Know-how for Horticulture™

Improved control of nematodes in carrot production

Dr. Frank Hay
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Final report for project VG99020 (June 2004)

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Project VG99020

Improved control of nematodes in carrot production

June 2004.

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Purpose of project:

This project aimed to develop improved methods of controlling nematodes in carrot production in Australia. It involved a national survey to identify the nematode species associated with carrot production in Australia and provided information on improved sampling techniques for nematodes, better understanding of host range and potential for rotation crops, alternative nematicides, the use of alternative methods to nematicides, and biodegredation of nematicides.

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Media summary

Carrot is an important vegetable crop in Australia, with some 330,000 tonnes produced annually from approximately 7500 ha, with a farmgate value of A$150 M. Worldwide, plant-parasitic nematodes are recognised as an important constraint to carrot production. Management of nematodes in carrots in Australia is heavily reliant upon the use of chemicals such as metham sodium and fenamiphos (Nemacur). Production of Nemacur in the USA is to cease in 2005. This, and the development of enhanced biodegredation in soils regularly treated with fenamiphos or metham sodium, suggests that alternative strategies will be required for nematode control in the future. A national project was conducted to determine the principal nematode species associated with carrot production in Australia and to develop improved methods of control. Surveys and field trials in five States determined that the main nematode species associated with yield and quality defects were root knot nematode (*Meloidogyne* spp.) and lesion nematode (*Pratylenchus* spp.). Carrot cyst nematode (*Heterodera carotae*) was not detected in the survey, confirming the absence of this important pest in Australia. Changes were suggested to current sampling and extraction methods to improve the accuracy of pre-plant counts for soil nematodes. Break crops were identified that can be grown prior to carrot to reduce populations of particular species of nematodes. Enhanced biodegredation of currently used nematicides was identified as an issue in carrot soils in some States. Alternative nematicides, including Telone and Telone C35 were trialled and found to be as effective as currently used nematicides. However, there are issues with environmental impact of nematicides, especially potential groundwater contamination. An integrated approach to nematode control based on pre-plant nematode counts to identify the need for nematicide and the use of break crops prior to carrot production is advocated.

Technical summary

Carrot is an important vegetable crop in Australia, with some 330,000 tonnes produced annually from approximately 7500 ha, with a farmgate value of A$150 M. Worldwide, plant-parasitic nematodes are recognised as an important constraint to carrot production. Management of nematodes in carrots in Australia is heavily reliant upon the use of nematicides such as metham sodium and fenamiphos (Nemacur). Production of Nemacur in the USA is to cease in 2005. This, and the development of enhanced biodegredation in soils regularly treated with fenamiphos or metham sodium, suggests that alternative strategies will be required for nematode control in the future. A national project was conducted to determine the principal nematode species associated with carrot production in Australia and to develop improved methods of control.

Survey for nematodes

The most widespread and damaging nematodes associated with carrot production were *Meloidogyne* spp. (mainly *M. javanica* and *M. hapla*) and *Pratylenchus* spp. (including *P. crenatus*, *P. penetrans*). Several other nematodes recorded as parasites of carrot (including *Paratylenchus* spp., *Paratrichodorus* spp., *Helicotylenchus* spp., *Hemicycliophora* spp.) were also present. These were considered of only minor significance to carrot production either due to limited distribution or low numbers, but may have an impact in particular cases. For example, *Hemicycliophora saueri* was identified as a significant cause of poor carrot yield and quality at one site in South Australia. The surveys confirmed the absence of carrot cyst nematode (*Heterodera carotae*) from Australia and of *Radopholus similis* from carrot production areas.

Pre-plant nematode counts

Conducting pre-plant nematode counts is a common method of assessing the risk that nematodes pose to the crop to be planted. Trials suggested that a composite soil sample taken from 40-50 core samples collected in a zig-zag

pattern are required per hectare to achieve a useful estimate of nematode numbers. Such a sampling scheme may appear to be prohibitively labour intensive for growers, but may be cost-effective in situations where it identifies that a costly nematicide application is not required. As many core samples as possible, from a wide cross-section of the field are required to ensure confidence in the result. The efficiency of extraction of *Meloidogyne* spp. from soil by the Whitehead tray technique was markedly increased by longer incubation times (up to 14 days) than are often currently used.

Effect of nematodes on yield

Two field trials in commercial crops were conducted in Tasmania to determine the effect of *Pratylenchus crenatus* on Kuroda carrot. Significant correlations were obtained between numbers of *Pratylenchus* in soil and/or roots shortly after planting and both shoot and root growth at that time, and yield at harvest. Yield differences between plots with low and high numbers of *Pratylenchus* were equivalent to 12.1 t/ha and 22.3 t/ha, with yield affected mainly by reduced plant density at the seedling stage and decreased size of tap root. *Pratylenchus* had minimal effects on quality of Kuroda carrot in these trials, although they have been associated with deformations of taproots in other studies.

In South Australia, nematode levels were monitored and yields of carrots and incidence of defects were assessed on three farms under conventional or organic management in which *Meloidogyne javanica* was present. Production of defective carrots was highest at the organically-managed farm, due to a high incidence of undersized and galled carrots. The most important carrot defects were galling, hairy roots, splitting, swollen tips, forking and undersized carrots. Nematodes with high multiplication rates over the carrot growing season were *Meloidogyne javanica*, *Hemicycliophora saueri, Pratylenchus neglectus*, *Helicotylenchus* spp., *Scutellonema brachyurum* and *Paratrichodorus* spp. Metham sodium at 300 L/ha was inadequate to provide effective control of nematodes, especially in early season crops. Levels of *M. javanica* at planting, mid-season and at harvest were correlated with incidence

of galled carrots, and were variously associated with incidence of forked, tip-swollen, stubbed, hairy, constricted, split and substandard carrots. At harvest levels of *H. saueri* were correlated with incidence of substandard carrots, and with hairy, constricted, forked, tip-swollen, stubbed and split carrots, suggesting that this nematode may also be a significant, and previously unreported, cause of carrot production loss in South Australia. The correlations between at planting levels of *H. saueri* and incidence of split (both a conventional and newly described type of split) and constricted carrots were particularly strong. Incidence of undersized carrots was correlated with at harvest levels of *P. neglectus*. Conventional methods of extracting nematodes (e.g. Whitehead tray) were reliable where high nematode levels existed at planting, however, they were unreliable for detecting *M. javanica* following soil fumigation until 14-17 weeks after planting.

Resistance/tolerance of carrot

A pot trial was established to determine the resistance of 5 common varieties of carrot to *M. javanica* and *M. hapla*. Stefano and Senior had higher gall ratings in comparison to Mojo and Red Hot when challenged with these nematode species, with Kendo Midi having a gall rating intermediate between these groups. This suggested Red Hot and Mojo may be more resistant to root knot nematode. Red Hot had a lower gall rating when challenged with *M. javanica* in comparison to *M. hapla*, suggesting greater resistance to the former species. The gall rating of Mojo was unaffected by the particular root knot nematode species. While promising, further work would be required to determine if the extent of resistance in current carrot varieties is such that this might be a useful management tool as part of an integrated control strategy.

Several carrot selections from USDA of Brasilia and Nantes crosses were shown to support significantly less multiplication of *M. javanica* and reduced galling on secondary roots. Development of varieties resistant to root knot nematode is promising but is dependent upon the development of resistant varieties with acceptable agronomic and market characteristics.

Break crops

Mixed species of *M. hapla* and *M. javanica* were found in Victoria and Western Australia in carrot crops. However, *M. hapla* was the dominant plant-parasitic nematode in Victoria, whereas *M. javanica* was the dominant root-knot nematode species in South Australia and Western Australia. Identifying the species present is important to ensure that a suitable non or poor host break crops is chosen. In pot trials, forage sorghum 'Jumbo' (*Sorghum halepense* x *sudanense*) was a poor host of *M. javanica* and non-host of *M. hapla*. Oats 'Taipan' (*Avena sativa*) and wheat 'Baxter' (*Triticum aestivum*) was a good host of *M. javanica* but a poor host of *M. hapla*. Maize 'DK689' was a moderate host of *M. javanica* and poor host of *M. hapla*. Depending on the species of root knot nematode present, forage sorghum, oats and wheat would be useful break crops to grow prior to carrot crops to reduce root knot nematode numbers. The biofumigant BQ mulch (*Brassica napus*) was a good host of *M. javanica* and moderate host of *M. hapla*. This demonstrates that biofumigant species may act as hosts of pathogens such as nematodes during the growing season and unless a good kill is achieved when incorporated may exacerbate pathogen levels. Alternatively, they may be grown during cooler months when *Meloidogyne* spp. will reproduce at a slower rate. Species such as tomato 'Tiny Tim' (*Lycopersicon esculentum*), broccoli 'Shogun' (*Brassica oleracea*), Radish 'Weedcheck' (*Raphanus sativa*) and Lucerne 'Rippa' (*Medicago sativa*) were all good hosts of both *M. javanica* and *M. hapla* and should be avoided prior to carrots when these nematodes are present.

Nematicides

The most commonly used chemical controls for nematodes in carrot production in Australia are the general soil fumigant metham sodium and the nematicide fenamiphos (Nemacur). There has been a steady reduction in the number of nematicides registered for horticultural use in the last 20 years due to their toxicity and environmental impact. This trend is continuing, with reports that Nemacur is not to be manufactured in the USA after 2005. In

addition, metham sodium and fenamiphos have been shown to be prone to enhanced biodegredation in which continual use leads to a build up of microorganisms in the soil that can rapidly break down the chemical, thereby markedly reducing its effectiveness. A study of 13 carrot soils collected from Victoria demonstrated enhanced biodegredation of fenamiphos in 5 soils, early development of biodegredation in another 5 soils and no evidence of biodegredation in the remaining 3 soils.

Several trials were conducted to determine the efficacy of nematicides in carrot production and to test alternatives to metham sodium and fenamiphos. Telone and Telone C35 have recently passed EPA special review and were registered in Australia in December 2001 for nematode control on vegetable cropping land. In a trial in South Australia, Telone C35 (1,3, dichloropropene and chloropicrin) at 520 kg/ha and Metham sodium at 300 L/ha applied pre-planting gave yields of 75.7 and 67.6 t/ha respectively, compared to 24.4 t/ha in the untreated control. In a trial in Western Australia, Telone at 130 kg/ha and Telone C35 at 270 kg/ha gave significantly higher percentage of export quality carrots by weight (64.1% and 65.2% respectively) than Nemacur (24L/ha) or untreated control (28.7% and 35.0% respectively). Results suggest that Telone and Telone C35 may be effective alternative nematicides to those currently used. However nematicides, including Telone and Telone C35, have the potential to contaminate groundwater if applied in sensitive areas (e.g. sandy soils of low percentage organic matter in areas of high rainfall or irrigation, where groundwater is near the surface).

Conclusion

Management of nematodes in carrots in Australia is heavily reliant upon the use of nematicides such as metham sodium and fenamiphos (Nemacur). Production of Nemacur in the USA is to cease in 2005. This, and the development of enhanced biodegredation in soils regularly treated with fenamiphos or metham sodium, suggests that alternative strategies will be required for nematode control in the future. Telone and Telone C35 have been identified as alternative chemicals in this project and are now registered

for use in vegetable systems in Australia. However, care must be exercised to ensure that these chemicals are used in an environmentally responsible manner, especially with respect to preventing ground water contamination. An integrated strategy for control of nematodes is advocated involving pre-plant nematode counts to determine the need for nematicide application, and suitable crop rotations or break crops to reduce nematode populations in soil prior to carrot. Other methods of control described in this report that may be useful in particular cases include manipulation of planting date.

1. Introduction

Carrot is an important vegetable crop in Australia, with some 330,000 tonnes produced annually, with a farmgate value of A$150 M. Worldwide, plant-parasitic nematodes are recognised as an important constraint to carrot production. Nematodes are also recognised as a significant problem in carrot production in Australia, however they have received little research attention in the past. Nematode control has been based mainly on chemicals. However, the withdrawl of nematicides from the market due to environmental and health concerns and the growing lack of substitutes indicates that alternative management strategies need to be sought. This project aimed to: a) identify or confirm the identity of nematode species associated with carrot production in each State in Australia, b) investigate sampling techniques and damage threshold populations, c) determine the host range of important nematode species as a means of developing suitable rotation practices or break crops, d) investigate chemical control and alternative control methods including biofumigant crops and tolerant/resistant varieties of carrot and, e) develop recommendations for integrated control of nematodes in Australia.

2. Carrot production in Australia

Carrot (*Daucus carota*) production in Australia has increased steadily in the last few years, with 258,000 tonnes produced in 1997 (Hill and McKay 2000). According to Australian Bureau of Statistics (ABS), in 2002 some 330,000 tonnes of carrots are produced in Australia, from 7500 hectares with a farmgate value of $A150M. Average yield of carrot in Australia is between 55 to 65 t/ha. The majority of carrot production in Australia is for the fresh market with 10% of carrot production turned into juice and frozen product (Caldwell 2002).

The percentage breakdown of carrot production per state is approximately, Victoria (34%), West Australia (27%), South Australia (14%), Queensland (8%), Tasmania (11%) and New South Wales (6%) (ABS statistics for 2002). Exports of carrot from Australia have also increased in recent years, with approximately 67,000 tonnes, worth $43.3M exported in 2001/02 (ABS statistics for 2002). Some 90% of exports are from Western Australia and are primarily of Nantes varieties to Asian markets (Malaysia, Singapore, Hong Kong and Thailand). In Tasmania, Kuroda carrots are produced over the summer for the Japanese market, although less so in recent seasons.

The main types of carrots grown in Australia include:

- *Dutch carrots*: small, sweet carrots approximately 5-8 cm long which are sold in bunches with leaves attached. Mainly produced in Victoria and New South Wales.
- *Imperator*: Long tapered carrots with a pointed growing tip.
- *Nantes*: Moderate taper with a cylindrical shape and rounded growing tip.
- *Nantes-Berlicum*: Similar to Nantes with a moderate taper and cigar shape.

- *Kuroda* or *Koyo*: Shorter than standard carrots with a wide shoulder that tapers to a rounded tip. This type has a high content of sugar and beta carotene.

2.1 Victoria

Victoria is the largest carrot producing state in Australia (some 34% of Australian production), with an annual production of 113,130 tonne (Australian Bureau Statistics, for 2002). Victoria is the major supplier of carrots to the domestic market for Australia, with most grown for set contracts with supermarkets. However, most regions export a proportion of their crop.

The main carrot growing regions of Victoria occur in the north (Murray Valley) and south of the state (Melbourne, Gippsland, East Gippsland, Mornington Peninsula and the Dandenong Ranges). Most of these regions have sandy soils. The number of growers and the area of carrots produced in these regions vary each year due to market dynamics, urban sprawl, costs of production, and an increased movement towards regions that allow year-round production. There is a trend in the Victorian industry toward fewer growers, each having larger farms and owning multiple farms in different regions. For example, it is not uncommon for individual growers to have one farm along the Murray in a climate that supports year-round production, and one farm on the Mornington Peninsula for the close location to Melbourne, where the grading and packing occurs.

The only heavy-soil carrot-growing region is found on the outskirts of Metropolitan Melbourne in the clay soils of the Dandenong Ranges near towns such as Silvan and Monbulk. Carrots in this region are often grown on steep slopes with fixed sprinkler irrigation. Most production is for the domestic market. This area was once a very intensive carrot-growing region. However, over the years growers have moved production to the sandy soil areas, usually the Murray or Mornington peninsula, but more recently east to Warragul and East Gippsland.

The Murray Valley is the only northern carrot-growing region in the state. The soil along the Murray is a sandy soil with very few slopes, and has a climate that supports the production of carrots all year round. This region supports few growers on very large farms, with close access to the Murray River for irrigation, using a mixture of mostly solid set and some centre pivot irrigation systems. These growers produce only one or two vegetables in their rotations and rely on the large size of their farms to ensure paddocks are fallowed during the rotation. This region is also home to Victoria's largest vegetable farms, and the adoption rate for technology integration into the farming system is high in the Murray region. Carrot production in this region is mainly for the domestic market, with many growers having set contracts to supply directly to the supermarkets.

The growers in the Gippsland region of Warragul and Thorpdale produce carrots on both sand at Warragul and the rich clay loams of Thorpdale. Thorpdale is traditionally a very important potato-producing region for Victoria, with some cropping occurring on steep slopes. Irrigation is usually from bore water. Some of these growers tend to lease land as part of their rotations to allow their own paddocks to fallow, and also to meet production demands.

Production of carrots in East Gippsland is on large farms with sandy soils and flat land. The production of vegetables at Lindenow (near Bairnsdale) has occurred for some time. Recent expansion of large-scale vegetable growing has occurred in areas such as Longford, with carrot production all year round. Irrigation is from river and bore water. Production in this region is for the domestic market and export. Some of the growers in this region grow, pack and process on site for both cut and peel or salad mixes.

The Mornington Peninsula is a very active agricultural region of Victoria and is situated only an hour's drive from metropolitan Melbourne. It is a region of urban encroachment, with many farms being sold and divided up into housing estates over recent years. This region supports a variety of growers from

large to small-scale growers. Some of the growers currently use the farm in this region to support their farms in the north until production in the north is well established. This region supports a diminishing number of 'bunch' crop growers, who produce baby or Dutch carrots for the domestic market. These growers are all concentrated at the top of the Mornington Peninsula in areas such as Pearcedale, Somerville and Fiveway. Growers of baby or Dutch carrots sow mostly cv. Mokum. These growers produce crops on very small plots, about one eighth of an acre, and plant the plots continually for shorter periods of time in comparison to large-scale producers. Larger scale carrot production occurs at the end of the Peninsula around Boneo. Here carrots are grown on relatively flat land in sandy soils. Irrigation is usually sourced from bores using fixed sprinklers at least three vegetables are usually grown in rotation. Production is mostly for the domestic markets due to the close proximity to Melbourne.

Many of the carrot producing regions in Victoria have the potential to produce carrots 12 months of the year. The most common variety sown for large carrot product is cv. Stefano. Most growers use the nematicide Nemacur as a precaution against nematode damage. Only one grower currently has regular pre-plant tests for nematodes and about half had never had a nematode count done for any carrot paddock.

2.2 Tasmania

Carrot production in Tasmania has increased rapidly in recent years with 21,000 tonnes produced from 459 ha in the 1995-1996 season and 35,000 tonnes from 616 ha in the 1999-2000 season (Australian Bureau of Statistics). Some 3773 tonnes of carrots were exported from Tasmania to other countries in 2000/2001 (McKay 2004). A large proportion of export carrots are to Japan of the variety Koyo 2, a Kuroda type. Most of the carrot production in Tasmania has been confined to the North West Coast spreading from Table Cape, Devonport, Moriarty, Sasafrass areas on mostly heavier Krasnozem

soils and in the North East around Scottsdale and Cressy on lighter soils. However, a considerable amount of production has recently moved to sandy soils on the East Coast (Swansea). Most growers are contracted by packing and processing companies, including Premium Fresh Tasmania Pty. Ltd, Field Fresh Tasmania, Harvest Moon Forth Farm Produce Pty. Ltd. for the fresh market or Simplot Australia Pty. Ltd. for processing. Much of the production process is controlled by the companies, with field officers provided to give advice to growers on agronomy during the growing season. The companies also arrange the planting and harvest of the crop. In some contracts, growers are paid only for first grade carrots. Therefore maintaining a high percentage pack out is very important. Irrigation has traditionally been by traveller irrigator, but in the last five years there has been a move towards Centre Pivot, Solid Set and Lateral Move irrigators. Irrigation monitoring is becoming more common with the use of tensiometers or Gopher equipment. The crop is graded and packed locally and most is exported to mainland Australia with some exports to Asia of Kuroda carrots by Field Fresh Tasmania. Premium Fresh Tasmania Pty. Ltd. have increased their production considerably in the last five years with a planned 30,000 t in 2003/2004 season and plans for 40,000 t in 2004/2005 season.

Root knot nematode is recognised as a sporadic problem in carrot production in Tasmania. Each year a few crops are severely affected. In the past companies have required a pre-plant nematode count prior to planting carrot. If root knot nematode is detected, a decision is made either to treat the field with nematicide or to abort planting the field. The most commonly used nematicide is fenamiphos (Nemacur). However, soil testing for nematodes is not always sensitive enough to detect root knot nematode. In some cases, crops have been severely affected by nematode damage when grown in fields in which root knot nematode has not been detected prior to planting. This has prompted some companies to apply Nemacur on all crops at planting.

Pratylenchus are also recognised by the local industry as a potential problem when present in ‘high’ numbers. However, the effect of *Pratylenchus* is not

well known. There are anecdotal cases where low yield of carrot has been associated with high numbers of *Pratylenchus* prior to planting and cases of fields with high pre-plant counts of *Pratylenchus* producing high yields. In general a decision is made to apply nematicide only if *Pratylenchus* numbers are considered 'high'. As a result, part of this project was directed towards quantifying the effect of *Pratylenchus* on carrot yield and quality in Tasmania.

2.3 Western Australia

In 2001/2002, Western Australia exported 67,000 tonnes of carrots worth $A43.3 (fob), accounting for 90% of Australia's carrot exports (Australian Bureau of Statistics). The major market for Western Australia carrots is Malaysia with other important markets including Singapore, Hong Kong, The United Arab Emirates, Thailand and Japan (McKay 2004). There is increased competition for export markets from China and New Zealand with some 20,900 t and 11,600 t respectively exported to Japan in 2000 (McKay 2004). The Western Australian carrot industry is based mainly on Nantes carrot varieties that have been bred in France and Holland. The variety 'Stefano' accounts for more than 90% of exports from Western Australia. Exports have increased over three-fold in the previous 10 years, with expansion mainly around the Gingin, Myalup and Capel areas (McKay 2004). Much of the production is on large farms and crops irrigated by Centre Pivots. The industry aims to produce high quality carrots year round. A feature of the industry is a lack of suitable rotation crops that can be grown profitably under irrigation. This has led to short or no rotations with a reliance on chemical control of soilborne pathogens and nematodes between carrot crops. The local industry has a strong research and development focus with the Carrot Association for Research and Development (CARD) formed in 1992 (McKay 2004).

2.4 Queensland

Carrot production in Queensland is currently centred around the eastern Darling Downs, Lockyer and Fassifern Valley areas. In 2002 there were 1,031 hectares under carrots production in these regions, producing 25,918 tonnes of carrots. Carrot production in Queensland is currently worth $20 million. The main cropping times are March-September although there are usually some crops in the ground somewhere in the region year round. Most of this land is fumigated before planting.

2.5 South Australia

South Australian produced 27,105 t of carrots in 1999 (Australian Bureau of Statistics) from 573 ha with a mean yield of 47.3 t/ha and a gross value of $21.7m. By 2002 gross value of production had risen to $36.3m making South Australia the third largest contributor to national production with 18.3% of total value. Gross unit value at $769/t was the highest in Australia (Australian Bureau of Statistics).

The older, established areas close to Adelaide on the Northern Adelaide Plains and the Adelaide Hills are still important producers of carrot. However, vegetable production, both generally and of carrots in particular, have shifted focus in recent decades to the Murray Mallee and Riverland areas. The latter areas feature the red sand to sandy loam soils that favour the production of premium vegetable crops but paradoxically also promotes the multiplication of important nematode parasites such as *Meloidogyne* spp.

The heavier soils of the older production areas, such as the red-brown duplex soils, have long been in production and have in some cases developed crop-limiting populations of soil-borne pathogens. A shift of production to the newer areas allowed the luxury, for a time at least, of growing vegetables in virgin soils. The smaller block sizes of the older areas and higher land costs

also impeded introduction of new technology. In contrast, centre-pivot irrigation has been introduced into the Murray Mallee and Riverland areas where abundant, relatively cheap land, usually with a cropping history of cereals only, has been available.

This shift into virgin soils has temporarily allowed good yields and quality of carrot crops despite the absence of a satisfactory crop rotation system. However, continued intensive production of carrots can be expected to lead to increased losses from soil-borne pathogens and nematodes. Short rotations or fallows are frequently practised and options for profitable rotation crops are limited. This remains the most important obstacle to achieving control of nematodes in carrot production in South Australia.

The most commonly used nematicidal chemicals used for carrot production in South Australia are preplanting soil fumigation with metham sodium and use of Nemacur®. Grower awareness of nematodes is low except in the case of *Meloidogyne* spp. due to the obvious nature of damage from these nematodes. Chemicals are often used as an insurance policy against the latter nematodes but monitoring of nematode populations is rarely conducted.

3. Nematodes parasites of carrot

Plant-parasitic nematodes are an important constraint to yield and quality of carrot worldwide. For example, Davis *et al*. (undated) ranked nematodes (mainly root knot nematode - *Meloidogyne* spp.) as the second most economically important carrot disease in the USA. Several species of nematodes are well known to reduce carrot yield and cause poor quality taproots. However, other factors may also cause malformations of the tap root of carrot, including *Pythium*, soil compaction, high water table, nutrition or insufficiently decomposed organic matter (Vrain and Belair 1981).

More than 90 species of plant-parasitic nematodes from several genera have been associated with umbelliferous crops, but few have been studied in detail (Davis and Raid 2002). In Australia, several species of nematodes were listed as associated with carrot (McLeod *et al*. 1994). This list has recently been updated (Nobbs 2003), with some of the records being generated from this project (Table 1).

Many of the nematode species associated with carrot in Australia are reported parasites of carrot in other countries. A fuller description of some of the main nematodes associated with carrot production follows.

3.1 Root-knot nematode (*Meloidogyne* spp.)

Root knot nematodes are generally considered the most important nematode parasites of carrot worldwide, being geographically widespread and capable of complete crop loss. Several species of root-knot nematode have been reported as parasites of carrot worldwide – *Meloidogyne arenaria*, *M. chitwoodi*, *M. fallax*, *M. hapla*, *M. incognita* and *M. javanica* (Davis and Raid 2002). Of these, all but *M. chitwoodi* are present in Australia (Nobbs 2003). In addition, there are two races of *M. arenaria*, three races of *M. chitwoodi* and four races of *M. inocognita* that have been identified by their reaction to differential hosts (Davis and Raid 2002).

Table 1. Plant parasitic nematodes associated with carrot in Australia (Nobbs 2003)

Nematode	State
Ditylenchus dipsaci	SA, WA
Helicotylenchus dihystera	QLD, TAS
Hemicycliophora sp., Hemicyliophora saueri	SA
Merlinius brevidens	VIC, TAS
Meloidogyne arenaria	NSW, TAS
Meloidogyne fallax	TAS
Meloidogyne hapla	NSW, SA, TAS, VIC
Meliodogyne incognita	NSW, WA
Meloidogyne javanica	NSW, QLD, SA, VIC, WA
Meloidogyne thamesi	NSW
Meloidogyne sp.	SA
Neodolichodorus adelaidensis	NSW
Paratrichodorus lobatus	SA
Paratrichodorus sp., P. minor, P. renifer.	SA
Paratylenchus sp.	SA, VIC
Pratylenchus crenatus, Pratylenchus neglectus	VIC, TAS
Pratylenchus penetrans	QLD, VIC, TAS, WA
Pratylenchus pratensis	VIC
Pratylenchus thornei	SA, TAS
Rotylenchus robustus	TAS, VIC
Scutellonema sp.	VIC
Tylenchorhynchus latus	SA
Tylenchorhynchus sp.	SA, TAS
Xiphinema monohysterum	SA[1]

[1] Associated with *Daucus glochidiatus* (native carrot)

Root knot nematode can be particularly damaging to carrot production. Slinger and Bird (1978) showed that only 58% of 'Spartan Premium' carrots grown in soil *M. hapla* were suitable for fresh market in comparison to 97% grown in nematode-free soil. In Canada, detrimental effects of *M. hapla* on carrot were noted at preplant densities of 2000 J2/L soil (Vrain *et al*. 1979). However, in another study in Michigan, preplant densities of only 200/L soil caused considerable reduction in marketable tap roots (Slinger and Bird 1978). Belair and Parent (1996) reported that in a field with *M. hapla*, carrot crops following two preceding carrot crops had a high degree of root galling and yielded only 2.2 t/ha with 7.3% marketable roots. In comparison, a carrot crop that was preceded by barley and onion yielded 56.8 t/ha with 88.9% marketable roots with only a small amount of root galling. Huang and Charchar (1982) reported that *Meloidogyne incognita* at 230 J2 + 2300 eggs/L

soil rendered carrot unmarketable, causing constrictions, digitations and cracking of taproots. Carrot roots attain full length during the first 2-3 weeks of growth and are most susceptible to *M. hapla* during this period (Yarger 1981).

Abawi *et al*. (1997) observed damage from *M. hapla* in all ten carrot crops surveyed in central and western New York in 1996. Carrots exhibited forking, galls on the carrot surface and fibrous roots, hairiness and stunting. The average incidence of damaged carrots ranged from 18-82%, resulting in 4-36% loss in marketable yield (Abawi *et al*. 1997). In field microplots, containing *M. hapla* at initial densities of 1, 2 and 8 eggs/ml the marketable yield of carrot cv. Oranza in an organic soil was reduced by 13%, 27% and 53% respectively and in a mineral soil by 26%, 68% and 77% respectively. From this, it was estimated that the threshold density for *M. hapla* to carrot was 0.4 eggs/ml of organic soil and 0.8 eggs/ml of mineral soil (Abawi *et al*. 2001b). In organic soil field microplots, Vrain (1982) demonstrated that marketable storage root weight was decreased by 36% at 20 eggs/100 ml, 59% at 40 eggs/100 ml, 75% at 80 eggs/100 ml, 92% at 160 eggs/100ml) and 89% at 240 eggs/100 ml. In pots, root and leaf weight and storage root length were significantly reduced at all inoculum levels, and percentage of forked roots was significantly increased (to 57% and 59%) at 160 and 240 eggs/100 ml soil respectively (Vrain 1982).

Most (if not all) commercial varieties of carrot are susceptible to *M. hapla*. Santo *et. al*. (1988) reported that Imperator Six Pak II, Pak More, Six Pak, Imperator 58, Top Pak, Gold Pak, Trophy, Charger, Nantes Amsterdam Minicor, Half-Long Nantes, Red Cored Chantenay, Hybrid Orlando Gold, Chancellor, Golden State and A Plus were all good hosts of *M. hapla*.

Establishing the economic threshold population density for root knot nematode in carrot has proven difficult. Vrain (1982) established an economic threshold of 9 *Meloidogyne hapla*/100 ml soil. However, Potter and Olthof (1993) noted that as carrot should be regarded as having a zero tolerance

threshold as it is deformed by root-knot nematodes and thus unmarketable. Stirling *et al*. (1999) considered pre-plant population densities in the range 0, 1-20 and >20 J2/200 ml soil to constitute a low, moderate and high risk to carrot crops respectively. However, they added that since carrots deformed by root-knot nematode are rejected in the market, a zero tolerance should be assumed until more definitive local information is available. Stirling *et al*. (1999) noted that in some cases, population densities as high as 60 J2/200 ml soil cause little damage.

The distribution of those root knot nematode species in Australia of known importance to carrot was reported by Nobbs (2003) as follows: *Meloidogyne arenaria* (NSW, QLD, SA, TAS, VIC, WA), *Meloidogyne fallax* (SA, TAS, VIC), *Meloidogyne hapla* (NSW, QLD, SA, TAS, VIC, WA), *Meloidogyne incognita* (NSW, NT, QLD, TAS, VIC, WA), *Meloidogyne javanica* (ACT, NSW, NT, QLD, SA, VIC, WA). *Meloidogyne fallax* was originally considered a race of *M. chitwoodi*, but has recently been recognised as a separate species (Karssen 1996, Beek *et al*. 1997) and it is possible that its distribution may be more widespread in Australia than currently recognised.

The general lifecycle of the root knot nematode consists of the nematode undergoing a moult within the egg and hatching as a worm-like second stage juvenile (J2). The J2 migrates through the soil, enters the root just behind the root cap and migrates to a preferred location near the vascular cylinder where it modifies plant cells to establish a sedentary feeding site consisting of hypertrophied, multinucleate giant cells. The nematode feeds within these cells for the remainder of its life, undergoing three more moults and enlarging into a mature pear-shaped female. The root becomes galled around the site of the developing female. Adult males may be formed after the 4th moult and can migrate and mate with females. However, females do not require males for reproduction. Depending on species and temperature, between 1 to 3 generations may be completed within a season (Davis and Raid 2002). Optimum temperatures for development range between 15-25^{o}C for *M.*

chitwoodi, *M. fallax* and *M. hapla*, and between 25-30^{o}C for *M. arenaria* and *M. javanica*. Thresholds for development are approximately 5^{o}C and 38^{o}C for *Meloidogyne* spp. (Davis and Raid 2002). Damage caused by root knot nematode is generally more severe in sandy-textured and muck soils compared to clay soils.

3.2 Lesion nematode (*Pratylenchus* spp.)

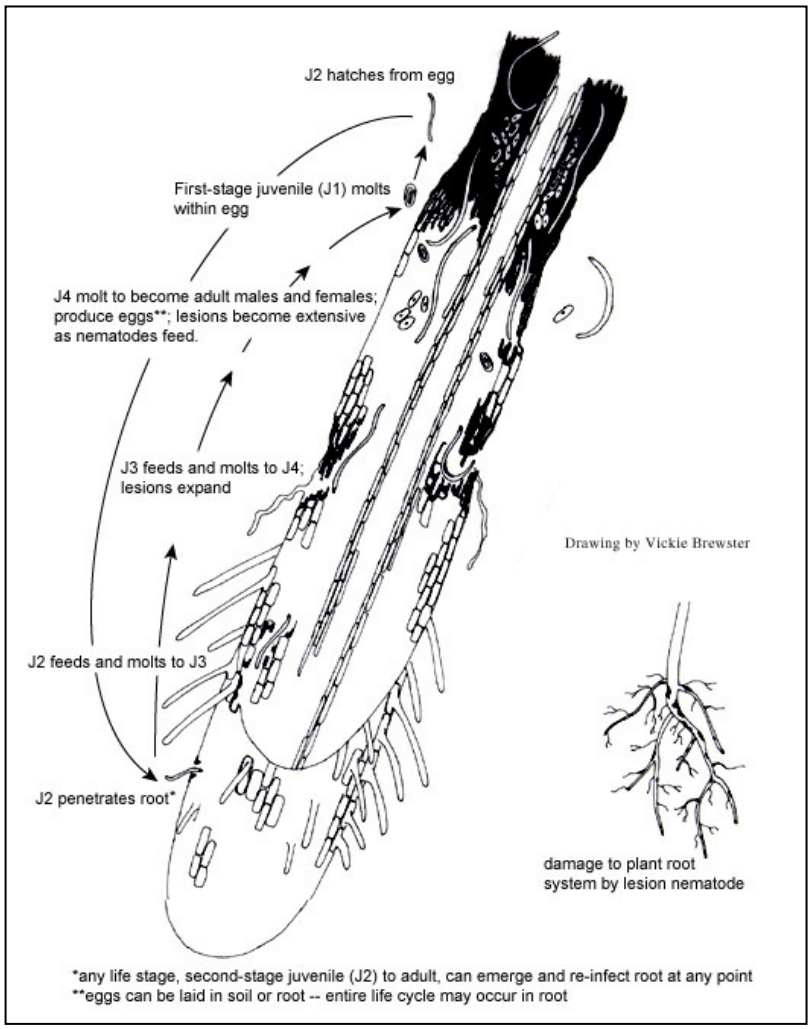
There are approximately 70 described species of *Pratylenchus*, that parasitise over 400 host plant species. Potter and Olthof (1993) reported that the threshold range for *Pratylenchus* in carrot was 30 to 180/100 g soil at planting, with moderate damage at about 100/100 g soil. Several species have been associated with damage to carrot. *P. crenatus* caused taproots to be branched and reduced, side roots with lesions and dead tips and above ground parts of the plant to be thin and chlorotic (Potter and Olthof 1993). *P. crenatus* has been noted as a cause of 'carrot sickness' resulting in patches of poorly growing thin, pale plants where the main root is small and often branched, other roots are short with lesions and dead tips (Loof 1991). Weischer and Brown (2000) reported that the damage threshold of *Pratylenchus crenatus* to carrots was 600 individuals/L soil as the initial population density. Carrot is also a host of *Pratylenchus neglectus* (Siddiqui *et al*. 1973) and of *Pratylenchus penetrans* (Kleynhans 1996). Coosemans (1975) reported than an initial density of 10 *P. penetrans*/100 ml soil caused 75% of carrots to be forked with multiplication of rootlets, while 100 *P. penetrans*/100 ml soil killed 40% of plants. Vrain and Belair (1981) showed initial nematode densities of 100/100 ml soil to delay carrot development but did not cause branching. However, at densities of 200 or 400 *P. penetrans*/100 ml, almost all carrots were branched, were smaller and weighed less than those from inoculated soil. Orion *et al*. (1988) reported that *P. mediterraneus* killed seedlings, resulting in poor stands and caused split carrots resulting from damage to root tips. Application of nematicide at seeding resulted in 50-65% control of the soil population and 38-45% increase in marketable carrot yield (Orion *et al*. 1988). Many *Pratylenchus* species can

be cultured on carrot taproot disks *in vitro*, which indicates that carrot is a host. For example, *Pratylenchus thornei* can reproduce on carrot disk cultures *in vitro*, with Rf=5.1 after 25 d and Rf=3619 after 100 d (Castillo *et al*. 1995). Note that Rf (reproductive factor) is a measurement of host suitability and is calculated as Pf (final population density)/Pi (initial population density) after a set time period.

Pratylenchus spp. are migratory endoparasites of plants, remaining as a mobile vermiform worm-like stage throughout the lifecycle and feeding inside of root tissue. Adults are approximately 0.4-0.7 mm long and 0.020-0.025 mm in diameter. As with most other plant parasitic nematodes, *Pratylenchus* form a first stage juvenile (J1) in the egg that moults to form a second stage juvenile (J2) (Fig. 1). The J2 hatches, moves through the soil and enters plant roots where it can begin feeding. The J2 grows and moults to form a third-stage (J3), a fourth-stage juvenile (J4) and finally an adult female (Fig. 1). Males are formed in some species (e.g. *P. penetrans*), but are absent or rare in others (e.g. *P. crenatus*). Females, either with or without fertilisation, lay their eggs singly or in groups inside roots with 1-2 eggs produced per day for *P. penetrans* (Mizukubo and Adachi 1997). Eggs can hatch within the root or are released into the soil as the root breaks down. The lifecycle of *P. penetrans* (egg deposition to egg deposition) is temperature dependent and takes 46, 38, 28, 26 and 22 days at 17, 20, 25, 27 and 30^{o}C respectively. The developmental zero degrees (^{o}C) and the effective degree days required for hatching, female emergence and the onset of egg-laying were estimated to be 2.7 and 200, 4.2 and 548, and 5.1 and 564 respectively (Mizukubo and Adachi, 1997). *Pratylenchus* remain mobile from the J2 stage onwards and can move inside of roots or through the soil to invade other roots. Nematodes enter roots in a radial direction anywhere along the root. Intracellular penetration is achieved by thrusting of the stylet and head, resulting in breakdown of cell walls. Nematodes then move into the cortex where they feed and reproduce. The endodermis remains unaffected, even when there are high population densities within the cortex. Nematodes tunnel through the cortex of the root as they feed, causing necrosis of the cortical cells and

brown discolouration of the surrounding cells (Agrios 1988). Lesions become sunken and elongated as cell walls collapse. Each lesion can contain more than one nematode. Frequently the eggs, juveniles and adults occur together as 'nests', which can be present in great numbers in roots of susceptible plants. Nematodes that hatch from eggs can continue feeding, thereby expanding the lesion or can emerge from the root to attack other roots. Enlarging lesions can join with others, eventually girdling the root. Necrotic cortical tissues of large lesions are sloughed off or are invaded by secondary

Figure 1. Life cycle of *Pratylenchus* spp. (Davis and MacGuidwin 2000).



fungi and bacteria that contribute to the death of the root distal to the affected region. Above ground symptoms of nematode feeding tend to be similar to symptoms of nutrient deficiency or water stress, with affected plants wilting under dry conditions or appearing chlorotic and stunted.

3.3 Needle nematode (*Longidorus* spp.)

Several species of *Longidorus* have been reported on carrot and celery in other countries. *L. africanus* occurs in Israel, the United States and Zimbabwe and was first identified as a pathogen of carrot in the Imperial Valley of California (Davis and Raid 2002). *L. elongatus* occurs in Europe and was reported by Hooper (1973) to cause severe damage to carrot. In Israel, *L. israelensis* and *L. vineacola* have been reported on carrot and *L. apulus* has been associated with damage to celery roots (Davis and Raid 2002). Weischer and Brown (2000) reported distortion of carrots (thumb like branch roots) and bending of tap roots caused by *L. israelensis*. Species of *Longidorus* occur in Australia, but have not been associated with damage to carrot (Nobbs 2003).

3.4 Pin nematode (*Paratylenchus* spp.)

Pin nematodes are ectoparasites which partially enter roots as they feed. Only one species, *Paratylenchus hamatus*, has been reported as pathogenic on carrot, celery and parsley in northern Europe and the United States. A second species, *P. projectus*, reproduces on celery but is not considered to be pathogenic (Davis and Raid 2002). Feeding by *P. hamatus* causes a ‘rat-tail’ appearance to carrot taproots as a result of reduced growth of secondary roots (Davis and Raid 2002). Initial population densities of 150-1000/100 ml of soil may cause low to moderate damage (Davis and Raid 2002). Weischer

and Brown (2000) reported that the threshold for *Paratylenchus hamatus* in carrot was 6000 individuals/L soil. Species of *Paratylenchus*, including *P. hamatus*, occur in Australia but have not been associated with damage to carrot (Nobbs 2003).

3.5 Sting nematode (*Belonolaimus* spp.)

Belonolaimus longicaudatus has been documented on carrot and celery in the United States. Carrot plants are typically stunted, chlorotic and wilted and young plants can die from heavy infection (Davis and Raid 2002). Necrotic lesions may be formed on roots that girdle the root. Carrot taproots can become stunted, forked and unmarketable (Davis and Raid 2002). Johnson (1998) reported that sting nematode causes serious damage on a wide range of vegetable crops, including carrot. Optimum conditions for *B. longicaudatus* are 28-30^{o}C with a life-cycle of approximately 28 days (Davis and Raid 2002). *B. longicaudatus* has been reported in New South Wales and Western Australia on crops other than carrot (Nobbs 2003).

3.6 Stubby root, Spiral and Stem nematodes

Paratrichodorus minor (syn. *P. christiei*) can cause stunting of the taproot of and forking of carrot. However, carrot is considered a relatively poor host of this nematode, and control is seldom necessary (Davis and Raid 2002). *P. minor* has been associated with carrot in South Australia (Nobbs 2003).

The spiral nematode, *Rotylenchus robustus* is widely distributed in Brazil, Canada, Egypt, Europe, India, Russia, the United States and Zaire, with a further species (*R. uniformis*) reported on carrot in the Netherlands (Davis and Raid 2002). High population densities of both species have been associated with stunting, yellowing and reduced yield of carrot and initial population densities of more than 100/100 ml soil are considered damaging (Davis and

Raid 2002). In Australia, *R. robustus* has been associated with carrot in Tasmania and Victoria (Nobbs 2003). It is not considered common in Tasmania, but was found recently (2003) in a pyrethrum paddock in the North West Coast of Tasmania (Hay pers. comm.), probably associated with weed hosts. *R. reniformis* has also been reported on carrot in China and nematicide treatment increased carrot yield by 10.2-23.5% compared to untreated (Liao *et al*. 1999)

The stem nematode (*Ditylenchus dipsaci*) has been reported to cause severe damage to carrot and celery in Italy (Greco 1993). Severe damage was also reported in Sicily (Schiliro *et al*. 1995) including stunted growth, foliar discoloration and wilting, giving rise to withering of the interior leaves and decay. *Ditylenchus dipsaci* has been reported on carrot in South Australia and Western Australia (McLeod *et al*. 1994, Nobbs 2003). Janssen (1994) reported different races of *D. dipsaci* to have differing pathogenicity to carrot, ranging from non-host to susceptible host.

3.7 Important nematode parasites of carrot not currently present in Australia

3.7.1 *Meloidogyne chitwoodi*

Two host races of *M. chitwoodi* have been reported (Santo *et al.* 1988). Populations of race 1 cannot reproduce on alfalfa (*Medicago sativa* L.), while most carrot cultivars are good to moderate hosts. Race 2 populations reproduce on alfalfa but not on most carrot cultivars (Table 2). Mojtahedi *et al*. (1988) demonstrated that isolates of both races from Oregon, Washington and Idaho varied in Rf from <0.01 to 10.7, with Rf > 2 considered a suitable host (Table 2). O'Bannon and Santo (1984) showed cv. Gold Pak to be a moderate host, cv. Half Long and cv. Imperator to be a poor host of *M. chitwoodi*.

Table 2. Host status of different varieties of carrot to different races of root knot nematode, *Meloidogyne chitwoodi* as measured by reproductive factor[1] (Rf) (From Santo *et. al*. 1988).

