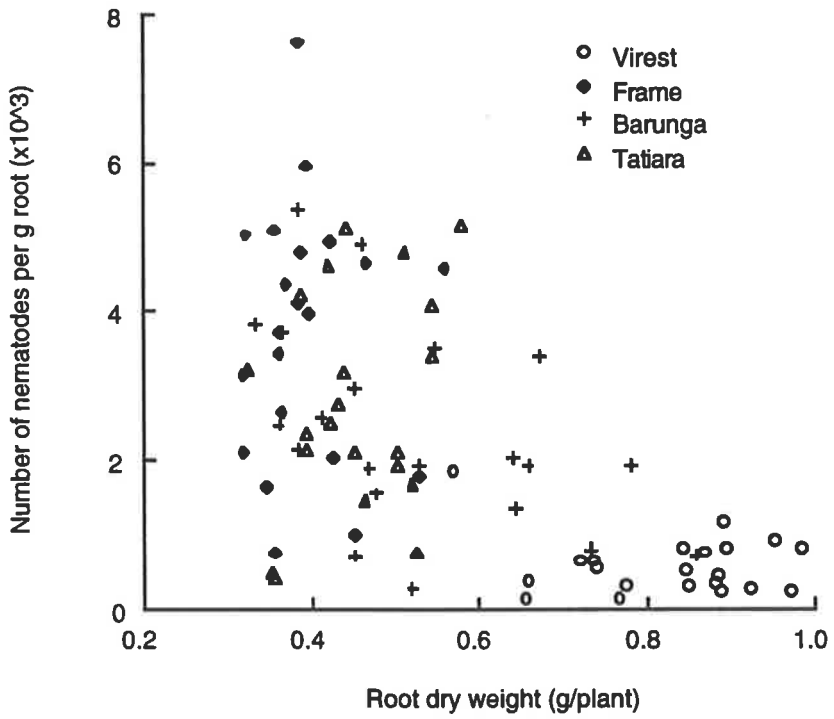
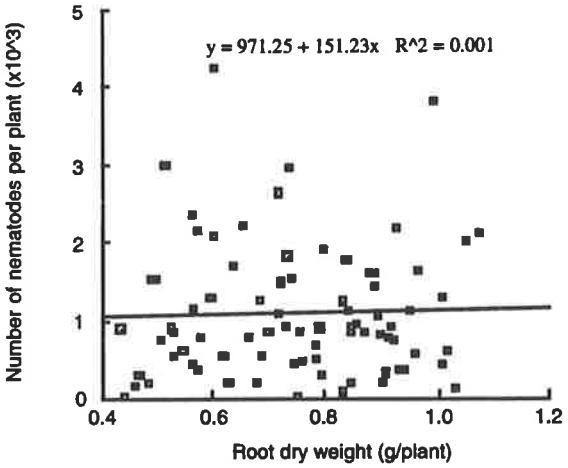


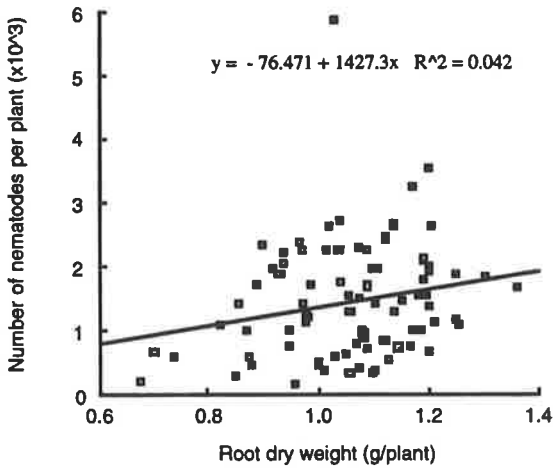
Figure 13.7 Regression between number of nematodes per plant and root dry weight of F_2 populations of (**a**) (Virest x Frame), (**b**) (Virest x Barunga) and (**c**) (Virest x Tatiara). Plants were grown for seven weeks in 650 g pots containing pasteurised soil and inoculated with 500 *P. neglectus*.

Figure 13.7

(a) (Virest x Frame)



(b) (Virest x Barunga)



(c) (Virest x Tatiara)

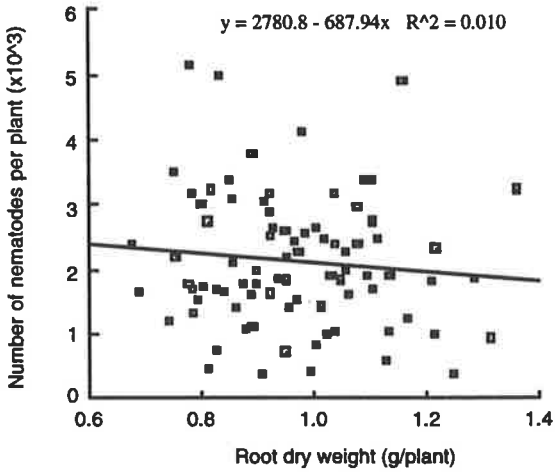


Figure 13.8 Relationship between number of nematodes per plant and root dry weight of parents and their F_2 populations.

(**a**) Virest and Frame and their F_2 population (Virest x Frame)

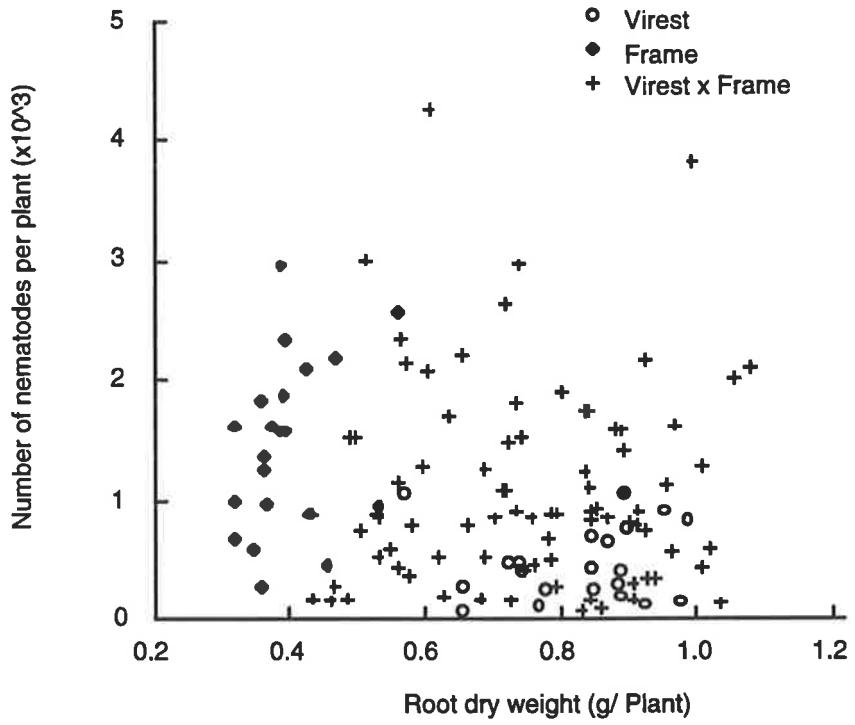
(**b**) Virest and Barunga and their F_2 population (Virest x Barunga)

(**c**) Virest and Tatiara and their F_2 population (Virest x Tatiara)

Plants were grown for seven weeks in 650 g pots containing pasteurised soil and inoculated with 500 *P. neglectus*.

Figure 13.8

(a) Virest, Frame and (Virest x Frame)



(b) Virest, Barunga and (Virest x Barunga)

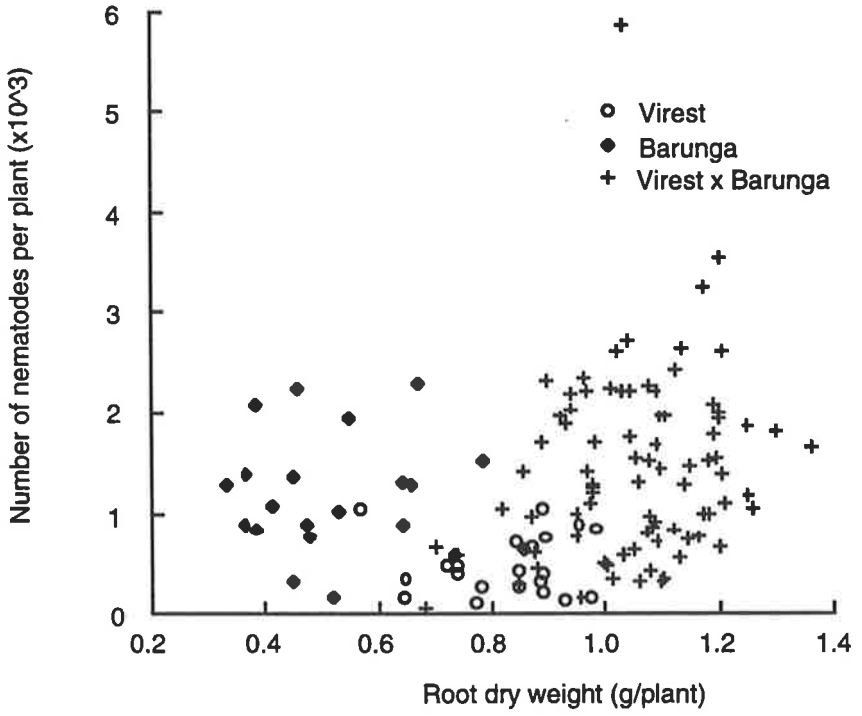


Figure 13.8 continued

(c) Virest, Tatiara and (Virest x Tatiara)