	Reproductive factor (Rf)	
Study and carrot variety:	Race 1 (non alfalfa)	Race 2 (alfalfa)
Santo *et al*. (1988)		
Hybrid A plus	2.1****	0*
Imperator Six Pak II	17.9****	0.8**
Pak More	14.4****	0.2**
Six Pak	12.3****	0.8**
Imperator 58	11.6****	0.01*
Top Pak	2.2****	0*
Nantes Amsterdam Minicor	10.6****	0.01*
Red Cored Chantenay	4.4****	0*
Hybrid Orlando Gold	10.5****	-
Hybrid Chancellor	6.4****	0*
Hybrid Golden State	4.7****	0.02*
Imperator Gold Pak	1.3***	-
Imperator Trophy	1.2***	0*
Imperator Charger	0.4*	0.01*
Gold Pak	-	0*
Half-long Nantes	-	0*
Mojtahedi *et. al*. (1988)		
Red Cored Chantenay	11.6****	<0.1**
Imperator 58	4.4****	<0.1**
Gold Pak	1.3***	0*

[1] Rf = final population density/initial population density.
- not assessed, * non-host, ** poor host, *** moderate host, **** suitable host

3.7.2 Carrot Cyst Nematode (*Heterodera carotae*)

Heterodera carotae was historically one of the causes of 'carrot sickness' in which infested fields displayed poor stands with chlorotic plants and poor yields (Nickle 1991). *H. carotae* is widespread throughout the carrot-growing areas of Europe and has been found in England, Ireland, Netherlands, Scotland, France, Italy, Switzerland, Germany, Sweden, Poland, Czechoslovakia, and Hungary (Mathews 1975, Greco 1986). It has also been reported from USSR, Cyprus and India (Greco 1986) and from Michigan,

USA, where it was found in 67% of carrot fields (Berney & Bird 1992). *H. carotae* has not been reported in other areas of North or South America (Johnson, 1998).

The host range of *H. carotae* is restricted to cultivated and wild carrot (*Daucus carota* L.), the wild relative (*D. pulcherrimus* (Willd.) Koch ex DC, and the umbelliferous weed *Torilis* spp. (Davis and Raid 2002). As with other cyst nematodes, eggs within the dead swollen body of the adult female (cyst) may remain viable for several years. The juvenile nematode undergoes one moult within the egg, which is stimulated to hatch in response to exudates from carrot roots (Davis and Raid 2002). The nematode hatches as a second stage juvenile (J2), which migrates towards the root in response to gradients of chemical attractants in the rhizosphere. The J2 penetrate the root just behind the root cap and migrate to be parallel to the central cylinder (stele) where they become sedentary and initiate the formation of a feeding site (syncytium) which acts as a transfer cell from which the nematode obtains water and nutrients. This syncytium is formed from partial cell wall degradation leading to large coalesced cells containing several nuclei. The nematode undergoes three more moults as it grows into a pear shaped adult female which deposits eggs internally. After egg deposition the adult female dies and the outer cuticle forms a protective cyst. One or two generations of *H. carotae* are typically completed within a season, with lower, optimum and maximum temperatures for hatching and development of 5^{o}C, 15-20^{o}C and 25^{o}C respectively. The threshold density for measurable damage is 80 eggs/100 ml soil with total loss of crop at densities above 6400 eggs/100 ml soil (Greco and Brandonisio 1980). A factsheet detailing symptomatology and lifecycle of this exotic pathogen has been produced by Agriculture Western Australia (Stansbury *et al*. 2001).

4. Sampling for nematodes

Sampling for nematodes in a short-term annual crop such as carrot is normally done by taking a number of soil core samples prior to planting, extracting the nematodes from a bulked composite sample and counting. Decisions with regard to whether the field is planted or not, or whether a nematicide is applied prior to or at planting are based on the nematode species present and their population density.

Sampling for nematodes is difficult as nematodes are rarely evenly distributed across a field. Furthermore, with any soil sample, the proportion of soil that can be examined is extremely small in relation to the size of the field. Therefore the result of any soil test can give only an estimate of the population density. Two critical decisions with regard to sampling are the size of the sampling unit and the number of core samples taken per sample unit. Experiments were conducted to determine the optimum sampling intensity.

4.1 Sampling intensity (Tasmania)

A 100m x 100m plot was marked out in a fallow field prior to carrot, and pegs placed at 25 m intervals along two parallel boundaries. Starting from one corner, a 'W' shaped pattern was walked across the field. Four different sampling regimes were used, collecting 2, 4, 8 or 16 soil cores per arm of the 'W', (i.e. 8, 16, 32 or 64 cores/ha). There were four replicates of each core number. Soil cores were taken with an Oakfield sampler and were 2.5 cm diameter to a depth of 20 cm. Nematodes were extracted over a period of 3 days from 400 ml of mixed soil using the Whitehead tray technique (Whitehead and Hemming 1965) and total nematodes counted. Figure 2 demonstrates the reduction in the coefficient of variability in the mean number of nematodes with increasing number of core samples. A sample of 40-50

soil cores per hectare was required to reduce variability to less than 20%, which is in agreement with other studies (e.g. Stirling *et al*. 1999). Such a sampling regime is often seen as prohibitively labour intensive. However, it is probably cost-effective in situations where it is identified that there is no need for a nematicide application where one would have been routinely made. This demonstrates that as many samples should be taken as possible to ensure an accurate estimate of the true population.

Figure 2. Relationship between number of core samples taken per hectare and the coefficient of variability around the mean number of total nematodes.

4.2 Sampling intensity (South Australia)

Four field experiments assessed the effects of sampling intensity on detection of *Meloidogyne javanica* in soil. Three fields were near Nuriootpa, under a

conventional management regime that included the use of metham sodium before planting. A further field was at Purnong with accredited organic status. In one of the conventionally managed fields, parallel experiments were conducted over the entire field, and in a single bed where previous sampling had detected the nematode. One of the conventionally managed fields was sampled after carrot harvest under a cereal cover crop while all other fields were sampled approximately one month before planting at a time when decisions on use of soil fumigants would need to be made. In each experiment, 8 replicates of either 10, 20, 40 or 80 core-samples were taken and subsamples (150 ml) were extracted on Whitehead trays (Whitehead and Hemming 1965) for 5 days after being thoroughly mixed. Second stage juveniles (J2) of *Meloidogyne* spp. were counted.

In the three conventionally managed sites, there was a clear relationship demonstrated between frequency of detection (reliability) and sampling effort (number of cores taken), indicating that the probability of detection was directly related to the sampling intensity (Table 3). Frequency of detection ranged from 25 to 100%. However, from a practical perspective, 80 core-samples generated volumes of soil that made sample collection and mixing a problem. In the field sampled after harvest, surveys of the previous carrot crop indicated that damage to carrots caused by the nematode was largely confined to several, small patches. Therefore, the subsequently demonstrated relationship between sampling intensity and probability of detection was not unexpected. In the organic field, no such relationship was found. Due to the high incidence of the nematode in this field, the frequency of detection approached or reached 100% under all sampling regimes. This demonstrated that in small fields with relatively uniform and/or heavy infestations, sampling intensity had little effect on reliability.

However, no clear relationship could be demonstrated between nematode density detected and sampling intensity. In none of the four experiments was there found a statistically significant relationship between these variables,

although in two of these experiments the trend was towards higher nematode densities at higher sampling intensity. These results indicated that over the range of sampling intensities used, increased effort was not necessarily rewarded with higher estimates of population densities, and cores taken

Table 3. The effects of sampling intensity (*n* = 8) on precision and reliability (frequency of detection) of estimates of soil densities of *Meloidogyne javanica* in 'conventional' (agrichemicals used) and certified organic South Australian carrot fields.

Site/Number of subsamples	Area (ha)	Farm type	Crop stage	Reliability (%)	*Meloidogyne* J2/300 mL ± S.E.*
Nuriootpa					
-Dam block	10	Conventional	Postharvest		
10				63	8.3 ± 3.8
20				88	17.6 ± 5.7
40				100	15.7 ± 2.6
80				100	21.0 ± 6.4
-Road block	13.6	Conventional	Preplanting		
10				50	2.5 ± 1.7
20				50	2.4 ± 1.3
40				63	1.3 ± 0.5
80				63	2.1 ± 0.6
-Bed	0.11	Conventional	Preplanting		
10				25	2.6 ± 2.2
20				50	7.6 ± 4.3
40				50	4.0 ± 2.4
80				88	6.1 ± 2.3
Purnong					
-Bed	0.11	Organic	Preplanting		
10				100	33.2 ± 15.8
20				100	25.8 ± 7.2
40				88	10.6 ± 2.3
80				100	22.6 ± 6.9

*within site means not significantly different by ANOVA ($P < 0.05$)

within small and infrequent areas of infestation could possibly be diluted by the many more cores taken within uninfested areas. Subdividing fields into smaller units for sampling is suggested as a means to limit this dilution effect and to provide more accurate information on nematode densities. Higher nematode densities were detected in the organic than in the conventionally-managed fields. In the latter, higher densities were found in the field sampled after harvest than in those sampled one-month before planting. This indicates that sampling should preferably be undertaken at or soon after harvest of the preceding susceptible crop (in addition to pre-planting) to maximise the probability of detecting nematodes.

4.3 Distribution of nematodes within fields (Tasmania)

Two field trials to determine the distribution of *Pratylenchus* in the soil were established on a property in Penguin, Tasmania in early February (Crop A) and November 2001 (Crop B), after emergence of the crops. The trials consisted of 42 plots arranged in a 6 x 7 lattice. In Crop A, plots were each 7 metres long by 3 beds (5.25 m) wide (each bed having two, double rows of carrots). In Crop B, plots were each 7 metres long by 4 beds (6.65 m) wide (each bed having 3 double rows of carrots). Soil samples were collected on 27/2/2001 (Crop A) and 20/11/2001 (Crop B) at 58 DAS. From 6 position in each plot, soil and plants (6) were collected with a trowel to a depth of 30 cm and samples bulked. Nematodes were extracted from 200 ml soil and chopped root samples by the Whitehead tray method (Whitehead and Hemming 1965). In Crop A at 58 DAS, lesion nematode occurred at a mean, minimum and maximum number per 200 ml soil of 106, 20 and 540 respectively and per gram dry weight of root of 109, 0 and 422 respectively. At 58 DAS in Crop B, lesion nematode occurred at a mean, minimum and maximum number per 200 ml of soil of 119, 10 and 354 respectively and per gram dry weight of root of 771, 201 and 2292 respectively.

Contour plots of average nematode numbers from each trial plot were produced using Sigmaplot Graphical Software, using the central position of each plot as the point of reference.

In Crop A, there was no significant correlation between numbers of *Pratylenchus* per 200 ml soil and per gram dry weight of root at 58 DAS (r=0.29). However, in the contour plots there was a patch of high numbers of nematodes in the soil and roots at the top centre of the trial site (Figure 3). In Crop B at 58 DAS, there was a significant correlation (r=0.59, P<0.01) between *Pratylenchus* in soil and roots. In the contour plots there was an area of higher population levels of *Pratylenchus* in the soil in the centre of the trial (Figure 4a) and in the roots at the bottom left hand corner of the trial (Figure 4b). The patchy nature of population levels across a relatively small distance can be seen in the contour plots (Figures 3 and 4), indicating the importance of taking an appropriate number of soil core samples from across the field to allow accurate determination of nematode numbers.

Figure 3. *Pratylenchus*/200 ml soil in soil (a) and per gram dry weight of roots (b) in Crop A, February 2001 at 58 DAS (carrots in rows running along X axis)

a) b)

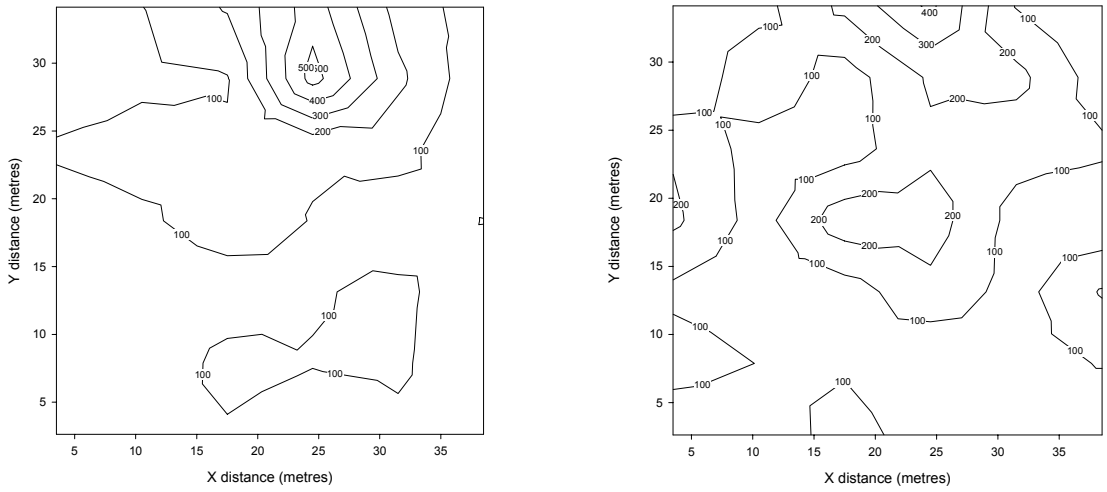
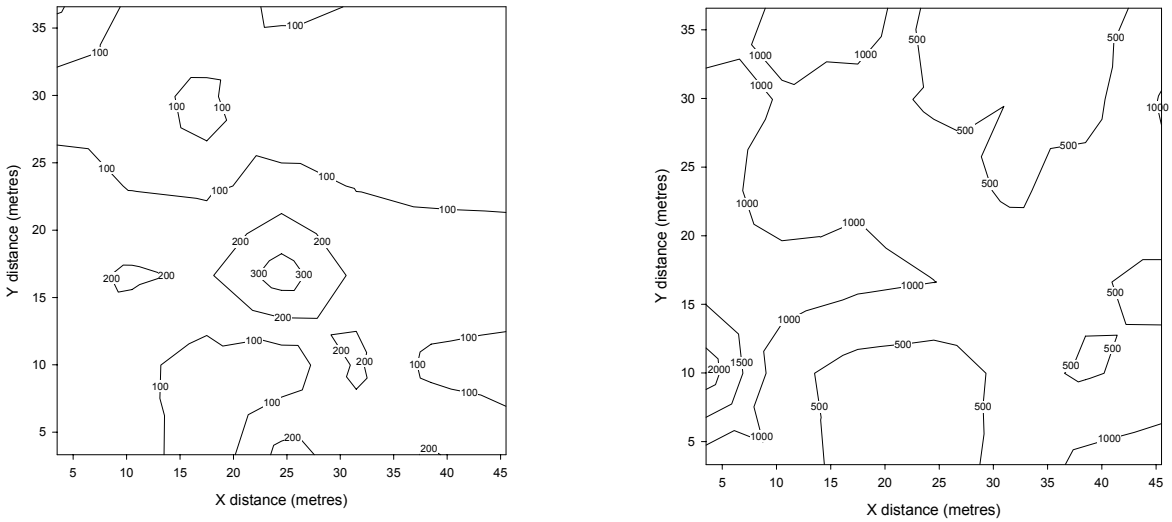


Figure 4. *Pratylenchus*/200 ml soil in soil (a) and per gram dry weight of roots (b) in Crop B, November 2001 at 58 DAS (carrots in rows running along X axis)

a) b)



4.4 Discussion

The precision of any estimate of nematode population density is improved by increasing the number of samples taken per sampling unit. Stirling *et al*. (1999) noted that the size of the sampling unit and intensity of sampling is dependent upon the value of the crop. Thus for crops valued at approximately $5000 per hectare (e.g. carrot), the sampling unit could constitute a size of 1 ha (Stirling *et al*. 1999). Stirling *et al*. (1999) indicated that as a general rule a single composite sample of 50 cores from an area of 0.5 to 1 ha would give a population estimate within 25% of the true mean. This agrees with results of trials above and suggests that a composite sample of approximately 50 core samples taken from 1 ha sections of the field would give a reasonable estimate of the true population density. As nematodes often have a patchy distribution, it is important that core samples are collected from across the

sampling unit. Pre-plant samples should therefore be collected along a zig-zag or 'W' pattern within the 1 ha sampling unit.

Core samples for vegetable crops are normally taken with a corer 2.5 cm diameter, or similar tool, to a depth of 20-30 cm. Belair (1998) suggested soil sampling for *M. hapla* in the upper 20 cm of soil in an organic soil. Some 60-68% of the *M. hapla* population occurred at 0-20 cm, with *M. hapla* found to a depth of 40 cm (Belair 1998). From each sampling unit, the 50 soil cores may be collected in a bucket, gently broken up, thoroughly mixed and a 500 g subsample sealed in a plastic bag and sent to a laboratory as soon as possible for extraction and counting. Care should be taken to ensure that samples are at all times kept at a moderate temperature (10-25^{o}C). Extremes of temperatures or extended periods between collection and processing can kill nematodes and thereby reduce the number extracted in the laboratory test.

5. Improving soil tests for nematodes (Western Australia)

A pre-plant nematode count is often used to indicate whether application of a nematicide is required. Nematodes are often extracted using the Whitehead tray technique, and a recommendation for treatment is made, if the number of plant pathogenic nematodes exceeds a pre-determined threshold.

Nematode counts on pre-plant soil samples can give an indication of the likely risk to the crop. However, there may be occasions where there is a poor relationship between the count and subsequent damage to the crop. There are a number of possible reasons for these false negatives: a) nematodes were dormant in the soil sample, and did not hatch during extraction, b) inappropriate handling of soil samples so that the nematodes died, c) the threshold levels are too high for local conditions, d) incorrect identification of plant pathogenic nematodes.

For *Meloidogyne*, the threshold for damage is often very low and the soil sampling and extraction technique may not be sensitive enough to detect population levels that are capable of causing substantial damage.

The efficiency of the extraction method used can have a bearing on the subsequent result. Laboratories quoting nematode numbers need to make clear what technique was used and quote the extraction efficiency, or provide numbers corrected for extraction efficiency. The Whitehead tray technique is most commonly used for nematode extraction and often has an extraction efficiency of around 50%. Some laboratories quote nematode numbers per weight of soil (e.g. per 200 g) and may correct for soil moisture, while others quote per volume of soil (e.g. per 200 ml). Unless the bulk density of the sample is known, it is difficult to convert between numbers quoted per volume of soil and those per weight. However in practice, the variation associated with bulk density conversions is generally less than the variation associated

with field sampling (Merrifield 1998). Therefore numbers per volume soil are an acceptable approximation of numbers per weight of soil (corrected for dry weight) for making management decisions (Merrifield 1998).

5.1 Example of poor correlation between pre-plant nematode count and seedling infection.

A site infested with root knot nematode at the Department of Agriculture, Western Australia, Medina Research Station, was used to investigate the association between nematode levels and carrot quality in crops sown in 2001 and 2002.

The effect of different periods of extraction on extraction efficiency of the Whitehead tray technique was tested for root knot nematode. Preliminary work had indicated a poor correlation between nematode numbers and seedling infection and carrot quality (see section 8.4). The site was seeded to carrots in January 2001, and the level of seedling infection determined in March 2001, using the trypan blue method (Sharma and Modiuddin 1993). Twelve seedlings were taken from each sampling point and incidence of infection assessed. Six soil samples from carrot grown on the Medina Research Station with high, moderate or low seedling infection were used. Three replicate samples of 200 g soil were extracted by the Whitehead tray method for several weeks, with the soil suspension being removed after 7 days and replaced with fresh water. Results show that many more nematodes were extracted between 1 and 2 weeks, than during the first week (Table 4). The number extracted between 0 and 1 week as a percentage of the total number extracted between 0 and 3 weeks was between 0% and 0.5% for 4/5 beds and 17% for 1/5 beds. This indicated that a period of 1 week extraction using the Whitehead tray technique was not long enough to extract the majority of *Meloidogyne* J2.

The field was left fallow following harvest of the carrot crop in May 2001 and used for similar studies in 2002. A pre-planting soil survey was conducted on 12/12/2001, after the site had been irrigated for 1 week. Soil samples taken from the same sampling points as in 2001 were extracted for 0 to 2 days and 2 to 4 days in Whitehead trays. The site was sown on 20/12/2001, and a seedling assessment using the trypan blue method of Sharma and Mohiuddin (1993) carried out on 29/1/2002. The results show that the pre-planting soil extraction was very poorly correlated (r=0.27) with seedling infection 7 weeks later. Seedling infection in 2001 was more highly correlated (r=0.65) with seedling infection in 2002.

Table 4. Number of *Meloidogyne* J2 recovered from 200 g soil after different times of extraction by the Whitehead tray technique.

		Meloidogyne J2/200 g soil recovered (standard deviation)		
Location and length of carrot bed	Level of seedling infection	0-1 week	1-2 weeks	2-3 weeks
Bed 8 (15 m)	Low	0.3 (0.6)	52 (35)	60 (33)
Bed 11 (55 m)	Low	0	0	0
Bed 4 (75 m)	Medium	1 (1)	75 (93)	136 (99)
Bed 13 (15 m)	Medium	0	41 (36)	55 (43)
Bed 3 (25 m)	High	346 (226)	810 (381)	865 (789)
Bed 5 (5 m)	High	0.3 (0.6)	52 (35)	60 (33)

In studies in Quebec, Belair (1998) also noted a poor correlation between densities of *M. hapla* in the soil as measured by the Baermann pan method (Townshend 1963), and the number of galls on tomato roots in a bioassay. The bioassay was a more sensitive than the Baermann pan method for detecting low numbers of *M. hapla* (Belair 1998). The poor correlation of nematode numbers and seedling infection in our study prompted an investigation of the extraction of *Meloidogyne* juveniles from soil. Six soil samples from areas of the Medina Research Station site with high, moderate or low seedling infection were taken in April 2002. Three replicate 200 g samples from each sampling site were extracted for 6 weeks, with the soil

suspension being removed at 7 day intervals and replaced with fresh water. Results show that many more nematodes were extracted between 1 and 2 weeks, than during the first week (Figure 5). This extended extraction was repeated with soil samples collected in July 2002. The original sampling positions were re-sampled, together with additional ones that spanned a range of nematode densities. The results from this winter sampling show that the total number of *Meloidogyne* J2 that were extracted is lower in winter than in autumn (Table 5, Figure 6). Although increasing the extraction time increased the number of nematodes recovered, the increase in the winter sample was not as great as that in the autumn sample (Figures 5 and 6). The log number of juvenile *Meloidogyne* recovered after 6 weeks was more closely correlated with the number recovered after 3 weeks than after 1 or 2 weeks (Table 6).

Table 5. Total number of *Meloidogyne* J2 extracted from soil samples taken from carrot grown at Medina Research Station, Western Australia.

	Bed									
	1-6	2-25	3-25	4-74	5-5	6-15	7-65	8-15	11-55	13-15
April	-	-	3375.8	408.9	288.4	-	-	245.8	0.5	120.5
July	89.4	10.1	15.4	5.1	18.6	20.9	11.7	3.1	0.3	3.6

Table 6. Correlations between log number of juvenile *Meloidogyne* extracted for different times (n=60).

Comparison	Correlation coefficent (r)
Extraction after 1 week with extraction after 6 weeks	0.497
Extraction after 2 weeks with extraction after 6 weeks	0.947
Extraction after 3 weeks with extraction after 6 weeks	0.986

Figure 5. The effect of duration of extraction on the cumulative number of *Meloidogyne* juveniles (J2) extracted from soil from different carrot beds in April 2002. Text in parentheses indicates the level of carrot seedling infection at these locations.

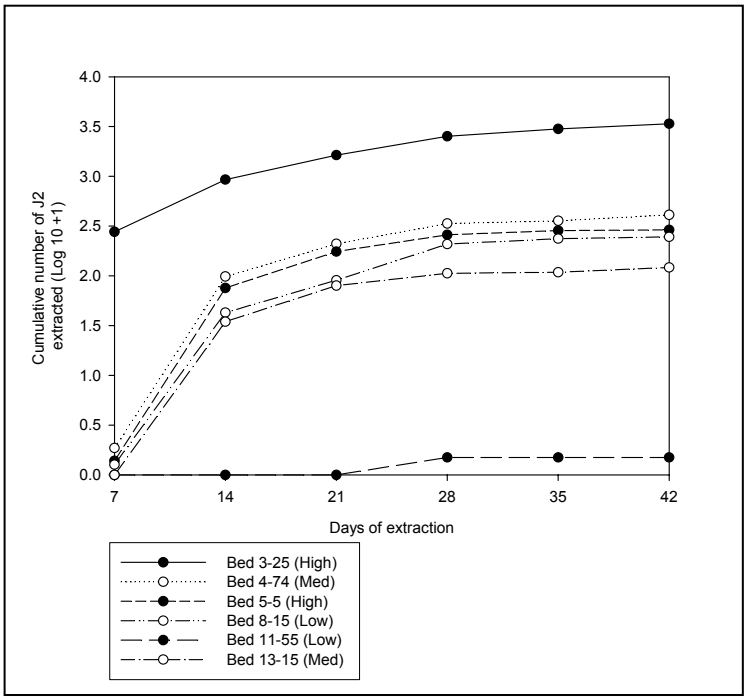
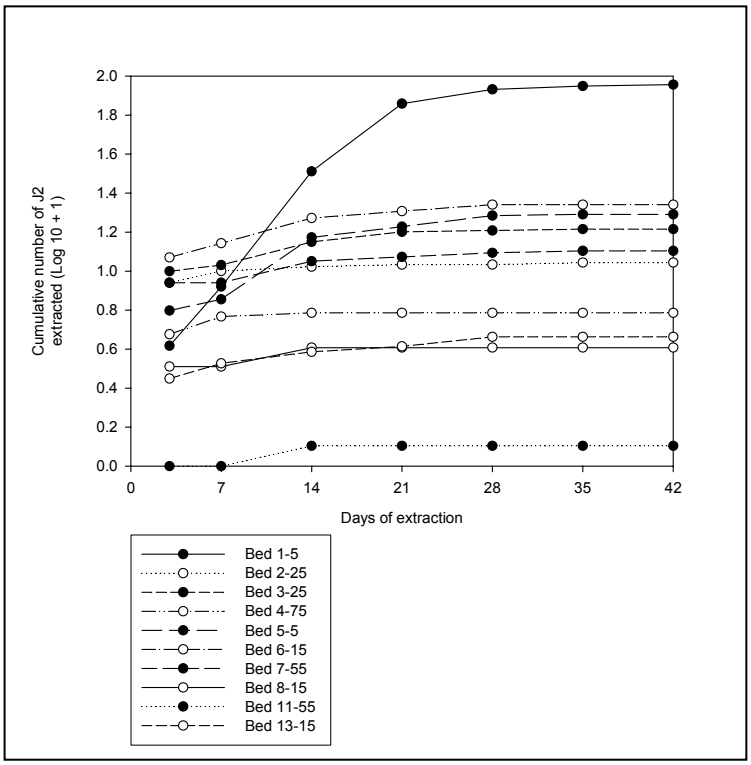


Figure 6. The effect of duration of extraction on the cumulative number of *Meloidogyne* juveniles (J2) extracted from soil from different carrot beds in July 2002.



Conclusions

In most cases, *Meloidogyne* J2 extracted by 14 days comprised a high percentage of the total number extracted over 42 days. A recommendation has been made to the AgWest Plant Laboratories to increase the length of time that soil samples are extracted from 4 days to 14 days, made up to two 7 day extractions, and to increase the number of replicate soil samples extracted from two to three.

6. Survey for nematodes in carrot crops in Australia

6.1 Tasmania 2000/2001 season.

A survey was initiated in late 2000 and early 2001 in conjunction with field officers from Simplot Australia Pty. Ltd., Harvest Moon Forth Farm Produce Pty. Ltd. and Field Fresh Tasmania Pty. Ltd. Soil samples were gathered from fields within one month after emergence of carrots. In each field, a soil and root sample was taken at approximately 30 metre intervals along every 10th bed. Nematodes were extracted by Whitehead tray method (Whitehead and Hemming 1965) from a 400ml sample taken from bulked soil and from weighed quantities of roots cut into 1cm lengths. Nematodes were counted under a microscope.

Pratylenchus were the main species associated with cropping soils in the Northern regions of Tasmania (Table 7). *Pratylenchus* were present on 28 of 33 crops surveyed at an average population density of 106/400 ml soil in carrot crops up to 5 weeks old (Table 7). They occurred at a wide range of population densities. Stunt nematodes (*Tylenchorhynchus* spp. and *Merlinius brevidens*) occurred on 14 farms and at an average population density of 11/400 ml soil early in the season (Table 7). Other genera occurred at low population density (on average less than 5/400 ml soil). *Paratylenchus*, stunt nematodes and *Helicotylenchus* are not known as significant parasites of carrots, especially at the low population densities observed. Second stage juveniles of cyst nematode (*Heterodera* spp.) were observed in soil extracts from 3 farms. However, there were no cysts observed on carrot root samples taken at the time of sampling and *Heterodera* J2 were not obtained from root extractions, indicating that the species was not *H. carotae*, a known parasite of carrot in other countries. It is probable that the *Heterodera* J2 observed in Tasmanian carrot fields were *H. trifolii*. *H. trifolii* is widespread in pastures in Tasmania and the J2 observed in this study were probably survivors from pasture rotations or surviving on clover weeds growing in the crop. Root knot

nematode was found infrequently, but is of concern because this nematode can be extremely damaging to carrot at low population densities. Root-knot nematode was associated with damage to approximately 10% of one crop surveyed and caused stunting of the taproot, proliferation of branch roots and produced characteristic galls on the feeder roots.

Fixed specimens of some genera of nematodes from 17 farms were sent to Dr. Jackie Nobbs, SARDI to identify to species. *P. crenatus*, *P. neglectus*, *P. thornei* and *P. penetrans* were confirmed from 14, 4, 2 and 1 out of 17 crops respectively (Table 8). A stunt nematode from one farm was identified as *Merlinius brevidens*. Second stage juveniles of *Meloidogyne* were identified as *Meloidogyne fallax* from 2 (possibly 3 of the farms). Up until now it has been assumed that the main *Meloidogyne* species in Tasmania was *M. hapla*, although *M. arenaria* has also been reported (Nobbs 2003). *Meloidogyne fallax* was first described from the Netherlands (Karssen, 1996) and has recently been reported from at least 6 sites in South Australia (Nobbs *et al*. 2001). It is thought this nematode has been in Australia for many years and is likely to be widespread, so it is not considered a quarantine issue within Australia. The presence of this nematode in Tasmania will be of concern to local growers as it can be particularly damaging to carrot and potato crops at higher population densities, causing a blistered appearance to the surface of potato tubers, similar in appearance to scab.

Table 7. Mean, maximum and minimum number of nematodes extracted per 400 ml soil from 33 Tasmanian carrot crops up to 5 weeks in age (November 2000 to February 2001).

	Pratylenchus	*Paratylenchus*	*Stunt*[1]	*Heterodera trifolii*	*Meloidogyne*	*Helicotylenchus*
Average	106.1	4.5	11.4	1.0	2.5	0.4
Maximum	627.0	40.1	76.0	17.9	12.0	5.8
Minimum	0	0	0	0	0	0
Number of crops in which present	28	8	14	3	5	1

[1] Includes *Tylenchorhynchus* spp. and *Merlinius brevidens*.

Table 8. Identification of nematode species present in carrot fields in Tasmania (2000/2001).

Farm and location	*P. crenatus*	*P. neglectus*	*P. thornei*	*P.penetrans*	*M. fallax*	*M. brevidens*
NS (Heybridge)	+					
MH (Sprent)		+			+	
MB (Kindred)	+					
C (Somerset)	+			+		
W (Forth)		+				
JP (Penguin)		+	+		?[1]	
SG (Wesley Vale)	+					
GR (Sulphur Creek)	+	+			+	+
DV (Penguin)	+					
PR (Sulphur Creek)	+					
DW (Burnie)	+					
IY (Sasafrass)	+					
K (unknown)	+		+			
R (Cressy)	+					
H (Kindred)	+					
RR (Thirlstane)	+					
B (Sassafrass)	+					
Total number	14	4	2	1	2 (3?)	1

[1] Potential *M. fallax*, but juvenile destroyed while making slide.

6.2 South Australia

Soil samples were collected from 29 fields between 2000 and 2002. Various genera were found associated with carrot in the South Australian survey. The genera and number of fields in which they were detected included: *Pratylenchus* (19)*, Tylenchorhynchus* (17)*, Meloidogyne* (12)*, Paratrichodorus* (12)*, Paratylenchus* (12)*, Xiphinema* (2)*, Morulaimus* (1)*, Hemicycliophora* (3)*, Scutellonema* (1)*, Macroposthonia* (3). Other genera present included*, Merlinius, Gracilacus*, and *Criconema*. Representative specimens were identified to species (Table 9) and deposited in the Waite Institute nematode collection. *Meloidogyne* were identified to species by morphology and by DNA techniques (J. Cobon pers. comm.). In addition, approximately 50 adult female *Meloidogyne* sp. from carrot roots collected from Nuriootpa, Blanchetown, Barmera, Purnong were identified by DNA techniques as *M. javanica.* (J. Cobon pers. comm.). Actual counts of particular nematodes on properties are presented (Table 10).

Table 9. Specimens collected from South Australian carrot crops deposited in Waite Institute, with accession numbers and DNA-identified *Meloidogyne* spp.

Accession no.	Species	Location
1137a	*Tylenchorhynchus latus*	Loxton
1137c	*Meloidogyne javanica*	Morgan
1153a	*Meloidogyne javanica*	Blanchetown
1153b	*Meloidogyne javanica*, *Tylenchorhynchus annulatus*, *Hemicycliophora saueri*	Nuriootpa
1153c	*Meloidogyne javanica*	Purnong
1156	*Pratyenchus neglectus*, *Merlinius brevidens*	Parilla
1159a, 1178a	*Hemicycliophora saueri*	Nuriootpa
1159b	*Scutellonema brachyurum*, *Gracilacus* sp.	Purnong
1187	*Hemicycliophora saueri*, *Scutellonema brachyurum*	Purnong
1164b	*Pratylenchus* sp., *Tylenchorhynchus* sp.	Virginia
1164c	*Pratylenchus thornei*, *Paratrichodorus* sp.	Virginia
1164d	*Paratylenchus* sp.	Virginia
1167a	*Pratylenchus* sp.	Virginia
1167b	*Pratylenchus thornei*	Virginia
1167c	*Pratylenchus* sp.	Virginia
1167d	*Pratylenchus thornei*, *Tylenchorhynchus* sp.	Virginia
1167e	*Pratylenchus thornei*, *Paratrichodorus renifer*	Virginia
1167f	*Pratylenchus* sp., *Paratrichodorus renifer*	Virginia
1167g	*Pratylenchus* sp., *Tylenchorhynchus* sp.	Virginia
1167h	*Tylenchorhynchus* sp.	Waterloo Corner
1168a	*Pratylenchus* sp., *Paratrichodorus minor*, *Criconema* sp.	Port Gawler
1168b	*Pratyenchus neglectus*, *Tylenchorhynchus* sp., *Xiphinema brevicolle*, *Gracilacus* sp., *Meloidogyne* sp.	Barmera
1168c	*Paratrichodorus* sp., *Tylenchorhynchus mashhoodi* (also includes *Heterodera schachtii* from previous crop of cabbage)	Virginia
1168d	*Hemicycliophora saueri*, *Paratrichodorus* sp.	Taylorville
1169b	*Pratylenchus* sp., *Meloidogyne* sp.	Virginia
1176h, 1178b	*Pratylenchus neglectus*	Barmera
1178a	*Hemicycliophora saueri*, *Paratrichodorus* sp.	Nuriootpa
1178b	*Helicotylenchus dihystera*, *H. pseudorobustus*, *Tylenchorhynchus* sp., *Paratylenchus* sp.	Barmera
1178c	*Paratrichodorus lobatus*	Waikerie
1178d	*Paratrichodorus* sp.	Waikerie

Table 10. Nematode species obtained from carrot crops between year 2000 and 2002.

Location	Date	Crop details	Plant parasitic nematodes*	
			Soil (No./300 mL)	Roots (No. /10 g FW)
Riverland				
Loxton (LRC)	Feb 00	Maturity	30 *Pratylenchus*, 324 *Tylenchorhynchus*	
Morgan (Buil)	Feb 00	Maturity (organic)	1370 *Meloidogyne*, 162 *Paratrichodorus*	*Meloidogyne javanica*
Barmera (Mas)	Nov 00	Maturity	4 *Pratylenchus*, 2 *Paratylenchus*, 4 *Tylenchorhynchus*	Nil
		Maturity	Nil	Nil
		At emergence	2 *Meloidogyne*, 27 *Pratylenchus*, 3 *Tylenchorhynchus*	Nil
		Hill	90 *Meloidogyne*, 30 *Pratylenchus*, 9 *Paratylenchus*, 3 *Tylenchorhynchus*, 3 *Xiphinema*	Nil
Barmera (Mas)	Jan 01	A		6.1 *Meloidogyne*
		B		23.0 *Meloidogyne*
		C		793 *Meloidogyne*
		D		1.1 *Meloidogyne*
		E		139 *Meloidogyne*
		Trial site A, B, C		Nil
	Mar 00	Maturity		216 *Pratylenchus*
Blanchetown (Plow)	June 00		392 *Meloidogyne*, 25 *Paratrichodorus*	
Blanchetown (Plow)	Aug 00	1-3	7 *Tylenchorhynchus*	
		4-6	11 *Paratrichodorus*	
		7-9	14 *Paratrichodorus*	
		10-13	Nil	
		E (maturity)	6 *Paratrichodorus*, 3 *Morulaimus,* 3 *Hemicycliophora*	
		R (maturity)	Nil	Nil
		R (maturity)	6 *Paratrichodorus*	Nil
Blanchetown (Plow)	Apr 01	A, B, C	A= 3 *Hemicycliophora*, B,C = Nil	
Blanchetown (Plow)	Jul 01	A	Nil	26.2 *Meloidogyne*
		B, C, D, E	Nil	Nil
		F	6 *Meloidogyne*	Nil
Bowhill/Murray Bridge	Sep 99	Spa	5 *Pratylenchus*, 10 *Tylenchorhynchus,* 15 *Paratylenchus*	

Location	Date	Crop details	Plant parasitic nematodes*	
			Soil (No./300 mL)	Roots (No. /10 g FW)
		11	17 *Pratylenchus*, 44 *Tylenchorhynchus*	
		15	55 *Meloidogyne*, 88 *Pratylenchus*	
		16	13 *Meloidogyne*, 117 *Pratylenchus*, 143 *Paratylenchus*	
Purnong (Bar)	Aug 00	2 (organic)	312 *Meloidogyne*, 12 *Scutellonema,* 6 *Tylenchorhynchus*	
		4 (organic)	138 *Meloidogyne*, 18 *Scutellonema,* 42 *Paratrichodorus*, 102 *Paratylenchus*	
Purnong (Bar)	Sep 00	Preplant (before 5 cultivations)	Mean densities (*n*=13):204.5 *Meloidogyne* (range 0-1072), 2.8 *Paratrichodorus*, 3.5 *Pratylenchus ,* 1.9 *Hemicycliophora*, 7.3 *Scutellonema,* 8.8 *Tylenchorhynchus*, 27.3 *Paratylenchus*, 0.9 *Xiphinema*, 2.1 *Macroposthonia*	
		Volunteer onions	2 *Pratylenchus*, 2 *Scutellonema*	1.3 *Pratylenchus*
		Volunteer onions	70 *Meloidogyne*, 4 *Tylenchorhynchus*, 4 *Paratylenchus*	1.1 *Macroposthonia*
		Weed (fumitory)	Nil	Nil
		Weed (fumitory)	18 *Meloidogyne*, 3 *Paratylenchus*	Nil
		Weed (neverdie)	*Meloidogyne javanica*	*Meloidogyne javanica*
Purnong (Bar)	Feb 01	Range of densities in 60 samples (mid-season)	*Meloidogyne*:, Not detected (38.3%), 1-30 (15%), 31-100 (21.7%), 101-500 (25%)	
Taylorville (Her)	Nov 00	A	2 *Paratrichodorus*	Nil
		B, C, D, E, F, G	B, C, D, E, F, G = Nil	B, C, D, E, F, G=Nil
		H (Stefano)	5 *Paratrichodorus*, 3 *Hemicycliophora*	Nil
		H (Red count)	25 *Paratrichodorus*	Nil
Taylorville (Her)	Jan 01	Stefano	Nil	0.1 *Pratylenchus*
		Kyoto	Nil	Nil
Waikerie (Del)	Jan 01	RKN-affected patch	2336 *Meloidogyne,* 4 *Paratrichodorus*	973 *Meloidogyne*
		Whole field	189 *Meloidogyne*, 5 *Pratylenchus*, 18 *Paratrichodorus*, 5 *Macroposthonia*	3 *Meloidogyne*
		Fallow field	42 *Paratrichodorus*, 4 *Macroposthonia*	-
	Apr 01	A	4 *Meloidogyne,* 67 *Paratrichodorus*	
		ID	624 *Meloidogyne,* 4 *Paratrichodorus*	

Location	Date	Crop details	Plant parasitic nematodes*	
			Soil (No./300 mL)	Roots (No. /10 g FW)
Waikerie (Ste)	Jan 01	Road	8 *Paratylenchus*	-
		Hill	21 *Paratrichodorus*	
		A (5-6 leaf)	70 *Paratrichodorus*	
		B	4 *Paratrichodorus*, 8 *Tylenchorhynchus*	
		C	2 *Pratylenchus*	
	Apr 01	A	11 *Pratylenchus*, 14 *Paratrichodorus*	0.7 *Pratylenchus*
		B	12 *Paratrichodorus*	Nil detected
		C	16 *Pratylenchus*, 4 *Paratrichodorus*	1.4 *Pratylenchus*
		D	14 *Pratylenchus*, 18 *Paratrichodorus*	Nil
Murtho (Kan)	Sept 00	P19	4 *Pratylenchus*, 4 *Tylenchorhynchus*	
		P17	Nil	
		P20	3 *Pratylenchus*	
		P10	Nil	
		P9	2 *Tylenchorhynchus*	
		P8	Nil	
Murtho (Kan)	Nov 00	A, B, C, D, E	A, B, D and E =Nil, C= 1 *Meloidogyne*	D, E = Nil
Murtho (Kan)	Jan 01	A, B, C	A, B, C = Nil	A, B =Nil
Murtho (Kan)	May 03			
		14C	2 *Pratylenchus*	
		14D	2 *Pratylenchus*, 2 *Paratylenchus*	
		17C	Nil	
		18A	Nil	
		23BC	840 *Pratylenchus*, 21 *Paratylenchus*, 7 *Tylenchorhynchus*	
		22K(1)	9 *Pratylenchus*	
		22K(2)	18 *Meloidogyne*, 6 *Pratylenchus*	
Murray Mallee				
Parilla (Py)	Jan 00	B3.1	88 *Pratylenchus*, 4 *Paratylenchus*, 8 *Tylenchorhynchus*	
		B3.2	72 *Pratylenchus*, 5 *Paratylenchus*	
		27	3 *Pratylenchus*, 3 *Paratylenchus*	
		28	4 *Pratylenchus*	

Location	Date	Crop details	Plant parasitic nematodes*	
			Soil (No./300 mL)	Roots (No. /10 g FW)
	00	Flame	20 *Pratylenchus*, 13 *Tylenchorhynchus*	
	00	Red hot	18 *Pratylenchus*, 84 *Tylenchorhynchus*	
	00	Pivot 4		
		East	120 *Pratylenchus*, 13 *Tylenchorhynchus*, 60 *Paratylenchus*	
		West	155 *Pratylenchus*, 13 *Tylenchorhynchus*	
		Hill	77 *Pratylenchus*, 13 *Tylenchorhynchus*	
Parilla (Py)	Jun 00	B8.1	140 *Pratylenchus*	Roots < 2mm: 11.7 *Meloidogyne* 5.7 *Pratylenchus*
		B8.2	100 *Pratylenchus*, 16 *Tylenchorhynchus*, 4 *Paratylenchus*	Tap roots: 0.5 *Tylenchorhynchus*
			-	Tap roots: nil
Parilla (Py)	Sept 00	B1	4 *Pratylenchus*, 8 *Tylenchorhynchus*, 88 *Paratylenchus*	
		B2	28 *Pratylenchus*, 4 *Tylenchorhynchus*, 32 *Paratylenchus*	
Parilla (Py)	Dec 00	B8	2 *Paratylenchus*	0.6 *Pratylenchus*
		T3	102 *Pratylenchus*, 4 *Paratylenchus*	
		B6	53 *Pratylenchus*, 11 *Tylenchorhynchus*, 7 *Paratylenchus*	
Parilla (Py)	Nov 99	Research Rd.	10 *Tylenchorhynchus*	
		27	41 *Pratylenchus*, 27 *Tylenchorhynchus*	
		28	9 *Pratylenchus*	
		29	440 *Pratylenchus*, 20 *Tylenchorhynchus*	
Parilla (Sob)	00	Unhealthy	3 *Meloidogyne*, 6 *Paratrichodorus*	
Barossa Valley				
Nuriootpa (Glow)	May 00	Maturity (infection lens)	1848 *Meloidogyne*, 44 *Hemicycliophora,* 6 *Tylenchorhynchus*	
		Volunteer carrots	Nil	Nil
		Volunteer carrots		Nil (cf. weeds: 3.3 *Pratylenchus*)
		Volunteer carrots		Nil (cf. cereal cover crop: 4.2 *Pratylenchus*)
		Volunteer carrots		Nil (or in weeds/cereals)