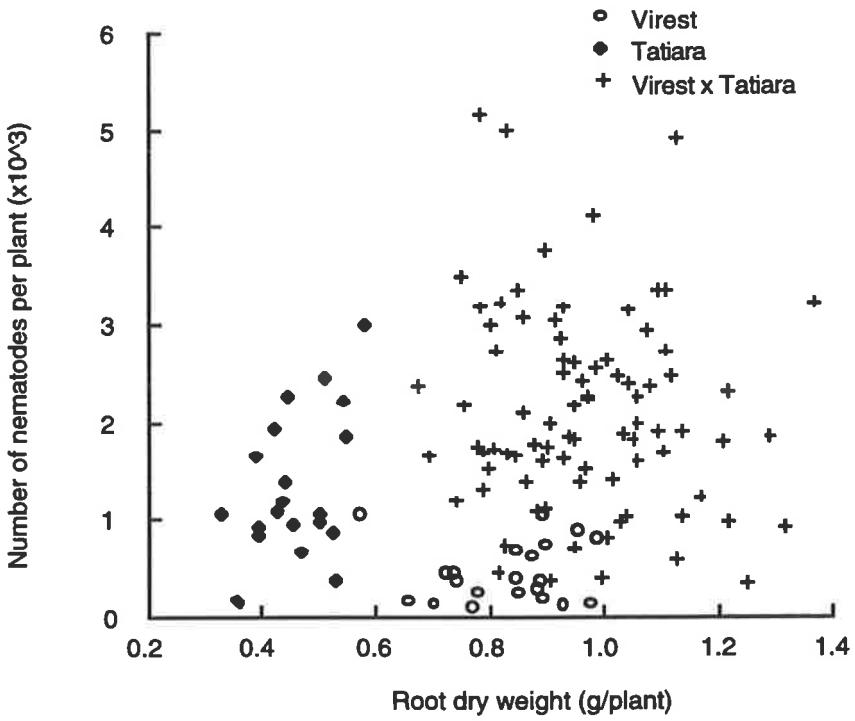
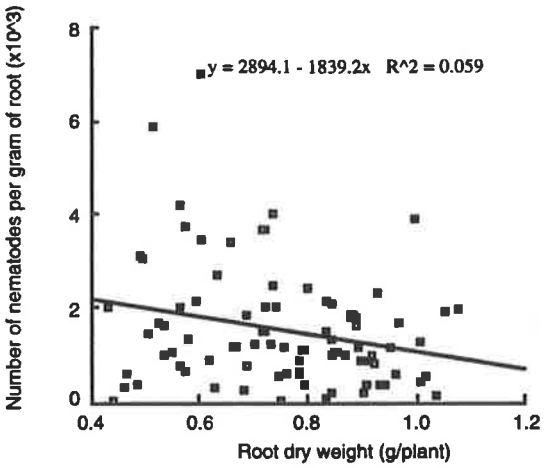


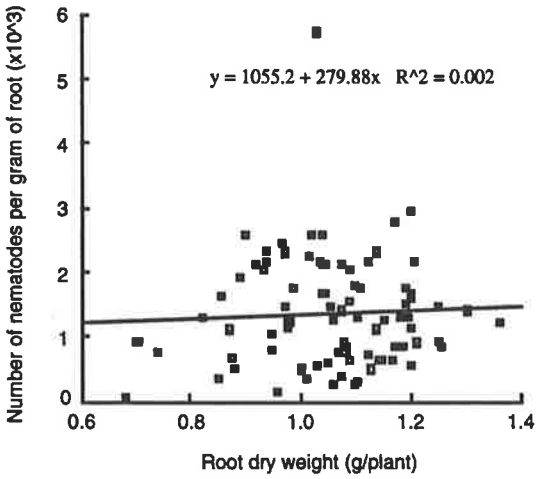
Figure 13.9 Regression between number of nematodes per gram of dry root and root dry weight of F_2 populations of (**a**) (Virest x Frame), (**b**) (Virest x Barunga) and (**c**) (Virest x Tatiara). Plants were grown for seven weeks in 650 g pots containing pasteurised soil and inoculated with 500 *P. neglectus*.

Figure 13.9

(a) (Virest x Frame)



(b) (Virest x Barunga)



(c) (Virest x Tatiara)

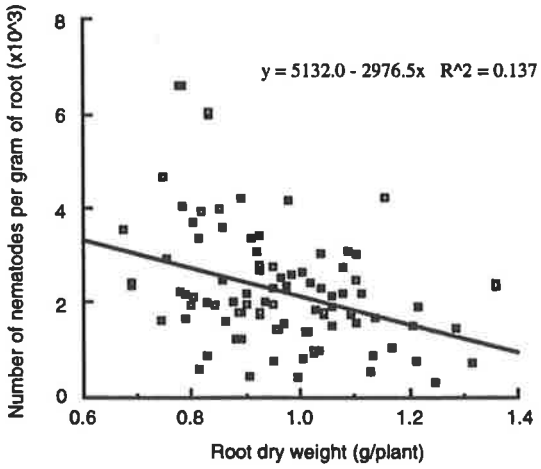


Figure 13.10 Relationship between number of nematodes per gram of dry root and root dry weight of parents and their F_2 populations.

(**a**) Virest and Frame and their F_2 population (Virest x Frame)

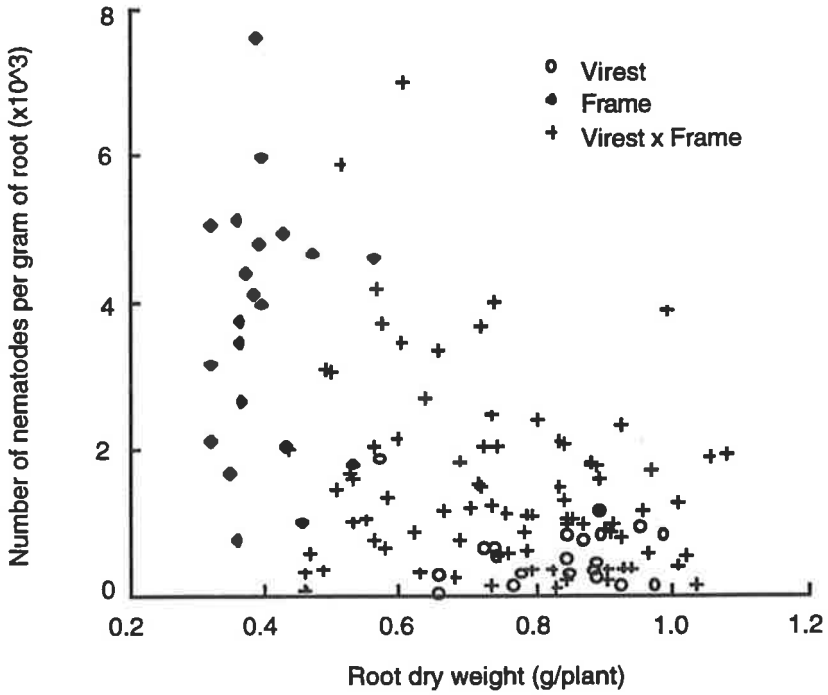
(**b**) Virest and Barunga and their F_2 population (Virest x Barunga)

(**c**) Virest and Tatiara and their F_2 population (Virest x Tatiara)

Plants were grown for seven weeks in 650 g pots containing pasteurised soil and inoculated with 500 *P. neglectus*.

Figure 13.10

(a) Virest, Frame and (Virest x Frame)



(b) Virest, Barunga and (Virest x Barunga)

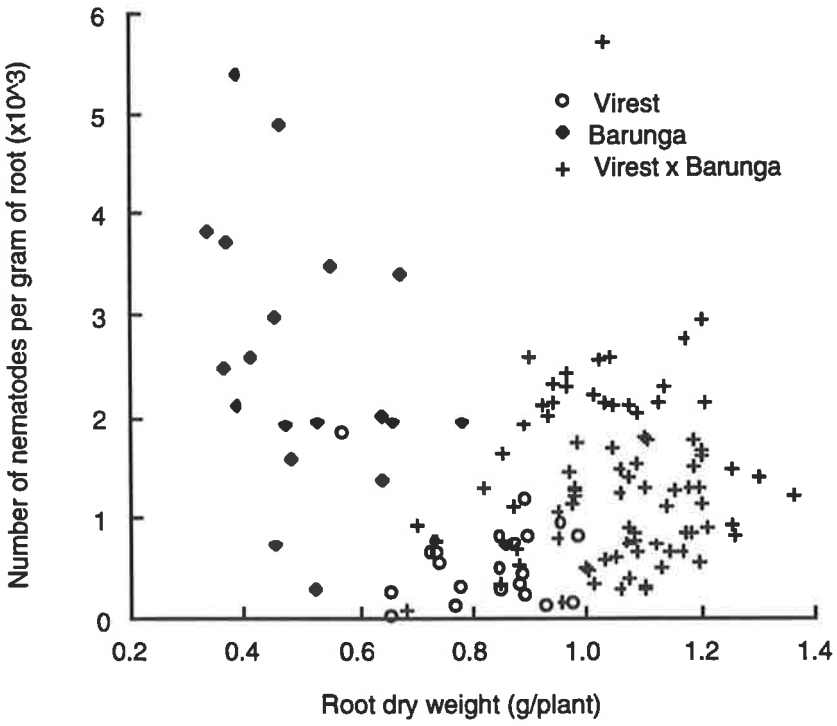


Figure 13.10 continued

(c) Virest, Tatiara and (Virest x Tatiara)

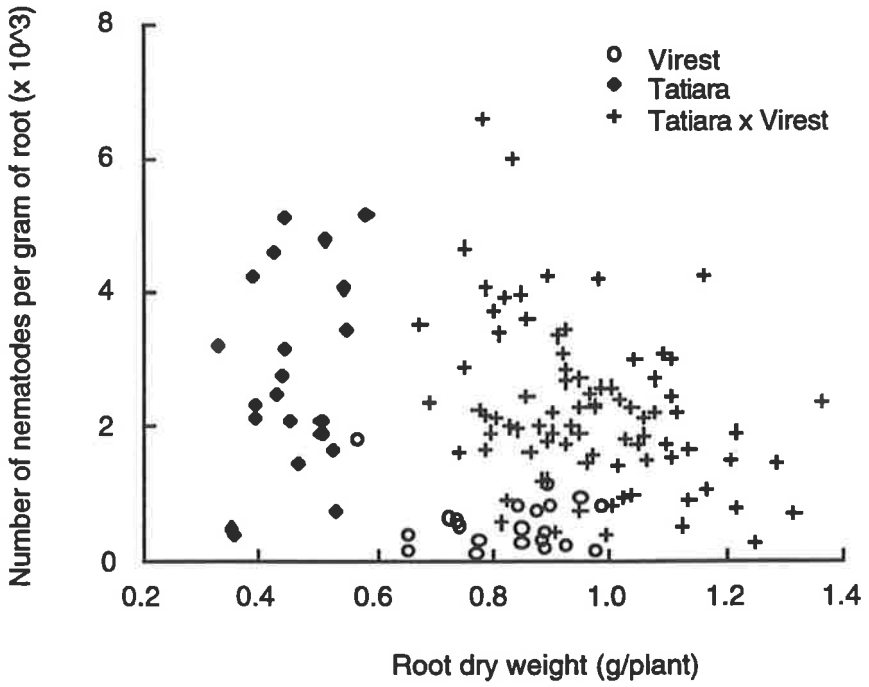


Figure 13.11 Frequency distributions of number of nematodes per plant of (**a**) (Virest x Barunga), (**b**) (Virest x Frame) and (**c**) (Virest x Tatiara) progenies and their corresponding parental varieties. Plants were grown for seven weeks in 650 g pots containing pasteurised soil and inoculated with 500 *P. neglectus*.

Figure 13.11a Virest, Barunga and (Virest x Barunga)

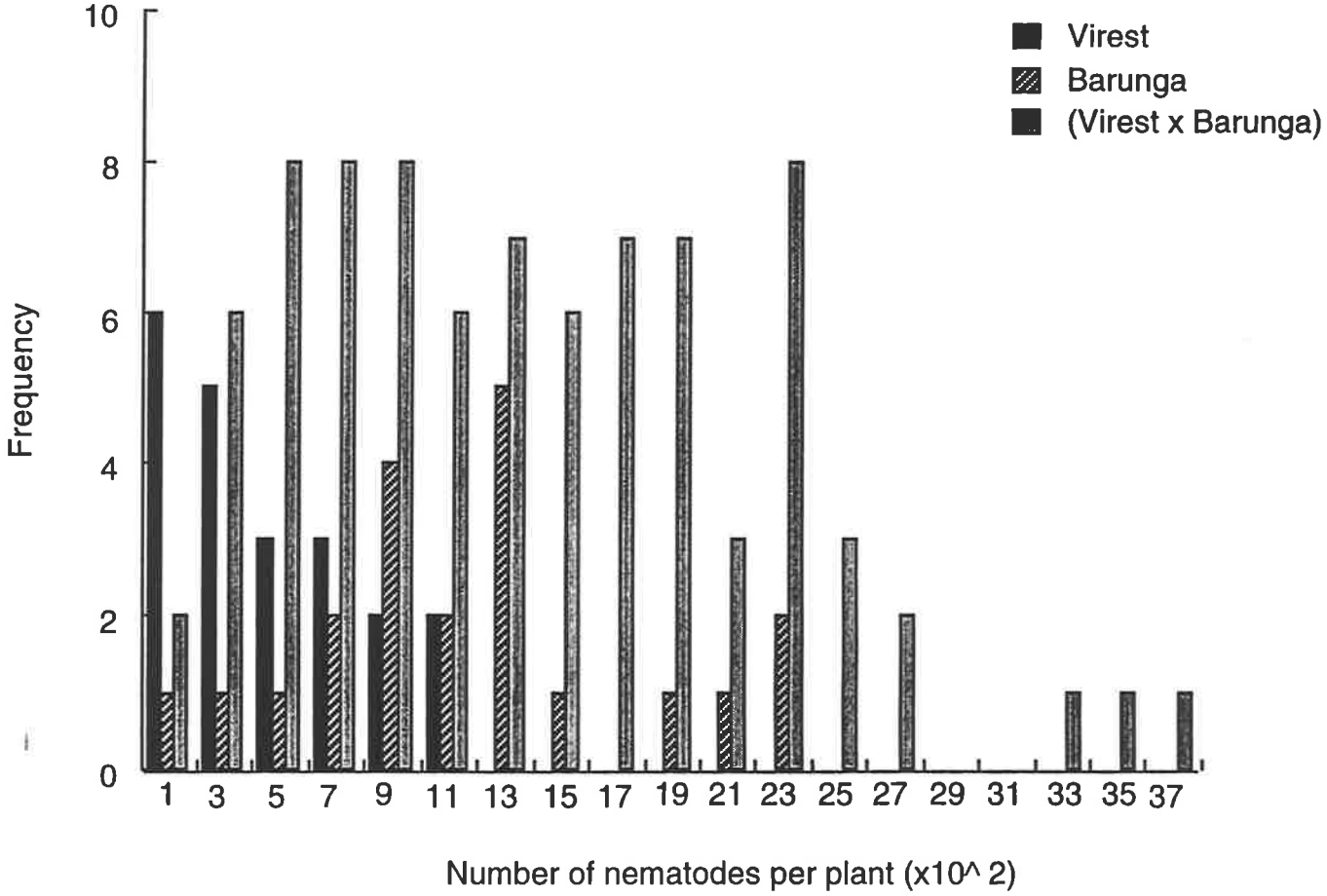


Figure 13.11b Virest, Frame and (Virest x Frame)