Location	Date	Crop details	Plant parasitic nematodes*	
			Soil (No./300 mL)	Roots (No. /10 g FW)
		Volunteer carrots		Nil (cf. cereals: 4.5 *Pratylenchus*)
		Volunteer carrots		9.5 *Pratylenchus* (nil in weeds/cereals
		Volunteer carrots		Nil (cf. weeds: 2.8 *Pratylenchus*)
		19 A-C	42 *Meloidogyne*, 11 *Paratrichodorus*, 4 *Hemicycliophora,* 53 *Tylenchorhynchus*	
		19 D-F	77 *Meloidogyne,* 59 *Tylenchorhynchus*	
		19 G-I	40 *Meloidogyne,* 5 *Tylenchorhynchus*	
		20 A-C	60 *Tylenchorhynchus*	
		19 D-F	50 *Tylenchorhynchus*	
		19 G-I	9 *Paratrichodorus*, 50 *Tylenchorhynchus*	
Nuriootpa (Glow)	Aug 00	Maturity	16 *Meloidogyne*, 6 *Paratrichodorus*, 63 *Hemicycliophora,* 11 *Tylenchorhynchus*	
		19 D	18 *Paratrichodorus*, 3 *Hemicycliophora*	
		19 E	11 *Paratrichodorus*, 3 *Hemicycliophora*	
		19 F	Nil	
		20 D	12 *Paratrichodorus*, 60 *Tylenchorhynchus*	
		20 E	12 *Tylenchorhynchus*	
		20 F	81 *Tylenchorhynchus*	
		Fallow	21 *Tylenchorhynchus*	
Nuriootpa (Glow)	Sept 00	Pre-fumigation	Mean densities (*n*=36): 19.7 *Meloidogyne* (range 0-189), 1.8 *Paratrichodorus*, 0.5 *Hemicycliophora,* 26 *Tylenchorhynchus*, 0.2 *Paratylenchus*	-
Nuriootpa (Glow)	Dec 00	3-5 leaf stage	7 *Tylenchorhynchus*	Nil detected
	Nov 00	Emergence	3 *Meloidogyne*	
		Post-emergence	6 *Meloidogyne*, 4 *Paratrichodorus*, 1 *Pratylenchus,* 1 *Paratylenchus*	
		Preplanting	5 *Meloidogyne*, 6 *Paratrichodorus*, 3 *Pratylenchus,* 1 *Hemicycliophora,* 11 *Tylenchorhynchus*	
		Fallow	21 *Meloidogyne*	

Location	Date	Crop details	Plant parasitic nematodes*	
			Soil (No./300 mL)	Roots (No. /10 g FW)
	Jan 01	2A		Nil
		2B		Nil
	Apr 01	Siegers. Rd.	12 *Tylenchorhynchus*, 6 *Paratylenchus*	2.9 *Pratylenchus*
Adelaide Plains				
Port Gawler (App)	Nov 00	Maturity	4 *Pratylenchus*, 11 *Paratrichodorus*	Nil
		Harvested	57 *Paratrichodorus*, 6 *Macroposthonia*	-
Port Gawler (Mus)	Nov 00	A	3 *Paratrichodorus,* 3 *Tylenchorhynchus*	Nil
		B	Nil	
Port Gawler (Mus)	Feb 01	A, B, C	-	A=Nil, B=0.03 *Pratylenchus* and C=0.02 *Meloidogyne*
Virginia (Nic)	Nov 99		22 *Pratylenchus*	
Virginia (Bnic)	Oct 00	Max	Nil detected	Nil
		Trans	12 *Meloidogyne*, 6 *Pratylenchus*, 2 *Tylenchorhynchus*, 2 *Paratylenchus*	Nil
Virginia (Zer)	Oct 00	Mojo (maturity)	4 *Tylenchorhynchus*	Nil
		Riccardo A	6 *Pratylenchus*	
		Riccardo B	24 *Pratylenchus*, 12 *Tylenchorhynchus*	
Virginia (Zer)	Nov 00	Short/Robert Rd. (maturity)	11 *Pratylenchus*	Nil
		Johns Rd. (maturity)	109 *Pratylenchus*, 21 *Paratrichodorus*, 4 *Tylenchorhynchus*	Nil
		Short/Penfield Rd. (Riccardo)	4 *Pratylenchus* , 16 *Tylenchorhynchus*	Nil
		Mojo (maturity)	4 *Pratylenchus* , 7 *Tylenchorhynchus*	Nil
Virginia (Trim)	Oct 00	Metham (maturity)	Nil	
		Nemacur (maturity)	27 *Pratylenchus*, 11 *Paratrichodorus*, 6 *Paratylenchus*	
Virginia (Trim)	Nov 00	Legoe Rd. (maturity)		1 *Pratylenchus*

Location	Date	Crop details	Plant parasitic nematodes*	
			Soil (No./300 mL)	Roots (No. /10 g FW)
Virginia (Kin)	Oct 99		Nil	Nil
Virginia (Co)	Sept 99		11 *Tylenchorhynchus*, 55 *Paratylenchus*	
Virginia (Mec)	Nov 00	Corona	16 *Pratylenchus*	
		Mojo (maturity)	2 *Pratylenchus*, 2 *Paratrichodorus*	Nil
Virginia (Mec)	Aug 01		7 *Tylenchorhynchus*	
Virginia (Mar)	Dec 01		24 *Pratylenchus*, 112 *Paratylenchus*	
Waterloo Corner (Cir)	01	N1, N2	N1=57 *Tylenchorhynchus,* N2=36 *Tylenchorhynchus*	
Waterloo Corner (Cir)	Nov 00	Symes Rd.	12 *Tylenchorhynchus*	
		Anjanto Rd. (Mojo)	4 *Tylenchorhynchus*	
		Anjanto Rd. (Riccardo)	Nil detected	
Waterloo Corner (DiF)	Nov 00	B1	2 *Pratylenchus* 18 *Tylenchorhynchus*	
		B2	2 *Tylenchorhynchus*	0.3 *Pratylenchus*
		A2 (22-24)	28 *Tylenchorhynchus*	
		A2 (25-30)	25 *Tylenchorhynchus*	
Adelaide Hills				
Ashbourne (Mer)	Dec 99	2 samples	Nil	
South East				
Rendlesham	Sept 99	Seed	5 *Pratylenchus*, 65 *Paratylenchus*, 45 *Tylenchorhynchus*	
Tantanoola	Sept 99	Seed	60 *Pratylenchus*, 108 *Paratylenchus*	
Kybybybolite (Mu)	Apr 02	Trial (maturity)	Mean densities (*n*=30):21.5 *Paratrichodorus* (range 0-179), 0.6 *Pratylenchus*	Mean densities (*n*=30): 7942 *Pratylenchus* (range 0-201,000)
	Jan 02	Volunteer weeds from carrot crop		Wireweed: nil Potato: nil Ryegrass: 6 *Pratylenchus*

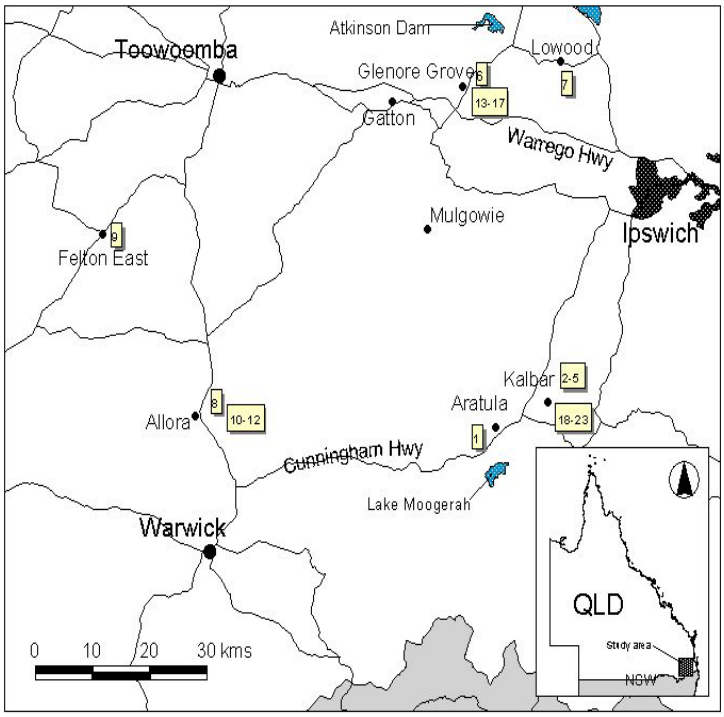
*soil cores and plant samples were taken using a systematic random sampling protocol except where otherwise indicated; individual growers often had more than one property and also divided fields into separate sections with different carrot cultivars/planting dates and these were sampled separately; samples were taken from carrot crops except where indicated, for example, from cereal rotation crops and broadleaved weeds between carrot crops; *Paratylenchus* sp. s.l. and *Tylenchorhynchus* sp. s.l.; methods of nematode extraction used were trays for soil and mist chamber for roots

6.3 Queensland

6.3.1 Sampling

Surveys were conducted in the three main carrot producing areas of south-east Queensland, which included the eastern Darling Downs region and the Fassifern and Lockyer Valleys (Figure 7). Field surveys were conducted to establish which plant-parasitic nematodes were associated with carrots grown in these areas and whether these nematodes had an effect on yield or quality. Soil and roots samples were collected in a 0.5 hectare area at each site. Fifty separate samples of soil and root were collected at random in a zig zag pattern across the fields. Soil samples were taken to a depth of 10-20 cm using a 2 cm diameter soil corer. Root samples were also collected where carrot plants were growing.

Figure 7. Map of areas sampled for plant-parasitic nematodes in carrots. Numbers displayed indicate sample locations (Table 12).



6.3.2 Methods

Soil extraction

Nematodes were extracted from 200 ml soil sub-samples using a modified Baermann funnel (Whitehead and Hemming, 1965) for 4-7 days. The soil was spread evenly over 2 ply tissue that was placed inside a 5 mm mesh basket. The basket was then placed in a tray and water added to just below the height of the soil. The soil mesh basket containing the soil and tissue was removed and discarded. The water in the tray was washed through a 38 µm sieve and the nematodes collected. The nematodes were backwashed into a 30 mL vial and stored at 10 ^{o}C until counted under a compound microscope at 40X magnification.

Root extraction

Root samples consisted of the whole root system for small carrot taproots or 1-2 cm pieces dissected from the root apex of larger taproots. To extract the nematodes from the root system the root samples were placed on 2 mm mesh inside a funnel inside a collection container and misted with water for 4-7 days (Hooper, 1986). The nematodes in the collection container were recovered on a 38 µm sieve and processed as described previously.

Root-knot nematode egg extraction

Roots are immersed in 1% NaOCl solution for three minutes to extract root-knot eggs. The suspension was then passed through a 38 µm sieve and rinsed with water to remove NaOCl residue (Stanton and O'Donnell, 1994). The number of eggs was quantified as previously described.

Bioassays

Bioassays of carrot soils were conducted for *Meloidogyne* spp. using tomato seedlings (*Lycopersicon esculentum* cv.Tiny Tim) planted in 1.5 litre sub-samples of soil and the root systems examined for galls after 5-6 weeks (Stirling *et al.*, 1999).

6.3.3 Results

Twenty-three sites in south-east Queensland in the three main growing areas, the Fassifern Valley, Lockyer Valley and eastern Darling Downs were surveyed for plant-parasitic nematodes between September 2001 and April 2003 (Table 11). Soil samples were examined from all fields and root samples from 18 fields. Plant-parasitic nematodes were detected in nine carrot fields. Nematodes were detected in the soil in eight fields and in the roots in three fields.

Lesion nematodes (*Pratylenchus* spp.) were detected in the soil of seven fields (Table 11). The highest number of lesion nematodes was detected in the Allora region of the eastern Darling Downs in April 2002. *Pratylenchus penetrans/P. brachyurus* were identified in the soil from field 10 and field 11 on the eastern Darling Downs (Table 11). However, there were no lesion nematodes detected in the roots of carrot plants from these fields.

Stunt nematode (*Tylenchorhynchus spp.*) was detected in the soil at five sites and in the roots of carrots at one site (Table 11). *T. capitatus/T. impar* was isolated at site 7 in the Lockyer Valley. Stunt nematodes (*T. capitatus/T. pratensis*) were extracted from the soil at site 10.

Spiral nematode (*Helicotylenchus dihystera*) was detected in the soil of three fields and in the roots at two sites (Table 2). Ring nematode (*Criconemella* sp.) was detected also in the soil at site 16. There were no root-knot nematode species detected in any carrot fields surveyed in southeast Queensland between 2001 and 2003.

6.3.4 Conclusions

Unlike many other carrot producing areas, plant-parasitic nematodes were found in very low numbers in South east Queensland and were unlikely to be limiting carrot production. Some farms used pre-plant fumigation to control soil borne pathogens but not specifically nematodes. This may contribute to low populations detected in carrot fields. However, many farms also use rotations and plant carrots only every five years in the same field. This would be expected to maintain populations of plant-parasitic nematodes at low levels.

Pratylenchus spp. was the most common plant-parasitic nematode from carrot fields isolated from 7 fields in the survey. One site on the eastern Darling Downs was found to have relatively high numbers of *Pratylenchus penetrans*/*P. brachyurus* in the soil 6 weeks after planting. However, further samples examined 19 weeks after planting, taken from poorly growing areas, indicated that nematodes were not affecting the carrot crop.

Root-knot nematode (*Meloidogyne* spp.), a problem in many carrot production areas, was not identified in the sites surveyed in south-east Queensland. Other genera detected in the survey were spiral nematode (*Helicotylenchus dihystera*), stunt nematodes (*Tylenchorhynchus* spp.) and ring nematode (*Criconemella* spp.).

Radopholus spp. was not found at any of the sites. This was particularly important as quarantine restrictions had been imposed on Queensland carrots entering Taiwan due to the presence of *R. similis*. By conducting the survey, a declaration that carrot producing areas in south-east Queensland were free from *R. similis* has allowed export of carrots to Taiwan to commence.

Table 11. Details of carrot fields sampled and nematodes identified in the eastern Darling Downs, Fassifern and Lockyer Valleys regions in 2001, 2002 and 2003.

Field number	Location	Township	Date sampled	Age of crop at sample time(weeks)	*Pratylenchus* spp.		*Tylenchorhynchus* spp.		*Helicotylenchus* spp.		*Criconemella* spp		*Meloidogyne* spp.
					Soil	**Root**	**Soil**	**Root**	**Soil**	**Root**	**Soil**	**Root**	**Bioassay**
1.	Fassifern Valley	Aratula	21/09/01	20	0	0	0	0	0	0	0	0	0
2.	Fassifern Valley	Kalbar	21/09/01	18	1	-	0	-	1	-	0	-	0
3.	Fassifern Valley	Kalbar	21/09/01	18	0	0	0	0	0	1	0	0	0
4.	Fassifern Valley	Kalbar	21/09/01	20	0	0	0	0	0	0	0	0	0
5.	Fassifern Valley	Kalbar	21/09/01	17	1	0	0	0	0	0	0	0	0
6.	Lockyer Valley	Lowood	6/04/02	5	0	0	0	0	0	0	0	0	0
7.	Lockyer Valley	Glenore Grove	6/04/02	4	0	0	21	0	0	0	0	0	0
8.	Eastern Downs	Allora	6/04/02	4	0	0	0	0	0	0	0	0	0
9.	Eastern Downs	Felton East	6/04/02	7	0	0	0	0	0	0	0	0	0
10.	Eastern Downs	Allora	10/04/02	6	70	0	6	0	0	0	0	0	0
11.	Eastern Downs	Allora	10/04/02	44	15	0	15	0	5	2	0	0	0
12.	Eastern Downs	Allora	10/04/02	7	1	0	0	0	0	0	0	0	0
13.	Lockyer Valley	Glenore Grove	5/09/02	17	35	0	2	1	1	0	0	0	0
14.	Lockyer Valley	Glenore Grove	5/09/02	16	0	0	0	0	0	0	0	0	0
15.	Lockyer Valley	Glenore Grove	5/09/02	22	0	0	0	0	0	0	0	0	0
16.	Lockyer Valley	Glenore Grove	11/09/02	19	3	0	2	0	0	0	1	0	0
17.	Lockyer Valley	Mulgowie	11/09/02	16	0	0	0	0	0	0	0	0	0
18.	Fassifern Valley	Kalbar	2/04/03	1	0	-	0	-	0	-	0	-	0
19.	Fassifern Valley	Kalbar	2/04/03	0	0	-	0	-	0	-	0	-	0
20.	Fassifern Valley	Kalbar	2/04/03	2	0	0	0	0	0	0	0	0	0
21.	Fassifern Valley	Kalbar	2/04/03	2	0	0	0	0	0	0	0	0	0
22.	Fassifern Valley	Kalbar	2/04/03	1	0	-	0	-	0	-	0	-	0
23.	Fassifern Valley	Kalbar	2/04/03	0	0	-	0	-	0	-	0	-	0

Nematode counts are indicated as the number of nematodes/200 mL of soil or number of nematodes/100 g of root or number of root-knot galls detected on tomato bioassays grown in 1.5 L of soil

6.4 Western Australia

A survey of nematodes in carrot crops was conducted during year 2000 and 2001. Additional details of nematode sampling are given (Table 12). A number of soils were screened for carrot cyst nematode (*Heterodera carotae*) an important pathogen of carrot in some other countries. This was found to be absent from carrot soils in Western Australia. *Meloidogyne* spp. were detected at 12/25 sites. Of the four sites at which *Meloidogyne* were identified to species, two sites had *M. javanica* and two sites had *M. hapla* (Table 12). *Pratylenchus* spp. were detected at 6/25 sites. At 7/25 sites, no plant parasitic nematodes were detected (Table 12).

Table 12. Nematode samples from carrot crops in Western Australia

Reference number	Date	Location	Crop	Sample	Symptoms	Nematodes	Comments
01/1186	18.9.00	Medina Research Station	Carrot	Carrots, soil	Root galls	*Meloidogyne javanica*	ID on perineal patterns
01/2003	6.11.00	Capel	Onion	Soil	None	7.6 *Pratylenchus* /100 ml soil	Adjacent to 01/2004, no Nemacur used
01/2004	6.11.00	Capel	Carrot, 18 weeks old	Soil	None	No plant pathogenic nematodes	Nemacur used before seeding
01/2005	6.11.00	Binningup	Ex carrot site	Soil	Carrot crop forked	0.4 *Pratylenchus* /100 ml soil	
01/2028	7.11.00	Medina Research Station	Ex carrot site	Soil	Carrots with galls	No plant pathogenic nematodes seen	Same site as 01/1186
01/2377	29.11.00	Augusta	Carrot	Carrots, soil	None	*Meloidogyne hapla*	ID by Jennifer Cobon
01/2378	29.11.00	Augusta	Carrot	Volunteer potato	None	*Meloidogyne hapla*	Similar to 01/2377
01/2543	12.12.01	Medina Research Station	Carrot (99MD2 plot 13)	Carrots	Root galls	*Meloidogyne javanica*	ID by Jennifer Cobon and on perineal patterns

Reference number	Date	Location	Crop	Sample	Symptoms	Nematodes	Comments
01/4020	22.2.01	Myalup	Carrot	Carrots, soil	Bulgy eyes	*Meloidogyne*	Nematodes dead. Sample to Jenny Cobon, but would not cut. Dissected females for perineal patterns.
01/4021	22.2.01	Myalup	Carrots	Carrots, soil	None		Mainly saprophytes
01/4627	19.3.01	Myalup	Ex carrot site	Rotting carrots, soil	Root galls	*Meloidogyne, Pratylenchus*	ID from soil sample
01/4629	20.3.01	Augusta	Carrot	Carrots, volunteer potato	Small galls on some lateral roots	*Meloidogyne*	Same site as 01/2377. Being processed.
01/5492	19.4.01	Myalup	Ex carrot site	Soil	Root galls	No plant pathogenic nematodes seen	
01/5493	19.4.01	Myalup	Ex carrot site	Soil	Root galls	No plant pathogenic nematodes seen	Same grower as 01/5492
01/5494	19.4.01	Myalup	Ex carrot site	Soil	Root galls	No plant pathogenic nematodes seen	Only 1st or 2nd carrot crop; symptoms patchy.
01/5495	19.4.01	Myalup	Carrot	Soil	Root galls	924 *Meloidogyne* /100 ml soil	Grower has a problem
01/5496	19.4.01	Baldivis	Carrot	Soil	Root galls	No plant pathogenic nematodes seen	Severe galling in sampled patches

Reference number	Date	Location	Crop	Sample	Symptoms	Nematodes	Comments
01/5497	19.4.01	Myalup	Ex carrot site	Soil	None	0.5 *Meloidogyne* /100 ml soil	
01/5498	19.4.01	Baldivis	Carrot	Soil	None	1.5 *Meloidogyne* /100 ml soil	
01/6066	9.5.01	Baldivis	Carrot	Soil	Root galls	107 *Meloidogyne* /100 ml soil	
01/6259	17.5.01	Gingin	Carrot	Soil	None	1.3 *Pratylenchus* /100 ml soil	Soil sample checked by elutriation for *Heterodera* cysts, none found
01/6260	17.5.01	Guilderton	Carrot	Soil	Stunting	717 *Pratylenchus* /100 ml soil	Soil sample checked by elutriation for *Heterodera* cysts, none found
01/6261	17.5.01	Gingin	Carrot	Soil	None	1 *Pratylenchus* /100 ml soil	Soil sample checked by elutriation for *Heterodera* cysts, none found
01/6262	17.5.01	Guilderton	Carrot	Soil	None	No plant pathogenic nematodes seen	
02/684	16.8.01	Myalup	Carrots	Soil and carrots	Root galls	*Meloidogyne hapla* 3 *Meloidogyne* /100 ml soil	ID by Jennifer Cobon

6.5 Victoria

A total of 27 carrot crops on 11 properties were surveyed in five separate regions. The following nematodes were identified in each region:

1. Dandenong Ranges: *Meloidogyne hapla, M javanica.*
2. Warragul*: Helicotylenchus spp., M. hapla, Paratylenchus spp., Helicotylenchus spp., Pratylenchus spp.*
3. Gippsland: *Pratylenchus spp. P. neglectus, M. hapla, Paratrichodorus spp., Helicotylenchus sp., Paratylenchus spp.*
4. Northern Murray: *Merlinius brevidens, Pratylenchus spp., P. neglectus, M. javanica, M. hapla, Paratrichodorus spp., Tylenchorhynchus spp.*
5. Mornington Peninsula: *Heterodera schachtii, Pratylenchus spp., Paratrichodorus spp.*

6.6 PCR identification of root knot nematode

6.5.1 Methods

Nematode samples were received from carrot fields from Western Australia, South Australia, Victoria and Queensland between 2000 and 2003. Specimens of female root-knot nematodes were received in 2% Triton – X extraction buffer. Alternatively, the roots from infected carrots were received and the females extracted at the Indooroopilly nematology laboratory of the Queensland Department of Primary Industries. The females were placed in extraction buffer and pulverized and frozen until required. A PCR-based diagnostic test was used to identify species of root-knot nematodes (Stanton *et al.* 1997*).*

6.5.2 Results

A total of 173 nematode samples from carrot fields were received for root-knot nematode identification (Table 13). Positive identifications using PCR techniques were made on 64% of the samples received, with 46% of samples not allowing amplification of DNA (Table 13). Only two species of root-knot nematodes were identified in the

survey, the most frequently identified root-knot nematode species was *Meloidogyne javanica*, 71% of positive identifications. However, 66% of the root-knot nematodes species from Victoria were identified as *M. hapla*. Whereas, 72% of the root-knot nematode species from Western Australia were identified as *M. javanica*.

Table 13. Positive identifications of root-knot nematode (*Meloidogyne* spp.) females made using PCR-based diagnostic test.

	Total samples received	***Meloidogyne javanica***		***Meloidogyne hapla***		**Number of samples that didn't amplify**
Western Australia	150	56	72%	22	28%	72
South Australia	8	5	100%	0	0%	3
Victoria	14	4	44%	5	66%	5
Queensland	1	1	100%	0	0%	0
Total	173	65	71%	27	29%	80

M. javanica was the most frequently identified root-knot nematode species from Australian carrot fields. All *Meloidogyne* spp. specimens received from Queensland and South Australia, where the DNA could be amplified, were identified as *M. javanica*. Populations of both *M. javanica* and *M. hapla* were identified in both Western Australia and Victoria. The majority of *Meloidogyne* spp. nematodes identified in Western Australia were *M. javanica* (72%), whereas, in Victoria, *M. hapla* was identified most frequently (66%).

The DNA of a number of samples that were received did not amplify possibly because there was insufficient DNA in the original sample to amplify (Stanton *et al.*, 1998), DNA was degraded, or the extracted females were too old to provide sufficient high quality DNA.

7. Effect of nematodes on carrot yield and quality.

7.1 Effect of *Pratylenchus* on yield and quality of Kuroda carrot in Tasmania (2000/2001).

7.1.1 Method

A field trial was established in a commercial field of carrot in Penguin, Tasmania (-41 11 latitude, 146 07 longitude) to examine the relationship between numbers of *Pratylenchus* and yield and quality of carrot. The field was sown on 1/1/2001 and the trial established shortly after emergence in early February 2001. The trial consisted of 42 plots arranged in a 6 x 7 lattice, each 7 metres long by 3 beds wide (approximately 5.25 metres) with each bed having two, double rows of carrots. Soil and root samples were obtained on 27/2/2001, 3/4/2001 and 26/4/2001 at 58, 93 and 123 days after sowing (DAS) respectively. The middle 3 metre section of the 7 metre long plots was left untouched for harvest purposes and samples during the season were collected from a 2 metre section at each end of the plots. Samples were collected at 58, 93 and 123 DAS and consisted of 6, 18 and 18 carrots and surrounding soil respectively removed with a trowel. Foliage was removed, blotted dry and fresh weight recorded. Dry weight of foliage was recorded after oven drying (100°C/24 hours). Roots were assessed for disfigurement (stunt, bent/twisted, forked, insect damage, other). Roots were washed, blotted dry and the fresh weight recorded before nematode extraction. For samples collected at 58 DAS, fine roots were chopped into 2 cm sections for extraction, while for those collected at 93 and 123 DAS, carrots were halved longitudinally and chopped into 5 cm lengths for extraction. Nematodes were extracted from roots by the Whitehead tray technique over 3 days (described below). After nematode extraction, roots were oven dried (100°C/24h) and dry weight recorded. Soil from each plot was mixed, and nematodes were extracted from 400 ml soil by the Whitehead tray technique (Whitehead and Hemming 1965) over a period of 3 days at room temperature. Soil was gently crumbled to a fine tilth and 200 ml sprinkled on the surface of tissues suspended in a lamington pan tray (30 cm length by 20 cm long by 3 cm depth) in a wire mesh basket. The tray was half filled with water and left at room temperature (16-21^{o}C). After 96 hours, the basket was removed from the tray and soil discarded. Nematodes that had migrated from the soil into the water, were retrieved by passing through a 20 µm sieve and material retained on the sieve was rinsed into a 60 ml Johns container.

Water that passed through the sieve was sieved for a second time and rinsate collected, to collect any nematodes that may have passed through the sieve. Nematodes were allowed to settle overnight (5^{o}C) and the supernatant slowly drawn off to leave approximately 30-40 ml suspension. A 4 ml aliquot was placed in a counting dish and nematodes counted and identified to genus at 50-100 x magnification.

On 3/5/2001 (130 DAS) the density of plants was assessed in the centre 3 metre section of each plot by counting the number of plants in 1 m transects taken along 3 beds. Plots were harvested by digging up 20 plants from each of 5 double rows from within the 3 metre centre section of each plot (100 plants/plot). Foliage was removed and discarded. Taproots were washed and graded into different categories.

Carrots were graded into categories (0-5) using a scale adapted from Belair and Boivin (1996), 0 = no lateral protrusions or stunting, 1 = taproot with 1-2 lateral protrusions <1cm long, 2 = taproot with 2-3 lateral protrusions, 1-3 cm long, no stunting, 3 = taproot with 3 or more lateral protrusions > 3 cm long, no stunting, 4 = taproot with 3 or more lateral protrusions > 3 cm long, moderate stunting, 5 = taproot with 3 or more lateral protrusions > 3 cm long, severe stunting. Carrots in category 0 or 1 were considered first grade suitable for export.

Carrots were also further graded into categories of small carrots (<10 cm long, <2.5 cm wide), forked, twisted, short cracks (<2cm), long cracks (>2cm), constricted, other damage (e.g. insect).

Correlation coefficient (r) was calculated between nematode numbers and measured variables and a t-test conducted for significance of association. For some variables having a significant correlation, a regression was conducted to examine the relationship more fully.

7.1.2 Results

Moderate numbers of lesion nematodes were present at the site along with low numbers of *Heterodera trifolii* and *Paratylenchus* (Table 14). The population density of

Pratylenchus did not increase in the soil over the season (Table 14). While moderate levels of nematodes were recovered from roots early in the season, very few were extracted at mid and late season (Table 14).

At 58 DAS there was a statistically significant negative correlation between the number of *Pratylenchus* per gram dry weight of root and the dry weight of shoots and roots of young plants (Table 15). This indicated that nematode feeding was having a deleterious effect on plant growth at an early stage (Figure 8). The mean root weight of plants in plots with the highest number of *Pratylenchus* (260.6/g dry weight root) had root systems less than half the weight of those predicted at 0 *Pratylenchus*/g dry weight root (Figure 8). However at 93 DAS there was no relationship between nematode numbers at 58 or 93 DAS and dry weight of roots (Table 15).

At harvest (130 DAS), numbers of *Pratylenchus* at various times were positively correlated with the weight of carrots in category 3 and category 4 (Table 15), suggesting that *Pratylenchus* feeding was contributing to stunting of carrot. In addition there was a significant positive correlation between *Pratylenchus*/g dry weight of root at 58 DAS and the weight of carrots in the twisted category. These relationships were also evident when the amount of carrots in particular categories were expressed as a percentage of the total weight or total number of carrots (Table 15).

The number of *Pratylenchus*/200 ml soil at 58 DAS was negatively correlated with the number of carrots/ha at harvest, the estimated total yield (t/ha) and the estimated yield of first grade carrots (t/ha). However, there were no relationships between *Pratylenchus* at any time and pack out (%) or average weight of carrots (Table 15).

A regression between *Pratylenchus*/200 ml soil at 58 DAS and estimated total yield (t/ha) at harvest predicted a yield of 56.0 t/ha and 43.9 t/ha at a population density of 0 and 223 *Pratylenchus*/200 ml soil respectively, a difference of 12.1 t/ha (Figure 9).

Table 14. Variation between plots in nematode numbers, yield and plant density.

	Average	Minimum	Maximum	Standard deviation
Pratylenchus				
/200 ml soil 58 DAS	86.9	19.9	223.1	46.3
/200 ml soil 93 DAS	75.6	9.2	258.4	53.2
/200 ml soil 123 DAS	80.0	14.8	245.0	59.3
/g dry weight root 58 DAS	99.3	0	260.6	75.8
/g dry weight root 93 DAS	0.02	0	0.20	0.04
/g dry weight root 123 DAS	0.02	0	0.36	0.06
Paratylenchus				
/200 ml soil 58 DAS	1.0	0	13	2.8
/200 ml soil 93 DAS	0.3	0	3.5	0.9
/g dry weight root 58 DAS	0.1	0	3.5	0.6
/g dry weight root 93 DAS	0	0	0	0
Heterodera trifolii				
/200 ml soil 58 DAS	0.4	0	5.7	1.4
/200 ml soil 93 DAS	0.1	0	0.3	0.5
/g dry weight root 58 DAS	0	0	0	0
/g dry weight root 93 DAS	0	0	0	0
Plants/m^2	42.2	32.8	52.2	4.7
Yield (t/ha)	53.1	39.6	70.4	7.7
First grade (% by weight)	71.8	51.8	86.9	9.4

Table 15. Correlations coefficients (r) between number of *Pratylenchus* in roots and soil at different times and growth, yield and quality of Kuroda carrot.

		Pratylenchus		
	/g dry wt. 27 Feb 58 DAS	/200 ml 27 Feb 58 DAS	/200 ml 3 Apr 93 DAS	/200ml 26 Apr. 123 DAS
58 DAS				
Dwt. tops/plant (g)	-0.43 (0.01)[1]	ns	-	-
Dwt. roots/plant (g)	-0.48 (0.01)	ns	-	
93 DAS				
Dwt. roots/plant	ns	ns	ns	-
% disfigured (n=18)	ns	ns	ns	-
Harvest May 130 DAS				
Fresh weight of carrots				
Category 0	ns	ns	0.32 (0.05)	ns
Category 1	ns	ns	ns	ns
Category 2	ns	ns	ns	ns
Category 3	0.38 (0.05)	0.34 (0.05)	0.27 (0.10)	0.34 (0.05)
Category 4	0.29 (0.1)	ns	ns	0.28 (0.05)
Category 5	ns	ns	ns	ns
Small (<10 cm length)	ns	ns	ns	ns
Forked	ns	ns	ns	ns
Twisted	0.44 (0.01)	ns	ns	ns
Short cracks	ns	ns	ns	ns
Long cracks	ns	ns	ns	ns
Constricted	ns	ns	ns	ns
Other abnormalities	ns	ns	ns	ns
Category 0-1	ns	ns	ns	ns
Category 2-5	ns	ns	ns	ns
Percentage of carrots in each category (by weight)				
Category 0	ns	ns	ns	ns
Category 1	ns	ns	ns	ns
Category 2	ns	ns	ns	ns
Category 3	0.34 (0.05)	0.32 (0.05)	ns	0.35 (0.05)
Category 4	0.27 (0.1)	ns	ns	ns
Category 5	ns	ns	ns	ns
Small (<10 cm length)	ns	ns	ns	ns
Forked	ns	ns	ns	ns
Twisted	0.39 (0.05)	ns	ns	ns
Short cracks	ns	ns	ns	ns
Long cracks	ns	ns	ns	ns
Constricted	ns	ns	ns	ns
Other abnormalities	ns	ns	ns	ns
Category 0-1	ns	ns	ns	ns
Category 2-5	ns	ns	ns	ns
Malformed [2]	ns	ns	ns	ns
Percentage of carrots in each category (by number)				
Category 0	ns	ns	ns	ns
Category 1	ns	ns	ns	ns
Category 2	ns	ns	ns	ns
Category 3	0.39 (0.05)	0.34 (0.05)	ns	0.33 (0.05)

Category 4	0.31 (0.05)	ns	ns	ns
Category 5	ns	ns	ns	ns
Small (<10 cm length)	ns	ns	ns	ns
Forked	ns	ns	ns	ns
Twisted	0.28 (0.1)	ns	ns	ns
Short cracks	ns	ns	ns	ns
Long cracks	ns	ns	ns	ns
Constricted	ns	ns	ns	ns
Other abnormalities	ns	ns	ns	ns
Category 0-1	ns	ns	ns	ns
Category 2-5	ns	ns	ns	ns
Malformed[2]	ns	ns	ns	ns
Carrots/ha [3]	ns	-0.42 (0.01)	-0.29 (0.1)	ns
Estimated total yield (t/ha)[4]	ns	-0.39 (0.05)	ns	ns
Estimated yield first grade carrots (t/ha)[5]	ns	-0.31 (0.05)	ns	ns
Pack out (% by weight)[6]	ns	ns	ns	ns
Average weight of a carrot (g)	ns	ns	ns	ns
Average weight of 0-1 carrot	ns	ns	ns	ns
Average weight other carrots	ns	ns	ns	ns

[1] Numbers in parentheses after correlation coefficients indicate level of statistical significance (P<)
[2] Total of forked, twisted, cracked, constricted and other abnormalities.
[3] Carrots/ha = (mean no. carrots/m row * metres of row per plot)/plot size (m^2)*10,000
[4] Estimated yield = (weight of carrots in a 100 carrot sample/100 carrots) * carrots/ha
[5] First grade considered as all carrots in categories 0 and 1.
[6] Weight of carrots in categories 0 and 1 as a percent of total weight.

Figure 8. Relationship between *Pratylenchus*/g dry weight of root at 58 DAS and the dry weight of root per plant (g) at 58 DAS.
Y=0.2469 + -0.0005 X, P=0.002, R^2=0.23, adjusted R^2=0.21

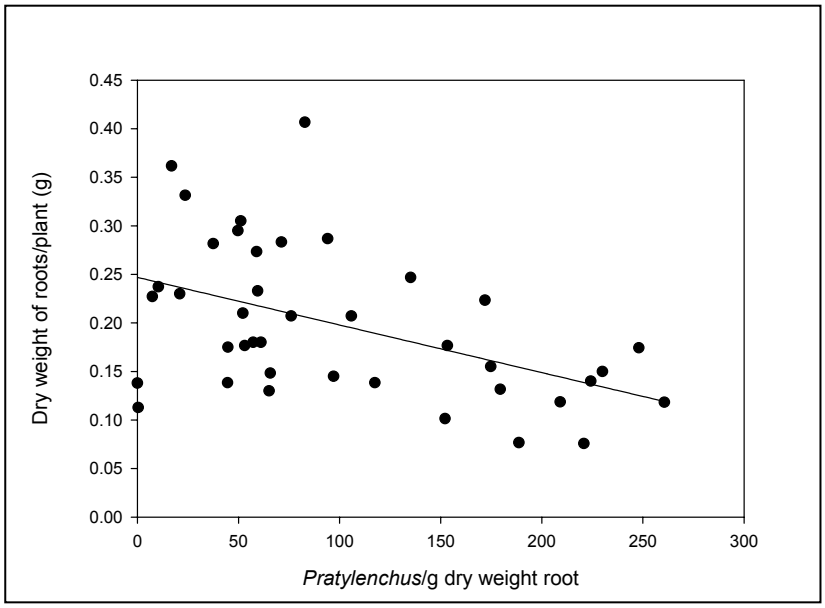
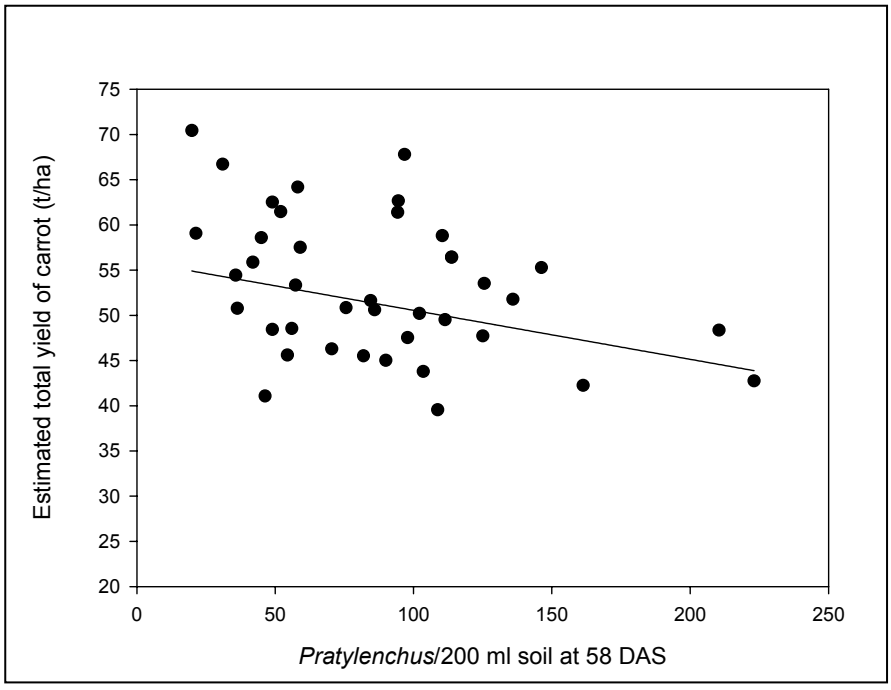


Figure 9. Relationship between *Pratylenchus*/200 ml soil at 58 DAS and the estimated total yield of carrot (t/ha) at 130 DAS.



7.2 Effect of *Pratylenchus* on yield and quality of Kuroda carrot in Tasmania (2001/2002).

7.2.1 Materials and methods

The trial site was located on a vegetable farm at Penguin, Tasmania (-41 11 latitude, 146 07 longitude) in a 4.8 ha field with a long history of cropping. The field had been planted with potato (*Solanum tuberosum*) followed by poppy (*Papaver somniferum*) prior to the carrot crop and had not been in carrots for over 5 years. The field had been cultivated by deep ripping, ploughing, and the seed bed prepared by rotary hoe and bed formed. Lime (2.5 t/ha) was bulk spread on 13/8/2001 and fertiliser (14-6-11) bulk spread at 0.9 t/ha on 13/9/2001. A Kuroda type of carrot, variety Coral 2 (CCO-018) was sown on 23/9/2001 with a precision seeder. On 23/10/2001 a peg was placed at each of 25 arbitrarily chosen sites across the field. One trowel depth of soil was collected at each peg. Nematodes were extracted from soil according to the Whitehead tray technique (Whitehead and Hemming 1965) as described previously (section 7.1).

Pre-emergent herbicides (Linuron @ 3 kg/ha and Stomp @ 2 L/ha) were applied on 24/9/2001. A further 4 applications of herbicides (Linuron and Gesaguard) were made between 1/11/2001 and 2/1/2002. The herbicide regime gave very good weed control throughout the season. The insecticide Chloropyrifos (4.8 L/ha) was applied for control of cutworm on 5/11/2001.

A trial was pegged out around an area found to have a high number of *Pratylenchus* spp. The trial consisted of 42 plots arranged in a 6 x 7 lattice. Each plot was 7 m long by 4 carrot beds wide (approximately 6.65 m). Each bed of carrots contained 3, double rows of carrots. Soil and plant samples were collected from 6 positions in each plot on 20/11/2001. Samples consisted of 1 trowel depth of soil and 2 carrot plants taken from a 2 m perimeter on each side of the plot. A central 3 m region was left intact for later harvest. Nematodes were extracted from 200 ml soil and all roots by the Whitehead tray technique described above. Foliage and roots following extraction was oven dried (80^{o}C for 48 h) and weighed.

The trial was harvested on 17/1/2001 (116 DAS), 4 days before commercial harvest. Plant density was recorded by counting the number of carrots in 1 m sections of rows at 4 arbitarily chosen places in the centre 3 m section of plots. Twenty carrots were removed from 5 arbitarily chosen locations in the centre 3 m section of each plot, bagged and kept in a coolroom (5^{o}C) overnight. Carrots were washed and graded into the following categories: forked (carrots with taproot in 2 sections of approximately equal size, twisted, cracks >5mm wide and >20 mm long, splits <5mm wide and >20 mm long, bolters (carrots which had formed a seed head), bent, and a 0-5 scale adapted from Belair and Parent (1996), as described previously. Carrots in categories 0-1 were considered first grade and were measured with a ruler for length and with calipers for diameter and graded according to size into <30 mm, 30-40 mm, 40-50 mm and > 50 mm diameter. Carrots in the 30-50 mm size range are considered optimum for export. The weight and number of carrots in each category was recorded.

Soil and root samples consisting of a trowel depth of soil and 1 carrot were collected at 116 DAS from 6 arbitrarily chosen locations in the outer 2 m perimeter of each plot as described previously. Nematodes were extracted from soil as above. Carrots were washed and cut in half longitudinally. Half of each carrot was discarded. Carrots were chopped transversely and chopped into pieces approximately 0.5-2 cm long by 0.2-1cm diameter with a food processor (Sunbeam LC045 Maestro Pro) fitted with a coarse cutting blade. Chopped carrot was weighed and nematodes extracted by Whitehead tray technique and sieving as described above, except mesh (2 mm) was substituted for tissue. After extraction, chopped carrot was dried (80^{o}C/96 h) and reweighed.

Correlation coefficient (r) was calculated between nematode numbers and measured variables and a t-test conducted for significance of association. For some variables having a significant correlation, a regression was conducted to examine the relationship more fully.

7.2.2 Results

Preliminary soil samples taken from 25 sites across the field on 23/10/2001 gave an average, maximum and minimum number and standard deviation of 186.9, 1189, 27.4,

and 260.5 *Pratylenchus*/200 ml soil respectively. The only other plant parasitic nematode present was *Paratylenchus* with an average, maximum and minimum number and standard deviation of 18.2, 96,0 and 29.9 *Paratylenchus*/200ml over the 25 sites.

The trial site was selected to encompass the highest count of lesion nematode (1189 *Pratylenchus*/200ml soil) in plot 23 and a low count (138 *Pratylenchus*/200 ml soil) in plot 7. At 58 DAS, the individual plot totals for *Pratylenchus* in the soil varied between 10.1 and 354.4/200 ml soil and for *Pratylenchus* in seedling roots varied between 200.8 and 2291.6/g dry weight of root (Table 16). By 20/11/2001, there appeared to be a large reduction in the number of *Pratylenchus* in the soil, with 213.8 and 55.5 *Pratylenchus*/200 ml soil recovered from plots 23 and 7 respectively. The fall in numbers in the soil can be attributed to migration of *Pratylenchus* into the developing carrot roots as 706.3 and 669.8 *Pratylenchus*/g dry weight were recovered from roots from plots 23 and 7 respectively at this time.