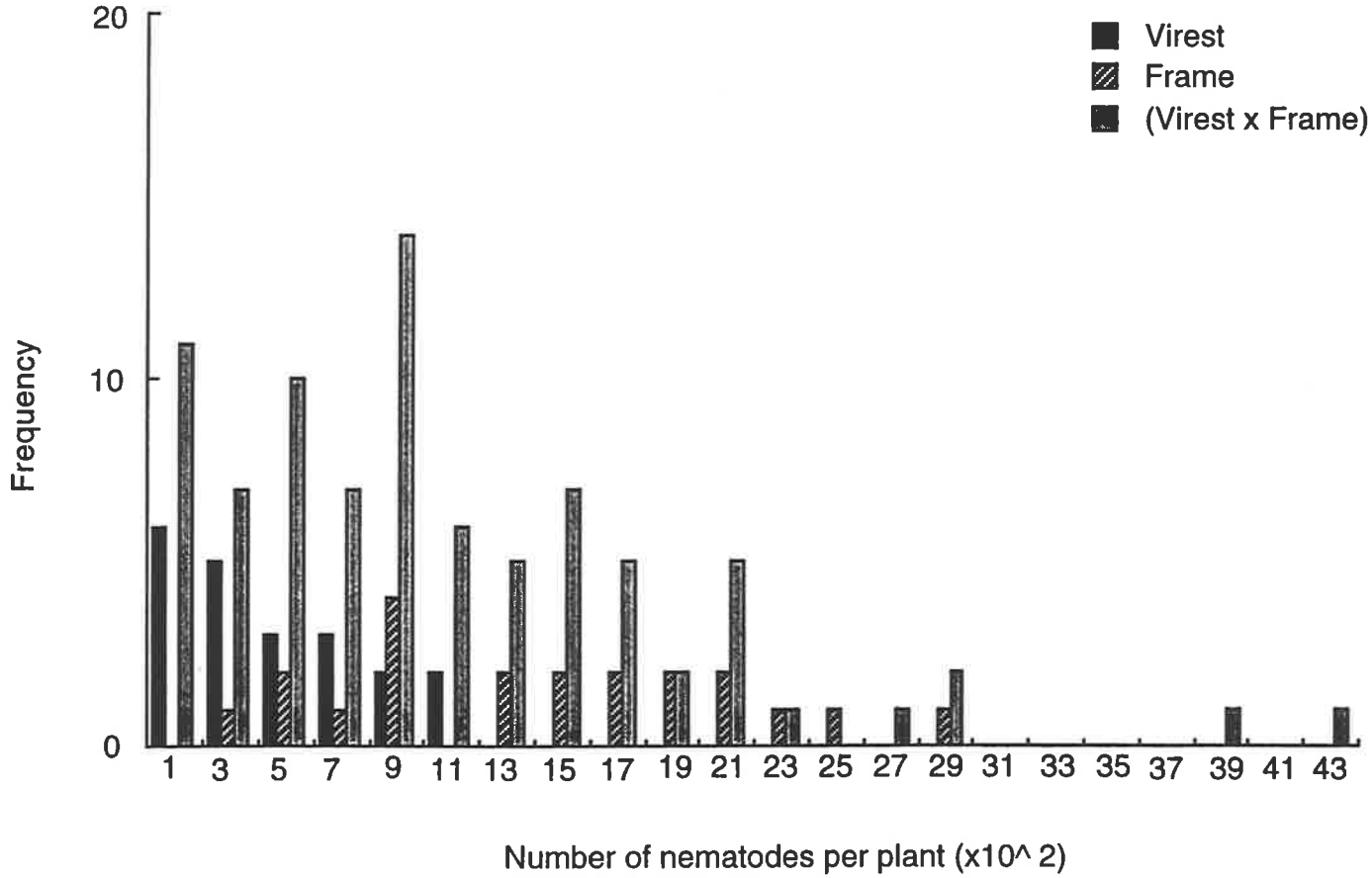


Figure 13.11c Virest, Tatiara and (Virest x Tatiara)

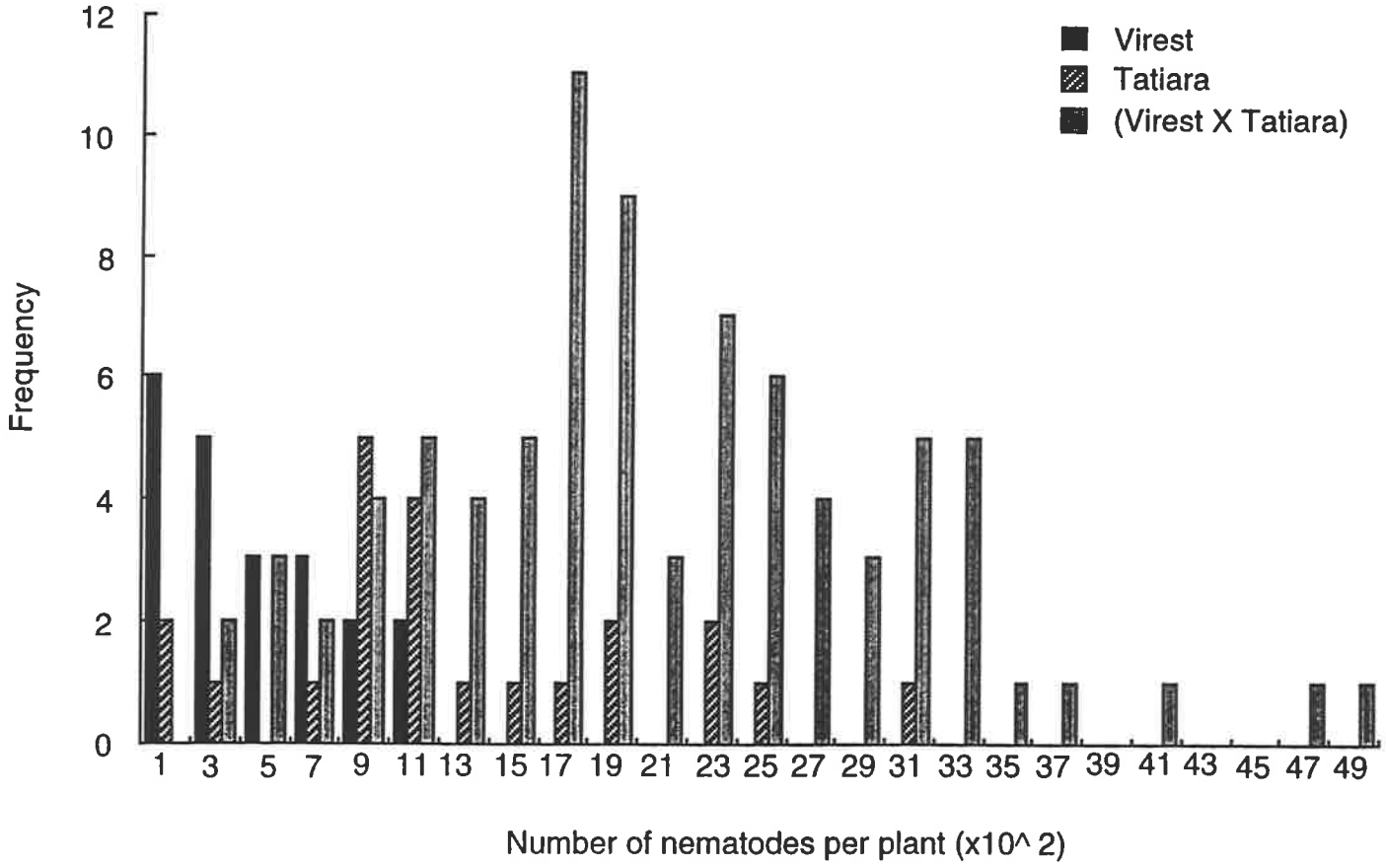


Figure 13.12a Virest, Barunga and (Virest x Barunga)

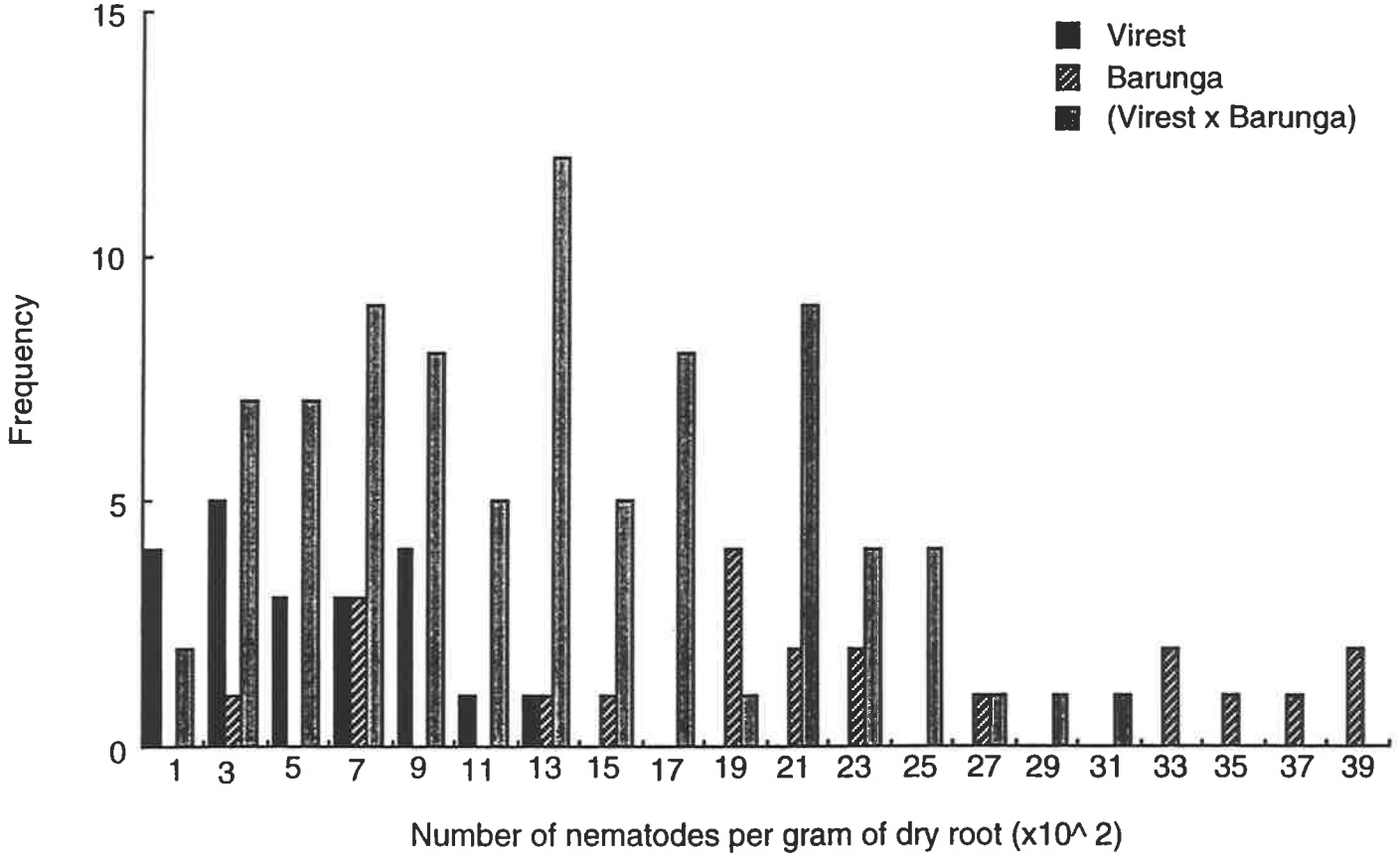


Figure 13.12 Frequency distributions of number of nematodes per gram dry root of (**a**) (Virest x Barunga), (**b**) (Virest x Frame) and (**c**) (Virest x Tatiara) progenies and their corresponding parental varieties. Plants were grown for seven weeks in 650 g pots containing pasteurised soil and inoculated with 500 *P. neglectus*.

Figure 13.12b Virest, Frame and (Virest x Frame)

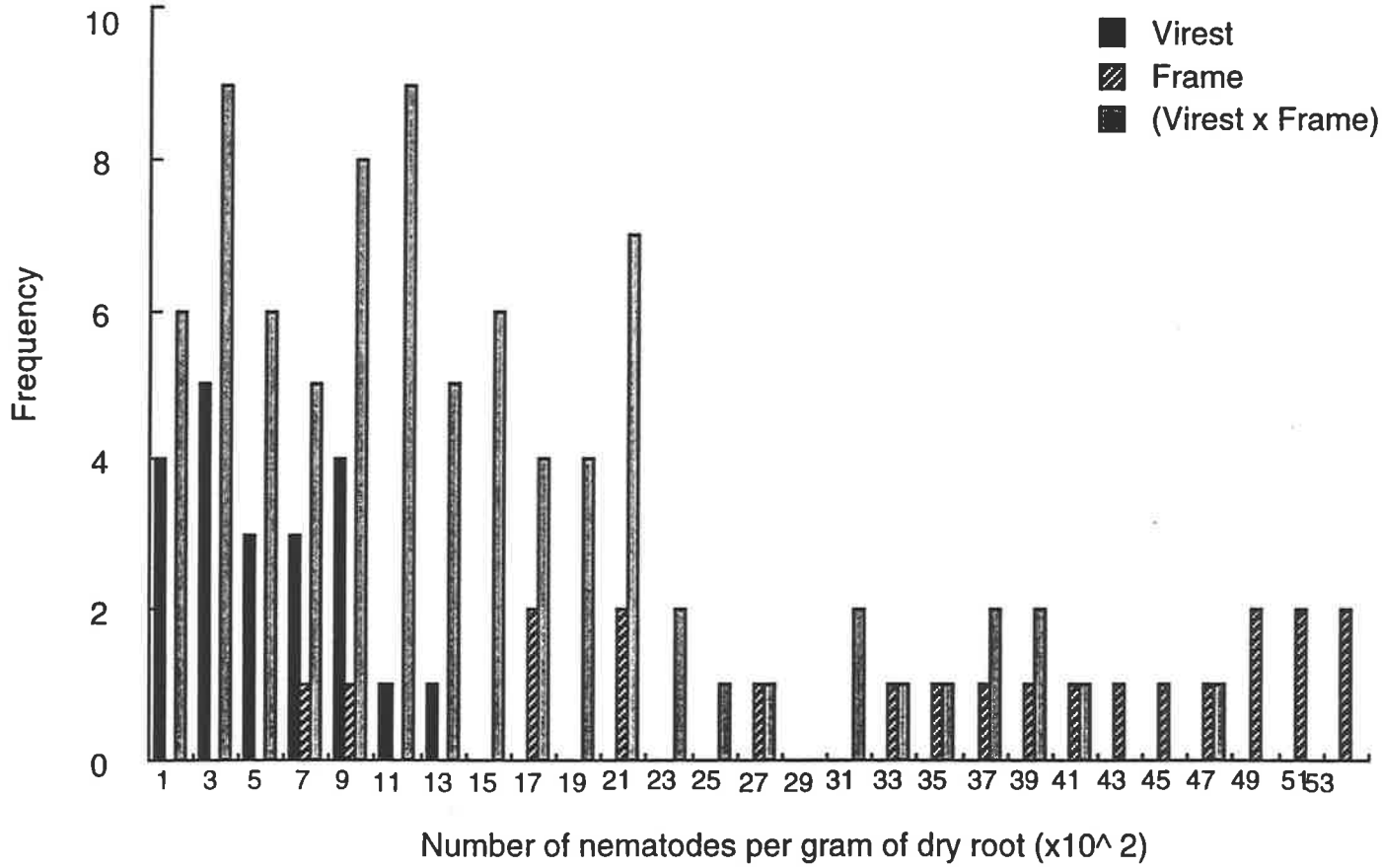
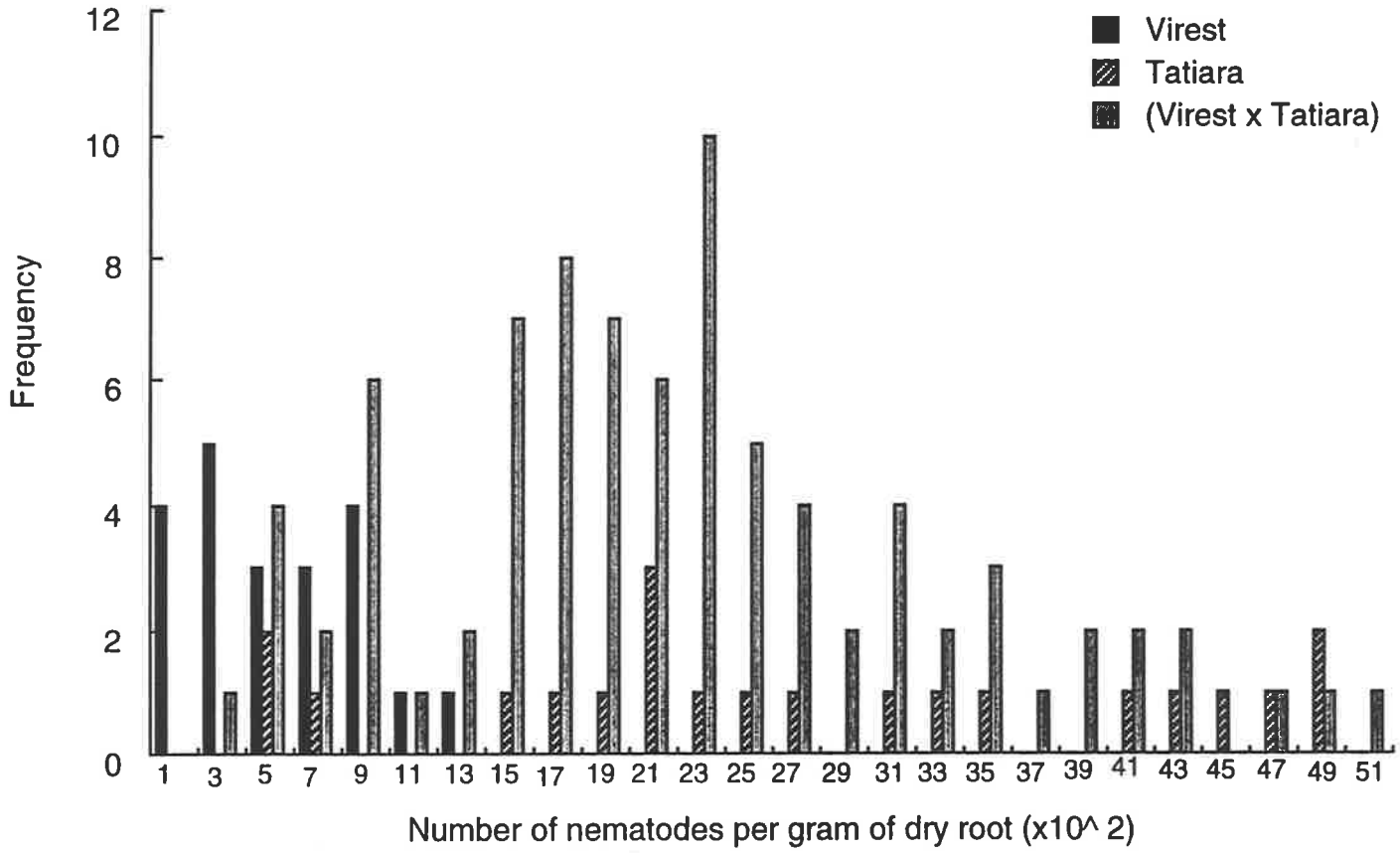