Table 16. Nematodes recovered from soil and roots on 20/11/2001 (58 DAS) and 17/1/2002 (116 DAS).

	Pratylenchus:			*Paratylenchus*
	-Females	-Juveniles	-Total	
20/11/2001				
Nematodes/200 ml soil				
Average	55.5	63.6	119.2	10.6
Maximum	151.9	202.5	354.4	81
Minimum	9.5	0	10.1	0
Std. dev.	32.5	48.3	73.4	16.6
Nematodes/g dry weight root				
Average	135.9	635.2	771.1	-
Maximum	399.4	1950.3	2291.6	-
Minimum	0	189.2	200.8	-
Std. Dev.	87.1	361.0	422.8	-
17/1/2002				
Nematodes/200 ml soil				
Average	not differentiated		76.0	2.1
Maximum			220.0	25.0
Minimum			0	0
Std. Dev.			51.3	6.6
Nematodes/g dry weight root				
Average	not differentiated		0.11	-
Maximum			0.61	-
Minimum			0	-
Std. Dev.			0.17	-

At harvest, *Pratylenchus* were recovered from carrot tap root samples in only 18 of 42 plots. The average number recovered over the 42 plots was 0.11/g dry weight of tap root with a maximum and minimum of 0.61 and 0/g dry weight of root (Table 17).

The number of *Pratylenchus* in roots at 58 DAS was negatively correlated with the dry weight of foliage and roots per plant at 58 DAS (Table 17). Regression analysis demonstrated that the average weight of roots per plant at the maximum number of *Pratylenchus* (1455/g dry weight of root), was less than half the weight of roots predicted with no nematode feeding (Figure 10). *Pratylenchus* in soil at 58 DAS were negatively correlated with the weight of carrots in categories 3 and 2-5. *Pratylenchus* in roots at 58 DAS were negatively correlated with the weight of carrots in category 4, the total weight of 100 carrots and with the weight in categories 0-1 and 2-5 (Table 17). Similar relationships were noted when nematode numbers were correlated with yield in different categories on a percentage basis (by weight and by number). *Pratylenchus* in soil at 116 DAS were negatively correlated with the percentage (by weight and by number) of split carrots. This relationship was difficult to explain. *Pratylenchus* in roots at 58 DAS were negatively correlated with the average weight and length of a carrot in categories 0-1 (Table 17 and Figures 11 and 12).

Table 17. Correlation coefficients (r) and probability (*P*=) levels between *Pratylenchus* populations at different times and yield and quality attributes of Kuroda carrot.

	Pratylenchus/ 200 ml soil 20/11/2001 58 DAS	*Pratylenchus*/g dry weight root 20/11/2002 58 DAS	*Pratylenchus*/ 200 ml soil 17/1/2002 116 DAS	*Pratylenchus*/ g dry wt. root 17/1/2002 116 DAS
20/11/2001				
Dry wt. foliage/plant (g)	ns	-0.45 (0.05)	ns	ns
Dry wt. root/plant (g)	ns	-0.48 (0.05)	ns	ns
17/1/2002				
Fresh weight of carrots				
Category 0	ns	ns	ns	ns
Category 1	ns	ns	ns	ns
Category 2	ns	ns	ns	ns
Category 3	-0.39 (0.05)	ns	ns	ns
Category 4	ns	-0.47 (0.05)	ns	ns
Category 5	ns	ns	ns	ns
Forked	ns	ns	ns	ns
Twisted	ns	ns	ns	ns
Cracked[1]	ns	ns	ns	ns
Split[2]	ns	ns	-0.38 (0.05)	ns
Bolters	ns	ns	ns	ns
Bent	ns	ns	ns	ns
Total fresh weight	ns	-0.40(0.01)	ns	ns
Total fresh weight 0-1	ns	-0.27 (0.10)	ns	ns
Total fresh weight 2-5	-0.36 (0.05)	-0.31 (0.05)	ns	ns
Total malformed	ns	ns	ns	ns
Percentage of carrots in each category (by weight)				
Category 0	ns	ns	ns	ns
Category 1	ns	ns	ns	ns
Category 2	ns	ns	ns	ns
Category 3	-0.40 (0.01)	ns	ns	ns
Category 4	ns	-0.47 (0.01)	-0.30 (0.1)	ns
Category 5	ns	ns	ns	ns
Forked	ns	ns	ns	ns
Twisted	0.28 (0.1)	ns	ns	ns
Cracked[1]	ns	ns	ns	ns
Split[2]	ns	ns	-0.36 (0.05)	ns
Bolters	ns	0.27 (0.1)	ns	ns
Bent	ns	ns	ns	ns
Percent in 0-1	ns	ns	ns	ns
Percent in 2-5	-0.35 (0.05)	ns	ns	ns
Percent malformed[3]	ns	ns	ns	ns
Percentage of carrots in each category (by number)				
Category 0	ns	ns	ns	ns
Category 1	ns	ns	ns	ns
Category 2	ns	ns	ns	ns
Category 3	-0.40 (0.05)	ns	ns	ns
Category 4	ns	-0.46 (0.05)	ns	ns
Category 5	ns	ns	ns	ns

Forked	ns	ns	ns	ns
Twisted	0.31 (0.05)	ns	ns	ns
Cracked[1]	ns	ns	ns	ns
Split[2]	ns	ns	-0.40 (0.01)	ns
Bolters	ns	ns	-0.30 (0.1)	ns
Bent	ns	ns	ns	ns
Percent 0-1	ns	ns	ns	ns
Percent 2-5	-0.38 (0.05)	-0.37 (0.05)	ns	ns
Percent malformed[3]	ns	ns	ns	ns
Average weight of a carrot in each category				
Category 0-1	ns	-0.36 (0.05)	ns	ns
Category 2-5	ns	ns	ns	ns
Others	ns	ns	-0.32 (0.05)	ns
All categories	ns	-0.36 (0.05)	ns	ns
Number of first grade in each size category				
<30 mm diameter	ns	ns	ns	ns
30-<40 mm	ns	ns	ns	ns
40-<50 mm	ns	ns	ns	ns
50 mm and above	ns	ns	ns	ns
Weight of first grade in each size category				
<30 mm diameter	ns	ns	ns	ns
30-<40 mm	ns	ns	ns	ns
40-<50 mm	ns	ns	ns	ns
50 mmand above	ns	ns	ns	ns
Average weight of a first grade carrot in each size category				
<30 mm diameter	ns	ns	ns	-0.27 (0.1)
30-<40 mm	ns	ns	ns	ns
40-<50 mm	ns	ns	ns	ns
50 mmand above	ns	ns	ns	ns
Average length of a first grade carrot (cm)	ns	-0.47 (0.05)	ns	ns
Average diameter of a first grade carrot (mm)	ns	-0.28 (0.1)	ns	ns
Plants/ha	-0.44 (0.05)	-0.38 (0.05)	ns	ns
Total yield (t/ha)	-0.35 (0.05)	-0.53 (0.01)	ns	ns
Total yield first grade	ns	-0.43 (0.01)	ns	ns
Total yield 2-5 (t/ha)	-0.41 (0.01)	-0.36 (0.05)	ns	ns
Packout (%)	ns	ns	ns	ns

1 Cracks >5mm wide
2 Cracks <5mm wide, >20mm long
3 Malformed is total of twisted, cracked, split, bolted and bent.

Figure 10. Relationship between *Pratylenchus*/g dry weight of root and dry weight of root per plant on 20/11/2001. Y=0.0398 + -1.417*10^{-5} X; P=0.0015, R^2=0.229, adjusted R^2=0.210.

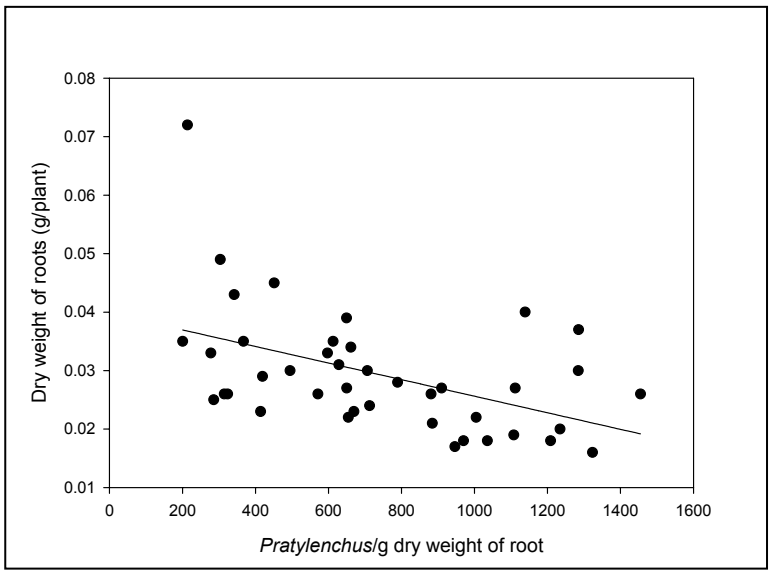


Figure 11. Relationship between *Pratylenchus* in roots at 58 DAS and mean length of a first grade carrot at harvest. Y=160.36 + -0.008 X P=0.002, R^2=0.21, adjusted R^2=0.19.

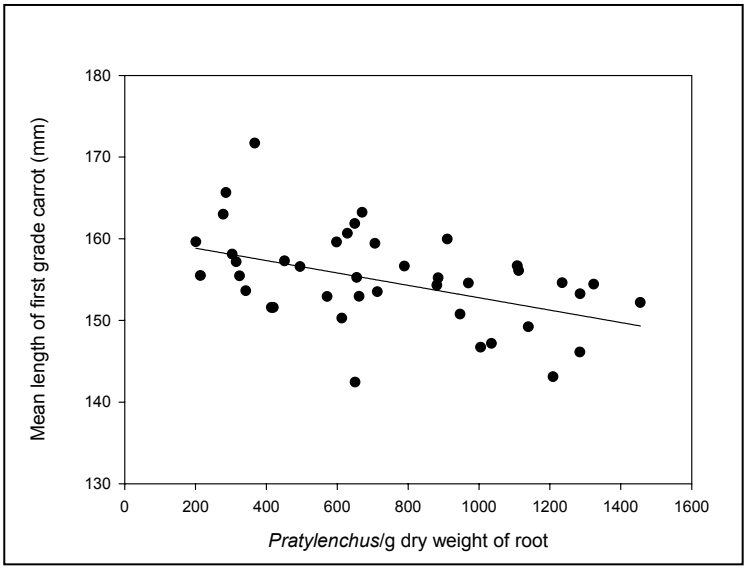


Figure 12. Relationship between *Pratylenchus* in roots at 58 DAS and mean weight of a first grade carrot at harvest. Y=117.57 + -0.01X P=0.03, R^2=0.11, adjusted R^2=0.10.

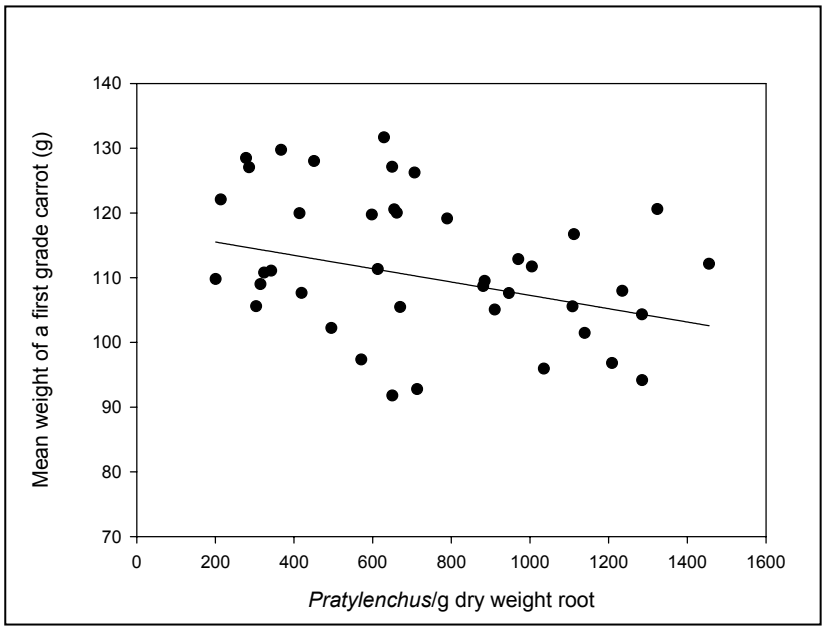


Figure 13. Relationship between *Pratylenchus* in soil at 58 DAS and estimated total yield of carrot at harvest. Y=65.09 + -0.045X P=0.002, R^2=0.21, adjusted R^2=0.19.

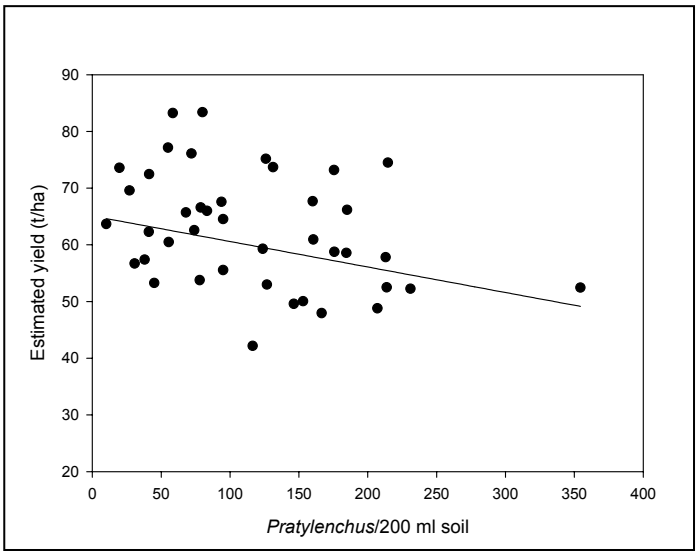
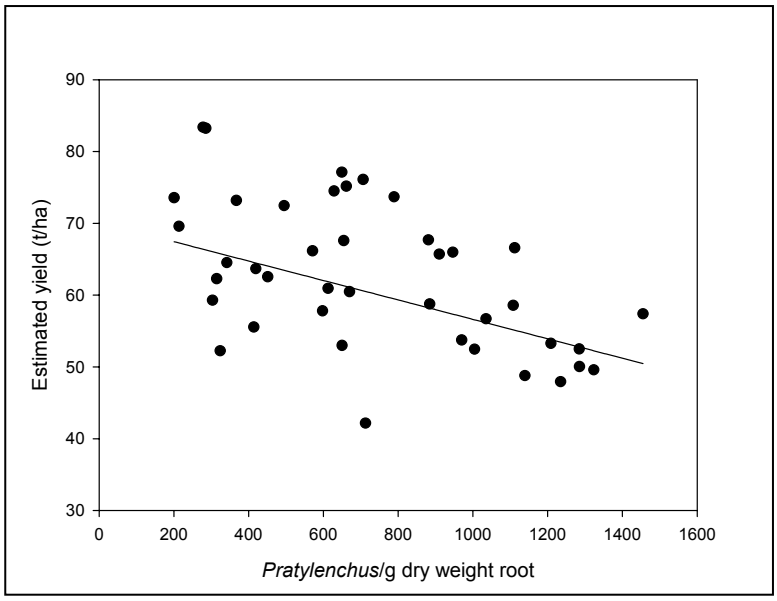


Figure 14. Relationship between *Pratylenchus*/g dry weight of root at 58 DAS and total yield of carrots (t/ha) at harvest. Y=73.802 + -0.0153 X; P=0.0003, R^2=0.285, adjusted R^2=0.267.



However, *Pratylenchus* were not correlated with the weight or average weight of first grade carrots in each of the different size ranges suggesting that the effect on weight was across all size ranges (Table 17).

Pratylenchus in roots and soil at 58 DAS were negatively correlated with plant density at harvest. Yield potential of each plot (t/ha) was calculated by multiplying plant density by the average weight of carrots in categories 0-1 and 2-5 from the 100 carrot sample (Table 17). *Pratylenchus* in soil and roots at 58 DAS were negatively correlated with total yield (t/ha), first grade (t/ha) and category 2-5, but had no effect on packout (%) (Table 17). Regression analysis predicted a total yield of 65.1 t/ha and 49.2 t/ha at 0 and 354.4 Pratylenchus/200 ml soil respectively, (the maximum number recorded in this trial), a difference of 15.9 t/ha (Figure 13). Similarly, regression analysis predicted a yield of 73.8 t/ha and 51.5 t/ha at 0 and 1455 *Pratylenchus*/g dry weight of root respectively, a difference of 22.3 t/ha (Figure 14). Results suggest that *Pratylenchus* reduced yield through reducing plant density at an early stage and reducing average carrot weight and length. *Pratylenchus* appeared to have little effect on carrot abnormalities. However, as with the first trial there were positive correlations with the

percentage (by weight and by number) of carrots in the twisted category. This indicated that *Pratylenchus* had little effect on the degree of malformation of carrots, but that they reduced the weight of carrots, the plant density and hence the yield (t/ha).

7.2.3 Discussion

Results suggested that the main impact of *Pratylenchus* on Kuroda carrot was at the early stages of seedling emergence. In both trials, high numbers of *Pratylenchus* were recovered from roots at 58 DAS and were correlated with reduced shoot and root growth at this stage. The significant negative relationship between *Pratylenchus* populations at 58 DAS and plant density at harvest in both trials suggested that *Pratylenchus* were also killing seedlings. However, the finding that *Pratylenchus* populations in the soil did not increase over the life of the crop, and of a large reduction in numbers extracted from roots beyond 93 DAS in trial 1 and at 116 DAS in trial 2, in comparison to 58 DAS suggested that Kuroda became resistant to nematode feeding with age. Although there were some relationships between *Pratylenchus* populations at various times during the season and abnormalities at harvest, overall these were minor and did not contribute to reduced pack out at harvest. In both trials, there was a positive correlation between *Pratylenchus* numbers and carrots in the twisted category implicating nematodes as the causal agent. However, the proportion of twisted carrots was small in comparison to the total amount of crop. Results suggested that the majority of carrots that survived early feeding were able to produce normal shaped taproots. Therefore the major impact of *Pratylenchus* on growth of Kuroda appears to be during early stages of growth prior to taproot formation by killing and stunting plants, rather than through causing abnormalities to the quality of the carrot. In trial 1, *Pratylenchus* populations had no effect on average carrot weight. However in trial 2, *Pratylenchus* at 58 DAS were negatively correlated with average carrot weight in some categories, suggesting that nematode feeding up until midseason had an effect on carrot size at harvest. The discrepancy between the two trials is probably due to the greater range of population densities of *Pratylenchus* in trial 2. In trial 2 at 58 DAS *Pratylenchus*/g dry weight of root ranged from 200.8 to 2291.6 while in trial 1 they ranged from 0 to 260.6.

Plant density at harvest was combined with the average weight of a carrot in a particular category from the 100 carrot sample taken from each plot to estimate potential yield of each plot (t/ha). The number of *Pratylenchus* at 58 DAS either in soil or roots was negatively correlated with total yield and yield of first grade carrots in both trials. The predicted yield differential between plots with no *Pratylenchus* at 58 DAS and those with the maximum number found in plots (223/200 ml soil) was some 12.1 t/ha in trial 1. In trial 2 the predicted yield loss between no *Pratylenchus* and the maximum number recorded at 58 DAS in soil and roots was 15.9 t/ha and 22.3 t/ha respectively.

7.3 Associations between carrot defects and nematodes in South Australia[1]

7.3.1 Introduction

Meloidogyne spp., especially *M. hapla* and *M. incognita*, reduce marketable yields of carrots (*Daucus carota*) due to galling, forking, fasciculation, constriction and stubbing of roots (Belair 1992; Huang and Charchar 1982; Roberts 1987; Yarger and Baker 1981). However, carrot defects can also be caused by other factors, especially environmental factors including compacted soils, rocky soils or undecomposed organic material (Rubatzky *et al*. 1999). In South Australia, carrot root and shoot growth was stimulated by preplanting fumigation (with ethylene dibromide) of soil infested with *M. hapla* and other nematodes (Walker 1990). Although local growers are aware of the link between galled roots and *Meloidogyne* spp., and use of fenamiphos or soil fumigants is common, awareness of the role of nematodes in production losses is generally low. There is little information locally on the associations between nematodes and carrot defects, and on their impact on marketable yields, particularly of nematodes other than *Meloidogyne* spp.

In this study, the associations between nematode levels and carrot defects and marketable yields were investigated at several conventionally-managed farms and one organically-managed farm. The aim was to increase knowledge of the negative impacts of nematodes on carrots in both conventional and organic production, and to focus research on nematodes of economic importance by identifying those associated with carrot defects in South Australia.

7.3.2 Methods

Location of trial sites

The conventionally-managed farms were located at Nuriootpa and Barmera. The organically-managed farm was located at Purnong, South Australia and had been certified organic by N.A.S.A.A. for the previous 12 years. Nuriootpa (138.99° longitude, -34.48° latitude) is situated 78 km north-west of Purnong (139.63° longitude, -34.85° latitude); Barmera (140.48° longitude, -34.24° latitude) is situated 102 km north-east of Purnong. At the Nuriootpa farm, carrots were grown in rotation with cereals on a sandy loam soil, pH 7.6. The field was fumigated 2 weeks before planting carrots with (soil-injected) metham sodium (423 g/L) at 300 L/ha. Inorganic fertilisers (N:P:K – 10:8.4:13.1 and 22.9:0:18.9) were used. The Barmera farm had a history of mixed vegetable production including carrots, cauliflower, cabbages, onions, melons and pumpkins. The soil type was a loam, pH 8.0. No fumigants or nematicides were used prior to planting carrots, but herbicides and inorganic fertilisers (N:P:K – 9:12:17) were used. At the Purnong farm, carrots were grown in rotation with onions, and cover crops of stock radish (*Raphanus sativus*) and fodder rape (*Brassica napus*). The farm had a sandy loam soil, pH 6.5. Weeds were controlled by cultivation and only approved organic fertilisers (N:P:K – 4:3:1.5) were used. Fertilisers were applied at total rates of 126:30:84 kg/ha and 222:296:420 kg/ha N:P:K at the Nuriootpa and Barmera farms respectively, and at rates of 79:59:30 kg/ha at the organic farm. All crops were irrigated as required from fixed overhead sprinklers. Growers managed their properties according to local industry standards and personal experience and no modifications were imposed on these in the setting up of field experiments to monitor impacts of nematodes on carrot production. Carrot seed, cv. Stefano (South Pacific Seeds Co.) at Barmera and Nuriootpa, and cv. Western Red (Arthur Yates & Co.; not treated with pesticides) at Purnong, was sown in all properties into double rows in raised beds, with bed spacings of approximately 1500 mm.

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[1] Results of this section have been submitted as an article to 'Australasian Plant Pathology'

Field experiment layout

Five field experiments were conducted (two each at the Nuriootpa and Purnong sites laid out in different parts of the same fields, and one at Barmera). Each experiment consisted of 30 plots, each 2 beds wide by 2 m-long. Planting dates were: Barmera, 10/11/2000; Nuriootpa experiment 1, 24/10/2000; Nuriootpa experiment 2, 8/1/2001; Purnong experiments 1 and 2; 18/12/2000. Mean monthly soil temperatures at a recording station at Loxton (24 km south-east of Barmera) at depth 10 cm from November 2000 to May 2001 were 24, 25, 28, 27, 20, 16 and 12°C respectively. Mean daily maximum air temperatures at the nearest recording stations (within 20 km) to Purnong and Nuriootpa are 0.1°C higher and 2.4°C lower respectively compared with Loxton. Climate statistics were obtained from the Bureau of Meteorology, Canberra (Kernich 1984). Carrots were harvested manually on 8 March at Barmera, on 2 May at Purnong, on 23 March at Nuriootpa (experiment 1) and on 30 May, 2001 at Nuriootpa (experiment 2). Harvests were timed at one week before date of commercial harvest.

Nematode sampling and carrot assessment

Soil was sampled from all plots in the first 1-3 days after planting ('at planting'), at 6 to 8 weeks after planting ('mid-season'), and at harvest ('at harvest'). In addition for the two Nuriootpa experiments, samples were taken at 14 to 15 weeks after planting, and again 17 weeks after planting in Nuriootpa experiment 2. Samples were taken 1 day after irrigation where possible to minimise variation in soil moisture, from depth 10-15 cm in the root zone approximately 2 cm from the edge of the carrots using a modified spade (10 subsamples per plot). A 150 mL sub-sample of each composite sample, containing both soil and fragments of secondary roots, was extracted on trays for five days at 22°C (Whitehead and Hemming 1965) and nematodes identified to genus and counted under a compound microscope. Selected specimens were deposited in the Waite Institute Nematode Collection (WINC) and further identified to species by Dr. J. Nobbs, SARDI Plant Research Centre, using morphological characteristics (Taylor and Sasser 1978). Female *Meloidogyne* spp. dissected from roots were also identified by J. Cobon (Qld DPI, Indooroopilly) using a PCR (polymerase chain reaction) diagnostic test (Stanton *et al.* 1997). Carrots (approximately 42 per plot) were dug from two 0.5m-lengths of double row from the centre of adjacent beds for each plot. Tap roots were washed and the incidence of (longitudinally) split roots, fasciculated (hairy) roots, constricted roots,

stubbed roots, and galled roots was assessed (Huang and Charchar 1982; Belair 1992). Severity of galling was scored on a scale of 0 (no galling) to 5 (severe galling) based on number of galls, whether galls were aggregated or not, and whether they occurred on secondary roots and/or tap roots (Huang and Charchar 1982). Carrots with a score of 1 (unaggregated galls on secondary roots only) or less were considered to be marketable; those with a score >1 were considered to be unmarketable, and those with a score of 1-5 were aggregated as total galled carrots. Carrots with all other defects were considered to be unmarketable. Carrots were also sized by maximum crown diameter and classed as either premium (or greater) size (crown diameter $\geq$ 25 mm) or "undersized" (crown diameter < 25 mm) based on local guidelines (C. Zerella pers. comm.). Undersized carrots were included with unmarketable carrots (although in fact they were acceptable in some markets) as total substandard carrots because they would fetch lower prices as they fell below the premium size range demanded by major retailers. Stunting is recognised as a symptom of *M. hapla* infection (Belair 1992). Additional types of defects (also classed as unmarketable) were observed during assessment, a) irregular splitting, a type of splitting differing from the more usual longitudinal fractures in the phloem parenchyma (McGarry 1993) in that it was shallower (up to 9 mm-deep), with irregular margins and tended to be more transverse in extension, and b) tip swelling, in which the tip of the tap root was bulbous and swollen. Numbers and fresh weight of marketable and substandard carrots were recorded, and numbers of individual defect types. After assessment, secondary roots and tips (< 2 mm in diameter) of tap roots from all carrots for each plot were dissected from the tap roots and extracted in a mist chamber (McSorley *et al*. 1984) for 5 days, and nematodes were identified to genus and counted**.**

A high level of weed control was achieved by the Nuriootpa and Purnong growers but caltrop (*Tribulus terrestris* L.), and to a lesser extent thornapple (*Datura* sp.), emerged early in the season at the Barmera site. The grower applied herbicide (linuron 1.2 kg/ha) and weeds were also removed manually from the experimental plots. At 5 weeks after planting carrots the roots from 10 plants of each weed were collected, washed free of soil and nematodes were extracted in a mist chamber for 5 days.

Statistical analysis

Numbers of substandard carrots and numbers of carrots with different defects or undersized carrots were expressed as percentage of total carrots harvested per plot. Stepwise regression and correlation analysis (Statistix® version 4.1, Analytical Software, Tallahassee, Florida), was conducted between incidence of substandard carrots and incidence of individual defects to determine which defects contributed most to variation between plots in frequency of substandard carrots. Linear regression (P<0.05) was conducted between nematode levels and incidence of substandard carrots and/or of individual defects to determine associations between nematodes and carrot defects. Data was combined for this purpose for Purnong experiments 1 and 2 which were planted and harvested on the same dates. Analysis of variance (P<0.05), or Student's t-test where appropriate, were conducted on carrot yields and numbers/ha (on a spacing of two double-rows per 1500 mm-bed) between field experiments, and between nematode levels and sampling date (weeks after planting) to identify nematodes undergoing population increase over the growing period. The Least Significant Difference test (P<0.05) was used to separate means. Before statistical analysis, nematode counts or numbers of carrots were transformed as ln (count + 1) or ln (count) respectively if plots of residuals or tests for non-additivity and normality indicated that this was required (Snedecor and Cochran 1980).

7.3.3 Results

Carrot yield

Total production of carrots (t/ha) was significantly higher in Nuriootpa experiments 1 and 2 than at the other farms, especially in experiment 2 (Table 18). Production of undersized and defective carrots was significantly lower in Nuriootpa experiment 2 and at Barmera than in other experiments (Table 18). Lowest production of non-defective carrots, and lowest mean carrot weight, was observed in the two Purnong experiments, while the highest mean carrot weight was observed in Nuriootpa experiment 2 (Table 18). In the latter experiment, emergence was reduced by very hot temperatures in January resulting in reduced stands but larger carrots. Total number of carrots per ha was significantly higher in the Purnong experiments than all other experiments (Table 18).

Nematode identification and levels

M. javanica was identified at the Nuriootpa and Purnong farms by both morphological characteristics and by a PCR-based test, and constituted a new South Australian record for carrots (WINC 1153c). *Hemicycliophora saueri* was identified from the Nuriootpa (WINC 1159a and 1178a) and Purnong (WINC 1187) farms; *Helicotylenchus dihystera* and *H. pseudorobustus* from the Barmera farm (WINC 1178b); *Pratylenchus neglectus* from the Barmera farm (WINC 1176h and 1178b); and *Scutellonema brachyurum* from the Purnong farm (WINC 1159b). With the exception of *H. dihystera*, which was previously recorded in Queensland, these are new Australian records for nematodes associated with carrots (McLeod *et al*. 1994).

High levels of *M. javanica* were detected in roots and soil at harvest of carrots in Nuriootpa experiment 1 and both Purnong experiments (Tables 19-21). Detection of this nematode at planting using the Whitehead tray method was reliable in the Purnong organically-managed experiments where initial levels were high, but was unreliable in the Nuriootpa experiments where soil had been fumigated and initial levels were low (Tables 20 and 21). Detection of *M. javanica* in the latter experiments was not reliable until 14 to 17 weeks after planting (Table 20). Preliminary sampling before fumigation had readily detected this nematode in the area where the field experiments were conducted (results not shown).

High levels of *P. neglectus* were detected in roots and soil at harvest at Barmera compared with levels of *Pratylenchus* spp. at other sites (Tables 19 and 21). Only low levels of *Pratylenchus* sp. were detected in soil throughout the growing season at Nuriootpa (<0.3 nematodes/150 mL of soil), and at Purnong experiment 1 (<0.8 nematodes/150 mL of soil). Levels of *M. javanica* and *P. neglectus* increased over the growing season reaching peak levels at harvest where these nematodes occurred (Table 21). Other nematodes which increased over the growing season of carrots were *H. saueri*, *Paratrichodorus* sp., *Helicotylenchus* spp., and *S. brachyurum*, although peak levels for *H. saueri* occurred in the first half of the season in 2 out of 3 experiments (Tables 20 and 21). *Macroposthonia xenoplax* levels did not increase significantly during the growing season (Table 21). Only low levels of *H. saueri* and *S. brachyurum*

were detected in carrot roots at harvest at the sites where these nematodes were present (Table 19). *Tylenchorhynchus* spp. *sensu lato* were also detected at all 3 farms, but at levels below those thought to be potentially damaging (Boag 1979).

Table 18. Numbers and yields of total and substandard (undersized and defective) carrots, and mean carrot weight, in field experiments at Nuriootpa, Barmera and Purnong

Field experiment	Undersized and defective carrots (t/ha)	Non-defective carrots (t/ha)	Total carrots/ha		Mean carrot weight (g)
			t	Ln (number)	
Nuriootpa					
Experiment 1	11.5	15.7	27.2	13.1	53.9
Experiment 2	6.0	36.7	42.7	12.5	156.7
Barmera	2.7	18.2	20.9	12.6	72.2
Purnong					
Experiment 1	9.3	13.0	22.3	13.6	29.7
Experiment 2	13.8	7.2	20.9	13.7	25.0
LSD (*P*=0.05)	2.3	3.4	3.4	0.1	8.9

Table 19. Population density of *Meloidogyne javanica*, *Pratylenchus* spp., *Hemicycliophora saueri* and *Scutellonema brachyurum* on carrot roots at harvest in field experiments

	Nematodes per gram dry weight of roots[A]			
	M. javanica	*Pratylenchus*	*H. saueri*	*S. brachyurum*
Nuriootpa				
Experiment 1	192.5 (2.7)	0.3 (0.1)	nd[B]	nd
Experiment 2	31.3 (1.3)	1.5 (0.3)	1.0	nd
Barmera	nd	4.0 (0.9)	nd	nd
Purnong				
Experiment 1	241.0 (2.8)	1.2 (0.3)	nd	nd
Experiment 2	51.0 (2.8)	0.2 (0.1)	0.1	0.3
LSD (P=0.05)	(1.0)	(0.4)	ns	-

[A] means in brackets are for ln-transformed data
[B] nd = not detected

Table 20. Population density of plant parasitic nematodes in soil in Nuriootpa experiments 1 and 2

	Nematodes per 150 mL of soil[A]				
Experiment 1	At planting	Weeks after planting 6	14	At harvest	LSD (*P*=0.05)
Meloidogyne javanica	nd[B]	nd	4.5 a[C]	174.3 b	–
Experiment 2		Weeks after planting 10	15	17	
Meloidogyne javanica	0.2	nd	nd	0.9	0.6	ns
Hemicycliophora saueri	0.1 (0.1)	66.3 (3.2)	33.6 (2.9)	23.6 (2.6)	24.7 (2.5)	(0.5)
Paratrichodorus sp.	0.2 (0.1)	4.0 (0.7)	3.0 (0.8)	3.4 (1.0)	1.1 (0.5)	(0.4)

[A] means in brackets are for ln-transformed data
[B] nd = not detected
[C] within-row means followed by a different letter are significantly different by t-test (*P*<0.05)

Carrot defects and nematode levels

Nuriootpa

Incidence of substandard carrots was much higher in experiment 1 than in experiment 2, mainly due to a higher incidence of undersized and galled carrots in experiment 1 (Table 22). Although undersized carrots were numerically most important in experiment 1, the defects most strongly correlated with variation in incidence of total substandard carrots were galling and hairyness (Table 22). The most common carrot defect in experiment 2 was irregular splitting, which was most strongly correlated with incidence of total substandard carrots (Table 22). Forking was associated with at harvest and mid-season levels of *M. javanica* in soil, and total galled carrots was associated with at harvest and mid-season levels of *M. javanica* in roots in experiment 1 (Table 23). A significant association was also found between mid-season levels of *M. javanica* in soil

Table 21. Population density of plant parasitic nematodes in soil at Barmera and Purnong.

	Nematodes per 150 mL of soil[A]			
	At planting	Mid-season	At harvest	LSD (*P*=0.05)
Barmera				
Pratylenchus neglectus	35.6 (4.7)	64.4 (3.9)	131.3 (3.3)	(0.3)
Helicotylenchus spp.[B]	1.3 (0.3)	2.9 (0.6)	24.8 (1.7)	(0.6)
Purnong				
Experiment 1				
Meloidogyne javanica	36.8 (1.7)	57.1 (2.1)	692.7 (4.2)	(0.7)
Hemicycliophora saueri	0.5 (0.1)	3.6 (0.4)	27.6 (1.4)	(0.5)
Scutellonema brachyurum	0.5 (0.1)	4.8 (0.4)	6.1 (0.9)	(0.4)
Paratrichodorus sp.	7.2 (0.8)	34.8 (1.5)	55.1 (1.8)	(0.4)
Macroposthonia xenoplax	7.0	7.3	7.1	ns
Experiment 2				
Meloidogyne javanica	316.6 (3.9)	183.7 (3.7)	844.9 (5.2)	(0.7)
Hemicycliophora saueri	6.9 (1.0)	197.8 (2.2)	156.3 (3.3)	(0.7)
Scutellonema brachyurum	13.9 (1.5)	47.8 (2.2)	83.1 (2.3)	(0.5)
Paratrichodorus sp.	19.3 (2.3)	42.5 (2.8)	33.4 (2.9)	(0.5)
Pratylenchus sp.	0.2 (0.1)	2.3 (0.5)	2.3 (0.6)	(0.3)
Macroposthonia xenoplax	5.0	6.5	5.7	ns

[A] means in brackets are for ln-transformed data
[B] *H. dihystera* and *H. pseudorobustus*

Table 22. Carrot defects associated with variation in total incidence of substandard carrots at Nuriootpa, Barmera and Purnong

Carrot defect category by field experiment	% of carrots (± SE) with defect	Correlation coefficient between defect and total substandard carrots
1) Nuriootpa		
Experiment 1		
Hairy	4.6 ± 0.8	0.49
Below premium size	34.6 ± 2.3	0.24
Gall index > 1	10.3 ± 2.5	0.72
Stepwise regression adjusted r^2	0.74	—
Total substandard	54.3 ± 2.6	—
Experiment 2		
Swollen tip	1.8 ± 0.5	0.11
Irregular split	6.7 ± 2.5	0.29
Below premium size	2.1 ± 0.5	0.04
Stepwise regression adjusted r^2	0.95	—
Total substandard	15.7 ± 2.9	—
2) Barmera		
Forked	6.9 ± 1.5	0.79
Split	0.6 ± 0.3	0.11
Below premium size	6.6 ± 1.2	0.51
Stepwise regression adjusted r^2	0.90	—
Total substandard	13.5 ± 1.7	—
3) Purnong		
Experiment 1		
Gall index > 1	1.0 ± 0.7	0.11
Hairy	0.5 ± 0.2	0.36
Below premium size	69.6 ± 2.6	0.94
Stepwise regression adjusted r^2	0.91	—
Total substandard	73.4 ± 2.3	—
Experiment 2		
Gall index > 1	22.5 ± 5.0	0.58
Split	1.1 ± 0.4	0.35
Below premium size	73.1 ± 2.2	0.34
Stepwise regression adjusted r^2	0.74	—
Total substandard	86.5 ± 1.8	—

Table 23. Incidence of carrot defects and total substandard carrots, and significant (P<0.05) linear regressions (with correlation coefficients) between defect incidence and levels of *Meloidogyne javanica*, *Pratylenchus* spp., *Hemicycliophora saueri* and *Paratrichodorus* spp. in soil or roots at Nuriootpa, Barmera and Purnong

Carrot defect category vs. nematode level by field experiment[A]	% carrots (± SE) with defect	Linear regression	
		Adjusted r^2 (correlation coefficient)	P
Nuriootpa			
Experiment 1			
% forking vs. Pf (*M. javanica* soil)	1.5 ± 0.4	0.32 (0.59)	< 0.001
vs. Pm (*M. javanica* soil)		0.17 (0.44)	<0.02
% roots gall index > 1 vs. Pm (*M. javanica* soil)	10.3 ± 2.5	0.40 (0.44)	0.0001
% total galled roots vs. Pm (*M. javanica* soil)	12.4 ± 2.7	0.28 (0.55)	<0.002
vs. Pf (*M. javanica* roots)		0.14 (0.42)	0.02
Experiment 2			
% splitting vs. Pf (*H. saueri* soil)	1.0 ± 0.6	0.29 (0.56)	0.001
% irregularly split roots vs. Pm (*H. saueri* soil)	6.7 ± 2.5	0.11 (0.38)	0.04
Barmera			
% stunted carrots vs. Pf (*Pratylenchus* soil)	6.6 ± 1.2	0.11(0.38)	0.04
Purnong (Experiments 1 & 2 combined)			
At harvest levels of nematodes			
% substandard carrots vs. Pf (*H. saueri* soil)	79.9 ± 1.7	0.07 (0.29)	0.02
vs. Pf (*H. saueri* + *M. javanica* soil)		0.05 (0.25)	<0.05
% forked roots vs. Pf (*H. saueri* soil)	4.5 ± 0.7	0.18 (0.44)	0.0005
vs. Pf (*M. javanica* soil)		0.23 (0.50)	0.0001
vs. Pf (*H. saueri* + *M. javanica* soil)		0.29 (0.55)	<0.0001
% irregularly split roots vs. Pf (*H. saueri* soil)	0.5 ± 0.2	0.30 (0.56)	<0.0001
% split roots vs. Pf (*H. saueri* soil)	0.6 ± 0.2	0.37 (0.62)	<0.0001
% constricted roots vs. Pf (*H. saueri* soil)	6.4 ± 2.1	0.21 (0.47)	0.0001
% hairy roots vs. Pf (*H. saueri* soil)	3.6 ± 1.0	0.13 (0.38)	<0.003
% tip-swollen roots vs. Pf (*H. saueri* soil)	0.8 ± 0.2	0.09 (0.33)	0.01
vs. Pf (*M. javanica* soil)		0.14 (0.39)	<0.005
% stubbed roots vs. Pf (*H. saueri* soil)	1.1 ± 0.2	0.15 (0.41)	0.001
% roots gall index > 1 vs. Pf (*M. javanica* soil)	11.8 ± 2.9	0.17 (0.43)	0.0006

vs. Pf (*H. saueri* soil)		0.05 (0.26)	<0.05
vs. Pf (*H. saueri* + *M. javanica* soil)		0.20 (0.46)	0.0002
% total galled roots vs. Pf (*M. javanica* soil)	31.5 ± 4.8	0.31 (0.56)	<0.0001
vs. Pf (*H. saueri* + *M. javanica* soil)		0.31 (0.57)	<0.0001
vs. Pf (*M. javanica* roots)		0.14 (0.40)	<0.002
Mid-season levels of nematodes			
% constricted roots vs. Pm (*H. saueri* soil)	6.4 ± 2.1	0.26 (0.52)	<0.0001
% forked roots vs. Pm (*M. javanica* soil)	4.5 ± 0.7	0.25 (0.51)	<0.0001
% stubbed roots vs. Pm (*M. javanica* soil)	1.1 ± 0.2	0.35 (0.60)	<0.0001
% hairy roots vs. Pm (*M. javanica* soil)	3.6 ± 1.0	0.07 (0.29)	<0.03
% roots gall index > 1 vs. Pm (*M. javanica* soil)	11.8 ± 2.9	0.12 ((0.37)	<0.004
% total galled roots vs. Pm (*M. javanica* soil)	31.5 ± 4.8	0.11 (0.35)	<0.006
At planting levels of nematodes			
% substandard carrots vs. Pi (*M. javanica* soil)	79.9 ± 1.7	0.14 (0.40)	<0.002
vs. Pi (All nematodes soil)		0.05 (0.41)	<0.05
% irregularly split roots vs. Pi (*H. saueri* soil)	0.5 ± 0.2	0.75 (0.87)	<0.0001
vs. Pi (*M. javanica* soil)		0.35 (0.60)	<0.0001
% split roots vs. Pi (*H. saueri* soil)	0.6 ± 0.2	0.78 (0.88)	<0.0001
% constricted roots vs. Pi (*M. javanica* soil)	6.4 ± 2.1	0.49 (0.70)	<0.0001
vs. Pi (*H. saueri* soil)		0.85 (0.92)	<0.0001
% forked roots vs. Pi (*M. javanica* soil)	4.5 ± 0.7	0.32 (0.58)	<0.0001
% hairy roots vs. Pi (*M. javanica* soil)	3.6 ± 1.0	0.33 (0.59)	<0.0001
% tip-swollen roots vs. Pi (*M. javanica* soil)	0.8 ± 0.2	0.06 (0.28)	<0.03
vs. Pi (*H. saueri* + *M. javanica* soil)		0.17 (0.43)	0.0006
% stubbed roots vs. Pi (*Paratrichodorus* sp. soil)	1.1 ± 0.2	0.08 (0.30)	<0.02
% roots gall index > 1 vs. Pi (*M. javanica* soil)	11.8 ± 2.9	0.36 (0.61)	<0.0001
% total galled roots vs. Pi (*M. javanica* soil)	31.5 ± 4.8	0.23 (0.49)	0.0001
vs. Pi (*H. saueri* + *M. javanica* soil)		0.31 (0.43)	0.0006

[A] Pi = at planting; Pm = mid-season, and Pf = at harvest levels in soil or roots

and incidence of galled carrots (> gall index 1) in experiment 1. Splitting (regular) was associated with at harvest levels of *H. saueri* in soil, and irregular splitting was associated with mid-season levels of *H. saueri* in soil (Table 23), but no association was found between incidence of substandard carrots or defects and at harvest levels of *Paratrichodorus* sp. in soil in experiment 2.

Barmera

Forked and undersized carrots were the most common defects at this site and these defects were most strongly correlated with variation in total incidence of substandard carrots (Table 22). Incidence of substandard carrots was associated with at harvest levels of *P. neglectus* in soil at Barmera (Table 23) but not with at harvest levels of this nematode in roots, or with at harvest levels of *Helicotylenchus* spp. in soil. Defects were not associated with at planting or mid-season levels of nematodes at this site. *P. neglectus* was detected in roots of caltrop and thornapple at levels of 4.3 and 2.8 nematodes per gram fresh weight respectively (compared with up to 2.1 nematodes per gram fresh weight of carrot roots at harvest).