Figure 13.12c Virest, Tatiara and (Virest x Tatiara)



13.4 Discussion

As discussed in previous chapters, the choice of the statistic can affect interpretation of the results. While, in general, it was argued that screening genotypes based on number of nematodes per plant rather than on number of nematodes per gram dry root was preferable, this ignores the problem of hybrid vigour in root growth in early generations which was observed in the experiment reported here. This hybrid vigour has made it difficult to discern the underl ying pattern of inheritance.

Variances and correlations

When considering the variances, we are confronted with several problems. In terms of number of nematodes per plant, plants with higher root growth could demonstrate a higher variance, while in terms of number of nematodes per gram dry root those with a higher root growth could have a lower variance, but only with the latter was a significant regression observed (Figures 13.3a and 13.3b). However, greater variances for number of nematodes per plant is to be expected for the F_2 populations, compared to their parents, due to the segregation for resistance to the nematode, with resistant plants showing a low and susceptible plants a high number of nematodes.

The negative correlation of variance of number of nematodes per gram of dry root with root growth was due to the higher root growth of F_2 populations together with a lower mean number of nematodes per plant. Furthermore, the variance of susceptible plants tends to be much greater than that of the resistant plants because of the "partial escape" of some replicates of susceptible genotypes (i.e. perhaps a limited initial invasion results in a low number of nematodes at the time of assessment).

The low expected variance for F_2 populations in terms of number of nematodes per plant, based on the one or two genes hypothesis (Table 13.1a), compared to those measured in the F_2s, was mostly due to a compratively low value of environmental variance, calculated from the parents. The observed variances in susceptible parents (Table 13.1a) compared to

that of the F_2 populations could be due to their lower root growth (Figure 13.2). Excluding Virest, a significant high positive correlation ($R^2 = 0.68$) was obtained between root dry weight and the variance in terms of number of nematodes per plant (Figure 13.5a). Any genetic explanation based on root dry weight must be treated with caution.

One of the assumptions in an analysis of variances is that the means should be independent of variances. In the case of number of nematodes, the transformation suggested by Proctor and Marks (1974), Ln (number of nematodes + 200), was appropriate, rendering the variances independent of means (Figure 13.4).

Genetic explanations

The genetic explanation based on number of nematodes per plant contrasted with that based on the number of nematodes per gram of root (Section 13.3). In the latter, the estimated number of genes was one (Virest x Tatiara), two (Virest x Frame) or several (Virest x Barunga). This was due to high values of (d) and (E). Since Virest yielded a significantly higher root weight than the other parents, the differences between the parents of each cross for number of nematodes per gram of dry root became large compared to their differences for number of nematodes per plant resulting in a higher value for (d). The problem of using number of nematodes per gram dry root was the significant negative correlation ($R^2 = 0.65$) between variances and root dry weights (Table 13.1b). Plants with higher root growth had a relatively low number of nematodes per gram of dry root and that made the genetic interpretation unconvincing.

Based on data transformation to Ln (number of nematodes per plant + 200) to make the variances independent of means, the genetic control of resistance of the crosses of (Virest x Frame) could be interpreted based on a single gene, that of (Virest x Barunga) based on both one or two genes and that of (Virest x Tatiara) based only on two genes. As argued in the previous section of this discussion, this transformation best meets the statistical requirements. Hence, the genetic explanations from this transformation must carry more

weight than those from other statistics. If a single gene in Virest confers resistance, the genetic situation for resistance to *P. neglectus* would resemble that to *P. thornei* (J. P. Thompson, pers. comm.) where in GS 50A a single gene confers resistance.

Biological factors

Excluding the resistant variety Virest, there was an insignificant positive correlation between the amount of root growth and the number of nematodes per plant (Figure 13.3a). Hybrid vigour expressed in root growth of F_2 populations (Figure 13.2) did not support markedly increased access of nematodes to roots or enhanced feeding, consequently the multiplication rate of nematodes in F_2 populations was similar to that in the parents. Inoculating plants with a low number of nematodes minimises the effect of root growth on nematode reproduction rate in the absence of a resistance mechanism, and it seems that in the results reported here nematode multiplication was virtually independent of the amount of food available in susceptible plants (Figure 13.3a).

A larger number of root hairs, as a result of the extensive root system in F_2 populations, could be expected to encourage a greater multiplication rate of the nematode as root hairs are preferred by nematodes for establishment and feeding (Dropkin, 1980; Chapter 12). However, one may argue that since the resistant parent (Virest) also had a larger number of root hairs compared to susceptible parents (Figure 13.2), therefore the resistance appears to have operated independently of root hair growth. This argument leads to the conclusion that the resistance mechanism is either expressed in the root hairs or that multiplication of the nematode on root hairs is insignificant compared to that which occurs after feeding in the cortical tissue.

Since only one resistant parent was used in all three crosses, the different genetic interpretation for the three crosses might have been a consequence of differences in the level of susceptibility of susceptible parents. Tatiara has shown a relatively lower number of nematodes than the other two susceptible parents in preliminary experiments (Vanstone

et al., 1994; A. J. Rathjen and P. F. Lonergan, unpublished data). Obviously these are minor background genetic effects which alter the multiplication rate of the nematode. It is not unexpected that these should confound the genetic explanation, especially in view of the large environmental variance in the system.

Further investigations

Obviously, the method of estimation of number of genes, applied using the expected variance, should be used cautiously, particularly when variances are positively or negatively dependent on factors such as root growth (Figures 13.6a and 13.6b). The hybrid vigour for root growth in F_2 populations is due to heterozygote genotypes. The amount of heterozygosity is reduced by 50% with any succeeding selfing generation for any heterozygote locus. With succeeding selfings, the offspring of any self fertilised individual plants of the F_2 and subsequent generation will increasingly resemble each other, within family heterozygosity and heterogeneity decreases and that between families increases. Therefore, further investigations such as analysis of F_3 families, where the observed heterozygosity for root growth is reduced, segregation for resistance and susceptibility enhanced and the between families variance increased, are needed. If some F_3 families have a distribution similar to that of the resistant parent, Virest, this will allow investigators to make a more precise conclusion regarding the number of genes controlling resistance to the nematode.

CHAPTER 14

GENERAL DISCUSSION

Root lesion nematodes attack root systems and result in symptoms similar to some other soil-borne pathogens, wilting, yellowing of lower leaves, senescence and stunting and alone or in combination with other diseases can result in large grain yield reductions within infected paddocks. Wheat is the major host of *P. neglectus* in South Australia and when sown into infected paddock is extensively damaged. While barley is less susceptible to the nematode than wheat, when it is grown on wheat stubbles a yield loss averaging 1t/ha has been observed (Eagles *et al.*, 1993). Medics, the most widely advocated of the rotational pasture plants with wheat in South Australia, are extensively damaged by the nematode resulting in poor growth and restricted nodulation (A. J. Rathjen, pers. comm.; Vanstone *et al.*, 1993a).

Greater top dry weight (up to 50%) following the control of the nematode by all methods adopted in the experiments reported in this thesis (Figures 4.4, 4.5a and 4.11) indicated that infection by the root lesion nematode *P. neglectus* can reduce top growth of infected plants. However, in some circumstances (Section 5.2) application of Temik® resulted in a lower grain yield. The benefits obtained from Temik® application depend on the nematode density at the time of sowing, soil fertility, other pathogens present in the soil and other environmental factors counteracting phytotoxic and other effects of the nematicide. When the soil is heavily infected and the texture more favourable for nematode movement as at Palmer (Section 5.2.3 E), nematode damage to plants will be more serious, so any method of reducing the nematode population will have a positive effect on plant growth (Chapter 4). In the experiments reported in Chapters 4 and 5, controlling the nematodes, using different treatments including Temik®, freezing or rapid drying, elevated the content of a number of nutrients such as N, P, K Ca, Mg and Mn (Table 4.5 and 4.7). The enhanced growth could be a result of improved nutrient uptake. Alternatively improved

water supply through the relatively undamaged root system could explain the increased yields.