Purnong

Incidence of substandard carrots was very high in both experiments at Purnong, due to a very high incidence of undersized carrots in experiment 1 and to both undersized and galled carrots in experiment 2 (Table 22). The defects most strongly correlated with variation in incidence of total substandard carrots were undersized carrots in experiment 1 and galled carrots in experiment 2 (Table 22). The highest levels (22.5%) of galled carrots of any site occurred in experiment 2 (Table 22). Variation in incidence of substandard, forked, hairy, constricted, stubbed, tip-swollen and both irregularly and regularly split carrots were associated with at harvest levels of *H. saueri* in soil (Table 23). At harvest levels of this nematode were also weakly associated with incidence of galled carrots (> gall index 1) but galling was more strongly associated with at harvest levels of *M. javanica* in soil and roots (Table 23). Total galled carrots was also associated with soil (and root) levels of *M. javanica* at harvest. Combining levels of *M. javanica* and *H. saueri* did not strengthen this association to any significant degree (Table 23). At harvest levels of *M. javanica* in soil were also associated with incidence of forked and tip-swollen roots (Table 23). Incidence of substandard carrots and defects

were not associated with at harvest levels of *S. brachyurum* and *Paratrichodorus* sp. in soil. Variation in incidence of both substandard and of galled carrots (> gall index 1) were associated with at harvest levels of *M. javanica* in soil in experiment 2 (P <0.03 and <0.001 respectively).

At planting and mid-season levels of *M. javanica* were associated with incidence of forked, hairy and galled (total galled and gall index > 1) carrots. At planting levels of *M. javanica* were also associated with incidence of irregularly split, constricted and tip-swollen roots, and mid-season levels were associated with incidence of stubbed roots (Table 23). Combining levels of *M. javanica* and *H. saueri* did not increase the strength of association with total galled carrots. At planting and mid-season, levels of *H. saueri* in soil were associated with incidence of constricted carrots. At planting levels of *H. saueri* were also associated with incidence of regularly and irregularly split carrots, and combined levels of this nematode and *M. javanica* were more strongly associated with incidence of tip-swollen carrots than *M. javanica* alone (Table 23). Incidence of stubbed carrots was also weakly associated with at planting levels of *Paratrichodorus* spp. Incidence of substandard carrots was associated with at planting levels of *M. javanica* and with all plant parasitic nematodes combined (Table 23).

7.3.4 Discussion

Total production of defective carrots was highest at the farm using organic production techniques, however, this was offset by the higher prices this grower received and by a greater tolerance of size variation by consumers of organically-grown produce. A large proportion of carrots grown organically, or in experiment 1 at Nuriootpa under conventional production techniques, fell below premium size ranges. This was due in part to higher seeding rates as reflected in the higher plant densities in these field experiments, and the Nuriootpa grower intended reducing plant spacing in subsequent seasons because of the low carrot size observed in experiment 1. The effect of plant stand on carrot size was clearly seen in experiment 2 at Nuriootpa, where emergence of late season carrots was reduced by very hot temperatures in January, and average carrot weight was greatly increased as a result. Production of both non-defective and total carrots was much higher in this experiment suggesting that reduced seeding rates

could be beneficial for early season carrots. Lower rates of NPK fertilizer were used at Purnong under organic production and this may also have contributed to lower carrot size here, as carrot size is known to increase with increasing rates of N up to about 150-180 kg/ha (Hochmuth *et al*. 1999). Total N applied at this site was only a half of this and fell well below rates used at the two farms under conventional production. Production of substandard carrots was highest in the experiments at Purnong and in experiment 1 at Nuriootpa, mainly due to the incidence of undersized carrots.

Levels of *M. javanica* in roots at harvest were highest in the experiments at Purnong and in experiment 1 at Nuriootpa and levels were significantly lower in experiment 2 (late season carrots) at the latter site. As soil levels of this nematode were non-detectable or near non-detectable in both Nuriootpa experiments it is likely that environmental conditions, particularly soil temperatures, were more favourable for nematode population increase in the early season carrots. Mean soil temperatures dropped sharply between March and April and were below thresholds for larval movement of this nematode (Wallace 1966) during May when late season carrots were harvested. Soil levels of *M. javanica* at harvest were similarly much higher in experiment 1 compared with experiment 2 at Nuriootpa, and production losses due to this nematode exceeded 10% in experiment 1 whereas losses were negligible in experiment 2. This suggests that nematode control is more important for early season than for late season carrots. Losses due to *M. incognita* in winter-grown carrots were found to be higher in early plantings (Roberts 1987) and postponing sowing summer-grown carrots reduced damage caused by *M. fallax* (Molendijk and Brommer 1998). However, early sowing of carrots was reported to reduce damage caused by *M. hapla* in Canada (Belair 1987). The latter observation may be explained by the lower soil temperatures experienced in Quebec compared with South Australia in spring. Production losses due to nematodes at Nuriootpa occurred despite preplanting soil fumigation with metham sodium. This was probably due to the low rate of fumigant used (300L/ha) rather than to enhanced biodegradation (Warton and Matthiessen 1999) as fumigation was limited to one application every two years. In potato, effective control of *Meloidogyne* spp. occurred at rates of at least 468 L/ha metham sodium, with 374 L/ha ineffective (Santo and Qualls 1984). Similarly a rate of 373 L/ha failed to provide season-long control of *M. incognita* in carrots (Hutchinson *et al*. 1999). The strategy

used by this grower was to apply only a sufficient rate of fumigant to achieve a level of nematode control that he was prepared to tolerate whilst avoiding excessive loss of beneficial soil micro-organisms. Although soil fumigation with methyl bromide can destroy mycorrhizae and retard the growth of carrots (Plenchette *et al*. 1983), this response has not been reported when metham sodium is used at normal rates prior to planting carrots. The latter fumigant tends to be less effective than methyl bromide (Hutchinson *et al*. 1999) and would pose less of a risk to mycorrhizae. Carrot response to soil fumigation is normally very positive where *Meloidogyne* spp. are present (Vrain *et al*. 1981; Hutchinson *et al*. 1999) and it would appear potentially beneficial for the Nuriootpa grower to increase rates of metham sodium to 500 L/ha before planting early season carrots.

Other nematodes displaying high multiplication rates (suggesting susceptibility of carrots) during the growing season were *Hemicycliophora saueri*, *Helicotylenchus* spp., *Scutellonema brachyurum*, *Pratylenchus neglectus* and *Paratrichodorus* spp. Weeds contributed to population increase of *P. neglectus* at Barmera as levels detected in roots of caltrop and thornapple were slightly higher than those in carrot roots, but levels in carrot roots at this site were higher than levels of *Pratylenchus* spp. at other sites. *P. neglectus* can be multiplied in carrot disk culture (Verdejo-Lucas and Pinochet 1992), also suggesting that carrots are able to host this nematode. Levels of *Macroposthonia xenoplax* did not increase significantly over the growing season, suggesting that carrots were a poor host of this nematode. No effects from this nematode were reported on carrots in an earlier study (Stapleton *et al*. 1987).

Carrot defects accounting for significant variation in the proportion of substandard carrots were: Nuriootpa – galling, hairy roots, splitting, swollen tips; Barmera – forking, undersized roots, splitting; and Purnong – undersized roots, hairy roots and galling. These were the most important defects affecting carrots at these farms, and anecdotal evidence suggests that (except for swollen tips which is a newly reported defect) they are generally the most commonly reported defects in South Australian carrot crops. Galling, forking, hairy roots, and stunting of roots have previously been associated with infection by *Meloidogyne* spp. (Belair 1992; Huang and Charchar 1982; Roberts 1987; Yarger and Baker 1981). Galled roots were only seen at sites where *M. javanica* was

found but the co-occurrence of *H. saueri* and *M. javanica* made it difficult to distinguish galls potentially caused by *H. saueri*. At harvest levels of the latter nematode were weakly correlated with galling. *H. similis* is reported to cause galls in carrots (McKewan 1979). At planting, mid-season and at harvest levels of *M. javanica* were more strongly correlated with incidence of galling, and were variously associated with incidence of forked, tip-swollen, stubbed, hairy, constricted, irregularly split and substandard carrots, suggesting that this nematode was a significant cause of production loss. At harvest levels of *H. saueri* were also correlated with incidence of substandard carrots, and with hairy, constricted, forked, tip-swollen, stubbed and split (regularly and irregularly) carrots, suggesting that this nematode may also be a significant, and previously unreported, cause of production loss of carrots in South Australia. Mid-season and at planting levels of this nematode were also associated with incidence of constricted carrots, and at planting levels were associated with incidence of split (regularly and irregularly) carrots. The correlations between at planting levels of this nematode and these defects were particularly strong (0.87-0.92). Further work is required to prove a causal link between *H. saueri* and these defects. These results suggest that the total nematode load needs to be considered and that nematodes apart from *Meloidogyne* spp. may be involved with defects such as forking. Incidence of undersized carrots was correlated with at harvest levels of *P. neglectus* suggesting a previously unreported association between this nematode and carrot stunting (although the role of weeds in this regard needs further study). *P. penetrans* caused stunting in carrots at all levels evaluated of up to 100 nematodes/100 mL of soil (Vrain and Belair 1981). Greenhouse and/or microplot experiments are required to demonstrate a causal relationship between these nematodes and carrot defects. The irregular splitting that was observed at some sites was not the usual type of harvest splitting (Sorensen and Harker 2000) which is related to diurnal phases of carbohydrate storage (Gracie and Brown 2000). This newly reported defect appeared to be related to nematodes, especially *H. saueri*.

Nematodes were shown to be associated with carrot defects and significant production losses to carrots in South Australia under both conventional and organic production techniques. Growers need to be aware of potential damage from nematodes in addition to *Meloidogyne* spp., including *Hemicycliophora* spp. and *Pratylenchus* spp. Loss of production due to defects such as galling, which is known to be caused by nematodes, was highest in one of the experiments under organic production techniques, and a

greater range of plant parasitic nematodes at potentially damaging levels occurred under organic production. Increased frequency of suitable rotation crops would assist nematode control in carrot production (Huang 1984), but would especially benefit growers engaged in organic production because they are unable to deploy chemical controls. However, most of the main carrot growers in South Australia are specialist carrot producers and unless profitable rotation crops can be found they are unlikely to reduce the frequency of carrot cropping.

Conventional methods of extracting nematodes were shown to be reliable where high levels of nematodes existed at planting, as in the farm under organic production techniques. However, conventional methods were unreliable for detecting *M. javanica* following soil fumigation until 14-17 weeks after planting. This nematode was readily detected in preliminary sampling before fumigation, and fields should therefore be sampled before fumigation to facilitate detection of *Meloidogyne* spp. Other nematodes such as *P. neglectus*, *Helicotylenchus* spp., *H. saueri* and *Paratrichodorus* spp. were detected more reliably at planting, even in fumigated soil in the case of the latter two nematodes. It appeared possible to predict the incidence of some carrot defects based on at planting levels of these nematodes at sites where high levels favoured detection. Improved techniques for detecting *Meloidogyne* spp. before planting are needed to minimise use of nematicides and fumigants.

7.4 Effect of *Paratrichodorus* and *Pratylenchus* on carrot production (South Australia)

Preliminary sampling of a commercial carrot field under centre pivot irrigation near Kybybolite in south-eastern South Australia identified the presence of *Paratrichodorus minor* and *Pratylenchus neglectus*. This afforded the opportunity to determine the effect of these nematodes on carrot production. The field was sown with carrot cv. Murdoch late November 2002 and harvested in early April 2002, and the soil type was a sandy loam. Nemacur 400® was applied 2 days after planting at 20 L/ha. A transect consisting of 30 plots was by Whitehead tray technique (Whitehead and Hemming 1965). Soil was sampled from each plot through the growing season and nematodes quantified. At harvest, carrot yields were determined and nematodes were extracted from both soil and root samples.

Table 24. Population dynamics of plant parasitic nematodes in a commercial carrot crop (cv. Murdock) at Kybybybolite, South Australia.

	Sampling date (weeks after planting)					
	1	5	13	15	19	l.s.d. (P<0.05)
Nematodes/300 mL soil ± S.E. (range)						
Paratrichodorus minor	1.8 ± 1.2 (0-35)	39.7 ± 14.4 (0-372)	40.6 ± 12.0 (0-288)	21.3 ± 6.5 (0-124)	21.5 ± 6.9 (0-180)	17.6
Pratylenchus neglectus	0 (0)	0.3 ± 0.2 (0-6)	4.2 ± 1.6 (0-39)	2.6 ± 1.3 (0-35)	0.9 ± 0.5 (0-10)	2.7
Nematodes/g fresh weight of root ± S.E. (range)	-	-	-	-	794.2 ± 676 (0-20100)	-
Mean carrot weight (g)	-	-	-	-	60.6 ± 2.2	-
Number of carrots/plot (1 m-length × 3 double rows)	-	-	-	-	93.2 ± 1.9	-
Marketable carrots (%)	-	-	-	-	86.9 ± 2.9	-
Non-marketable carrots (%)						
Soft rot[1]	-	-	-	-	8.7 ± 3.0	-
Hairy	-	-	-	-	3.5 ± 0.5	-
Forked	-	-	-	-	2.4 ± 0.3	-
Split	-	-	-	-	1.3 ± 0.3	-

[1]*Sclerotinia sclerotiorum* was a problem in some plots in a poorly drained area

Strong associations between nematode densities and yields or the incidence of carrot defects were not found in this trial, despite the presence of what were considered to be high and damaging densities of Stubby root nematode (*P. minor*) in some plots (Table 24). This nematode was not controlled effectively by use of Nemacur®. However, it is recognised that this *Paratrichodorus* is generally difficult to control using chemicals. The density of this nematode peaked mid-season as was observed at some other field sites in South Australia (Table 24).

Although soil densities of lesion Nematode (*P. neglectus*) remained low throughout the crop cycle, very high densities were present in carrot feeder roots at harvest in some plots indicating that the nematode was able to penetrate and multiply on carrot roots (Table 25). A weak association (adjusted r^2 = 0.32, P<0.05) was demonstrated using stepwise regression, between mean carrot weight and densities of *P. minor* and *P. neglectus* (mean carrot weight = 58.4 + 0.08**P. minor* (13 weeks) – 0.001* *P. neglectus* (roots)). The study suggested that these nematodes at the particular densities recorded were not contributing to reduced production of carrot at this site.

8. Pathogenicity of nematodes to carrot

8.1 Pathogenicity of *Pratylenchus* spp. to carrot (South Australia)

Lesion Nematodes (*Pratylenchus* spp.) are commonly encountered in carrot field soil. However, there is little information on the effects of these nematodes on carrot growth.

Potting soil was made up of equal parts of coarse and fine sands, pH 6.8, and was steam-disinfested prior to use. *P. neglectus* originally extracted from carrot roots from a carrot farm at Naracoorte, S.A. and *P. jordanensis* and *P. zeae* originally extracted from grapevine roots from vineyards at Angaston and McLaren Vale, S.A. respectively, were multiplied aseptically in carrot disk culture (Moody *et al*., 1973). Known densities of each species (40,000, 5,000 or 2,000 of *P. neglectus* and 5,000 of *P. jordanensis* or *P. zeae* per pot) in suspension were spread evenly over the surface of each inoculated pot immediately before seeds were placed on the surface of the soil and then covered with 6 mm-depth of clean potting soil. For the un-inoculated control, a similar volume of suspension from un-inoculated carrot disks was similarly spread over the surface of pots. At 6 weeks, emergence was recorded and seedlings thinned to five per pot. Roots of thinned seedlings were weighed and nematodes extracted for 5 days in a Seinhorst mist chamber (Hoopper 1970). Carrots were grown for 22 weeks from seeding and then harvested. Root and shoot weights were recorded, defects assessed, and nematode levels in both soil and feeder roots assessed.

Before statistical analysis, nematode densities were transformed (ln (density + 1)) if plots of residuals or tests for non-additivity and normality indicated that this was required. Randomised block designs were used and ANOVA, Fisher's protected LSD, t-tests (untreated vs. treated) or contingency tests (P=0.05) were conducted using Statistix (Version 4.1, Analytical Software, Tallahassee, Florida).

Mean dry root weight of thinned carrot seedlings (range 0.002-0.003 g/seedling, SE=0.0005) did not differ between uninoculated carrots and those inoculated with three *Pratylenchus* spp., however, nematodes of all three species were extracted from their roots (Table 25). Carrot emergence, incidence of forking, and shoot biomass

production were not significantly different in inoculated and uninoculated carrots, but carrot dry weight was stimulated by inoculation with *P. zeae* and by the 5,000 inoculum density only with *P. neglectus* (Table 25). Densities of *P. jordanensis* and *P. zeae*/g of carrot roots at harvest were significantly higher than those of *P. neglectus* including the higher inoculum density of 40,000 used for the latter species (Table 25). However, in roots of young seedlings (after at most one generation of the nematode life cycle had elapsed) densities of *P. jordanensis* were not significantly different from those of *P. neglectus* at the higher inoculum density of 40,000 but not at lower inoculum densities (Table 25).

Table 25. Effects of inoculation with *Pratylenchus neglectus*, *P. jordanensis* and *P. zeae* at varying inoculum densities, on densities of *Pratylenchus* sp. in roots of both thinned seedlings and mature carrots, and in soil, and on carrot emergence, growth, and per cent forked carrots in disinfested potting soil[1]

Pratylenchus sp./inoculum density	Carrots (%)		*Pratylenchus sp.*[2]			Dry weight (g)	
	Emerged	Forked	/g root		/175 ml soil	Carrot	Shoots
			Seedling	Mature			
Uninoculated	78	4	–	–	–	2.41 c	1.0
P. neglectus							
40,000	77	nd	5.6 ab (358)	1.1 b (7)	(7)	2.44 c	1.1
5,000	81	nd	3.7 b (110)	1.8 b (19)	(3)	3.11 a	1.2
2,000	78	nd	nd	1.3 b (12)	nd	2.80 abc	1.0
P. jordanensis 5,000	88	4	7.1 a (1,457)	4.6 a (267)	(9)	2.57 bc	1.1
P. zeae 5,000	84	nd	5.0 b (192)	4.0 a (112)	(6)	2.98 ab	1.2
LSD	ns	ns	1.9	2.0	ns	0.51	ns

[1] Means followed by the same letter in the same column are not significantly different at $P = 0.05$; nd = not detected; ns = not significant.
[2] Transformed data [ln(x + 1)] used in statistical analysis, with original means in parentheses.

All three *Pratylenchus* spp. apparently entered roots of carrot seedlings in large numbers, but little reproduction of *P. neglectus* appeared to have occurred as this nematode was detected at low densities in secondary roots from mature carrots, suggesting partial resistance. However, carrots were more susceptible to both *P.*

jordanensis and *P. zeae*. Although the latter species were not shown to suppress carrot growth in pots, they may have the potential to be damaging in the field especially in association with fungal pathogens. The *P. neglectus* population used in this study was isolated originally from carrot roots, but may have been primarily associated with cereal cover crops.

8.2 Pathogenicity of *Meloidogyne* spp. to carrot (Victoria)

The primary nematodes causing problems to the growth of carrots in Victoria are two species of root knot nematode (*Meloidogyne hapla* and *Meloidogyne javanica*). Pre-plant testing and an understanding of the significance of nematode population numbers are the keys to management and predicting suitability of a paddock for carrot production. The aim of this trial was to relate nematode numbers to carrot marketability and economic loss. Maximising marketability of carrots can be achieved by managing nematode population to below the threshold before planting.

Materials and Methods

The trial was established in a glasshouse. Seeds of five common carrot varieties (Stefano, Senior, Kendo Midi, Red Hot and Mojo) were germinated in 8 inch diameter pots containing 2.5 kg of potting mix. After 4 weeks the plants were thinned to 3 plants per pot. Plants were fertilised with Thrive® (Yates) as required throughout the trial.

Each carrot variety was inoculated with two species of root knot nematode (*Meloidogyne hapla* and *Meloidogyne javanica*) at 0, 10, 20, 40, and 80 nematodes per 100g soil. The pots were infected with eggs and J2. Eggs and J2 of each root knot species were retrieved from heavily galled roots of tomato plants reared in the greenhouse. Root knot nematode inoculum was produced as follows. Tomato plants (1 month old) with first true leaves (approximately 10 cm tall) were transplanted into a pot containing sterile media (below) and infected root material was placed around the roots of the host plant. A media of 50% sand/50% potting mix was used. Sand was sterilised in an autoclave then sieved through a 2 mm mesh. Pots were maintained in the greenhouse at temperatures above 25^{o}C. Pots were hand watered as required and nutrients were supplied as Phostrogen liquid fertiliser every two weeks. When host

plants become root bound and exhibited obvious galling (3 months after inoculation), roots were cut off and used to inoculate new host plants or extracted for experiments (below). Other species tested included tobacco, chilli and Impatience as host plants. No galling was evident on the tobacco and chilli, and only small, less frequent galling occurred on the Impatience. Roots were shaken in 1% sodium hypochlorite solution for 4 minutes (Hussey and Barker 1973) to extract eggs. The suspension was then passed through a 75 µm sieve to collect debris and a 38 µm sieve to collect eggs and J2. The eggs were rinsed with water and the suspension transferred to a tray to allows eggs to hatch over several days. During inoculation, hatched J2 were introduced to the root zone of each plantlet and watered in lightly.

The trial was harvested approximately 18 weeks after inoculation. The gall rating of Belair and Boivin (1996) was used to determine a level of galling associated with marketability.

Results

Variety had a significant effect on the gall rating (Table 26) with Mojo and Red Hot having significantly lower gall ratings averaged across all population densities of nematodes than other varieties. Stefano and Senior had significantly higher gall ratings, with Kendo Midi having an intermediate gall rating.

Table 26. Effect of variety on gall rating (*P*<0.001, LSD = 0.26).

	Mojo	Red Hot	Kendo Midi	Senior	Stefano
Mean gall rating	1.5 c	1.5 c	1.9 b	2.2 a	2.3 a

Means in row followed by same letter are not significantly different at *P*=0.05.

Initial population density had a significant effect (*P*<0.001) on gall rating with an average rating across both species of 3.0 at 80 root knot nematodes per 100 g soil. On average, across population densities and varieties, *M. hapla* had a significantly (P<0.001) higher gall rating (2.06) compared to *M. javanica* (1.69). The interaction between variety and

initial population density was statistically significant (P<0.001), reflecting the less rapid increase in gall rating at low population densities in Mojo for *M. hapla* and Mojo and Red Hot for *M. javanica* (Figures 18 and 19). The interaction between variety and nematode species was statistically significant (P=0.02). Mojo and Senior had a similar average gall rating when inoculated with *M. hapla* or with *M. javanica* (Figure 20). However, Kendo Midi, Red Hot and Stefano had higher average gall ratings when challenged with *M. hapla* in comparison to *M. javanica*.

The variety Red Hot performed well in the presence of both species of *Meloidogyne*. Soil and root counts were constantly lower with Red Hot than other varieties and Red Hot maintained the highest root growth and least fibrous root growth and forking and maintained a constant foliage growth. Senior and Stefano appeared to have the least taproot growth and Mojo had the highest root weight in the presence of nematodes. Mojo displayed a constantly high but even growth and weight of foliage for both nematode species and at all infection rates. Kendo Midi displayed a much higher root count compared to the rest of the varieties when infected with *Meloidogyne hapla*. Red Hot and Stefano had a lower root count compared to the other varieties when infected with *Meloidogyne javanica*. Stefano had higher soil nematode counts, with Mojo and Red Hot both having the lowest soil counts of nematodes when infected with both species of nematodes (results not shown).

According to the gall rating used, carrots become unmarketable at a rating of 3 when 50-100 galls occurred on secondary roots, some coalesced and light forking was evident. Figure 15 shows the threshold where unmarketability resulted was approximately 18 nematodes /100g soil for varieties Stefano and Senior when infected with *Meloidogyne hapla*. The threshold for *Meloidogyne javanica* for varieties Stefano and Senior was between 60-70 nematodes/100g soils as shown in Figure 16. The varieties Kendo Midi, Red Hot and Mojo did not reach a gall rating of 3 at the highest inoculation rate of 80 nematodes/100grams of soil.

Figure 18. Gall rating of varieties infected with *Meloidogyne hapla*.

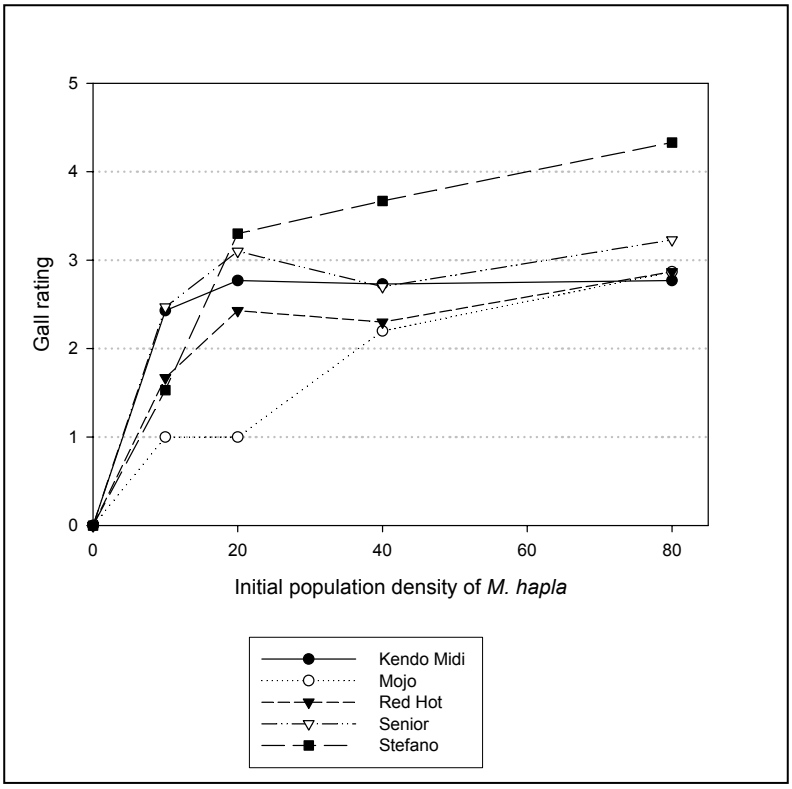


Figure 19. Gall rating of varieties infected with *Meloidogyne javanica*

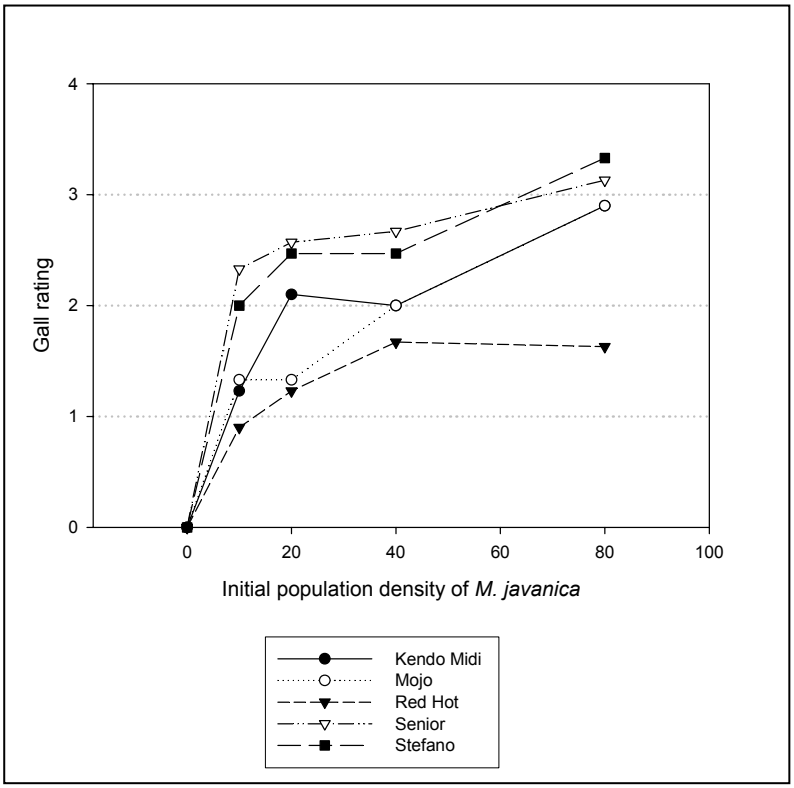
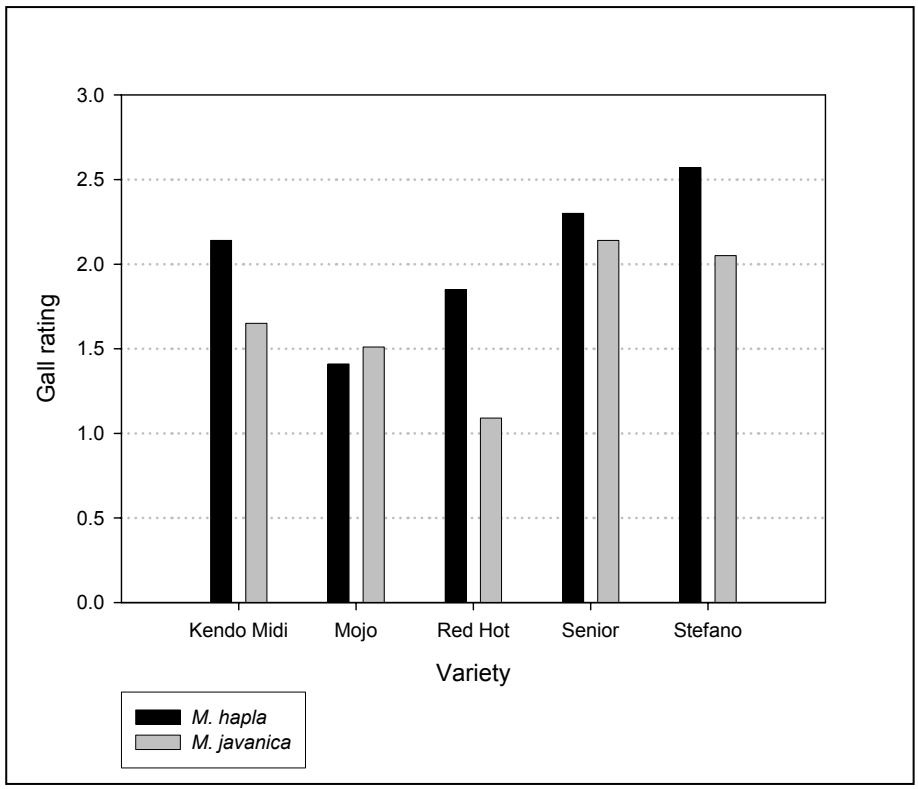


Figure 20. The interaction between variety and *Meloidogyne* species on gall rating (*P*=0.02, LSD = 0.37).



8.3 Pathogenicity of *Meloidogyne* spp., *Pratylenchus* spp. and *Radopholus similis* to carrot (Queensland).

The aim of this trial was to test the pathogenicity of six nematode species on carrots.

Materials and Methods

Carrot seeds (*Daucus carota* c.v. *Stefano*) were planted in 1.5 L of UC-mix on 27 September 2002. The plants were thinned to 20 seedlings/pot. Four weeks after planting the pots were inoculated with either *Meloidogyne hapla, M. incognita, M. javanica, Pratylenchus neglectus, Pratylenchus thornei* or *Radopholus similis*. Carrots were inoculated with 500, 2,500, 5,000 or 10,000 nematodes per pot for each nematode species. Each inoculation level and species was replicated 5 times. Five control pots were left uninoculated.

The carrot plants were harvested 42 days after inoculating with nematodes. The shoots were dried in an oven for 7 days at 70°C and then weighed. The roots from pots inoculated with *Meloidogyne* spp. were washed in water to remove UC mix, blotted dry and weighed. The roots were then soaked in 1% NaOCl for three minutes to extract root-knot eggs (Hussey and Barker 1973). The nematode suspensions were passed through a 38 μm sieve and rinsed with water to remove NaOCl.

The roots from pots inoculated with *Pratylenchus* spp. or *R. similis* were washed to remove the UC-mix and then placed in a misting chamber for 4 days. The roots were blotted dry and weighed. The nematodes were recovered from suspension using a 38 μm sieve.

Results

M. hapla had the highest reproduction rate on carrots after 6 weeks at each inoculation level in comparison to other nematodes (Table 27). The reproduction rate of *M. hapla* was, however, not significantly higher than on the roots of pots inoculated with *M. javanica* at the 2,500 and 5,000 inoculum level (Table 27). Compared with other nematode species, *P. neglectus* and *R. similis* had the lowest reproduction rate on carrot roots in pots inoculated with 2,500, 5,000, and 10,000 nematodes per pot (Table 27). The final number of *P. neglectus, P. thornei* and *R. similis* recovered from the roots of carrot plants was consistently less than initial populations added to the pots. This suggested that carrots were poor hosts for these nematodes.

There was no significant difference in dry shoot weights between the six nematode species inoculated at 500, 2,500 or 5,000 nematodes per pot. (Table 27). *M. hapla* inoculated with 10,000 nematodes per pot significantly reduced shoot weight relative to the untreated plants, although it was not significantly lower than the shoot weights of plants inoculated with 10,000 *R. similis* per pot. Pots inoculated with 10,000 *M. javanica* had the highest shoot weight, although this was not significantly higher than the untreated, or carrots inoculated with *M. incognita*, *P. neglectus* and *P. thornei* (Table 27).

Fresh root weights were not significantly different between nematode species for pots inoculated with 500 or 2,500 nematodes (Table 27). When inoculated with 5,000 nematodes per pot *M. javanica* had the highest root weights, significantly greater than the untreated plants, but was not significantly different to *P. neglectus*. *P. thornei* had the lowest root weight at an initial inoculum number of 5,000 nematodes per pot but this was not significantly reduced relative to the untreated control. At 10,000 nematodes per pot, the root weights of carrots inoculated with *M. javanica* were again the highest, but not significantly different to *M. incognita*, *P. neglectus* and *P. thornei* at the same inoculum level. Carrots inoculated with *M. hapla* and *R. similis* had the lowest root weight at 10,000 nematodes per pot but not significantly lower than untreated, *P. neglectus* and *P. thornei* (Table 27).

M. hapla had the highest reproduction rate on carrot at four inoculation levels; 500, 2,500, 5,000 and 10,000 nematodes in 1.5 L of soil. Inoculation of carrot plants with *M. hapla* resulted in the lowest shoot weight at 10,000 nematodes per pot. *M. hapla* was the only nematode to significantly reduce carrot root and shoot weights with increasing initial nematode populations. The amount of *M. hapla* inoculum at planting accounted for 87% of the decline in shoot weight of carrots (Shoot weight = $– 2.88x10^{-6}$ P_i + 0.08818, $P<0.05$). Similarly, the amount of *M. hapla* inoculum at planting accounted for 72% of the decline in root weight of carrots (Root weight = $- 2.01x10^{-5}$ P_i + 1.1285, $P<0.05$). The final population of *M. hapla* in the roots could explain 69.5% of the reduction in shoot weight of carrots (Shoot weight = $-9.67x10^{-7}P_f$ + 0.092, $P<0.05$) but was not significantly related to the reduction in root weight of carrots. However, a multiple correlation of the initial and final numbers of *M. hapla* could explain 96.3% of the reduction in carrot root weight (Root weight = 1.078 – $4.27x10^{-5}P_i$+$8.93x10^{-6}P_f$, $P<0.05$). This suggested that *M. hapla* was the most pathogenic nematode species tested on carrot.

M. javanica had a similar reproduction rate to *M. hapla* when 2,500 eggs were inoculated per pot. However, at other population levels the reproduction rate was not as high as *M. hapla*. This suggested that the pathogenicity of *M. javanica* was not as high as *M. hapla* on carrot roots under the conditions of this trial. Additionally, there was no correlation between nematode numbers and the weight of roots and shoots of carrot

Table 27. The effect of nematode species and number of nematodes added per pot (P(i)=500,2500,5000,10000) on the number of nematodes detected in the roots six weeks after inoculation and on shoot dry weight and root fresh weight.

Nematode species and initial population	**Number of nematodes per 100 (g) fresh weight root ln(x+1)**	Shoot dry weight per plant (g)	Root fresh weight per plant (g)
500/1.5 l pot			
Untreated	0.0 a (0)	0.092	1.07
M. hapla	8.95 f (7707)	0.081	1.13
M. incognita	6.42 d (613)	0.077	1.17
M. javanica	7.07 e (1175)	0.083	1.3
P. neglectus	0.37 ab (0.4)	0.084	0.96
P. thornei	1.55 c (4)	0.093	1.01
R. similis	1.24 bc (2)	0.097	0.98
2500/ 5 l pot			
Untreated	0.00 a (0)	0.092	1.07
M. hapla	9.6 d (14764)	0.081	1.12
M. incognita	7.04 c (1140)	0.078	1.08
M. javanica	8.77 d (6437)	0.084	1.27
P. neglectus	0.76 a (1)	0.077	0.96
P. thornei	3.92 b (49)	0.092	1.04
R. similis	1.03 a (2)	0.10	1.09
5000/1.5 l pot			
Untreated	0.00 a (0)	0.092	1.07 ab
M. hapla	10.07 e (23622)	0.077	1.06 ab
M. incognita	8.10 d (3293)	0.079	1.11 ab
M. javanica	9.14 de (9320)	0.087	1.36 c
P. neglectus	1.70 b (4)	0.092	1.18 bc
P. thornei	5.06 c (156)	0.074	0.96 a
R. similis	2.45 b (10)	0.095	1.09 ab
10,000/5 l pot			
Untreated	0.00 a (0)	0.092 bc	1.07 ab
M. hapla	10.22 e (27446)	0.058 a	0.90 a
M. incognita	8.41 d (4491)	0.088 bc	1.26 bc
M. javanica	9.09 d (8865)	0.11 c	1.3 c
P. neglectus	3.24 b (24)	0.086 bc	1.08 abc
P. thornei	5.80 c (329)	0.083 bc	1.08 abc
R. similis	3.38 b (28)	0.076 ab	0.94 a

Means with the same subscript within the same species are not significantly different from each other (P = 0.05). Numbers in parenthesis are back transformed (e^x-1) means of number of nematodes/100 g root.

plants 6 weeks after inoculation. The root weight of the *M. javanica* infected carrot plants may have been heavier relative to the untreated plants due to the formation of root-knot galls. There was no significant reduction in shoot weight of carrot plants

infected with *M. javanica* relative to the untreated plants, which suggested that the formation of galls was not impacting on the shoot growth.

P. neglectus, *P. thornei* and *R. similis* all had significantly lower reproduction rates on the roots relative to the *Meloidogyne* species. When carrots were inoculated with 10,000 nematodes of each *P. neglectus*, *P. thornei* and *R. similis* there was no significant reduction in shoot weight relative to the untreated control. However, *P. thornei* was able to significantly reduce the weight of roots when 5,000 nematodes were added to each pot.

Conclusion

Compared to other species, *M. hapla* had the highest reproduction rate on carrot at the 500, 2,500 and 10,000 inoculation level, indicating that carrot was a particularly good host for this species. The lowest shoot weight occurred with *M. hapla* and *R. similis* when 10,000 nematodes were added to each pot indicating that these species were pathogenic. *R. similis* significantly reduced root weight at 10,000/pot, relative to the untreated control. The reproduction rates of *R. similis* and *P. neglectus* were significantly lower, relative to other nematodes at 5,000 and 10,000/pot. Although it had the highest reproduction at 2,500 and 5,000/pot, *M. javanica* appeared to be the least pathogenic nematode in this trial. Significantly higher carrot root weights were recorded in treatments with *M. javanica* at 5,000 and 10,000/pot in comparison to the untreated.

Some known aggressive parasites of carrot such as *M. javanica* had no impact on carrot shoot and root growth in this experiment. However, pot trials are often not a true reflection of the field situation, as plants in the former situation can receive sufficient nutrient and water to compensate for the effects of nematodes. It is important to correctly identify the nematode species, as this will affect the level of risk associated with carrot management options. *M. hapla* appears to be more damaging than the other plant-parasitic nematodes. However, only the shoot and root weight was assessed. Nematodes significantly reduce the quality of carrot production causing forking and taproot deformations, which would significantly reduce profitability. This was not assessed in determining the pathogenicity of the various plant-parasitic nematodes.

8.4 Relationship between root knot nematode populations and carrot quality (Western Australia).

Root knot nematode is becoming an increasingly important problem for Western Australian carrot growers. Infected carrots are unmarketable because of forking and galling. The purpose of this investigation was to conduct a preliminary study to determine the relationship between root knot nematode populations and carrot quality.

A site at the Medina Research Station that was partly infested with root knot nematode was used to investigate the association between nematode levels and carrot quality in crops sown in 2001 and 2002. The site was seeded to carrots in January 2001, and the level of seedling infection determined in March 2001, using the trypan blue method (Sharma and Modiuddin 1993). Twelve seedlings were taken from each sampling point and incidence of infection assessed. At harvest (May 2001), the yield and quality of mature carrots was assessed in a 1 m long plot centred on the same sample points as the seedling survey. As expected, the marketable yield was inversely correlated with the proportion of infected seedlings (Table 28).

Table 28. Association of incidence of seedling infection with quality of the mature 2001 crop.

Parameter	r
Total number of carrots	-0.34
Proportion of export quality carrots	-0.81
Proportion of marketable carrots (export + shorts)	-0.80
Proportion of forked and misshapen carrots	0.73

The infested site was left fallow after the carrot crop was harvested in May 2001, and then part of the site was used for similar assessments in 2002. A pre-planting soil survey was conducted on 12/12/01, after the site had been irrigated for 1 week. Soil samples taken from the same sampling points as in 2001 were extracted for 0 to 2 days and 2 to 4 days in Whitehead trays. The site was sown on 20/12/01, and a seedling assessment using the trypan blue method (Sharma and Mohiuddin 1993) carried out on 29/1/02. The mature crop was harvested on 9/4/2002. Carrots were assigned to

grades of export market quality, short marketable, and the various reject grades of oversize, undersize, forked, misshapen, bulgy eyes, splits. Seedling infection was inversely correlated with export market quality (r=-0.48) and correlated with bulgy eyes (r=0.47).

8.5 Resistant varieties (South Australia)

Carrot varieties have been shown to vary in their tolerance/resistance to nematodes, including *M. hapla* (Yarger and Barker 1981). Recently, carrot breeders have produced lines with simply inherited dominant resistance to *M. javanica* (Simon *et al*., 2000) opening up the possibility of using resistant cultivars to restrict nematode multiplication.

The U.S. Department of Agriculture-developed carrot selections with resistance against *Meloidogyne* spp. were compared with a susceptible cultivar to determine their potential for use in soils infested with local populations of *M. javanica*.

Six selections 636-7, -8 and -13; 637-5, -14 and -15 (inbreds from a cross of Brasilia and Nantes inbred, obtained from P. Simon, USDA Vegetable Crops Research Unit, Madison, Wisconsin) and a susceptible cv., Baby, were planted in the organic farm soil and grown as described in section 9.3.

Root and shoot biomass production, nor incidence of carrot forking, did not significantly differ between resistant selections and the susceptible control cv. Baby (Table 29). Secondary roots of the susceptible cultivar were more galled, and had significantly higher densities of *M. javanica* J2 than all resistant selections. *M. javanica* was not detected in roots of selections 636-8 and 636-13 and *M. javanica* densities did not build up in soil growing resistant selections as it did with the susceptible cultivar, however, densities of *H. saueri* and *S. brachyurum* in soil were not significantly different between resistant selections and the susceptible cultivar (Table 29).

The resistant carrot selections had useful levels of resistance to a local population of *M. javanica* but their marketability and consumer acceptance will need to be fully

evaluated. They were also not resistant to other nematodes present in this soil, including *H. saueri* and *S. brachyurum.*

Table 29. Effects of resistant selections (USDA) and a susceptible carrot cultivar on densities of *Meloidogyne javanica*, *Hemicycliophora saueri* and *Scutellonema brachyurum,* and on carrot growth, root galling and percent forked carrots in an organic farm soil

Carrot cv. /selection	*M. javanica**		Nematodes/175 mL soil		Carrot		Dry weight (g)	
	J2/g root	J2/175 mL soil	*H. saueri*	*S. brachyurum*	Gall index[2]	% fork	Carrot	Shoots
Susceptible								
cv. Baby	7.8 a[1] (3,203)	7.2 a (4,752)	1.7 abc (6.6)	(6)	3.8 a	28	5.3	1.6
Resistant								
637-5	1.0 b (36)	0.2 b (0.4)	2.3 ab (16.6)	(4)	0.8 b	12	4.8	1.5
637-14	1.2 b (72)	0.3 b (0.8)	2.0 ab (10.0)	(11)	0.6 b	8	4.7	1.3
637-15	1.1 b (56)	0.4 b (1.1)	2.8 a (18.6)	(11)	0.8 b	16	5.4	1.7
636-7	0.9 b (15)	0.5 b (1.9)	0.9 bc (2.6)	(12)	0.2 b	16	4.7	1.4
636-8	nd	0.2 b (0.3)	0.3 c (0.7)	(6)	0.2 b	24	4.2	1.2
636-13	nd	nd	2.4 ab (19.4)	(5)	0.2 b	16	4.4	1.4
LSD	2.9	1.4	1.5	ns	–	ns	ns	ns

Transformed data [ln(x + 1)] used in statistical analysis, with original means in parentheses

[1] Means followed by the same letter in the same column are not significantly different at $P = 0.05$; nd = not detected; ns = not significant..