14.1 Yellow leaf symptom

The presence of root lesion nematode is often associated with yellow leaf symptoms, which are a cause of a reduction in plant photosynthesis and as a result plant yield. It was therefore possible that the intensity of yellow leaves in different varieties is an indication of the extent of tolerance to the nematode. Based on the experiments conducted in the glasshouse (Chapter 4) and under field condition (Chapter 5), or using pasteurised soil with aseptic nematodes and examining different genotypes (Chapter 8), there are four factors involved in the yellow leaf symptoms, including extent of nematode invasion, deficiency of nitrogen and to some extent phosphorus, varietal differences (genotype) and water stress. The effect of these factors on the extent of yellow leaf symptoms could be summarised as below;

Nematode presence

Nematodes increased the level of yellow leaf symptoms expression as in both the Temik® and freezing treated pots plants were greener than in the no control of nematode treatment (Plate 4.2a). The effect of nematodes on the expression of symptoms was greater in some genotypes than in other genotypes (Figure 8.6). The relative concentration of nutrients in roots of plants growing at Palmer was higher compared to that of plants growing at Roseworthy (Tables 5.5 and 5.7 and Tables 5.6 and 5.8). Nematode density was also greater at Palmer than Roseworthy. Although different environmental factors might be responsible for the difference of the root/shoot concentration at these two sites, there are reports that plants infected with a high population of nematodes, due to restricted water absorption, are not able to translocate the nutrients from the roots to shoots efficiently (Orion *et al.*, 1984). Nematodes through wounding the roots increase the opportunity for infection by fungi and bacteria (Taheri

et al., 1995). Fungi and bacteria may reduce the ability of plant to absorb some ions including Mn (Pedler, 1994).

Fertiliser

It seems that the symptom of yellow lower leaves is highly related to nitrogen deficiency and to a less extent to that of phosphorus (Figure 5.2 and Table 5.3). In Chapters 4 (Plate 4.2b) and 5 (Table 5.3) it was revealed that application of nitrogen or N+P fertiliser could reduce the symptoms of yellow leaves of plants growing in nematode infested soil. Although the yellow leaf symptoms were not merely confined to nitrogen deficient plants, nitrogen application alone could remove most of the symptoms (Plates 4.2b). Where N+P was applied to the soil of nematode infested fields, the symptoms of yellow leaves were almost completely removed (Figure 5.2). Nitrogen fertiliser, through enhancing root growth (Section 4.2.3.1), stimulates the root system to proliferate extensively through the soil environment, giving a greater access of roots to other nutrients. Uptake of P, K, Ca, Mg, Mn, Na and S was facilitated by nitrogen application as plants treated by nitrogenous fertilisers had a significantly higher concentration of these nutrients in their shoots compared to those supplied with fertilisers lacking nitrogen (Tables 4.9 and 5.3).

Nitrogen application also demonstrated a nematicidal effect. The application of calcium nitrate significantly ($P<0.05$) reduced the number of nematodes both per gram of dry root and per pot (Table 8.3). While a lower number of nematodes per gram of root in plants receiving nitrogen could be accounted for by a significantly higher root growth stimulated by the nitrogen fertiliser, but the significantly lower number of nematodes per pot in the plants treated with additional nitrogen (Table 8.3) can only be explained by the nematicidal effects of nitrogen. This again confirms the result of Kimpinski *et al.* (1976) and Vanstone *et al.* (1993c).

Genotype

Molineux appears inherently more susceptible to expressing the yellow leaf symptoms and less efficient in absorbing nitrogen than Spear and Janz (Tables 5.5 and 5.6). Physiological factors such as cytokinin production or the rate of nutrient translocation could be also involved in the lower level of expression of the yellow lower leaf symptoms in Spear than Molineux. Janz and Spear were more efficient in absorbing nitrogen compared to Molineux as they showed a higher concentration of N in their shoots than Molineux (Tables 5.5 and 5.6). Molineux also showed less efficiency in absorbing phosphorus from the soil (Table 5.6). Plants with greater efficiency in nitrogen and phosphorus absorption may be less sensitive to a reduction of the root's ability in nutrient uptake resulting from nematode infection. Genotypes with the higher root growth rate were greener and had a significantly greater shoot fresh weight (Figure 8.7b). These plants, by more thoroughly exploring the soil volume and having a lower nematode density in their roots, would have a higher portion of healthy roots compared to the heavily infected plants, and would likely be more efficient in taking up nutrients from the soil.

Water stress

Water uptake is reduced due to the damage caused to the roots by nematodes. Water stress was not investigated in the experimental work for this thesis, but it is often observed that plants infected with nematodes are more likely to wilt than uninfected plants.

Yellow leaf symptoms are of little use in screening for tolerance to the nematode as Molineux was susceptible to expressing the symptoms even in the absence of nematodes (Figure 8.6). In general the yellow leaf symptoms are often an indication of the presence of root lesion nematodes in the field through their reducing the uptake of nitrogen and phosphorus. However, other factors such as plant inefficiency in

absorbing nitrogen or soil deficiency in nitrogen and/or phosphorus are also involved in the expression of the symptoms.

14.2 Resistance

The most promising and most economical method of nematode control is through growing resistant varieties. These varieties, if available and also tolerant to the nematode, would result in better yields on nematode infested soil without increasing the nematode population and would lessen the chance of spreading the infestation to unaffected soil. Developing resistant varieties through screening wheat varieties or searching for resistance sources in species closely related to bread wheat and translocating the gene(s) to adapted local varieties would allow farmers to produce high-yielding crops in heavily infested soils.

Screening conditions

Sampling time is an important factor in measuring multiplication rate for screening resistance genotypes to root lesion nematodes. Host factors affecting nematode multiplication are varietal characteristics including the rate of growth, time of maturity and extent of tolerance, and abiotic factors include nutrition and water availability in the soil (Vanstone and Nicol, 1993; Fisher, 1993).

The rate of nematode multiplication depends on the initial nematode population introduced into the root system, the rate of root growth and varietal characteristics such as resistance and tolerance. The maximum density of nematodes in the roots of intolerant or resistant varieties is less than tolerant varieties. In general, the required time for nematodes to reach to the maximum density is negatively correlated with the initial population and positively with root growth. At a low initial density there is little constrain on the multiplication of nematodes and, providing the host grows reasonably well, minimal damage occurs due to the nematode attack. In these conditions, differences in the rate of multiplication in different varieties will be expressed, so it is

possible to screen effectively for resistance. Depending on the soil physical characteristics and the size of the pot, the suitable initial number for differentiation between varieties could be different. In pot experiments with 650 grams soil, a population around 500 nematodes (including eggs and all larvae stages) is adequate (J. M. Fisher, pers. comm.). The work in this thesis confirmed this suggestion (Chapters 12 and 13).

A population of nematodes consisting of a single biotype is vital for screening for resistance, otherwise the mixture of different species or different biotypes of the nematode may result in failure to detect genotypes with resistance for specific biotypes. As an example of this at the species level, the wheat variety GS 50A while resistant to *P. thornei*, is susceptible to *P. neglectus* (Chapter 10). As many South Australian soils contain a mixed population of both species, GS 50A would have as high number of nematodes as varieties susceptible to both species.

Terminating pot experiments at around eight weeks after inoculation and counting number of nematodes per plant has proved suitable for ranking genotypes for nematode reproduction ability or resistance (Chapter 8).

The choice of statistics will affect the results. For screening to detect resistant varieties, the number of nematodes per g of root (Dennis *et al.*, 1989; Marull *et al.*, 1990) or number of nematodes per plant (Townshend, 1989) have been used. Nematode multiplication rate is affected by root growth (Dennis *et al.*, 1989). Faster growing roots will always appear more resistant when judged on the number of nematodes per gram (Chapter 8), even though this character may have no influence on multiplication of the nematode and hence on the number of nematodes present in the soil to invade a subsequent crop. As the economic damage by nematodes is predominantly related to the size of the population invading the seedlings of a subsequent crop, the number of nematodes per plant is a more appropriate estimate of resistance for an agricultural crop than the number per gram of root (J. M. Fisher, pers. comm.). Screening for resistance

on the basis of the number of nematodes per gram of root could result merely in selecting plants with a higher root growth rate (Figure 8.2a and 8.2b).