[2]Gall index of secondary roots on 0-5 scale after Sasser *et al. (*1984)

9. Control of nematodes through rotation or break crops

9.1 Effect of fallow period on *Pratylenchus* populations (Tasmania).

The 42 plots that constituted the field trial in section 7.2, were sampled on 17 January 2002 and commercial harvest of the field occurred on 21 January 2002. The field was cultivated by deep ripping in early February 2002 and left fallow. Nematode numbers were re-assessed on 6 July 2002 by taking a sample of soil with a trowel to a depth of 20 cm at each of 6 arbitrarily chosen locations in each plot. Nematodes were extracted by Whitehead tray technique over 3 days at room temperature and recovered on a 25 μm sieve. There was little change in the numbers of *Pratylenchus* between 17 January 2002 and 6 July 2002 (Table 30). On average the number of *Pratylenchus*/200 ml in each plot declined by 15.1/200 ml. The maximum decline was 168.8/200 ml in one plot and the maximum increase between the two time periods was 108.9/200 ml (Table 30). This suggested that the weeds present in the field were acting as hosts for *Pratylenchus* and allowed the nematode to maintain its populations between crops. The presence of preferred host weed species in some plots and not others may account for the increase in nematodes in some plots. This highlighted the importance of maintaining a weed-free fallow period if this strategy is to be used for control of nematodes. An example of a weed species that allows the survival of a nematode pathogenic to carrot is given in section 9.6. Mani (1999) studied the survival of *Pratylenchus jordaniensis* in a fallow field after harvest of alfalfa. Pratylenchus survived in dry roots/root residues under field conditions for 100 days and in the fallow field for 380 days. Under laboratory conditions nematodes survived for 270 days in wet soil

Table 30. Change in lesion nematode (*Pratylenchus*) populations (no./200 ml soil) in 42 plots (each 6.7 x 7 m) during a carrot crop (Koyo) and following a fallow period after harvest. (DAP = days after planting).

	Pratylenchus/200 ml soil on:			
	20 November 2001 52 DAP	17 January 2002 110 DAP (harvest)	6 July 2002	Change between harvest and fallow
Mean	119.2	76.0	60.9	-15.1
Standard deviation	73.4	51.3	46.0	56.5
Maximum	354.4	220.0	187.5	n/a
Minimum	10.1	0	8.9	n/a

9.2 Host range of *Pratylenchus penetrans* (Tasmania)

Introduction

Nematode control in vegetable crops is often based on reducing the initial population density to a level that is sufficiently low prior to planting that the nematode populations is unable to build up to an economically damaging density under the subsequent crop. One method of achieving this is to grow green manure plants prior to the crop that are poor or non-hosts of the particular species of nematode. Green manures are often sown in Tasmania to provide ground cover over winter as a means of reducing soil erosion from slopes and adding organic matter to the soil. Similarly a pasture rotation is sometimes used in Tasmania after a cropping sequence. The objective of this trial was to screen a number of forage species for their ability to host *Pratylenchus penetrans*.

Materials and methods

Red ferrosol Kraznosem soil was pasteurised (62^{o}C for 1 hour) and 400 ml placed into white plastic pots (0.5 L). Five seeds of a range of forage species (Table 3) were planted into each pot on 20/7/2001 and maintained in a greenhouse at 21^{o}C day/10^{o}C night. *P. penetrans* were obtained from a field site which had been regularly monitored over 3 years and shown to contain *P. penetrans* only. Nematodes were extracted from soil over 24 hours by Whitehead tray technique (Whitehead and Hemming 1965), concentrated by sieving and counted as described previously (section 7.1). On 7/8/2001, four pots of each plant species were inoculated with an aliquot of a nematode suspension containing (150 *P. penetrans*/pot). One pot was left uninoculated. Plants were between the 1-2 true leaf stage at the stage of inoculation. Plants were harvested on 14/1/2002. Nematodes were extracted from the whole root mass and soil of each pot by Whitehead tray technique over 3 days at room temperature. Nematode counts were subjected to transformation (Log n+1) to normalise data prior to analysis of variance.

Results

No nematodes were recovered from the uninoculated pots, indicating that pasteurising had been successful in killing any plant-parasitic nematodes initially present in the

Table 31. Recovery of *Pratylenchus penetrans* from different host plants at 6 months after inoculation with 150 *P. penetrans*/pot.

Plant (variety)	*P. penetrans* recovered/pot Transformed means[1] and (actual means)	
White clover (Irrigation)	0 (0)	a[2]
White clover (Kopu)	0 (0)	a
Phalaris (Maru)	0 (0)	a
Perennial ryegrass (Impact)	0.320 (4.5)	ab
Chicory (Puna)	0.352 (6.1)	ab
Cocksfoot (Tekapo)	0.732 (14.6)	abc
Perennial ryegrass (Jackaroo)	0.846 (9.6)	abcd
Tetraploid perennial ryegrass (Quartet)	0.905 (12.2)	abcd
Brome (Bareno)	0.913 (37.2)	bcd
Lotus (Maku)	1.006 (16.4)	bcd
Short rotation ryegrass (Flanker)	1.079 (22.0)	bcde
Cocksfoot (Kara)	1.110 (27.8)	bcdef
Serradella (Spectra)	1.130 (27.1)	bcdefg
Red clover (Pac 19)	1.141 (25.1)	bcdefg
Tall fescue (Advance 542)	1.265 (37.6)	cdefg
Short rotation ryegrass (Concord)	1.348 (24.8)	cdefgh
Caucasian clover (Endura)	1.494 (111.0)	cdefghi
Annual ryegrass (Surrey)	1.608 (132.5)	cdefghi
Tall wheat grass (Dundas)	1.701 (62.7)	defghi
Strawberry clover (Onward)	1.974 (115.2)	efghi
Tetraploid perennial ryegrass (Winterstar)	1.982 (122.6)	fghi
Oats (Enterprise)	2.015 (191.8)	fghi
Subterranean clover (Leura)	2.030 (116.2)	ghi
Barley (Dictator)	2.184 (172.7)	hi
Lucerne (Prime)	2.302 (232.4)	i

[1] Data transformed log (x+1) prior to analysis.

[2] Transformed means followed by same letter are not significantly different ($P<0.05$)

soil. Results suggested that some of the white clover varieties tested were non-hosts for *P. penetrans* (Table 31). There were large differences between ryegrass varieties, with Impact, Jackaroo and Quartet having very few *P. penetrans* and Flanker having significantly higher numbers.

Discussion

The multiplication of *P. penetrans* on any of the hosts in this pot trial was poor with fewer nematodes being extracted than originally put into the pot. However, the experiment did illustrate that there were large differences in the ability of these plant species and varieties within plant species to host *P. penetrans*.

9.3 Use of cover crops, organic amendments and nematicides for control of *M. javanica* (South Australia)

Introduction

Four experiments (experiments 1-4) were conducted in the greenhouse to test the effect of cover crops or organic amendments on populations of *M. javanica* and carrot growth. Soils used in experiments were collected from either an organic farm (agrochemicals not used for thirteen years) or a 'conventionally-managed' carrot farm (agrochemicals used, including soil fumigation with metham sodium before planting carrots). Soils were collected after harvest of carrots. Soil was thoroughly mixed and stored in sealed containers at 10°C until use. Both soil types were sandy loams with similar characteristics (Table 32). To establish initial (population) densities of nematodes, five replicate 175 mL sub-samples were extracted for five days on trays (Whitehead and Hemming, 1965) just before starting experiments. The organic farm soil used in experiment 1 (section 9.3.1) contained 108.2±15.2 (SEM) of second-stage juveniles (J2) of *M. javanica*/175 mL of soil, and 7.5±3.5 and 4.6±0.8 of *Hemicycliophora saueri* and *Scutellonema brachyurum* respectively. The conventionally-managed farm soil used in experiments 2 and 3 (sections 9.3.2 and

Table 32. Nutrient contents (NPK) of organic amendments, and of organic and conventionally-managed farm soils used in pot experiments, and soil pH.

Experiment/soil	Nitrogen (mg/kg)		Total N (%)	P (mg/kg)	K (mg/kg)	pH [1]
	NO_3	NH_4				
Experiments 1-2						
JPLF[2]	181	54	2.7	774	20,194	-
Poultry manure	115	6,358	4.0	4,976	16,645	-
Experiments 3-4						
JPLF	nt[3]	nt	2.6	258	26,060	-
Poultry manure	nt	nt	2.4	1,572	19,680	-
Organic farm soil	8	1	0.1	54	260	7.9
Conventional farm soil						
Experiment 3	19	1	0.1	64	193	7.8
Experiment 4	17	1	0.1	74	180	7.8

[1] 1 : 5 soil : 0.01 mol $CaCl_2$/L (w/v)
[2] Johnson's Pure Lucerne Fertiliser
[3] nt = not tested

9.3.3), contained 184.5±20.3 (SEM) and 716.8±35.1 of *M. javanica*/175 mL of soil respectively, and 76.6±15.4 (SEM) and 14.3±2.6 of *H. saueri*/175 mL of soil respectively. Soil was dispensed into 175-mm pots (2.5 L soil/pot). There were five replicate pots per treatment in each experiment arranged in a randomised block design on a greenhouse bench. Greenhouse temperature was maintained at 22±8°C, and pots were fertilised every three weeks with a complete aqueous fertiliser (NPK, 27:5.5:9%) unless otherwise indicated, and watered as required. Before planting, carrot seed was coated with Apron XL350ES (Syngenta; 350 g/L metalaxyl-M) fungicide (2 μl/g seed) to control damping-off caused by pythiaceous fungi. Carrot seed (20/pot) cv. Baby (Arthur Yates), unless otherwise indicated, was planted at a depth of 6 mm. After three weeks, per cent emergence was recorded and seedlings thinned to five/pot. Carrots were grown for 21 weeks after seeding before harvesting, except for experiment 2 (section 9.3.2) where a fifteen-week growing period was used. Fresh weight and dry weight (three days at 70°C) of roots and shoots were recorded. Galling was assessed either by counting the number of galls on secondary roots or by using a 0-5 root gall index for either secondary roots (Sasser *et al.,* 1984) or primary and secondary roots (Huang and Charchar, 1982). Nematodes were extracted from a 175 ml subsample of soil/pot on Whitehead trays as described previously, and from carrot secondary roots from each pot in a mist chamber as described previously. Roots were subsequently dried to determine nematode densities/g dry weight of roots.

9.3.1 Cover crops

Introduction

The effect of fallowing on numbers of *M. javanica* was compared with three cover crops (a *Brassica* sp. 'biofumigant' cover crop cv. Dwarf Essex, and two sudangrass/sorghum hybrids, cvs. Supergraze and Jumbo) followed by soil incorporation as green manures.

Experiment 1 was conducted in the organic farm soil. Forage sorghum (*Sorghum bicolor*) cultivars cv. Jumbo (sorghum × sudangrass hybrid) and cv. Pacific supergraze (sweet sorghum × sweet sorghum hybrid were used in this experiment. Both cultivars are regarded as having high cyanogenic glucoside levels, especially when young.

Rapeseed (*Brassica napus*) cv. Dwarf essex was also used as it is regarded as having high glucosinolate levels. Six seeds/pot were planted, and the stand was thinned to three plants/pot (or 125 plants/m^2) after fourteen days. After weighing shoots, whole plants were coarsely chopped into pieces (25 mm-length) 58 days after seeding and before plants had flowered, and mixed into the potting soil. A 1 g-subsample of roots/pot was retained, examined under a dissecting microscope for presence of galls and *M. javanica* egg masses, and placed in a mist chamber for extraction of nematodes. Six weeks after incorporation of green manures, two cores (each containing 26 mL of soil) were taken from each pot just before planting carrot seed to assess nematode densities using Whitehead tray extraction (Whitehead and Hemming 1965). At this time plant residues had largely decomposed, with only fibrous material remaining. A fallow soil not planted to green manure crops was also included in this experiment as a control, and was kept moist throughout the growing period of the manure crops.

Results

Fresh shoot weight production of green manure crops was 25.9±2.1 (SEM), 18±1.1 and 15.4±0.8 t/ha (LSD=4.9) for cvs. Dwarf essex, Jumbo and Supergraze respectively. Abundant, small galls with large (external) egg masses were observed on roots of cv. Supergraze, small galls with small egg masses (within roots) were less commonly observed on roots of cv. Dwarf Essex, but galling or egg -masses were not observed on a sub-sample of roots of cv. Jumbo. However, *M. javanica* juveniles were detected in roots of all three green manure crops, but densities were significantly higher in cv. Supergraze roots than in cv. Jumbo roots (Table 33). This nematode was detected in green-manured soil at planting of carrots, but not in unmanured, fallow soil, and densities of *M. javanica* J2 in soil were significantly higher after incorporation of cv. Supergraze residues than after the other two cvs. (Table 33). No significant difference in carrot emergence rate between treatments (range 59-69%, SEM=7) was observed. Carrots grown in unmanured, fallow soil or in soil manured with cv. Jumbo residues were significantly less galled, and had lower densities of *M. javanica* J2 in roots compared with the other two green manure cultivars. In addition, *M. javanica* was not detected in soil from the former two treatments (Table 33). The mean density of *S. brachyurum* at carrot harvest was 3.4, 1.5, 4.5 and 4.4 per 175 ml soil (SEM=1.8) for

Dwarf essex, Jumbo, Supergraze treatments and the untreated control respectively, with no significant differences. *H. saueri* was detected (1.5/175 mL of soil) at harvest only in soil previously amended with Supergraze residues.

Table 31. Effects of green manure crops on densities of *Meloidogyne javanica* in both manure and carrot crops, and on carrot growth, number of root galls and per cent forked carrots in an organic farm soil

Green manure crop	*M. javanica**				Carrot		Dry weight (g)	
	J2/g root		J2/soil volume in carrots					
	Manure crop	Carrot	At planting	At harvest	Galls	% fork	Carrot	Shoots
Fallow	–	0.2 b (0.3)	nd	nd	0.1 b	12	1.2 a	0.6
Dwarf essex	4.6 ab[1] (1,361)	7.4 a (2,390)	0.6 b (1.2)	(87)	5.6 a	4	1.3 a	0.6
Jumbo	1.2 b (8)	0.3 b (0.9)	0.3 b (0.6)	nd	0.3 b	8	1.3 a	0.7
Supergraze	8.9 a (30,689)	6.8 a (5,556)	3.7 a (44.6)	(240)	6.0 a	8	0.9 b	0.6
LSD	4.5	1.6	1.1	ns	2.7	ns	0.2	ns

* Transformed data [ln(x + 1)] used in statistical analysis; original means in parentheses; roots of green manure crops sampled just before incorporation into soil; soil sampled from carrots just before planting (26 mL/pot) and at harvest (175 mL/pot).

[1] Means followed by the same letter in the same column are not significantly different at $P = 0.05$; nd = not detected; ns = not significant.

None of the three green manure cultivars tested was resistant to *M. javanica*. Although egg masses or galling were not observed on roots of cv. Jumbo, low densities of *M. javanica* juveniles were detected in roots. All green manures resulted in higher densities of this nematode in soil before planting carrots than simply leaving soil fallow. Green manuring can be expected to provide other benefits to soil such as increased levels of organic matter. The *B. napus* cultivar and the sorghum cv. Jumbo resulted in significantly lower densities of *M. javanica* in soil than cv. Supergraze, and the latter can not be recommended as a green manure crop in soils infested with this nematode as it resulted in reduced carrot weight. Despite their incomplete resistance, the *B. napus* cultivar and sorghum cv. Jumbo, did not result in reduced carrot weight, and carrots grown after the latter green manure crop were no more galled than those grown in fallowed soil. In addition, cv. Jumbo did not result in higher densities of *M. javanica* in the succeeding carrot crop. Therefore soghum cv. Jumbo may have some potential for

use as a green manure especially if it can be grown for a reduced period not allowing significant nematode multiplication. However, when used as a green manure crop, cv. Jumbo does not control *Pratylenchus jordanensis* as it hosts this nematode (Stirling *et al*., 1995). Johnson *et al.* (1992) reported that *M. javanica* did not enter or reproduce on roots of rape including cv. Dwarf essex in the first two crops but that a few females with eggs were found in a third crop. However, the *M. javanica* population used in this experiment did reproduce on a single crop of this cultivar, although only small galls were observed with small egg masses inside of the roots. This suggested possible differences due to nematode population.

9.3.2 The effect of lucerne or poultry manure soil amendments compared with nematicides.

Introduction

Soil amendments as follows were mixed with potting soil fourteen days before carrot seed were planted: Johnson's pure lucerne (*Medicago sativa*) fertiliser®, (Johnson's, Kapunda, South Australia) in the form of 4 mm-diameter pellets and poultry manure (Attunga Garden Products, Dandenong), applied either singly at 20 t/ha or together at 10 t/ha each. Granular nematicides, fenamiphos at 9.6 kg/ha (Yates Nemacur Granular Nematicide, 50 g ai/kg, Yates) and cadusafos at 30 kg/ha (Rugby 100G, 100 g ai/kg, Crop Care) were applied to the soil surface, lightly incorporated and irrigated in, 14 days before planting carrot seed. An untreated control receiving neither nematicide nor soil amendment was also included in the experiment. Soil in all pots was kept moist before planting carrot seed.

Results

Carrot emergence was suppressed by both the organic amendments and by cadusafos (Table 34). However, carrot dry weight in soil with organic amendments (4.2±0.3g) was significantly higher (P<0.01) than for carrots grown in soil without amendments (3.2±0.2g). Soil treatment did not cause significant differences in shoot biomass production or incidence of carrot forking (Table 34). All soil treatments reduced densities of *M. javanica* J2/175 mL of soil and root gall index. All treatments except poultry manure alone reduced densities of *M. javanica* J2/g of root (Table 34). *S.*

brachyurum was detected only in untreated, fenamiphos-treated or lucerne-amended soils at 0.9±0.9, 1.2±0.8, and 0.8±0.5 (SE)/175 mL soil respectively.

Table 34. Effects of lucerne and poultry manure soil amendments, and nematicides on densities of *Meloidogyne javanica*, and on carrot emergence, growth, root galling and per cent forked carrots in an organic farm soil

Soil treatment	Carrots			*M. javanica*[*]		Dry weight (g)	
	% emerged	Gall index	% forked	J2/g root	J2/175 ml soil	Carrot	Shoots
Untreated	85 a	3.4 a	nd	7.9 a[1] (3,103)	7.5 a (2,088)	3.3	1.8
Lucerne	54 b	0.8 b	25	0.8 b (5.0)	0.8 b (3)	4.7	1.6
Poultry manure	44 b	1.2 b	8	7.4 a (5,298)	0.5 b (3)	4.2	1.1
Lucerne + manure	39 b	0.6 b	nd	nd	0.7 b (2)	3.6	1.1
Cadusafos	50 b	0.2 b	16	0.4 b (0.9)	nd	3.4	1.4
Fenamiphos	85 a	1.0 b	4	2.0 b (172.5)	2.2 b (25)	2.9	1.3
LSD	22	–	ns	2.8	1.9	ns	ns

[*] Transformed data [ln(x + 1)] used in statistical analysis; original means in parentheses
Gall index of secondary roots on 0-5 scale after Sasser *et al. (*1984)
[1] Means followed by the same letter in the same column are not significantly different at $P = 0.05$; nd = not detected; ns = not significant.

9.3.3 Comparison of lucerne and gypsum soil amendments.

Introduction

Potting soil from the conventionally managed farm was mixed with fertilisers/soil amendments 65 days before seeding in the following combinations, and additional fertiliser was not subsequently applied: 3 g/pot of complete (NPKS, 8:3.7:10:13.8) fertiliser (equivalent to 100 kg N/ha); 24 g of Johnson's pure lucerne fertiliser (JPLF)/pot (equivalent to 10 t/ha), and 0.1 g urea (46% N, equivalent to 20 kg N/ha); 24 g of JPLF/pot (10 t/ha) and 6.0 g of gypsum (94% Ca $SO_4.2H_2O$), equivalent to 2.5 t/ha, and 0.1 g urea (20 kg N/ha); and 48 g of JPLF/pot (20 t/ha) and 24.1 g of gypsum (equivalent to 10 t/ha) and 0.1 g urea (20 kg N/ha). Potting soil was kept moist during this period.

Results

Carrot emergence and *M. javanica* J2/g of root were not significantly different between soil treatments, but carrot root and shoot biomass production was stimulated by all treatments, significantly higher shoot production was observed with the highest rate of JPLF and gypsum compared with all other treatments (Table 35). Root galling was suppressed by all treatments but carrot forking was not observed. Soil treatments did not reduce densities of *M. javanica* J2/g of root but the highest rate of JPLF and gypsum reduced density of *M. javanica* J2/175 mL of soil (Table 35). Densities of *H. saueri*/175 mL of soil were not significantly different between treated and untreated soils (56.1±37.7 and 16.4±4.3 SE) respectively.

Table 35. Effects of lucerne and gypsum soil amendments, and inorganic fertilisers on densities of *Meloidogyne javanica*, and on carrot emergence, growth, root galling and per cent forked carrots in a conventionally-managed farm soil.

Soil treatment (lucerne/gypsum t/ha)	Carrots % emerged	Gall index[1] A	Gall index[1] B	*M. javanica* J2/g root	J2/175 ml soil	Dry weight (g) Carrot	Dry weight (g) Shoots
Untreated + NPK	72	2.0 a[2]	2.3 a	(47)	3.9 a[3] (55)	0.12 b	0.08 c
(10/0) + N	56	1.2 b	1.6 b	(15)	3.4 a (42)	0.26 a	0.25 b
(10/2.5) + N	62	1.2 b	1.7 b	(18)	3.6 a (39)	0.29 a	0.25 b
(20/10) + N	67	1.2 b	1.6 b	(30)	1.5 b (7)	0.34 a	0.39 a
LSD	ns	–	–	ns	1.4	0.11	0.09

[1] Gall index (0-5) after (A) Sasser *et al.* (1984) and (B) Huang and Charchar (1982)
[2] Means followed by the same letter in the same column are not significantly different at $P = 0.05$; ns = not significant.
[3] Transformed data [ln(x + 1)] used in statistical analysis with original means in parentheses

9.3.4 The effect of lucerne and poultry manure soil amendments

Introduction

Six rates/combinations of poultry manure (2.5-5 t/ha) and lucerne residues (6-15 t/ha) were compared with three control treatments (nitrogenous or complete fertilizer ± fenamiphos (Nemacur®) for control of *M. javanica*. Amendments were mixed with soil before sowing carrots (Yates cv. Baby). There were five replicate pots per treatment combination. Potting soil (conventionally-managed farm soil) was mixed with

fertilisers/soil amendments four weeks before seeding as follows, and additional fertiliser was not subsequently applied: 3 g/pot of complete (NPKS, 8:3.7:10:13.8) fertiliser (equivalent to 100 kg N/ha), and treated with fenamiphos as described above seven days before planting carrot seed; 24 g of JPLF/pot (equivalent to 10 t/ha) and 12 g of poultry manure/pot (equivalent to 5 t/ha); 14.4 g of JPLF/pot (6 t/ha) and 9.6 g of poultry manure/pot (4 t/ha); 24 g of JPLF/pot (10 t/ha) and 6 g of poultry manure/pot (2.5 t/ha); 36 g of JPLF/pot (15 t/ha) and 6 g of poultry manure/pot (2.5 t/ha); 24 g of JPLF/pot (10 t/ha), and 0.52 g urea (46% N)/pot (equivalent to 100 kg N/ha); 36 g of JPLF/pot (15 t/ha), and 0.52 g urea/pot (100 kg N/ha) ; 0.52 g urea /pot (100 kg N/ha); and 3 g/pot of complete (NPKS, 8:3.7:10:13.8) fertiliser (equivalent to 100 kg N/ha). Potting soil was kept moist during this period. Carrots were grown for 22 weeks. At harvest carrots were assessed for galling and other defects, root and shoot weights and nematode levels in soil and on feeder roots were assessed.

Results

Inorganic fertilisers, fenamiphos or organic amendments did not reduce carrot emergence or increase incidence of carrot forking (Table 36). The organic amendments significantly reduced root galling as measured by the index of Huang and Charchar (1982). Four of the six organic amendment-treatments suppressed densities of *M. javanica* J2/g of root compared with the two NPK-treated (untreated and fenamiphos-treated) soils. All of the organic amendment-treatments apart from 6 t/ha JPLF + 4 t/ha poultry manure suppressed densities of *M. javanica* J2/175 mL of soil compared with fenamiphos-treated soil (Table 36). Carrot dry weight was significantly higher (P<0.0001) in soils with organic amendments (0.32±0.03, mean±SE) than in soil without organic amendments (0.07±0.01, mean±SE). Carrot dry weights were significantly and consistently higher when rates of JPLF of 15 t/ha but not lower were used compared with unamended soil (Table 36). Shoot biomass production was stimulated by all organic amendment-treatments (compared with both untreated and fenamiphos-treated soils) and carrot biomass production was stimulated by all organic amendment treatments except the 6 t/ha JPLF + 4 t/ha poultry manure, and the 10 t/ha JPLF + 2.5 t/ha poultry manure treatments (Table 36). Densities of *H. saueri*/175 mL of soil were not significantly different between unamended soils and soils with organic amendments (19.4±7.2 and 16±5.2 SE) respectively.

Table 36. Effects of lucerne and poultry manure soil amendments, fenamiphos and inorganic fertilisers (N, NPK) on densities of *Meloidogyne javanica*, and on carrot emergence and growth, root galling and per cent forked carrots in a conventionally-managed farm soil[1]

Soil treatment (lucerne/poultry manure, t/ha)	Carrots				*M. javanica*		Dry weight (g)	
	%		Gall index[1]		J2/g root	J2/175 ml soil	Carrot	Shoots
	Emerged	Forked	A	B				
Untreated + N	82	6.6	2.0	2.8 a[2]	9.4 ab[3] (17,564)	5.8 ab (423)	0.11 d	0.24 e
Untreated + NPK	84	nd	2.3	2.9 a	10.4 a (39,219)	5.7 abc (318)	0.05 d	0.16 e
Fenamiphos + NPK	77	0.2	1.6	3.2 a	10.9 a (60,723)	6.5 a (698)	0.06 d	0.12 e
JPLF 10 t/ha/ Poultry manure 5 t/ha	74	nd	1.2	0.9 b	8.0 bc (4,052)	3.5 d (53)	0.35 abc	0.69 abc
JPLF 6t/ha/Poultry manure 4 t/ha	81	nd	1.0	1.7 b	8.0 bc (4,460)	4.9 abcd (227)	0.19 cd	0.72 ab
JPLF 10 t/ha/Poultry manure 2.5 t/ha	70	nd	1.2	2.0 b	7.8 c (3,008)	3.8 d (81)	0.21 bcd	0.57 bcd
JPLF 15 t/ha/ Poultry manure 2.5 t/ha	81	nd	1.2	0.9 b	7.8 c (5,759)	4.2 bcd (77)	0.38 ab	0.86 a
JPLF 10 t/ha/Poultry manure 0 t/ha + N	82	4.0	1.0	1.2 b	7.4 c (4,384)	3.6 d (353)	0.31 abc	0.43 d
JPLF 15t/ha/Poultry manure 0t/ha + N	83	6.0	1.2	0.8 b	7.8 c (3,900)	3.9 cd (216)	0.47 a	0.52 cd
LSD	ns	ns	ns	–	1.5	1.8	0.18	0.18

[1] Gall index (0-5) after (A) Sasser *et al.* (1984) and (B) Huang and Charchar (1982)
[2] Means followed by the same letter in the same column are not significantly different at $P = 0.05$; nd = not detected; ns = not significant.
[3] Transformed data [ln(x + 1)] used in statistical analysis; original means in parentheses

9.3.5 Discussion of use of amendments

Organic amendments (Johnson's pure lucerne fertiliser and poultry manure) suppressed carrot emergence when applied fourteen days before planting but not if planting was delayed for at least four weeks. A longer period (65 days) used after application of high rates of JPLF and gypsum (because of the potential for generation of high levels of toxins such as hydrogen sulphide) also did not suppress carrot emergence. However, further research is needed to determine safe waiting periods under varying conditions of soil types, conditions and temperatures. Johnson's pure lucerne fertiliser alone or combined with urea, poultry manure or gypsum consistently increased carrot weight without increase in incidence of carrot forking, and often reduced both carrot galling and *M. javanica* densities. Since lucerne is itself susceptible to this nematode, growing it as

a green manure crop instead of using it as soil amendment could risk increasing the nematode population, although some cultivars have resistance to some *Meloidogyne* populations (Griffin and Gray, 1995). Increasing costs, both economic and environmental, and reduced availability of fumigants and nematicides could, however, make use of JPLF a practical proposition for farmers. Poultry manure alone also reduced galling and *M. javanica* densities in soil (but not roots). These organic amendments had the potential to replace inorganic fertilisers as indicated by these pot experiments, at least partially offsetting their costs. Johnson's pure lucerne fertiliser at or above 15 t/ha appeared to have good potential to stimulate carrot growth and reduce damage from *M. javanica*. However, field testing will be required to confirm the results of these pot experiments, in particular to confirm the absence of increased forking or other adverse effects. Buried crop debris has been implicated as a contributory cause of carrot forking (Rubatzky *et al*., 1999) but pelletised lucerne fertiliser may be less likely to cause problems. Lucerne soil amendments have shown potential to reduce plant disease caused by soil-borne fungi (Asirifi *et al*., 1994; Nam *et al*., 1988; Okamura, 2000). However, lucerne as a green manure was not effective against *P. penetrans* as it hosted this nematode (Abawi and Widmer, 2000).

In the organic farm soil, JPLF and poultry manure provided the equivalent of 270-540 and 400-800 kg N/ha respectively, and 260-520 and 120 kg N/ha respectively in the conventionally-managed farm soil. Very high rates of chicken manure (66 t/ha containing 3.3 t/ha N) have been used to control potato diseases and nematodes (Conn and Lazarovits, 1999), and up to 48 t/ha containing 900 kg N/ha to control *M. incognita* in ginger (Stirling, 1989). Rates of 8t/ha (dry weight) were found to be effective against *Meloidogyne* spp. on tomato (Chindo and Khan, 1990), and 11 t/ha was effective against *P. penetrans* (Abawi and Widmer, 2000). Rates over 20 t/ha may run risks of polluting water by leaching or soil erosion (Sumner *et al*., 2002), although Maynard (1993) showed that chicken manure can be applied for three successive years at rates high enough (112 t/ha) to supply the fertiliser requirement of most vegetables without excessively contaminating groundwater with nitrate.

Fenamiphos effectively controlled *M. javanica* in the organic farm soil with an initial density of 108 J2/175 mL but was ineffective in the conventionally managed farm soil

with a higher initial density of 717 J2/175 mL of soil. Cadusafos was highly effective in controlling this nematode but it suppressed carrot emergence, and would need extended waiting periods between application and planting if it were ever to be used in carrot production.

9.4 Rotation crops to control *Meloidogyne* and *Pratylenchus* spp. (Queensland)

Introduction

Common green manure crops grown in south-east Queensland include Lab Lab purpea and forage sorghum. Lucerne is also commonly grown in rotation to carrots. Vegetable crops grown in rotation with carrots include broccoli, onions, sweet corn and tomatoes. Field crops such as wheat, corn and oats are also grown in rotation to carrots. The time between successive carrot crops in south-east Queensland varied between three and five years, with longer fallows being common. When two carrot crops were grown in succession, soil fumigants such as metham sodium were used to control nematode and fungal diseases.

A pot trial was established to determine the resistance of nine rotation crops to four plant-parasitic nematode species; *M. javanica*, *M. hapla*, *P. thornei* and *P. neglectus*. Rotation crops were planted into 140 mm pots containing 1.5 litres of UC-mix. Each treatment and nematode combination was replicated four times. A 'bare' treatment was also established involving four replicate pots inoculated with each species of nematode, but with nothing planted.

The rotation crops were inoculated two weeks after planting with either 10,000 *M. javanica* or *M. hapla* eggs, or 2,000 *P. thornei* juveniles or 1,500 *P. neglectus* juveniles. Nematodes were extracted from the roots of the plants 6-7 weeks later. Root-knot nematode eggs were extracted by soaking the roots in a 1% NaOC1 solution for 3 minutes (Hussey and Barker 1973). Root-lesion nematodes were extracted by misting the roots in Baermann funnels for 4 days. In the 'bare' treatment, lesion nematodes and root knot nematode J2 were extracted by Whitehead tray technique (Whitehead and Hemming 1965) for 4 days, from a 200 ml subsample of UC mix.

Results

Forage sorghum was the most resistant rotation crop to the two root-knot nematode species (*M. javanica* and *M. hapla*), significantly more resistant than any of the other crops evaluated (Table 37). However, the maize cultivar was as resistant to *M. hapla* as the forage sorghum (Table 37). The mustard and radish varieties were as susceptible as the tomato standard to the two root-knot nematode species (Table 37). Additionally oats was as susceptible as tomatoes to *M. javanica* and broccoli and lucerne was as susceptible as tomato to *M. hapla* (Table 37). All rotation crops investigated were more resistant to the two lesion nematode species (*P. thornei* and *P. neglectus*) than wheat, the susceptible standard (Table 37).

Table 37. Resistance to plant-parasitic nematodes (*Meloidogyne javanica, M. hapla, Pratylenchus thornei* and *P. neglectus*) of rotation crops with potential for carrot production systems in south-east Queensland.

Scientific Name	Common Name	Cultivar	Nematodes in 100g of fresh root							
			M. javanica		***M. hapla***		***P. thornei***		***P. neglectus***	
Lycopersicon esculentum	Tomato	Tiny Tim	680,102	e	80,821	c	477	ab	463	a
Triticum aestivum	Wheat	Baxter	217,509	cde	40	b	24,100	e	13,766	c
Brassica napus	Mustard	BQ mulch	529,664	de	10,909	c	698	b	4,104	b
Sorghum halepense x sudanense	Forage sorghum	Jumbo	239	a	0	a	613	ab	952	a
Brassica oleracea	Broccoli	Shogun	49,020	bc	81,633	c	387	ab	3,677	b
Raphanus sativa	Radish	Weedcheck	226,386	cde	80,016	c	301	a	952	a
Zea mays	Maize	DK689	5,767	b	20	a b	2,100	c	820	a
Medicago sativa	Lucerne	Rippa	76,114	cd	97,733	c	5,114	d	2,778	b
Avena sativa	Oats	Taipan	80,016	cde	111	b	9,508	d	3,010	b

Means with the same subscript are not significantly different from each other (P=0.05). Nematode data presented are back-transformed means (e^x-1), after analysis of variance using transformed data (1n(x+1)).

From the bare treatment, the number of nematodes extracted from the UC mix for *M. javanica*, *M. hapla*, *P. thornei* and *P. neglectus* was 0, 2, 133 and 13/1.5 L UC mix respectively. This indicated higher numbers of lesion nematodes survived relative to root-knot nematode.

No rotation cultivar was resistant to all four nematode species, *M. javanica*, *M. hapla, P. thornei* and *P. neglectus*. It is therefore important to determine which nematodes occur at the site prior to selecting a green manure break crop. Lucerne, and broccoli are commonly used as rotations with carrots. Lucerne was found to be an intermediate host for both *Meloidogyne* spp. and *Pratylenchus* spp. Broccoli was an intermediate host to *Pratylenchus* spp. and *M. javanica* and a good host to *M. hapla*.

Forage sorghum was found to be the most resistant of the crops tested to *M. javanica* and *M. hapla*. Weedcheck was the most resistant to *P. thornei* and *P. neglectus*. However, wheat was the most susceptible rotation crop to the two species of *Pratylenchus* and therefore should not be used as a rotation with carrots.

The poor survival of root-knot nematode in the 'bare' treatment may be due to the eggs being extracted from the egg masses using a bleach solution, which may make the eggs more susceptible to desiccation. However, the results indicate that in a bare fallow in the absence of plants, nematodes that parasitise the roots of carrots are able to survive for 6 to 7 weeks.

9.5 Biofumigation species for control of *Meloidogyne* and *Pratylenchus* spp. (Queensland)

Introduction

A pot trial was used to determine if the incorporation of Brassica spp. leaf material was able to reduce the numbers of plant-parasitic nematodes infecting carrots. River sand (1.5 kg) was weighed and dispensed into plastic bags and then put inside 140-mm diameter pots. The sand was inoculated with *Meloidogyne hapla, M. javanica, Pratylenchus neglectus or P. thornei* at 10,000, 10,000, 2,000, and 2,000 nematodes respectively on 5/2/2003.

Fumus leaf (*Brassica juncea*), Weedcheck leaf (*Raphanus sativa*), Wheat leaf (*Triticum aestivum*) and Wheat chaff were added to river sand mix one day later to each pot at 5% (w/w). Fumus and Weedcheck plants had been growing for eight weeks and wheat plants had been growing for 30 days prior to incorporation. The leaves and petioles from the plants (75 g per pot) were macerated in a Waring Blender with 150 g of water. This mixture was added to plastic bags containing the river sand that had been inoculated with nematodes. The contents within the bag were mixed for one minute to provide an even distribution of organic matter within the soil. Each nematode species and organic amendment combination was replicated five times. Five untreated pots were left uninoculated as untreated controls. Water (150 g) was added to each pot and the contents of the bag mixed for one minute as before.

Following mixing, the soil within the plastic bags was placed inside the pots and allowed to sit for seven days. After the seven days, the contents within plastic bags were emptied back into the pots and left for a further three days. The pots were then planted with 30-day-old carrot plants at 10 plants per pot.

Carrot plants were harvested 65 days after being replanted. The shoots were dried in an oven for one week at 70°C and then weighed. The roots from carrot plants were washed in water to remove the sand mix, blotted dry and weighed. Roots from carrot plants inoculated with *Meloidogyne* spp. were then immersed in 1% NaOCl solution to extract root-knot eggs. The roots from plants inoculated with *Pratylenchus* spp. were placed in a water mister for seven days as previously described.

Results

All four sources of organic matter were effective in reducing *M. hapla* numbers in carrot roots compared to the untreated soil (Table 38). Wheat leaf and wheat chaff incorporated to the sand mix were also able to significantly reduce *M. javanica* numbers in roots compared to the untreated control. However, the addition of the wheat chaff significantly reduced the fresh weight of carrot roots in all nematode treatments (Table 38).

There were significantly more *P. neglectus* in carrot roots in the wheat chaff treatment compared to the untreated control (Table 38). The addition of wheat leaf, Weedcheck and Fumus were not able to significantly reduce *P. neglectus* in roots compared to the untreated control. No treatment was able to significantly reduce populations of *P. thornei* in the roots compared to the untreated control.

Table 38. The efficacy of incorporating Brassica leaf and other organic matter into sand mix to reduce nematode numbers in carrot roots and the effect on shoot weight and root weight.

Nematode species and treatment	Nematodes in 100 g of fresh root		Shoot dry weight per plant (g)		Root fresh weight per plant (g)	
M. hapla						
Fumus	2	a	0.32	cd	3.90	bc
Weedcheck	0	a	0.29	bc	3.21	b
Wheat Leaf	0	a	0.40	d	4.78	c
Wheat Chaff	2	a	0.18	a	0.91	a
Untreated	180	b	0.23	ab	2.71	b
M. javanica						
Fumus	1211	d	0.34	n.s.	3.96	bc
Weedcheck	53	bc	0.29	n.s.	2.99	b
Wheat Leaf	5	ab	0.37	n.s.	4.67	c
Wheat Chaff	0	a	0.25	n.s.	1.51	a
Untreated	811	cd	0.25	n.s.	3.55	bc
P. neglectus						
Fumus	0	a	0.29	bc	2.75	bc
Weedcheck	0	a	0.30	bc	1.98	ab
Wheat Leaf	0	a	0.36	c	3.58	c
Wheat Chaff	10	b	0.17	a	1.10	a
Untreated	1	a	0.22	ab	2.55	bc
P. thornei						
Fumus	6	n.s.	0.33	c	4.36	c
Weedcheck	2	n.s.	0.25	b	2.92	b
Wheat Leaf	1	n.s.	0.40	c	4.62	c
Wheat Chaff	6	n.s.	0.14	a	1.05	a
Untreated	11	n.s.	0.22	b	2.72	b

Means with the same subscript are not significantly different from each other (P=0.05). Nematode data presented are back-transformed means (e^x-1), after analysis of variance using transformed data (1n(x+1)).

Adding wheat chaff to the soil caused a reduction in the root weight of carrots compared to the untreated control. The addition of wheat chaff also significantly reduced the shoot weight in all treatments, except when the pots were inoculated with *M. javanica*. The addition of wheat leaf tended to increase the weight of carrot roots and shoots relative to the untreated control for all nematodes (Table 38). Similarly, the addition of fumus

leaf tended to increase root and shoot weights, although the effect was not as consistent as the addition of wheat leaf (Table 38).

Fumus had a high level of glucosinilate (56.8 µmole of 2-propenyl glucosinolate per gram of fumus leaf), while Weedcheck had a low level of glucosinolate (0.7 µmole of 4-methyl-sulphur-butyl glucosinolate and 5.5 µmole 4-methyl-thiobutyl glucosinolate per gram of leaf). There was no glucosinolate detected in the leaves of wheat.

No form of organic matter was consistently able to control both *Meloidogyne* spp. and *Pratylenchus* spp. Wheat chaff was more effective than Brassica leaves at controlling *M. javanica*. However, the addition of wheat chaff significantly reduced the root weight of carrot plants relative to the untreated control. The reduction in root weight may be due to an immobilisation of nutrients or an increase in fungal pathogens, due to the high carbon status of the chaff. The addition of carbon may have been responsible for suppressing root-knot nematode numbers due to a change in the biology of the soil allowing an increase in nematode antagonists.

There was no consistent reduction in nematode populations using Brassica species for biofumigation. Although, the Brassica cultivars, Weedcheck and Fumus, were effective in reducing the number of *M. hapla* recovered from carrot roots, they were no better than wheat leaf or wheat chaff. There were high levels of 2-propenyl glucosinolate per gram of leaf in the Fumus tissue but this was not able to give effective nematode control when carrots were planted. There were very low levels of glucosinolates in the leaves of the Weedcheck, which may explain the poor level of control following its incorporation into the soil

Improved carrot shoot growth resulted when wheat leaf was incorporated in the pots inoculated with *M. hapla*, *P. neglectus* and *P. thornei*. Wheat leaf also improved root growth when incorporated with soil in pots inoculated with *M. hapla*, and *P. thornei*. The improved shoot and root growth following incorporation of wheat leaf appeared to be independent of nematode control. Therefore, improved plant growth could be attributed to improved plant nutrition following the incorporation of the wheat leaf.

9.6 Pigweed (*Portulaca oleracea* L.) as a host of *Meloidogyne javanica* in carrot in South Australia.

Pigweed (*Portulaca oleracea*), also known as Purslane, is an Australian native plant (Jackson and Jacobs 1985), which has been used in the past as a "bush" food. However, this species is also regarded as a cosmopolitan weed. A heavy infestation of Pigweed occurred in a summer-fallowed bed on an organic vegetable farm at Purnong in the Murray Lands region of South Australia, under moist soil conditions arising from the drift of sprinkler irrigation from adjacent beds under vegetable production. Root systems of this weed were examined and small galls and egg masses were observed on fine roots associated with the presence of adult female root knot nematodes (*Meloidogyne* sp.). Perineal patterns were used to identify the species as *M. javanica* (Taylor and Sasser 1978) and a sample of 50 specimens was deposited in the Waite Institute Nematode Collection (accession number 1181). The previous spring, heavily galled carrot (*Daucus carota*) roots were dug from the same bed and female nematodes dissected from galls were also identified as *M. javanica* (accession number 1153c). Two samples consisting of 20 to 50 females dissected from galled carrot and *P. oleracea* roots were identified as *M. javanica* using a multiplexed mtDNA PCR (polymerase chain reaction)-based diagnostic test (Stanton *et al.* 1997).

This is a first Australian record for *M. javanica*, or any *Meloidogyne* spp., on *P. oleracea* (McLeod *et al.* 1994), and indicates that this weed should be regarded as an alternative host in the development of management systems for *Meloidogyne* spp. in vegetable production. Many common broad-leaved weeds are able to host these nematodes, maintaining levels of *Meloidogyne* spp. in fallow ground. On conventional farms, *P. oleracea* is commonly controlled using herbicides, but on organic farms it is difficult to control using only cultivation. This work has been published elsewhere (Walker *et al*. 2002). *P. oleracea* was recorded as a host of *M. arenaria*, *M. incognita* (Tedford and Fortnum 1988), and *M. hapla* (Belair and Benoit 1996) in North America. This plant exists in several different forms (Jackson and Jacobs 1985) but it is not known if these forms vary in their reactions to different *Meloidogyne* spp.