Genetic variability of resistance within bread wheats and in related species

Due to insufficient resistance in adapted wheats against particular pests or diseases, resistance has often been sought in varieties that are poorly adapted to the area in which the breeding program is being carried out. Before resistance had been identified in *T. aestivum* in screening conducted in association with the Waite wheat breeding program, some closely related species of bread wheat were examined in the work reported in this thesis. Durum varieties and triticales demonstrated a lower (42 and 47%, respectively) nematode multiplication, (Table 9.2) than the wheat varieties. All lines with rye chromosomal material demonstrated a comparatively low number of nematodes compared to the wheats (Figures 10.1a and 10.1b). Durum wheats and triticales examined here have two genomes (A and B) in common and both lack the D genome. This suggests that different alleles within the D genome might be responsible for susceptibility to the root lesion nematode. The lower number of nematodes per plant of Tahara and Abacus triticales could be also due to some activate alleles in the rye genome (R). Since triticales have both the durum and rye genomes, these genomes may have a complementary effect in increasing resistance to the nematode.

Abacus, consisting of rye and tetraploid wheat (durum) genomes, could be used either as a rotational crop or as a donor parent to incorporate the *P. neglectus* resistance gene (or genes) into commercial local wheat varieties. This needs great effort as wheat-rye translocation would have undesirable gene(s) with deleterious effects (Section 2.1.3). Among the many wheat-rye translocations, only 1B/1R translocation has been commercially acceptable, so the prospect of transferring the resistance from rye to wheat is not promising on historical grounds.

Fortunately, there appears to be sufficient resistance to *P. neglectus* in wheats such as Persia 20 and Virest, exotic materials from Iran and Italy respectively (Chapter 11). A significantly lower number of nematodes was counted for Persia 20 and repeatedly for Virest and this suggests the existence of resistance gene or genes to *P. neglectus* in these varieties.

14.3 Mechanism of resistance

Resistance is measured by the ability of the nematode to penetrate, feed, develop and reproduce on its host and could operate at any time during the nematode's life cycle. Resistant varieties (Persia 20 and Virest wheat and Abacus triticale) showed non-significant lower nematode penetration (Table 12.1) than Spear, the susceptible check variety.

Mostly, resistance in plants is expressed after nematode invasion, resulting in larvae being unable to complete development and reproduce (Cotten, 1970; Cook *et al.*, 1974). The time of moulting and commencement of egg laying was delayed in the resistant genotypes compared to the susceptible (Chapter 12; Plate 12.2). Resistant plants may lack substances necessary for the development and reproduction of the nematodes, or contain them in insufficient amounts which results in a failure of nematodes to reach maturity (Giebel, 1982).

A significant result, which is illustrated in Plate 12.3, was the phenomenon of dead cells surrounding the nematodes in the resistant plants which could be based on plant tissue hypersensitivity to nematode infection. Hypersensitive reaction is a rapid response of the plant to the nematode that prevents development of the nematode and its feeding site. The host-parasite interaction can stimulate, in the host, biochemical reactions that cause histological changes, such as host cell necrosis. Necrosis then occurs around the nematode, walling it off and either delaying development or causing the nematode to die. In resistant plants the necrotic area, therefore, is confined to the cells surrounding

the nematode (Plate 12.3), whereas in the susceptible plants necrosis occurs slowly and expands to neighbour cells (Trudgill, 1991).

Further work is needed to confirm the mechanism of resistance to *P. neglectus* as the results presented here had a high level of statistical variation. Understanding the mechanism of resistance would help investigators to conduct screening experiments with more efficiency. Penetration rate in resistant and susceptible plants need to be thoroughly examined by developing a method to give an equal chance for all parts of root to be penetrated by the nematodes. It will reduce the variability between replicates and in turn will lessen the experimental error resulting in differentiation between genotypes more accurately. Nematode development in resistant and susceptible plants needs to be studied by examining the development of the sexual organs of the nematodes in the resistant and susceptible genotypes. In case of significant changes in nematode development in resistant plants, understanding the causal agent(s) will facilitate screening resistant varieties at laboratory level. The time of egg laying needs to be re-examined by monitoring the eggs in both roots and the soil.

14.4 Genetics of resistance

The probability of success in a breeding program aimed at incorporating a specific character is inversely correlated to the number of genes by which the character is controlled. So questions of great importance to a breeder are how many genes are involved and how can the gene effects be recognised unambiguously. An understanding of the genetic control of resistance of wheat to *P. neglectus* therefore would facilitate the breeding of resistant varieties.

Almost all the genetically identified resistances used in breeding programs are controlled by single, dominant major genes (O'Brien and Fisher, 1979; O'Brien *et al.*, 1980; Sidhu and Webster, 1981). Resistance of maize to *P. zeae* and *P. brachyurus* is due to two dominant genes with an additive effect (Savazaki *et al.*, 1988). In Queensland (J. P. Thompson, pers. comm.)the resistance of the Gatcher selection GS 50A to *P. thornei* is controlled by a single, dominant gene.

Due to lack of time available for this thesis, genetic studies were undertaken using F_2 populations. Otherwise, as discussed in Section 13. 4, F_2 derived families in the F_3 or double haploids lines would have been preferred. As discussed previously (Chapter 8), the choice of the statistic can affect the interpretation of the results. While in general it was argued that screening genotypes based on number of nematodes per plant rather than number of nematodes per gram dry roots was preferable, the hybrid vigour for the root growth in the F_2 populations made it difficult to discern the underl ying pattern of inheritance using this statistic.

Because of the continuous distribution of nematodes numbers (Figures 13.11a-c and 13.12a-c) (due to varying penetration ratios and multiple generations) it was not possible to define the genetic ratios directly. Therefore variances were used to identify the number of genes involved in the resistance to the nematode in Virest. However variances were highly correlated with the means for number of nematodes. Plants with higher root growth could demonstrate a higher variance for number of nematodes per plant, but a lower variance in terms of number of nematodes per gram of dry root (Figures 13.3a and 13.3b). The variance of susceptibles tends to be much greater than that of the resistants because of the "partial escape" of some replicates of susceptible genotypes i.e. perhaps a limited initial invasion resulting in a low number of nematodes at the time of assessment. These made the genetic interpretation based on both number of nematodes per plant or per gram of root unconvincing. However the transformation suggested by Proctor and Marks (1974), Ln (number of nematodes + 200) was appropriate to rendering the variances independent of means (Figure 13.4).

Based on data transformation, the genetic of resistance of the crosses of (Virest x Frame could be interpreted based on single gene, that of the (Virest x Barunga) based on both one or two genes and that of the (Virest x Tatiara) only based on two genes. In accordance with that for *P. thornei,* the inheritance of resistance to *P. neglectus* could

be simple. The variation in the estimated number of genes was due to differences in susceptibility level of susceptible parents (Figure 13.2).

Improving inoculation method by introducing the nematodes to roots more evenly and counting un-extracted nematodes in the roots after misting as well as extracted ones will help overcome the inaccuracies. To investigate the genetic of resistance, examining F_3 families or double haploid lines are preferred. With any succeeding selfing 50% of heterozygosity is reduced. Selfing will also eliminate the effect of hybrid vigour expressed in the F2's examined here.. Producing double haploid lines, an alternative method to continued selfing, would also facilitate differentiation of resistant plants due to the absence of any heterozygote genotypes.

14.5 Conclusion and future work

Although application of nitrogen could be recommended to farmers to overcome the yield lost caused by root lesion nematodes, a high rate of nitrogen is required to satisfactorily reduce nematodes. To have the maximum yield plants should be supplied with additional phosphorus as well as the nitrogen. Unfortunately this will reduce nematicidal effect of the nitrogenous fertiliser as well-fertilised plants leave a higher nematode population to invade the next crop (Thompson, 1989; Chapters 4 and 5).

Either of the triticales Tahara and Abacus could be used as a rotational crop with wheat, or if necessary, as a donor parent for transferring the gene or genes responsible for resistance to the nematode to cultivated wheat.

It was demonstrated that two lines of bread wheat (Virest and Persia 20) have a satisfactory level of resistance to the nematode. Transferring the gene or genes from these lines, although is easier than from rye or triticale, would be difficult as both are poorly adapted to southern Australia and have many undesirable genes. To restore recurrent parent, depending on its similarity to donor parent, several backcrosses will

be necessary. In each generation plants should be carefully examined for the rate of nematode reproduction and resistant plants are only used for next crosses.

Improved methods of screening resistant varieties are needed. The inoculation method needs to be improved to minimise variation between replicates and a method to non-destructionaly identify resistant plants need to be established. Using twin pots with half the plant roots in each, one of which is inoculated, and harvesting the inoculated half could enable the investigators to extract nematodes and save plants for seed production and further crossing. Mixing the aseptic nematodes with pasteurised soil evenly before transplanting the seedlings into the pots, although damaging a percentage of nematodes, could reduce the variability between replicates.

Further work is necessary to find the chromosomal location of the resistant gene(s). This would help the investigators to map the gen(s) and identify linkages with molecular markers to aid in the transfer of these genes to adapted commercial varieties. The development of molecular markers for identification of resistant genotypes enables investigators to screen large numbers of plants more accurately.

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