10. Chemical control

Nematicides have been one of the most widely used methods of controlling nematodes in carrot production. Their advantages are that they are a 'quick' and relatively 'easy' approach to nematode control, and can often be cost-effective. Disadvantages of nematicides include cost, need for specialised equipment, generally high toxicity, impact on the environment and development of enhanced biodegredation in which continual use leads to the build up of soil microflora able to rapidly break the chemical down to harmless constituents.

Abawi *et al*. (2001a) reported that in one trial, the percentage of unmarketable carrots was 43.3%, 18.8%, 0.3% and 0.5% in the untreated, Vydate applied as in furrow drench, Vydate broadcast and Vydate drench + broadcast treatments respectively. The cost of the broadcast treatment (US $110/ac.) led to an increased profit of (US $1434/acre). However, Vydate was not cost-effective in fields with low infestations of *M. hapla* (Abawi *et al*. 2001a).

Becker *et al*. (1997) indicated that soil fumigation was often used in California to reduce root knot nematode populations to maintain profitable levels of carrot production. Telone II (1,3 D) is often used. However the total amount that can be applied in California is legally limited, as is the amount per township, which can lead to shortages (Becker *et al*. 1997). The fumigant Methyl bromide is being phased out under the Montreal Protocol due to its effects on ozone depletion. Methyl iodide has been trialled as a replacement to Methyl bromide. Methyl iodide decomposes more quickly in the atmosphere and is unlikely to cause ozone depletion. Hutchinson *et al*. (1999) reported on trials to control *Meloidogyne incognita* in carrot production in California. Methyl iodide at various rates (112-336 kg/ha) and methyl bromide (112 and 224 kg/ha) were applied to tarped beds by hot-gas fumigation and compared with a non-treated control, metam sodium (373 L/ha) applied through overhead irrigation and 1-3 dichloropropene (112 L/ha) commercially shank applied. Methyl bromide, methyl iodide and 1,3-D effectively reduced *M. incognita* populations over the season at all rates tested. Plots fumigated with methyl bromide or methyl iodide produced 161% and 181% more marketable carrots without nematode damage than untreated plots respectively.

Hutchinson *et al*. (1999) suggested that methyl iodide was therefore an effective alternative to methyl bromide for nematode control in carrot production.

Anon (1998) reported that fenamiphos (Nemacur) at 400g/L applied at 20 L/ha to nematode infested plots gave a 12% higher marketable yield of fresh carrots than untreated plots.

Vrain *et al*. (1981) compared several fumigant and non-fumigant nematicides for control of *M. hapla* on carrot in Quebec. These included non-fumigant organophosphates (isazophos, fosthietan and fenamiphos) and carbamates (carbofuran, oxamyl, aldicarb, Bunema M) and fumigants Telone II and D,D (1,3-dichloropropene), Telone C17 (1,3-dichloropropene + chloropicrin) and Volex (1,3-dichloropropene + methylisothiocyanate). The percentage of marketable roots (by number) was 25.6% for untreated, 70.5-83.2% for isazophos, 74.8-74.9% for fenamiphos, 76.1-78.2% for carbofuran, 84.5% for oxamyl, 85.5-86.9% for fosthietan, 74.4-84.2% for aldicarb, 58.2-71.7% for Vorlex, 75.5-78.5% for Telone II and 73.0-83.0% for DD.

Belair and Fournier (1997) reported on control of *M. hapla* in carrot in an organic soil with plant bed treatment with 1,3-D. Soil was treated with 1,3-D at 56 and 112 L/ha, mixed with a rototiller in a 15cm band over the row, or injected 20 cm deep with a single shank behind the rototiller at 56 L/ha. The latter treatment gave the lowest galling indices and highest yield of marketable carrots (66.7 t/ha) in comparison to the untreated control (5 t/ha). Rototiller incorporation was less effective than shank injection, even at twice the rate (Belair and Fournier 1997). In a second trial, 1,3-D was injected at 40 L/ha through a single shank at 20 cm deep. Treatment reduced galling and increased the number of marketable carrots (68.7 t/ha) in comparison to the untreated (11.8 t/ha). It was concluded that plant bed treatment with 1,3-D was an effective alternative to broadcast treatment for control of root knot nematode in carrot production on an organic soil (Belair and Fournier 1997).

Few studies have been conducted to demonstrate control of *Pratylenchus* on carrot with nematicides. In Israel, Orion *et al*. (1988) applied fenamiphos (Nemacur) 40% EC formulation at 15 and 30 l/ha or Nemacur granular formulation at 60 and 120 kg/ha at

seeding time. Nematicide application resulted in between 50-65% control of *Pratylenchus mediterraneus* populations and an increase of between 38-45% in marketable carrot yield compared to the untreated control.

Several trials were conducted to examine the efficacy of existing and newly registered nematicides for control of nematodes in carrot production in Australia.

10.1 Comparison of nematicides and fallow treatments for control of *M. javanica* (Queensland)

A pot trial was established to determine if fallow treatments had the same efficacy at controlling plant-parasitic nematodes as pre-plant fumigation or post-plant non-volatile nematicide treatments. Tomatoes (*Lycopersicon esculentum* cv. Tiny Tim) were planted on 15/2/2002 in 1 kg of river sand and watered as needed. Plants were inoculated with 1,000 *M. javanica* eggs, 7 days after planting. The tomato tops were removed 42 days later leaving the root system intact in the soil. Pots were then replanted with a fallow crop or replanted with tomatoes. Pots receiving a fallow treatment were planted with either Weedcheck (*Raphanus sativus* cv. Weedcheck) or Sorghum (*Sorghum halepense* x *sudanense* cv. Jumbo). The fallow crops were allowed to grow for 9 weeks before being harvested. At the end of the fallow period the fallow crops and tomatoes were harvested and the tops cut finely. Plant tops (50 g) per kg of soil (5% w/w) were incorporated into the sand within the pots.

The metham sodium (468 g methyl isothiocyanate per 1 kg) treatment was applied at 25 µL of product per pot (equivalent to 10 µg active ingredient per g of soil) 7 days before replanting carrots. The metham sodium was mixed with water and applied using a pipette 2 cm below the soil surface.

Three-week old carrot seedlings were transplanted into the soil 14 days after harvesting the fallow crop. Four carrot plants were replanted into each pot. Seven days after replanting the carrots, the nematicides Nemacur 400® and Rugby 200 CS® were applied to the pots to achieve a rate of 10 µg active ingredient per g of soil.

Carrots were harvested 70 days after transplanting carrot seedlings. Juvenile *M. javanica* were extracted from the roots by placing in a misting cabinet for seven days as previously described. Each treatment was replicated six times.

Results

Nematodes were not detected in the roots of carrots grown in the Nemacur 400® and the metham sodium treatments (Table 39). Although, low numbers of nematodes were extracted from the roots of carrots grown after sorghum, it was found to be equally as effective in reducing nematode numbers as nematicides (Table 39). Rugby 200 CS® was not able to significantly reduce the number of nematodes in the roots of carrots relative to the untreated control, but was not significantly worse than Nemacur 400® or metham sodium at controlling nematode numbers (Table 39).

Weedcheck did not suppress root-knot nematode numbers in the roots of the carrot plants relative to the untreated control (Table 39). Weedcheck was not as efficacious as Nemacur 400®, Rugby 200 CS® or sorghum. This was probably due to Weedcheck being a moderate host to root-knot nematode and the biofumigation effect from incorporating the leaves not being sufficient to significantly reduce nematode numbers.

Table 39. Root-knot nematodes in the roots of carrots grown in pots that were fallowed with Weedcheck or Sorghum, or treated with nematicides.

Treatment	Rate	Nematodes per 100g of root	
Untreated	Nil	630	bc
Nemacur 400®	10 µg a.i./g soil	0.0	a
Rugby 200 CS®	10 µg a.i./g soil	180	abc
Metham sodium	10 µg a.i./g soil	0.0	a
Sorghum	50 g / g soil	80	ab
Weedcheck	50 g / g soil	1180	c

Means with the same subscript are not significantly different from each other (P=0.05). Nematode data presented are back-transformed means (e^x-1), after analysis of variance using transformed data (1n(x+1)).

10.2 Efficacy of non-fumigant nematicides for control of *M. javanica* (Queensland)

Introduction

A pot trial was established to determine the efficacy of non-fumigant nematicides for control of root-knot nematodes on carrots. Carrot seeds (*Daucus carota* cv. All Seasons) were planted into 1.5 kg of coarse sand in 150 mm diameter pots on 12/11/2001. The number of carrots in each pot was thinned to five plants per pot and inoculated with 5,000 eggs of *M. javanica* 14 days after planting.

Five different formulations of nematicide were applied to the soil surface (Table 40) of each pot to achieve a concentration of 10 μg of active ingredient per g of soil, seven days after nematode inoculation (3/12/2001). All of the nematicides except Rugby 100 G® were liquid formulation and were applied using a micropipettor, as an undiluted application. The Rugby 100 G® granules were weighed into a glass vial and sprinkled onto the soil surface. Nematicides were watered into the pots with 20 mL of water following application.

Carrots were fertilised with 3 g of Osmocote mini (16:8:11) and watered daily. All plants were kept in a glasshouse at temperature between 25 – 31 ^{o}C. Plants were harvested 9 weeks after nematicide application (30/1/2002), by washing the roots free of any adhering sand and cutting the tops off of the plants. The tops were dried in an oven at 70 ^{o}C for five days before being weighed. Nematodes were extracted from carrot roots by placing in a misting cabinet for seven days as described previously.

Results

Three nematicides, Nemacur 400®, Rugby 200L® and Vydate 240L®, significantly reduced the number of nematodes in the roots of carrots relative to the untreated plants (Table 41). Nemacur 400® was significantly better at reducing nematode numbers in the roots of carrots than all other nematicide treatments and had the highest shoot weight per plant (Table 41). There was no difference amongst treatments in the fresh weight of roots of carrots.

Table 40. Characteristics of nematicides evaluated for control of root-knot nematode (*M. javanica*) in carrots.

Treatment	Formulation	Active ingredient	Quantity of product per pot to achieve 10 μg ai per g of soil
Untreated	Nil	Nil	Nil
Nemacur 400®	Liquid	400 g / L	37.5 μL
Nemacur 240 GS®	Liquid	240 g / L	62.5 μL
Rugby 100 G®	Granule	100 g / kg	150 mg
Rugby 200 L®	Liquid	200 g / L	75 μL
Vydate 240 L®	Liquid	240 g / L	62.5 μL

Table 41. Efficacy of nematicides to reduce root-knot nematode numbers and effects on shoot dry weight and root fresh weight of carrots.

Treatment	Nematodes per g of root (ln(x+1))		Shoot dry weight per plant (g)		Root fresh weight (g)	
Untreated	1407	d	0.90	ab	3.74	n.s.
Nemacur 400®	4	a	1.50	c	3.29	n.s.
Nemacur 240 GS®	347	cd	0.98	ab	3.39	n.s.
Rugby 100 G®	420	cd	0.79	a	3.74	n.s.
Rugby 200 L®	41	b	1.10	b	3.43	n.s.
Vydate 240 L®	185	bc	1.05	ab	4.03	n.s.

Means with the same subscript are not significantly different from each other (P<0.05). Data are presented are back-transformed (exp(x)-1) after analysis of variance using the transformation (1n(x+1)).

Rugby 200 L® was able to reduce nematodes and caused a slight increase in the shoot weight relative to the untreated plants.

There was no significant difference in the nematode numbers recovered and the shoot dry weight between Vydate 240 L® and Rugby 200 L®. However, the efficacy was slightly less than Rugby 200 L® in this trial. Nemacur 240® and Rugby 100 G® were not able to significantly reduce nematode numbers or improve shoot dry weight of carrots relative to the untreated control (Table 41). The efficacy of these chemicals may have been hampered by poor redistribution after application.

General discussion

Cadusafos would be worthy of further investigation for nematode control in carrots as an alternative to fenamiphos, which is currently a registered treatment throughout Australia. In this study, cadusafos was applied to developed carrot seedlings and no adverse effects of the chemical on seedling growth were observed. However it should be noted that in other trials, the active ingredient cadusafos has been associated with reduced emergence of carrot when applied as a granular formulation at or before seeding (section 9.3.2). If this nematicide were to be developed for carrot production, a suitable pre-plant withholding period would need to be determined. In addition, cadusafos as Rugby 200 L® gave better control of root-knot nematode than the granular formulation (Rugby 100G®) possibly through more even chemical distribution within the soil (section 10.2). Nemacur 400® was significantly better than other nematicides tested in section 10.2 for reducing *M. javanica* numbers and increasing shoot dry weight.

The use of sorghum as a rotation crop was as effective at reducing nematodes numbers as the nematicides Nemacur 400® and metham sodium. A sorghum rotation would help to reduce chemical costs where root-knot nematodes were expected to be a problem. Weedcheck would not be recommended as rotation treatment with carrots where root-knot nematode was a problem, due to its ability to host the nematodes.

In an integrated nematode management system for carrots, nematicides should only be applied if necessary, as a post plant operation. It is important to rotate nematicides to slow the onset of enhanced biodegradation and reduce the use of broad-spectrum biocides.

10.3 Effects of pre-planting fumigation on carrot yields and quality and nematode population dynamics (South Australia)

Introduction

A fumigation experiment was set up in a commercial carrot field near Nuriootpa, Barossa Valley. Soil was fumigated on 9/11/2001, 3 weeks before carrot seed cv. Stefano was planted. Two different fumigants were compared, metham sodium applied

by Australian Fumigation P/L by soil injection using a Rumpstad® rig (at rates of either 300 or 525 L/ha) or with the grower's self-constucted rig (at 300 L/ha), and Telone C35® using a Rumpstad® rig (at 50 g/m^2). An area was left unfumigated to act as a control. The grower treatment (metham sodium at 300 L/ha) was found the previous season to give incomplete control of *Meloidogyne javanica* and other nematodes, but the grower preferred to apply relatively low rates of fumigant in the belief that this reduces deleterious effects on beneficial soil organisms, and to accept a certain amount of nematode damage. This field experiment was set up with the aim of comparing the efficacy of such lower rates of metham sodium with a higher rate more normally recommended, and to compare this fumigant with a newly registered fumigant, Telone C35®. After treatment, soil was compacted by a roller on the fumigant rig and afterwards irrigation by overhead sprinklers was applied to seal in the fumigant. The fumigants were applied (on 9/11/2001) 3 weeks before seeding.

To compare extraction techniques for *Meloidogyne* spp., soil samples were taken 3 days before fumigation and again 6 days before seeding. Samples were mixed evenly and divided into aliquots for extraction using a DNA-based method, a baiting method, a tray extraction method and a decanting/sieving method (as outlined in the footnote to table 31). The DNA-based test for *Meloidogyne* spp. was conducted by SARDI Root Disease Testing Service using 400 g of the same soil sample as used for baiting, decanting/sieving and tray methods. The tomato bioassay was conducted similar to the methods of Hutchinson *et al*. (1999). A tomato seedling (cv. Grosse Lisse) was transplanted into 400 g of the test soil and grown in a greenhouse (minimum 22°C) for 6 weeks. Roots were gently washed and then placed in 1 L beakers containing about 300 mL of 0.015% phloxine B solution for 15 min. Numbers of egg masses and/or adult female *Meloidogyne* sp. were then counted.

Soil samples were taken repeatedly during the growing season (from central sub-plots) in 7 replicate plots of each treatment to monitor nematode population dynamics. Nematodes were extracted from soil using Whitehead tray technique (Whitehead and Hemming 1965). At harvest, nematodes were also extracted from feeder roots by mist incubation.

Carrot emergence (per m of double-row) and number of expanded leaves were determined 8 weeks after seeding. At harvest, carrot yields were determined from central sub-plots consisting of 1 m lengths of a double-row. Mean carrot weight and incidence of carrot defects were also assessed. All data were analysed using Analysis of Variance (P<0.05) and the associations between nematode densities and carrot yields/weight and incidence of defects were investigated using Stepwise linear regression (P<0.05).

Results

Telone C35 fumigation markedly increased mean carrot size, especially crown diameter and weight (Table 42). This resulted in much higher total yields, but also a higher proportion of defective (mainly hairy and forked) carrots (Table 42). The latter may have been associated with the heavy growth stimulation and consequent excessive vigour and size of carrots in Telone C35-treated ground. An earlier harvest date may possibly have reduced hairy root incidence and increased marketable yields. Metham sodium also stimulated carrot growth, especially carrot length, and yields (Table 42). However, a lower rate than normally recommended was more effective in this regard, especially when applied by a professional contractor using a Rumpstadt injector, and gave the highest overall marketable yield.

Although no visual evidence of phytotoxicity was observed, the number of expanded leaves on plants grown in soil fumigated with the higher rate of metham was significantly below that of other treatments at 8 weeks after planting (Table 42). This indicated despite the November fumigation and planting dates when soil temperatures would generally be expected to be warm that the 3-week wait between fumigation and planting was too short and that residual chemical was adversely affecting carrot growth.

Soil fumigation had no significant effect on plant stand (Table 42), suggesting that damping-off was not a major factor at this site. Nonetheless, plant stand (at harvest) was lowest in unfumigated ground. Soft rot was more common in carrots in untreated soil (Table 42). Although the incidence of soft rot was relatively low at harvest, it may have been associated with reduced carrot size.

After fumigation, densities of *Meloidogyne* spp. were very low (Table 42) and detection was difficult. The DNA method also failed to detect the nematode at that time. Detection of low densities was most reliable using the baiting method, however, this method was laborious and time consuming. Baiting detected the nematode both before fumigation and, in the case of metham applied at 300 L/ha by the grower, after fumigation (Table 42). In all cases, low levels (1-2 egg masses/females per tomato root system) were detected. Initially the DNA method failed to detect the nematode in any of the preplanting soil samples. However, the test was repeated with increased sensitivity and the nematode was then detected in a single sample taken before fumigation (Table 42). The (improved) DNA method was therefore more reliable than tray and decanting/sieving methods for detecting low densities of nematodes. In the single sample taken before fumigation in which the nematode was detected by this method, the density was estimated at 8 *Meloidogyne* spp./400 g of soil.

Following fumigation, galled roots and *Meloidogyne* spp. were detected only in the treatments with a low rate (300 L/ha) of metham indicating that this rate was insufficient to control this nematode (Table 42). Despite this, the nematode density was insufficient to cause rejection of significant numbers of carrots due to galling. *Meloidogyne* spp. were not detected in soil fumigated with Telone C35® or with the high rate of metham.

Densities of *Paratrichodorus* sp. reached high and potentially damaging levels in some plots (Table 42). Although soil densities of *Pratylenchus* sp. remained low throughout the growing season, high levels were observed in feeder roots, especially in soil treated with lower rates of metham.

Table 42. Effect of pre-plant fumigation treatments on carrot yield and quality and nematode populations.

Yield parameters	Soil fumigant injected (applicator, rate)					LSD (*P*=0.05)
	Telone C35 (P)[1] 520 kg/ha	Metham sodium (P)[1] 525 l/ha	Metham sodium (P)[1] 300 l/ha	Metham sodium (G)[1] 300 l/ha	Untreated	
Carrot emergence and growth (8 wks)						
Plants/m double-row	37.6	34.4	32.7	32.1	31.1	n.s.
Number of expanded leaves	4.0 a	3.3 b	4.1 a	4.1 a	4.2 a	0.6
Carrot size						
Carrot weight (g)	148 a	110 b	138 a	121 b	58 c	16
Crown diameter (mm)	37 a	31 b	32 b	32 b	25 c	3
Length (mm)	177 ab	166 b	184 a	183 a	134 c	15
Total yield						
t /ha	75.7 a	60.4 bc	67.6 b	58.3 c	24.4 d	8
Number/ha	520,408	546,938	506,122	489,795	487,755	ns
Marketable yield						
t /ha (by subtraction)	51.1	48.1	55.0	46.4	21.2	-
Carrot defects (%)						
Forked	9.2	2.0	1.6	5.0	0.4	
Constricted	3.3	2.3	3.9	2.8	3.6	
Bent	3.2	5.5	3.1	3.8	1.5	
Split	3.1	4.3	2.4	5.1	3.1	
Hairy	13.6	6.4	7.6	3.9	2.3	
Soft rot	0	0	0	0	2.3	
Total	32.5 a	20.4 b	18.7 b	20.5 b	13.1 b	8.8
Nematode densities						
Preplanting						
Meloidogyne						
Prefumigation (3d)						
a) DNA test[2]	0%	0%	0%	14%	0%	-
b) Baiting[3]	0%	28%	0%	14%	0%	-
c) Tray	0%	0%	0%	0%	14%	-
d) Decant/sieving	0%	0%	0%	0%	0%	-
Postfumigation (6 d before planting)						
a) DNA test[2]	0%	0%	0%	0%	0%	-
b) Baiting[3]	0%	0%	0%	14%	0%	-
c) Tray (200 g)	0%	0%	0%	0%	0%	-
d) Decant/sieving (200 g)	0%	0%	0%	0%	0%	-
***Paratrichodorus* sp./150 mL of soil**						
a) prefumigation	3.9	nd	3.3	1.0	1.3	
b) preplanting	nd	nd	nd	nd	0.6	
c) 6 wk postplanting	nd	0.6	5.9	2.0	72.6	
d) 16 wk	nd	nd	1.8	0.6	28.8	
e) 22 wk	nd	0.5	0.6	1.0	18.1	
f) 27 wk	0.3	0.6	2.6	3.3	23.4	
g) 29 wk	nd	nd	0.7	0.7	4.9	
Range	0-11	0-5	0-9	0-12	0-250	
LSD (*P*=0.05)						15.0
Pratylenchus						

Yield parameters	Soil fumigant injected (applicator, rate)					LSD (*P*=0.05)
	Telone C35 (P)[1] 520 kg/ha	Metham sodium			Untreated	
		(P)[1] 525 l/ha	(P)[1] 300 l/ha	(G)[1] 300 l/ha		
sp./150 mL of soil						
a) prefumigation	1.1	nd	1.2	0.5	0.4	
b) preplanting	nd	nd	nd	nd	0.3	
c) 6 wk postplanting	nd	0.2	1.8	0.7	3.9	
d) 16 wk	nd	nd	0.6	0.2	2.9	
e) 22 wk	nd	0.2	0.2	0.5	2.8	
f) 27 wk	0.2	0.2	1.nd	1.1	3.0	
g) 29 wk	nd	nd	0.3	0.3	1.2	
Range	0-18	0-4	0-9	0-4	0-2	
LSD (*P*=0.05)						0.7
***Pratylenchus* sp./g DW root**						
a) 29 wk	15.8	12.9	66.3	126.7	13.3	n.s.
Range	0-69	0-72	0-440	0-720	0-49	
***Meloidogyne* sp./150 mL of soil**						
a) prefumigation	nd	nd	nd	nd	0.2	
b) preplanting	nd	nd	nd	nd	nd	
c) 6 wk postplanting	nd	nd	nd	nd	nd	
d) 16 wk	nd	nd	nd	nd	nd	
e) 22 wk	nd	nd	nd	nd	nd	
f) 27 wk	nd	nd	2.9	2.9	nd	
g) 29 wk	nd	nd	0.7	1.9	nd	
Range	0	0	0-12	0-14	0-2	
LSD (*P*=0.05)						n.s.
***Meloidogyne* sp./g DW root**						
a) 29 wk	nd	nd	8.0	25.7	nd	n.s.
Range	0	0	0-56	0-144	0	

[1] P = applied by professional contractor using Rumpstadt injector; G = applied by grower using own injector

[2]DNA-based test results are given as proportion of positive detections (*n*=7).

[3]Tomato bioassay results are given as proportion of positive detections (*n*=7).

Within-row means followed by the same letter are not significantly different (*P*=0.05); n.s. = not significantly different; nd = not detected.

10.4 Efficacy of nematicides in carrot production (Western Australia)

A component of the approach to developing control measures for nematodes in carrots is, at least in in the short term, to identify alternative chemical control measures. In Western Australia, the failure in some situations of metham sodium and fenamiphos (Nemacur) to provide reliable nematode control has provided impetus for this activity. There are few nematicides available in Australia, which can be considered potentially suitable for use on carrots.

10.4.1 Efficacy of carbofuran for control of nematodes in carrots

The carbamate pesticide carbofuran (Furadan 100G, Crop Care Aust.) is registered in Australia for pest control in rice and tobacco and nematode control in sugar cane. The National Registration Authority via the Crop Protection Approvals process indicated a permit might be possible for preplant use of carbofuran on carrots.

An experiment to test the efficacy of carbofuran was carried out at Medina Research Station. The site on Karrakatta sand had a history of *Meloidogyne* spp.infection in carrots. Treatments included an untreated control, Furadan 100G at 50 kg/ha, Furadan 100G at 100 kg/ha and Nemacur at 24 L/ha. A randomised block design with four replicates was used. Treatments were applied and incorporated to 15 cm depth with a rotary hoe immediately prior to sowing the carrots (cv Stefano) with a precision airseeder on 24/12/2001. Standard fertiliser and herbicide programs were applied.

Six weeks after sowing, seedlings were sampled from 2 x 1m lengths of row from each plot. Lateral and taproot infection was assessed by checking the presence of egg masses using the trypan blue staining method (Sharma and Mohiuddin 1993). Results are shown (Table 43). At this stage of the crop the trend was for Furadan to reduce nematode infection compared to both the control and Nemacur. The final harvest of the site was on 9/5/2002. Carrots were washed and graded from 4 x 1m lengths of row (1.5 m^2) from each plot (Table 44). Nematicide had no statistically significant effect on yield or anomalies at this site. A high degree of variability among plots was noted at harvest and this may be related to the variable distribution of nematodes on the site.

Table 43. The incidence of *Meloidogyne* infection on carrot seedlings.

Treatment	Taproot (%)	Taproot and/or lateral root (%)
Control	39.8	72.2
Furadan 50 kg/ha	5.3	28.6
Furadan 100 kg/ha	6.9	31.3
Nemacur 24 L/ha	16.9	65.4
Probability	*P=0.112*	*P=0.056*

Table 44. The effect of nematode control treatments of the yield and grade of carrots.

Treatment	Plant density (/m^2)	Total yield (t/ha)	Marketable (Export + short) (%)	Misshapen (%)	Forked/ stumped (%)	Bulged eyes (%)
Control	64	50.6	22.4	22.0	8.0	39.4
Furadan 50 kg/ha	55	46.7	30.8	17.2	7.2	39.3
Furadan 100 kg/ha	59	48.0	40.6	11.6	5.1	38.9
Nemacur 24 L/ha	65	54.4	12.7	15.5	5.1	60.9
Probability	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*

The trend was for Furadan to improve marketable percentage by decreasing the proportion of missshapen and forked or stumped roots (Table 44). There was a trend for Nemacur to increase the proportion of roots with bulged eyes compared to the control. More work would be needed to determine whether Furadan would make a useful option for nematode control in carrots.

10.4.2 Efficacy of Telone and Telone C35 for control of nematodes in carrot.

In December 2001, both Telone and the Telone/chloropicrin mixture Telone C35 were registered in Australia for nematode control on vegetable cropping land. In Western Australia, Telone C35 became available before Telone and commercial scale trials commenced in late 2001 with Telone C35. Both products require injection into the soil using a tyned injection rig. Telone is of greater interest as a nematicide for carrots as it is less than half the cost per hectare of Telone C35.

Myalup site

On a commercial vegetable farm near Myalup area south of Perth, Telone C35 was applied to an area with a history of nematode problems. A control area was left untreated within the block and both treated and untreated areas have been monitored for nematode infection. A contractor applied Telone C35 at 270 kg/ha on 31/12/2001

and carrots (cv Stefano) were sown into the area on 23/1/2002. Plant samples were taken on 26/2/2002 and again on 19/3/2002. At the first sampling, very few *Meloidogyne* egg masses were detected with the trypan blue stain however by the second sampling high levels of nematode infection were observed (Table 45). Telone C35 markedly reduced nematode infection on this site.

Table 45. The effect of Telone C35 application on the incidence of *Meloidogyne* infection on carrot seedlings sampled on 19/3/2002. (Standard errors in parenthesis)

Treatment	Taproot (%)	Taproot and/or lateral root (%)
Control	26.4 (25.4)	48.7 (38.2)
Telone C35	1.2 (2.9)	2.2 (3.5)

Gingin site

Telone has recently been trialed by growers for nematode control in carrots. On one property near Gingin north of Perth, high levels of *Pratylenchus* sp. had been damaging to previous carrot crops. Telone was applied at 100 kg/ha, however the application was not uniform and some rows within the crop were growing on treated areas while others appeared to receive little chemical. The crop developed a stripped appearance with stunted yellow rows (-Telone) amongst vigorous green rows (+Telone). Soil and plant samples were taken from these areas and nematodes (*Pratylenchus*) extracted and counted (Table 46).

Table 46. Number of *Pratylenchus* recovered from carrot roots and soil in areas treated or untreated with Telone.

	No. of *Pratylenchus* in:	
	Soil (per 100 ml)	Roots (per g dry root)
- Telone	106	14,962
+ Telone	0	295

Numbers of *Pratylenchus* extracted from roots were over 50 times higher in the untreated area while soil numbers averaged 0 from the treated soil and over 100 per 100 ml from the untreated soil.

10.4.3 Efficacy of Telone and Telone C35 at different rates for control of Meloidogyne hapla

Introduction

The aim of this experiment was to compare the efficacy of Telone®, and two rates of Telone® C-35 for control of root knot nematode, on a commercial carrot farm. The experiment was conducted at a commercial property 250 km south of Perth. The site was badly affected by root knot nematode, and previous sampling had established that the species present was *Meloidogyne hapla*.

The trial was a fully randomised block with four treatments replicated four times. The treatments were: a) Telone® (a.i. 945 g/kg 1,3-dichloropropene) at 130 kg/ha, b) Telone® C-35 (a.i. 615 g/kg 1,3-dichloropropene + 345 g/kg chloropicrin) at 270 kg/ha, c) Telone® C-35 (a.i. 615 g/kg 1,3-dichloropropene + 345 g/kg chloropicrin) at 185 kg/ha, d) Control.

Each experimental plot comprised one bed, 1.8 m wide by 18 m long. There was a 9 m buffer between the plots. The treatments were applied on 13/1/2003, 40 days before seeding. Pelleted carrot seed, cv. Stefano, was sown with an Agricola air seeder on 22/2/2003, to give four double rows per bed, with a target density of 60 plants/m^2. Fertiliser and weed control were as per standard commercial practice.

Each plot was soil sampled on 26/2/2003, 6 weeks after treatment and 4 days after seeding. The soil samples were stored at 4 ^{0}C for 14 days before being extracted for nematodes. Three 200 g sub-samples from each soil sample were extracted by the Whitehead tray method for 7 days (Whitehead and Hemming, 1965). After this time the nematode suspension were removed and then the soil was re-extracted for a further 7 days. This re-extraction was repeated once more.

Seedling infection was determined by collecting two seedlings at 1 m intervals from each outside double row of the experimental plot. They were taken on 7/4/2003, 45 days, after seeding. Egg masses were stained with 0.25 % trypan blue (Sharma and Mohiuddin, 1993).

Carrots were assessed at maturity (136 days after seeding), by hand harvesting five 1 m lengths of row from the middle two double rows of each plot. The carrots were placed in onion bags, machine washed, and then stored in a cold room at 1° C until they were assessed.

The quality of the bulk crop was assessed by number and weight into the following categories: export marketable (>150 mm long, 25-50 mm crown diameter), short marketable (120-150 mm long, 25-50 mm crown diameter), undersized (<120 mm long, or <25 mm diameter), oversize (>50 mm crown diameter), forked, misshapen, prominent eyes, split.

The statistical software Genstat version 4.2 was used to analyse the results, using transformed or untransformed data as appropriate. The number of nematodes in 200 g of soil was analysed on untransformed data. At the final harvest the incidence of symptoms was analysed on arcsine transformed data using block/treatment/sub-sample as the block stratum and treatment as the treatment stratum.

Results

Soil sampling 6 weeks after the fumigation treatments were applied showed that even though there were more nematodes in the untreated control, there were not significantly more than in the other treatments (Table 47).

Between 38 and 44 seedlings per plot were assessed for nematode infection 45 days after seeding. Egg masses were seen on both lateral and tap roots of some of the seedlings. Although there was a higher incidence of infection in the control treatment, it was not significantly different from the other treatments (Table 48).

Table 47. Number of juvenile *Meloidogyne* extracted from soil 6 weeks after treatment and 4 days after seeding.

Treatment	Number of nematodes /200 g soil
a) Telone® at 130 kg/ha	0.07
b) Telone® C-35 at 270 kg/ha	0.14
c) Telone® C-35 at 185 kg/ha	0.42
d) Control	4.98
Significance	Not significant

Table 48. Comparison of the proportion of carrot seedlings (cv Stefano) with root knot nematode egg masses 45 days after seeding.

Treatment	Proportion of seedlings with egg masses (%)
a) Telone® at 130 kg/ha	0.0
b) Telone® C-35 at 270 kg/ha	3.2
c) Telone® C-35 at 185 kg/ha	1.9
d) Control	34.1
Significance	Not significant

At the final harvest, carrots were assessed for size and quality by both number and weight (Table 49 and 50). There were similar numbers of carrots in each treatment, but the weights of carrots in the Telone® and Telone® C-35 treatments were significantly greater than the untreated control. There were a significantly greater proportion of carrots in the export marketable and short marketable quality categories in the Telone® and Telone® C-35 treatments compared with the control. There were significantly fewer forked carrots in these treatments, compared with the control (Tables 49 and 50).

Table 49. Comparison of the number of mature carrots (cv Stefano) and proportion of different grades harvested from different nematicide treatments.

Treatment	Number	Export market (%)	Short market (%)	Undersize (%)	Forked (%)	Misshapen (%)	Prominent eyes (%)
a) Telone®, 130 kg/ha	59.1	34.4[a]	43.0[a]	17.5	0.4[a]	1.6	0.1
b) Telone® C-35 270 kg/ha	60.9	34.5[a]	36.9[a]	20.6	0.8[a]	3.5	0.1
c) Telone® C-35, 185 kg/ha	58.9	35.8[a]	34.4[a]	21.3	0.5[a]	4.2	0.1
d) Control	59.3	7.6[b]	12.2[b]	10.1	48.2[b]	4.9	0.1
Significance	n.s.	**	*	n.s.	*	n.s.	n.s.

**=*P*<0.01, *=*P*<0.05, n.s.=*P*>0.05
Comparison of numbers was made on untransformed data, comparisons of proportions were made on arcsine transformed data, untransformed data are presented. Within columns, values with the same superscript are not significantly different (P>0.05).

Table 50. Comparison of the weight of mature carrots (cv Stefano) and proportion of different grades harvested from different nematicide treatments.

Treatment	Weight (kg)	Export market (%)	Short market (%)	Undersize (%)	Forked (%)	Misshapen (%)	Prominent eyes (%)
a) Telone®, 130 kg/ha	5.57	45.3[a]	40.8	9.3	0.3[a]	1.2	0.1
b) Telone® C-35 270 kg/ha	5.92	46.1[a]	34.6	11.5	0.7[a]	3.2	0.1
c) Telone® C-35, 185 kg/ha	5.78	47.6[a]	32.1	11.1	0.5[a]	4.1	0.2
d) Control	4.64	10.7[b]	13.3	5.8	48.6[b]	4.6	0.1
Significance	*	**	0.08	n.s.	*	n.s.	n.s.
LSD (P<0.05)	0.90						

**=*P*<0.01, *=*P*<0.05, n.s.=*P*>0.05
Comparison of weights was made on untransformed data, comparisons of proportions were made on arcsine transformed data, untransformed data are presented. Within columns, values with the same superscript are not significantly different (P>0.05).

10.4.4 Efficacy of Telone, Telone C35 and Nemacur for control of Meloidogyne javanica*.*

The aim of this experiment was to compare the efficacy of Telone®, Telone® C-35 and Nemacur® for control of root knot nematode, on a commercial carrot farm. The experiment was conducted at a commercial property 45 km south of Perth. The normal rotation practice involved a winter potato crop followed by summer carrots.

The site was badly affected by root knot nematode, and previous sampling has established that the species present is *Meloidogyne javanica*. The trial was a fully randomised block with four treatments replicated four times. The treatments were: a) Telone® (a.i. 945 g/kg 1,3-dichloropropene) at 135 kg/ha, b) Telone® C-35 (a.i. 615 g/kg 1,3-dichloropropene + 345 g/kg chloropicrin) at 270 kg/ha, c) Nemacur® 400 (a.i. 400 g/L fenamiphos) at 24 L/ha, d) Control.

Each experimental plot comprised three beds, 1.5 m wide by 24 m long. The outside beds were treated as buffers. There was a 12 m buffer between the plots. The treatments were applied on 18/12/2002, 30 days before seeding. Pelleted carrot seed, cv Stefano, was sown with an Agricola air seeder on 18/1/2003, to give three double rows per bed, with a density of 66 plants/m^2. Fertiliser and weed control were as standard commercial practice.

The trial site was soil sampled on 24/10/2002 and the soil was stored at 4^0C for 6 days. Three 200 g sub-samples from each soil sample were extracted by the Whitehead tray method for 7 days (Whitehead and Hemming, 1965). After this time the suspension removed and then the soil was re-extracted for a further 7 days. This re-extraction was repeated once more.

The central bed of each plot was soil sampled on 7/2/2003, 51 days after treatment and 13 days after seeding. The soil samples were stored at 4 ^{0}C. Three 200 g sub-samples were extracted for a total of 21 days by the Whitehead tray method, as described above. The first replicate samples were set up 6 days after collection, the second and third replicates were set up 12 days after collection.

Infection of seedling roots was determined by collecting two seedlings at 1 m intervals from each buffer row of the central bed on 10/3/2003, 45 days, after seeding. Egg masses were stained with 0.25 % trypan blue (Sharma and Mohiuddin, 1993).

Carrot yield and quality was assessed at maturity (129 days after seeding), by hand harvesting five 2 m lengths of row from the middle row of the centre bed of each plot. The carrots were placed in onion bags, machine washed, and then stored in a cold room at 1^{o}C until they were assessed. The quality of the bulk crop was assessed by number and weight into the following categories: export marketable (>150 mm long, 25-50 mm crown diameter), short marketable (120-150 mm long, 25-50 mm crown diameter), undersized (<120 mm long, or <25 mm diameter), oversize (>50 mm crown diameter), forked, misshapen, prominent eyes, veined, split.

The statistical software package Genstat version 4.2 was used to analyse the results using transformed or untransformed data as appropriate. The number of nematodes in 200 g of soil was analysed on untransformed data using block/treatment/sub-sample and treatment and treatment as the treatment stratum. At the final harvest the incidence of symptoms was analysed on arcsine transformed data using block/treatment/sub-sample as the block stratum and treatment as the treatment stratum.

Results

Soil sampling from the experimental site 8 weeks before the nematode control treatments were applied gave a mean of 9.3 nematodes per 200 g soil. Only juvenile (J2) *Meloidogyne* were observed, and all but one was extracted in the first 7 days.

Soil sampling 6 weeks after the fumigation treatments were applied showed that even though there were more nematodes in the untreated control, there were not significantly more (P>0.05) than in the other treatments (Table 51). Between 40 and 48 seedlings per plot were assessed for nematode infection 45 days after seeding. Egg masses were seen on both lateral and tap roots. The incidence of infection was significantly higher in the fenamiphos and control treatments than in the Telone® and Telone® C-35 treatments (Table 52).

Table 51. Number of juvenile *Meloidogyne* extracted from soil 51 days after treatment and 13 days after seeding.

Treatment	Number of nematodes /200 g soil
a) Telone®	0.07
b) Telone® C-35	0.00
c) Fenamiphos	0.07
d) Control	0.29
Significance	n.s.

Table 52. Comparison of the proportion of carrot seedlings (cv Stefano) with root knot nematode egg masses 45 days after seeding.

Treatment	Proportion of seedlings with egg masses (%)
a) Telone®	6.1
b) Telone® C-35	3.4
c) Fenamiphos	36.0
d) Control	39.5
Significance	***
LSD (P<0.05)	14.9

***=*P*<0.001

At the final harvest, carrots were assessed for size and quality by both number and weight (Tables 53 and 54). There were similar numbers of carrots in each treatment, but the weight of carrots in the Telone® C-35 treated plots was significantly greater than in the fenamiphos and control treatments. There was a greater proportion of carrots of export market quality in the Telone® and Telone® C-35 treatments than in the fenamiphos and control treatments. There was a greater proportion of carrots with bulgy eyes in the fenamiphos treatment than in the Telone® and Telone® C-35 plots.

The trial was also rated for the incidence of cavity spot, because the property had a history of this disease. The incidence of cavity spot was significantly lower in the Telone® C-35 treatment than in the fenamiphos and control treatments (Table 53 and 54).

Table 53. Comparison of the number of mature carrots (cv Stefano), proportion of different grades and proportion of carrots with cavity spot.

Treatment	Number	Export market quality (%)	Short market (%)	Undersize (%)	Forked (%)	Misshapen (%)	Prominent eyes (%)	Veined (%)	Incidence of cavity spot (%)
a) Telone®	64.1	56.5[a]	7.1	2.1	1.9	26.0	1.2[a]	0.2	2.5[ab]
b) Telone® C-35	67.1	58.6[a]	8.2	2.3	1.3	20.6	2.9[ab]	0.8	0.2[a]
c) Fenamiphos	67.9	24.7[b]	6.0	3.7	5.9	23.7	23.8[c]	1.2	7.3[b]
d) Control	64.5	29.2[b]	4.5	1.9	5.9	26.4	15.1[bc]	2.1	9.7[b]
Significance	n.s.	*	n.s.	n.s.	n.s.	n.s.	*	n.s.	*

*=P<0.05, n.s.= not significant P>0.05
Comparison of numbers was made on untransformed data, comparisons of proportions were made on arcsine transformed data, untransformed data are presented. Within columns, values with the same superscript are not significantly different (P>0.05).

Table 54. Comparison of the weight of mature carrots (cv Stefano), proportion of different grades and proportion of carrots with cavity spot.

Treatment	Weight (kg)	Export market quality (%)	Short market (%)	Undersize (%)	Forked (%)	Misshapen (%)	Prominent eyes (%)	Veined (%)	Incidence of cavity spot (%)
a) Telone®	7.99	64.1[a]	4.3	0.6	1.4	22.4	2.0[a]	0.1	2.8[ab]
b) Telone® C-35	8.83	65.2[a]	4.9	0.8	1.1	17.2	4.5[ab]	1.0	0.2[a]
c) Fenamiphos	7.37	28.7[b]	4.1	1.2	4,8	19.1	30.8[b]	0.1	7.7[b]
d) Control	7.02	35.0[b]	3.3	0.8	4.8	22.5	19.4[b]	0.1	9.9[b]
Significance	*	*	n.s.	n.s.	n.s.	n.s.	*	n.s.	*
LSD (P<0.05)	1.07								

*=P<0.05, n.s.= not significant P>0.05
Comparison of weights was made on untransformed data, comparisons of proportions were made on arcsine transformed data, untransformed data are presented. Within columns, values with the same superscript are not significantly different (P>0.05).

10.5 Enhanced biodegredation of nematicides in Victoria

Introduction

Traditionally in Australia, nematodes have been controlled using pre-plant fumigant nematicides such as methyl isothiocyanate or non-fumigant nematicides such as Nemacur 400®. These products have been useful tools for managing nematodes in carrot production. However, repeated applications to the same area have led to enhanced biodegradation, in which there is build-up of populations of micro-organisms in the soil which are able to rapidly break down the nematicide into non-nematicidal byproducts. Non-fumigant nematicides are generally nematastatic in that they do not kill nematodes but paralyse them (Bunt 1987). Non-fumigant nematicides therefore need to be active for long enough in the soil that the nematode starves to death or that nematode activity, feeding and reproduction are delayed sufficiently in relation to the crop development to prevent economic damage. Non-fumigant nematicides are generally active in the soil for six to eight weeks, but this can be reduced to less than two weeks in soils where enhanced biodegradation has occurred.

Two commonly used nematicides in the Victorian carrot industry are Nemacur 400® (400g.L^{-1} fenamiphos) a nematicide-insecticide, applied to control nematodes and soil borne insects and Telone C-35® (615g.kg^{-1} 1,3 – dichloropropene and 345g.kg^{-1} chloropicrin) a pre-planting soil fumigant. Nemacur® is more persistent than Telone® being active in the soil for several weeks (Tomlin 1997, p.504) whereas Telone® has a metabolic half-life of 1.5 hours (Tomlin 1997, p.367). This trial aimed to determine the efficacy and extent of enhanced biodegradation of Nemacur 400® and Telone C-35® in 13 carrot producing soils from Victoria.

Methods

A bioassay was established to determine the extent of enhanced biodegradation of two nematicides used in carrot production in Victoria. Soil was sampled from 13 carrot-producing fields in Victoria (supplied by Deborah Keating, NRE Victoria) and sent to DPI Queensland for analysis. Each soil sample was divided into 5 sub samples so that they could have different treatments applied and follow a bioassay protocol for testing for enhanced biodegradation (Pattison *et al.*, 2000). The treatments applied to the soil were: a) Untreated soil (UTC), b) Sterilised (121^{o}C for 15 minutes) + Nemacur (SN), c) Unsterilised + Nemacur (USN), d) Sterilised (121^{o}C for 15 minutes) + Telone (ST), e) Unsterilised + Telone (UST).

Nemacur 400® was applied to achieve a concentration in the soil of 10 µg fenamiphos per g soil. Telone-C35® was applied to achieve a concentration in the soil of 40 µg 1,3-dichloropropene per g soil. The nematicides were spread over the soil surface of the sub-samples before it was thoroughly incorporated by shaking and mixing in a plastic container.

All samples were then stored in plastic food containers with a loose fitting lid inside polystyrene boxes with water in the bottom at room temperature (20-35^{o}C). Samples (35 g) were taken from each at 0, 2, 4 and 6 weeks after nematicide application. Three mung bean (*Vigna mungo*) seeds were planted into each 50 ml vial containing the 35 g of soil from each sub sample. Sieved river sand was then placed on the top of each vial to allow for a better seed to soil contact and watered with 5 ml before being placed in the glasshouse.

Four days after planting, the vials were inoculated with 1 mL of distilled water containing approximately 500 *Radopholus similis*. The inoculum was obtained from carrot cultures hosting *R. similis* (Moody *et al.*, 1973). Following inoculation, the mung beans were kept in the glasshouse and watered with distilled water each day as required until harvest, 7 days later. At harvest the plastic vials were washed out with water to expose the mung bean roots, which were then cut into pieces no longer than 2cm and placed onto a sieve inside a funnel and cup and misted for 5 days with water. After 5

days the samples were placed under the microscope so that any *R. similis* could be quantified.

An average for each treatment at the end of the 6th week of the trial was used to determine the percentage control of each treatment in a soil relative to the untreated control. No statistical analysis was possible as the small amount of soil received did not allow replication. To determine the degradation status for the treatments in each soil, the following assessment criteria was used:

- Enhanced biodegradation was defined as when nematode recovery in the unsterile Nemacur® (USN) treated soil was greater than or equal to 75% of untreated control (UTC).
- Advanced biodegradadation was defined as when nematode recovery in the USN was less than 75% but greater than 20% of UTC.
- No biodegradation was defined as when nematode recovery in the USN was less than or equal to 20% of UTC

Results

Five carrot producing soils (GWSBR, PTP, GSP, LP25 and BC4) were found to have enhanced biodegradation of Nemacur 400®, using the criteria of enhanced biodegradation occurring if nematodes recovery in the unsterile soil was 75% or greater of the untreated control (Table 55). The five soils that were determined to have enhanced biodegradation had low recovery of nematodes when the soil was sterilised and Nemacur 400® (SN) added to the soil (Table 55). This confirms that loss of nematicide efficacy was due to a biological cause.

Five carrot producing sites, LP21, DBS, CBP, GBH and PBP, were found to have greater than 20% nematode recovery relative to the untreated soil, when nematodes were added to Nemacur 400® treated, unsterile soil (Table 55). This was considered to be advanced biodegradation of Nemacur 400®, as the product would not be expressing its full efficacy in these soils to control plant-parasitic nematodes. Again, the recovery of nematodes in the sterile Nemacur 400® treated soil (SN) was less than 10.4% of the untreated soil,

suggesting that the cause of the reduced control of nematodes was due to biological degradation of the chemical.

Nemacur 400® applied to soil from three sites, BB3, GSH and CLP was found to have similar efficacy in both the sterile and unsterile soil (Table 55). This suggested that there was no biodegradation of Nemacur 400® at these sites and that the efficacy of the chemical was preserved. Nemacur 400® applied to these three sites would be expected to give good control of plant-parasitic nematodes.

Table 55. Percentage nematode recovery averaged over a 6 week period relative to the untreated control (UTC) for 13 carrot growing soils with sterile and un-sterile soil treated with Nemacur 400® (sterile Nemacur® SN, unsterile Nemacur® USN) and Telone C-35® (sterile Telone® ST, unsterile Telone® UST).

Degradation	Site code	Nematodes recovered relative to untreated control (%)				
		UTC	USN	SN	UST	ST
Enhanced biodegradation	GWSBR	100	157.7	10.3	131.0	279.3
	PTP	100	126.8	0.0	45.1	45.1
	GSP	100	111.4	7.9	20.4	97.7
	LP25	100	94.1	0.0	46.1	27.9
	BC4	100	75.3	1.1	13.5	31.4
Advanced biodegradation	LP21	100	60.8	0.0	65.5	18.7
	DBS	100	54.7	9.0	76.0	83.0
	CBP	100	44.1	2.9	35.0	11.0
	GBH	100	32.8	10.4	66.4	37.6
	PBP	100	21.9	5.5	79.4	72.6
No biodegradation	BB3	100	15.5	0.8	6.3	13.6
	GSH	100	14.2	6.4	95.7	248.2
	CLP	100	3.6	2.3	51.8	45.2

Telone C-35® lost its effectiveness from week 0 in the majority of soils. However, this was expected, as Telone® is a soil fumigant and therefore only has a short active life in the soil. The bioassay method was not an effective method for soil fumigants, as it could not determine if biodegradation was occurring (Table 55).

Discussion

Five sites growing carrots were found to have enhanced biodegradation of Nemacur 400®, with greater than 75 % nematode recovery in unsterile, Nemaur 400® treated soil relative to untreated soil. Nemacur 400® would not be expected to be efficacious at reducing nematode numbers at these five sites due to enhanced biodegradation. The decomposition of Nemacur 400® probably occurred before its nematicidal products could reduce nematode numbers in the carrot crop.

A further five sites had advanced biodegradation of Nemacur 400® with between 20 and 75% nematode recovery in the unsterile, Nemaur 400® treated soil relative to untreated soil. Some reduction in nematode numbers in the field could be expected in these soils. However, Nemacur 400® applied to these sites would not be expected to have maximum efficacy.

Only three soils from the 13 carrot producing sites in Victoria had what would be considered “normal” efficacy of Nemacur 400®, with less than 20% of nematodes recovered in unsterile, Nemacur 400® treated soil relative to untreated soil. This suggested there was no biodegradation of Nemacur 400® at these sites and that the efficacy of the chemical was preserved. Nemacur 400® applied to these three sites would be expected to give good control of plant-parasitic nematodes.

The sterilisation of the soil improved the efficacy of Nemacur 400®at 10 sites, which suggested that the degradation of Nemacur 400® was due to a biological cause and not due to chemical degradation. In contrast this could not be demonstrated for Telone C-35®.

The bioassay was not a useful technique for determining the amount of enhanced biodegradation of Telone C-35®. Because of the short persistence of Telone® in the soil, it was difficult to distinguish between chemical and biological degradation of the active ingredients of Telone C-35®. Smelt *et al.* (1996) reported that there was a very fast degradation of Telone® despite infrequent applications of this soil fumigant in the past. In their study they also

measured the rapid decline of Telone® within 3-6 days of application in soils treated with the fumigant once or twice before and in soils that have previously been untreated (Smelt *et al.*, 1996). This short persistence, a metabolic half-life of 1.5 hours (Tomlin, 1997) in the soil was why the bioassay method (Pattison *et al.*, 2000) was not effective in determining the degradation rate of Telone C-35®. Telone C-35® causes nematodes to die through contact rather than starvation and therefore has a different mode of action to Nemacur 400®. Telone C-35® does not need to persist in the soil like organophosphate nematicides, such as Nemacur® to reduce nematode populations.

11. Summary of strategies for control of nematodes in carrot production

11.1 Assessing nematode population density

Pre-plant count

Any management decision for nematodes is dependent upon determining which nematodes are present and how many. Stirling (2000) adopted an approach of nematode monitoring which consisted of i) an initial intensive sampling phase to develop an understanding of the nematode status of the farm and ii) a regular monitoring program to provide information on specific fields. It was estimated that vegetable fields could be monitored for up to $175/ha. Assuming costs of nematicide and fumigant of $800-$1200/ha, growers could save as much as $1000/ha when results suggested nematicide was not required (Stirling 2000). This pattern of monitoring was far more intensive than currently employed.

A useful strategy for the grower may be to conduct a nematode count prior to planting and a nematode count later in the season (near harvest) to determine whether populations of particular nematodes are increasing or decreasing in particular fields under particular crops. The limit of detection for species such as root knot nematode on carrot is often above the threshold population that can cause economic damage. However, a late season sampling would increase the probability of detection (as nematodes would have had opportunity to reproduce) and provide information for a subsequent crop in the rotation. It should be noted that a late season sample should also involve extracting nematodes/eggs from roots as a significant proportion of the population of endoparasitic species may be feeding inside roots at this time. Ferris *et. al*. (1994) reported that damage to potato caused by *Meloidogyne chitwoodii* and *Pratylenchus neglectus* was predicted more reliably from nematode counts conducted the previous autumn in comparison to the spring just before planting. Sampling carrot plants from different parts of the field

later in the season would also allow the grower to locate patches of nematode infestation. Staining root tissue can also improve detection (e.g. Sharma and Mohiuddin 1993, Thies *et al*. 2002). Patches of nematode infestation could then be monitored through the rotation and/or be selectively treated with nematicide when another susceptible crop is to be established.

A bioassay involving counting the number of galls developing on tomato cv. Rutgers has been found to be more sensitive at detecting low populations of *M. hapla* in carrot soils than soil extraction of nematodes (Belair 1998). In addition, a highly significant relationship ($r = -0.97$, $P<0.01$) was found between marketable carrot yield in the current year and bioassay gall counts from the previous year of onion in a microplot test (Belair 1998). Bioassays are labour intensive, time consuming and are therefore not always practical for pre-plant testing of soils immediately prior to carrot. However, soil samples could be taken at the end of the previous crop and tested by bioassay as suggested by Belair (1998). This would allow sufficient time for the results of the bioassay to be used in the subsequent carrot crop and would stand a higher chance of detecting low populations. In addition, in cooler climates the result may be available in time for the grower to make a decision with regard to the need for a break crop in the autumn/winter period prior to carrot the next spring.

Shortcomings in pre-plant nematode counts include inaccuracies due to soil sampling and extraction technique and the laborious nature of identifying individual nematodes to species by traditional microscopy. Furthermore there is difficulty in setting a damage threshold population density, as the relationship between nematode numbers and amount of crop damage may fluctuate with environmental conditions, host susceptibility and nematode aggressiveness. One difficulty particular to carrot and other sensitive crops is that the limit of detection of root knot nematode is often above the population density at which economic damage can occur. This encourages risk adverse growers to apply nematicide on an insurance basis, whether it is required or not. In some situations, increased effort in sampling to increase the accuracy of the pre-plant nematode count would more than offset the cost of an

insurance nematicide. While DNA based methods of detecting soil borne pathogens such as nematodes are becoming commercial (e.g. the Cereal Root Disease Testing Service run by SARDI and Aventis CropScience), such techniques will also suffer from the inherent inaccuracy of soil sampling and establishment of meaningful thresholds. The advantage of DNA based extraction and detection techniques might lie (ultimately) in their ability to detect lower densities of nematodes in a given soil sample than current methods. A further advantage will lie in their ability to more easily determine which species is present, especially when it is considered that DNA techniques will be able to identify eggs and juvenile nematodes, which is not currently possible by microscopy. Species identification will enable the grower to more easily manage a nematode problem by rotation or by the use of suitable non-host break crops. However, this will require good information regarding the host range of particular species. Unfortunately this information is not always available as host status to a particular nematode can vary with variety within a plant species.

Estimating risk to succeeding crops from damage in current seasons crop.

Risk of nematode damage to the current carrot crop can be estimated from soil counts of nematodes conducted in the preceding crop. A variation on this is to estimate risk of nematode damage to the current crop from assessing damage that has occurred in the preceding crop. Belair and Boivin (1988) worked out the relationship between a rating for root damage caused by *Meloidogyne hapla* and yield loss in carrot. Their rating scale was based on diagramatic representation of damaged roots on a 0-5 scale. The scale involved: 0=no galling, 1=1-10 galls on secondary roots with taproot unaffected, 2=10-50 galls, none coalesced, taproots with light forking, marketable, 3=50-100 galls on secondary roots, some coalesced, light forking, unmarketable, 4=more than 100 galls, many coalesced, severe forking and stunting, unmarketable, 5=more than 100 galls, mostly coalesced, severe stunting, unmarketable. Belair and Bovin (1988) assumed an average production of 40 t/ha at CAN$60/t (CAN$2400/ha) and nematicide costs at CAN$960/ha. They estimated that a 40% yield loss was equivalent to the

cost of control i.e. the economic threshold. From experimental trials, Belair and Bovin (1988) determined a straight-line relationship between the average gall rating in plots and the amount of yield loss in those plots, and determined that an average gall rating of 1.6 was equivalent to a 40% yield loss in the current season. They also determined the relationship between gall ratings in the previous crops and gall ratings in current crops. In their study an average gall rating of 0.65 in the previous crop led to an average gall rating of 1.6 in the current crop. Belair and Boivin (1988) then developed a sequential sampling technique in which carrot samples were taken from a carrot field nearing harvest and each rated for root damage (above) as they were collected. A cumulative damage rating was kept during the sampling. If the cumulative damage rating was greater than an upper limit for that particular number of samples then the average gall rating was assumed to be above 0.65 and nematicide treatment of the succeeding crop was recommended. If the cumulative damage rating fell below a lower limit for that number of samples then the average gall rating was assumed to be below 0.65 and the sampling was ended. In this case nematicide for the succeeding crop was not considered economically viable. If the cumulative damage rating was between the upper and lower limit for a particular number of samples then sampling continued. This technique minimised the often labour intensive nature of sampling, with a minimum of 10 and a maximum of 72 samples required per unit area.

This technique would be appropriate where carrots are grown in close rotation and where an IPM specialist or agronomist could take samples prior to harvest. Considerable work is required to derive the relationship between damage in the current crop and that in the succeeding crop and to establish a damage threshold in the current crop, above which there is a need to treat the succeeding crop.

Instead of actively sampling a crop for damage near harvest time, an alternative strategy would be to determine i) the proportion of carrots at grading rendered unmarketable by nematode damage which equated to the cost of nematicide application and ii) determine the relationship between the

proportion of the crop rendered unmarketable by nematode damage at grading in the preceding crop and that in the current crop. The level of damage in the preceding crop at grading could then be used to determine the need for a nematicide application prior to the succeeding crop. However, this would require research to determine the reliability of the relationship between nematode damage between one season and the next, and a reliable method of recognising nematode damage at grading, in comparison to other defects.

11.2 Resistant varieties of carrot

Breeding work overseas is showing promise in developing varieties resistant to root knot nematode. However, it is likely to be some time before resistant varieties with desirable market characteristics are commercially available. Currently, market demand in Australia is for a relatively few varieties of carrots. Limited testing of currently available varieties has suggested some differences in susceptibility to root knot nematode. Further testing would be necessary to determine whether some of the currently available varieties have a useful level of resistance.

11.3 Fallow (bare fallow and break crops)

The principle of a fallow period is to deny nematodes of a food source, so that they starve to death in the absence of a host crop. This can be done by using a bare fallow or planting a non-host break crop. Alternatively a suppressive crop that directly reduces populations (e.g. biofumigation) can be employed.

Bare fallow

It has been generally accepted that populations of root knot nematode decline by 75% or more per year. Cultivating the soil over this time will increase mortality but increases the costs of the fallow. Huang and Porto (1988) examined the survival of *M. javanica* and *M. incognita* in soil without a host plant. More than 75% of the initial nematode population of both species died during a 1-2 month fallow, and less than 10% survived after 3-4 months in soil of water content between 22-38%. Carrot yield in soil which had been fallow

for 1, 5, 9 or 13 weeks were 77%, 35%, 31% and 46% lower respectively than those in soil treated with methyl bromide. Fallow of 1 year reduced the population of *M. fallax* by 95%, but this was not sufficient to prevent damage in subsequent crops (Brommer 1996). Less damage to carrot was possible when crops were sown later in the spring.

Break crops used overseas

For economic reasons and for soil conservation it is more desirable to grow a non-host break crop over this time. Abawi *et al*. (2001b) reported that a two-year rotation with a non-host or antagonistic crop could greatly decrease populations of *M. hapla* and reduce damage to subsequent crops including carrot. They reported all grain crops tested were non-hosts, and that sudangrass (cv. Trudan 8), rapeseed (cv. Jupiter), marigold (cvs. Polynema and cv. Nema-gone) and several accessions of white clover were also effective against this nematode. It should be noted that contrary to this study, white clover is a known host of *M. hapla*. Suppression by green manure of sudangrass and white clover was due to production of hydrogen cyanide during decomposition in the soil (Abawi et al. 2001b). Similarly, Sorghum is also able to suppress some nematodes, which may be due to the production of hydrogen cyanide (Widmer and Abawi 2002).

Guyton *et al*. (1989) reported that populations of *M. hapla* J2 increased and carrot yield decreased with 37 months of continuous carrot cropping. *M. hapla* J2 populations following 29 months of Haifa and common white clover (*Trifolium repens*) were higher after both subsequent carrot crops. Significantly fewer *M. hapla* J2 and higher carrot yields were obtained when the nematode-resistant lucerne varieties Nevada Synthetic XX and Nevada Synthetic YY were grown before carrot.

The influence of previous crop rotation on populations of *M. hapla* and subsequent carrot yield and quality in Quebec was studied by Belair and Parent (1996). They investigated seven 3-year sequences of crop rotation in a muck soil involving barley, carrot, onion or weedy fallow, all with carrot as the third-year crop. Carrot monoculture, two seasons of weedy fallow or

carrot followed by onion resulted in high population densities of *M. hapla* and severe damage to carrot in the third year. Barley followed by onion, or onion followed by barley led to low population densities of *M. hapla* and provided the highest yields of carrot in the third year (56.8 t/ha and 47.2 t/ha marketable carrots respectively). This compared with only 2.2 t/ha marketable carrots in the third year of the carrot monoculture. A single crop of barley preceding carrot reduced *M. hapla* population density and led to 73% marketable carrots compared to 7% in carrot monoculture. High population densities of *M. hapla* and high proportion of damaged carrots following the weedy fallow emphasised the importance of an effective weed management program for successful use of crop rotation for nematode control. Leroux *et al*. (1996) also studied various 3-year rotations of onion, barley or weedy fallow, all with carrot as the final third year crop, in comparison to a monoculture of carrot. Total carrot yield increased by 35-50% and marketable yield by 17-25 fold when barley was included in the rotation, due to a reduction in population density of *M. hapla*. The onion-barley-carrot rotation provided high yield and good quality of carrots and good weed control, including the weed species *Bidens cernua*, a good host of *M. hapla*.

It is important to recognise that weed species may also act as hosts of nematodes. Belair and Benoit (1996) reported that 21/32 weeds commonly found in organic soils in Quebec were hosts for *M. hapla*. *M. hapla* had a higher reproduction factor on 16 of these than it did on carrot. Weeds which supported the highest reproduction and galling were *Bidens cernua*, *B. frondosa*, *B. vulgata*, *Polygonum scabrum*, *Sium suave* and *Thlaspi arvense*. *Capsella bursa-pastoris*, *Chrysanthemum leucanthemum*, *Gnaphalium uliginosum*, *Stellaria media* and *Veronica argestis* supported moderate galling and moderate reproduction of *M. hapla*. *Chenopodium album*, *C. glaucum*, *Erysimum cheranthoides*, *Polygonum convolvulus*, *Portulaca oleracea* and *Rorippa islandica* supported low reproduction and had low galling. *Eupatorium maculatum* and *Thalictrum pubescens* had no distinct galling but supported low to moderate reproduction of *M. hapla*. Non-weed hosts of *M. hapla* were *Amaranthus retroflexus*, *A. artemisiifolia*, *Echinochloa crusgalli*, *Erysimum cheranthoides*, *Oenothera parviflora*, *Panicum capillare*, *Setaria*

glauca, *S. viridis*, and *Solidago canidensis* (Belair and Benoit 1996). The weed *Senecio vulgaris* formed galls, but no eggs or J2 of *M. hapla* were recovered (Belair and Benoit 1996). Weed control is therefore essential where rotation or break crops are utilised to manage nematode populations.

In Ontario, Rye (*Secale cereale*) is often planted in autumn after vegetables as it is winter hardy and establishes quickly (McKeown and Potter 2001), and is not a host for *M. hapla* (Potter and Olthof 1993). However, *S. cereale* is a host for *Pratylenchus penetrans* (Olthof 1980).

McLeod (1994) investigated sward clovers and grasses and other cover crops in vineyards for control of nematodes including *M. incognita* and *M. javanica* (Table 56). Varieties of ryegrass, chewings fescue, oats and marigold were found to be useful cover crops that supported no or little multiplication of these root knot nematode species. Mercer (1997) tested the host status of several species of legume against four species of root knot nematode. *M. hapla* and *M. javanica* caused few galls (2-3% of the root system) on *Trifolium glomeratum* and *Trifolium semipilosum* respectively (Mercer 1997), suggesting that these may be useful break crop species. Diamond *et al*. (1991) advocated avoidance of continuous carrot crops and rotation of non-hosts of *M. hapla* such as barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), timothy (*Phleum pratense*) and annual ryegrass (*Lolium multiflorum*) and the elimination of forage legumes from the rotation.

Wang and McSorley (2001) reviewed cropping systems for nematode management and identified several plant species which were poor hosts or suppressive to *Meloidogyne* spp. (Table 57). Several legume species (cowpea, crotalaria, joint vetch and sunn hemp) have additional use in nitrogen management. Hagan *et al*. (1998) also reported on crops suppressive to *Meloidogyne* spp. (Table 58). Less is known about the host range of *M. fallax*. Brommer (1996) reported that Italian ryegrass, potato and carrot were good hosts, while maize and other cereals were poor hosts.

Table 56. Increase in populations of *Meloidogyne* spp. (*M. incognita* and *M. javanica*) under interrow cover crops in a vineyard (Mcleod 1994).

Crop	Variety	Increase in *Meloidogyne*
Strawberry clover	O'Conners	50 x
White clover	Kopu, Tahora	>10 x
Subterranean clover	Seaton Park, Trikkala, Woogenellup	6-9 x
Ryegrass	Brumby, Citation II, Concord, Duet, Jazz, KV, Tetila, Wimmera	0
Chewings fescue	Tudor creeping, Shadow chewings, Victory chewings	0
Oats	Swan, Wallaroo	0
Triticale	Tahara	3 x
Crucifers	Hobsons forage rape, Humus green manure rape, Rauola oilseed radish	3-10 x
Marigold	African King, French Minuet	0

Table 57. Examples of rotation crops suppressive against species of *Meloidogyne* (Wang and McSorley 2001)

Break crop	*Meloidogyne* spp. affected
Crotalaria (*Crotalaria spectabilis*)	*M. arenaria, M. incognita*[1]
Jointvetch (*Aeschynomene americana*)	*M. arenaria, M. incognita*
Maize (*Zea mays*)	*M. arenaria, M. hapla, M. incognita*.
Marigold (*Tagetes* spp.)	*M. hapla, M. incognita, M. javanica*
Oat (*Avena sativa*)	*M. arenaria, M. hapla, M. incognita*
Rye (*Secale cereale*)	*M. hapla, M. incognita*
Sunn hemp (*Crotalaria juncea*)	*M. incognita, M. javanica*
Sorghum (*Sorghum bicolor*)	*M. arenaria, M. incognita*, *M. javanica*
Velvet bean (*Mucuna pruriens*)	*M. arenaria, M. incognita*
Wheat (*Triticum aestivum*)	*M. incognita*

[1] Note Hagan *et al*. (1998) reported American Jointvetch to be suppressive to *M. arenaria* only, and to allow the reproduction of *M. incognita* and *M. javanica*.

Table 58. Break crops suppressive to *Meloidogyne* spp. (Hagan *et al*. 1998)

	Root knot nematode species:			
Suppressive crop	*M. incognita*	*M. arenaria*	*M. hapla*	*M. javanica*
French marigold (*Tagetes patula*)				
'Tangerine'	**	**	**	-
'Happy days'	-	-	-	**
'Lemondrop'	**	-	-	-
'French dwarf double'	-	-	-	-
Chrysanthemum (*Chrysanthemum morifolium*)				
'Escapade'	**	-	-	-
Castor bean (*Ricinus communis*)				
'Bronze king'	**	-	-	-
'Hale'	-	**	-	-
Partridge pea (*Cassia fasciculata*)	-	**	-	-
Crotalaria (*Crotalaria spectabilis*)	**	**	-	**
Velvetbean (*Mucuna deeringiana*)	**	**	-	**
Common vetch (*Vicia sativa*)				
'Cahaba White'	**	**	-	**
'Vantage', 'Nova II', 'Vanguard', 'Warrior'	-	**	-	-
Rapeseed (*Brassica napus*)				
'Jupiter', 'Cascade', 'Elena', 'Indore', 'Humus', 'Bridger', 'Dwarf Essex'	**	-	-	**
Sesame (*Sesame indicum*)	-	**	-	-

** = high level of nematode suppression, - = no suppression or no data available

Castor bean (*Ricinus communis*) greatly reduces survival of *M. incognita, M. arenaria* and some species of lesion nematodes (Hagan *et al.* 1998). However, Castor bean must be plowed under before seed is set as seed is poisonous (a single seed is sufficient to kill humans or livestock). Partridge pea (*Cassia fasiculata*) can reduce populations of *M. arenaria* but its effects on other species are not known (Hagan *et al*. 1998). However, Partridge pea produces small, hard seed that make this forage a potential weed problem. Crotalaria (*Crotalaria spectabilis*) is highly resistant to a broad range of root knot nematodes (Tables 57, 58, 59), but should be grown as a green manure as its tops and seed are toxic to livestock and humans. Velvetbean (*Mucuna deeringiana*) has been shown to reduce populations of several root knot species (Tables 57, 58). It can be incorporated as a green manure, allowed to mature before the tops are cut down with a disk, or cut as hay for cattle and other livestock (Hagan *et al*. 1998).

Varieties of common vetch (*Vicia sativa*) are resistant to several root knot nematode species (Table 58) and can be used as a winter cover crop, but may become a weed problem if allowed to set seed (Hagan *et al*. 1998).

Jackbean (*Canavalia ensiformis*) is a cover crop grown as a source of nutraceuticals, pharmaceuticals and industrial products (Walker and Morris 2002). It is an effective rotational crop or cover crop in semi-temperate regions. Jackbean is used to produce compounds such as concanavalin, which is used in medicine and is also nematicidal (Walker and Morris 2002). Incorporation of Jackbean tissues into soil reduced root knot nematode galling on tomato by up to 76%, although there was wide variation between Jackbean accessions in their effectiveness (Walker and Morris 2002).

McSorley (1999) also assessed several potential cover crops species (Table 59), again highlighting that many of the species described above were effective break crops for *M. arenaria*, *M. incognita* and *M. javanica*.

Table 59. Host status of potential cover crop species to *Meloidogyne* spp. (McSorley 1999)

Crop	*M. arenaria* Race 1	*M. incognita* Race 1	*M. javanica*
Castor (*Ricinus communis*)	0	0	0
Cowpea (*Vigna unguiculata* 'Iron Clay')	0	0	0
Crotalaria (*Crotalaria spectabilis*)	0	0	0
American Jointvetch (*Aeschynomene americana*)	0	0	0
Marigold (*Tagetes minuta*)	0	*	0
Sesame (*Sesamum indicum* 'Paloma')	*	0	0
Sunn hemp (*Crotalaria juncea* 'Tropic Sun')	0	*	0
Pearl Millet (*Pennisetum typhoides* 'Tifleaf II hybrid')		**	
Japanese millet (*Echinochloa frumentacea*)	**	**	**

* = minimal egg mass production, **=substantial egg mass production, 0 = no reproduction.

Recommendation of break crops for *Pratylenchus* spp. is difficult due to their wide host ranges and number of species. There has been considerable effort in the Australian grains industry to develop cereal varieties with resistance to *Pratylenchus thornei* and *Pratylenchus neglectus*. Where these nematode species are identified as causing problems in vegetables, then break crops incorporating resistant cereal varieties are likely to be of benefit. As mentioned above, in Ontario, Rye (*Secale cereale*) is often planted after vegetables as it is winter hardy break crop (McKeown and Potter 2001), and is not a host for *M. hapla* (Potter and Olthof 1993). However, *S. cereale* is a host for *Pratylenchus penetrans* (Olthof 1980). Jagdale *et al*. (2000) determined that hybrids of forage and grain pearl millet (*Pennisetum glaucum*) reduced populations of *P. penetrans*, more so than rye (*Secale cereale*) and grain sorghum (*Sorghum bicolor*) and recommended further work to develop hybrids of pearl millet as break crops for lesion nematode. Jagdale *et al*. (2000) noted that in the literature several species of sorghum (*S. bicolor*, *S. vulgare* and *S. sudanense*) were poor hosts of nematodes while their study and that of others showed sorghum to be good hosts. They recommended that further testing of species and varieties of sorghum were required to identify useful varieties of Sorghum against *P. penetrans*.

The limited work in this project on host range of *P. penetrans* in Tasmania suggested a number of plant species which may be useful break crops (Table 31), however there were differences between varieties within a species. For example, there was significantly lower numbers of *P. penetrans* recovered from perennial ryegrass ‘Impact’, ‘Jackaroo’, and ‘Quartet’ than from ‘Winterstar’. There is a need to confirm what species and varieties are most useful as break crops. Hay *et al*. (2002) planted plots of different species in a field with *P. penetrans*. High numbers of *P. penetrans* (9933-16,915/g dry weight of root) were obtained from Green bean ‘Montano’, Green pea ‘Onward’, Tic bean and Shaftal clover. Lower numbers (910-2679/g dry weight of root) were obtained from Blue lupin, Japanese millet, Rye corn, Onion and Carrot. Oats supported 598 *P. penetrans*/g dry weight of root and the lowest numbers (179-215/g dry weight of root) were obtained from Forage sorghum and Ryegrass ‘Nui’. LaMondia (1999) reported that highest

populations of *P. penetrans* were obtained from soil and roots of Garry oat, lowest populations from Triple S sorgho-sudangrass (*Sorghum bicolor* x *S. sudanense*) and Saia oat (*Avena strigosa*) and intermediate from strawberry, buckwheat and Humus canola (*Brassica napus* and *Brassica campestris*). Saia oat was suggested as a rotation crop to reduce *P. penetrans* numbers prior to strawberry (LaMondia 1999). Diamond *et al*. (1999) reported that high populations of *P. penetrans* after hay crops (red clover and timothy) or potato crops could cause problems in succeeding carrot crops. Kimpinski *et al*. (1988) suggested that annual ryegrass (*Lolium multiflorum*) harboured lower numbers of lesion nematode than red clover or timothy and could be rotated with carrot where *P. penetrans* was a problem. Florini and Loria (1990) suggested that rye, wheat and sorgho-sudangrass would be better rotation crops than oat or corn to reduce *P. penetrans* populations prior to susceptible potato crops.

Marigolds

Marigolds have long been known for their suppressiveness against some species of nematodes. The demand for sustainable and environmentally responsible methods of managing nematodes has led to resurgence in interest in marigolds (Ploeg 2002). Marigold has been shown in some situations to be as effective as soil fumigation in controlling nematodes. At a field site with *Meloidogyne incognita*, yield of tomato (*Lycopersicon esculentum* 'Pixie') following *Tagetes patula* 'Single Gold', Tagetes hybrid 'Polynema' or methyl iodide fumigation was 156%, 151% and 171% respectively as a percentage of the tomato yield following a bare fallow, with no significant differences between marigold treatments or fumigation (Ploeg 2002). In comparison yield of tomato 'Pixie' following a susceptible tomato 'Peto98' was only 39% of that following a fallow. Galling of roots of tomato 'Pixie' was least following fumigation or Tagetes patula 'Single Gold', with slightly more galling following Tagetes hybrid 'Polynema', consistent with the observation that 'Polynema' allows some reproduction of *M. incognita* at high soil temperatures (Ploeg 2002).

Reynolds *et al*. (2000) studied the use of *Tagetes* spp. for the control of *Pratylenchus penetrans* in susceptible crops such as Tobacco (*Nicotiana tabacum*) in Ontario. Field plots of *Tagetes patula* 'Creole' and *T. erecta* 'CrackerJack' were compared as rotation crops with the traditional cover crop of rye plus fumigation prior to tobacco. A marigold density of 20 plants/m^2 reduced *P. penetrans* populations below the economic threshold for the rotation crop year and the two following years. Tobacco yield was increased by 197 kg/ha by marigold in comparison to rye plus fumigation. With establishment rates for *T. patula* and *T. erecta* of 45% and 56% respectively to achieve 20 plants/m^2 cost US $221/ha for *T. patula* and US $ 294/ha for *T. erecta*. This suggested that marigold rotation for control of root lesion nematode was a functional alternative to chemical fumigation (US $484/ha) for tobacco production (Reynolds *et al*. 2000).

Ploeg and Maris (1999) reported that the suppression of *M. incognita* differed amongst 6 marigold cultivars and 5 soil temperatures. *Tagetes signata* (syn. *T. tenuifolia*) 'Tangerine Gem' and the Tagetes hybrid 'Polynema' allowed reproduction and root galling when grown at 30^{o}C and were not recommended for control of this nematode in warm environments. When grown at 20-30^{o}C soil temperature, *Tagetes patula* 'Single Gold' and 'Tangerine' and *T. erecta* 'Flor de Muerto' significantly reduced root galling and nematode infestation in a subsequent tomato crop. When grown at 10^{o}C-15^{o}C, only *T. erecta* 'Crackerjack' reduced *M. incognita* on a subsequent tomato crop. It was suggested that marigolds should be grown at soil temperatures above 15^{o}C to ensure suppression of *M. incognita* in succeeding crops and that the nematode suppression of some *Tagetes* spp. may be prevented at high soil temperatures.

Biofumigation species for control of nematodes.

Biofumigation refers to the suppression of soil-borne pests and pathogens by the release of biocidal compounds when tissues of Brassica crops are incorporated into the soil. Much of the biocidal activity is thought to occur from hydrolysis of glucosinilates (GSL's) into isothiocyanates (ITC's) in the soil, although other biologically active compounds including nitriles and

thiocyanates are also released (Rosa and Rodriguez 1999). Glucosinilates are a naturally occurring class of sulphur compounds that occur in plants of the families Capparaceae, Brassicaceae (Cruciferae), Koeberliniaceae, Moringaceae, Resedaceae and Tovariaceae (Rosa 1997). Approximately 100 distinct glucosinilates have been identified and recently reviewed (Brown and Morra 1997). However, the biofumigation effect of these compounds also appears to arise from the combined action of a cocktail of volatile S-containing compounds produced during the decomposition of brassica tissues and not just the activity of ITC's. For example in addition to 2-phenyl ethyl GSL (2-PE GSL), Indian mustard has been shown to release a range of other non-GSL derived volatile sulphur compounds which also have biocidal activity including methanethiol, dimethyl sulphide, carbon disulphide and dimethyl disulphide (Matthiesen and Kirkegaard, 1999). There has been less work done with biofumigant crops with regard to nematode control than with fungal pathogens. Dr. Greg Walker (SARDI) has demonstrated the biocidal activity of a range of brassicas against citrus nematode (*Tylenchus semipenetrans*). In pot experiments, significant reductions in the populations of citrus nematode larvae were observed following incorporating with brassicas in comparison to untreated. Humus rape was most effective with a 78% and 81% reduction in nematode numbers following incorporation of 80 and 40 g/kg soil respectively (Matthiessen and Kirkegaard 1997). Stirling and Stirling (2003) achieved a significant reduction in *M. javanica* populations in soil amended with 8.5-17.0 t/ha dry matter of biofumigant Brassica. Several varieties of rapeseed (*Brassica napus*) were listed by Hagan *et al*. (1998) as being suppressive to root knot nematode species when incorporated into soil (Table 58). However, some varieties allow nematode reproduction during the growing season. Hagan *et al*. (1998) recommended planting in the fall and incorporation as a green manure 2-3 months later. Mature rapeseed (6 months old) had little effect on root knot nematode populations.

Stirling (1999a,b) noted that while brassicas are growing, the roots are not toxic to root-knot nematodes. Under favourable conditions, root knot nematode populations can increase on biofumigant brassicas by 20-60 times in 6-8 weeks. Since many of the newly produced nematodes may not be

killed when the crop is ploughed in, the brassica crops may increase rather than decrease nematode problems on the next crop in the rotation. Stirling (1999b) overcame this problem by growing biofumigant crops in the winter in Queensland when soil temperatures were lower and root knot nematode took some months to complete a generation. Stirling (1999a) suggested that under the growing conditions of south east Queensland, crops sown in early June would yield approximately 5t/ha dry matter by the end of August, sufficient for biofumigation purposes. Stirling (1999a) suggested that in cooler climates of southern Australia the period in which brassicas could be grown was likely to be longer, possibly from mid May to late September. This should be less of a problem in cooler regions of Australia, or where control of lesion nematode (*Pratylenchus* spp.) is required, as this nematode has a slower rate of multiplication than root knot nematode.

Potter *et al*. (1998) reported suppressive effects of glucosinilates in Brassica vegetative tissues on root lesion nematode (*Pratylenchus neglectus*). *P. neglectus* was found to reproduce well on canola varieties tested, often generating numbers comparable with those following a susceptible wheat crop. However, plants producing higher root levels of 2-PE GSL show greater resistance to invasion and multiplication by *P. neglectus*. Plants with high levels of 2-PE GSL also had a stronger biofumigation effect against *P. neglectus* (Potter 2001). Work is currently continuing on breeding biofumigant crops with resistance to nematodes.

Pattison (2003) reported that biofumigant Brassicas Weedcheck (*Raphanus sativus*), Nemfix (*Brassica juncea*), BQ Mulch (*B. napus/B. campestris*) and Fumus (*B. juncea*) had some resistance to root knot nematode (*Meloidogyne javanica*). Weedcheck was the most resistant biofumigant tested, however all were able to host some nematode multiplication and therefore had the potential to carry nematodes over to the following crop if a good kill was not achieved following incorporation. In comparison to untreated soils, the incorporation of leaf tissue of BQ Mulch, Fumus, Nemfix and Weedcheck at rates between 0.03 and 0.05 g leaf tissue per gram soil were able to

significantly reduce the number of root knot nematodes recovered from roots of tomato which were subsequently planted (Pattison 2003).

Glucosinilate content has been found to vary greatly amongst plant species and even cultivars within a species. There has been much work to breed cultivars with high levels of glucosinilates. In Australia, this has led to the development of a blend of brassicas in association with CSIRO, which is marketed as 'BQ Mulch' (Matthiessen and Kirkegaard 1998). Similarly, Ag-Seed research in a joint venture with Agriculture Victoria has released cultivars of mustard (*Brassica juncea*) as part of the 'Fumus' product range (Matthiessen and Kirkegaard 1999). There are also variations in glucosinilate content between plant parts, with some species having high contents in root tissue and others having high levels in both root and foliar tissues. For example, mustard shoot was more toxic than mustard root tissue because it contained higher levels of short-chain propenyl GSL. However, fodder rape root material is more potent than shoot tissue because it contains 2-PE GSL rather than the less toxic long-chain aliphatic GSL's in shoots (Matthiessen and Kirkegaard 1998). Differences in glucosinilate content between years, growing seasons and even during a single day have been reported (Rosa 1997). Plant maturity may also have a large impact on glucosinilate content. Kirkegaard *et al*. (1996) reported that canola and Indian mustard tissues at maturity had only a slight suppressive effect on several fungal pathogens. However, at flowering, they contained higher levels of 2-propenyl and 2-PE ITC's and enhanced their suppressive effect. The amount of plant material incorporated into the soil will also have an impact on the amount of ITC released in the soil. Conversion of GSL to ITC in the soil is also an important determinant of the success of biofumigation. For example Matthiessen and Kirkegaard (2001) reported that after incorporation of 'BQ Mulch' and 'Fumus' into the soil the maximum ITC concentration measured in soil did not exceed 1.0 nmol/g, which was only some 1% of the ITC potentially available in shoots at the time of incorporation. Further studies showed that disruption of plant tissue by freezing increased release efficiency from <1% to 26%. Wetter soil also maintained higher ITC concentrations (Matthiessen and Kirkegaard 2001). Matthiessen and Kirkegaard (2002 a,b) demonstrated that

incorporation of fodder rape and mustard into soil by mulching and immediate rotary hoeing, increased ITC concentration in the soil by 5-10x compared with rotary hoeing alone or mulch left on the surface. Rotary hoeing and mulching alone caused only a brief and low level release of ITC, with concentration dropping significantly after 2 hours. However, in the mulched and rotary hoed treatment, higher concentrations of ITC were present earlier with a slower decline over a period of 102 days. Irrigation following treatment at 2 and 7 days after incorporation produced a further release of ITC's after irrigation. However, where mulched plant material was left on the soil surface and watered immediately after the mulching operation, a large release of ITC's was noted at 2 hours after watering, especially in the mustard treatment. The concentration of ITC's was 100 nmol/g soil. Metham sodium produces a methyl ITC concentration of approximately 400 nmol/g soil in the top 30 cm of soil. The release of ITC by the mustard in this trial was comparable, especially when considered that many of the ITC's released from Brassicas are up to 10 times more toxic than MITC (Matthiessen and Kirkegaard 2002a,b, Kirkegaard *et al*. 1999). The results suggested that mulching is important to ensure best release of GSL and conversion to ITC and that watering of mulched residue left on the surface can be an effective strategy for maximising release. From estimates of the amount of GSL in plant tissue and the amount of ITC in soil in these experiments, it was calculated that the conversion efficiency was 30-40% (Matthiessen and Kirkegaard 2002a,b).

Potter (2003) demonstrated variations in root gluconsinilate levels in genetically identical double haploid canola grown in pots containing gamma sterilised soil from different locations. There was also variation between the soils in the toxicity of freeze-dried tissue containing similar amounts of glucosinilate to *Pratylenchus*, with higher toxicity in soils containing high levels of silt compared to low levels (Potter 2003). Tissue toxicity was increased with increasing fluoride concentration in soil. Factors such as minerals in the soil may therefore influence the toxicity of biofumigants to nematodes and other soil-borne pathogens.

One of the concerns with biofumigation is that at least part of the biocidal activity is similar to that of applying the soil fumigants metham sodium, vapam or basamid, i.e. the release of ITC into the soil. Metham sodium which releases MITC in the soil has been shown to suffer from enhanced microbial breakdown following repeated use which renders it ineffective (Matthiessen and Warton 2000). It is possible that biofumigant plants, which rely on the same mechanism of activity, may be susceptible to this same problem (cross-degredation). Matthiessen and Kirkegaard (1999) point out that biofumigant plants contain a mixture of ITC compounds and other biocidal compounds and are likely to be less susceptible to this effect than application of pure chemicals such as MITC from metham sodium. However, Matthiessen and Warton (2002) were able to demonstrate that the efficiency of release of ITC from mustard meal and the pesticidal effect of mustard meal on white fringed weevil was markedly reduced in a soil which exhibited enhanced biodegredation of MITC from metham sodium applications compared with a soil which had no history of fumigation. Matthiessen and Warton (2002) noted that risk of the development of enhanced biodegredation was greater in sandy soils of high pH and that an integrated approach to pest management was required to avoid over-reliance on metham sodium.

There are potential problems in achieving successful biofumigation in cooler regions. Subbarao and Hubbard (1996) recommended incorporation should occur when soil temperatures are at least 20^{o}C to achieve maximum efficiency with amendments. This has prompted some investigators to use biofumigation in association with solarisation. For example, Gamliel and Stapleton (1993) reduced numbers of viable propagules of *Sclerotium rolfsii* and *Pythium ultimum* by incorporating leaf and stem residues of dried and ground green cabbage (*B. oleraceae* var. *capitata*) at 2% w/w. However, effectiveness was increased by heating the soil to 38^{o}C, with viable propagules reduced by up to 95% within 14 days. However, solarisation greatly adds to the costs of treatment. Another potential problem to using biofumigant crops in cooler regions is that the availability of ITC in the soil depends on the soil physical properties. The amine and sulphydryl groups of ITC can react irreversibly with clay and organic matter (Wood 1975) and allyl-

ITC may react irreversibly with clay and organic matter (Kawakishi and Kaneko 1985). Therefore amendments in soils with higher levels of clay or organic matter are likely to have less biocidal activity than those applied to lighter soils (Rosa and Rodriguez 1999). However, recent trials by Serve-Ag research have shown in a red ferrosol soil in Tasmania that Fumus and BQ Mulch sown in July and mulched into the ground in October/November were able to reduce tip burn, bacterial rot and *Sclerotinia* in lettuce (Anon 2002). This indicates that biofumigation can be an effective strategy in cooler regions.

11.4 Planting date

Adjustment of planting date has been shown overseas to be an effective management strategy for root knot nematode on carrot, by planting when soil temperatures are sufficiently low that nematode juveniles are inactive in soil (Davis and Raid 2002). For example, a delay in autumn planting in California until soil temperatures fall below 18^{o}C avoids significant root infection by *M. incognita*. Similarly in Quebec Canada, early Spring plantings in May when soil temperatures range between 6-8^{o}C increased marketable yield by 20-50% in soils infested with *M. hapla* in comparison to mid-June plantings when soil temperatures have risen to 15^{o}C. This is related to the temperature dependent nature of the lifecycle of nematodes. (Table 60). Postponement of sowing by one month has also been shown to reduce quality damage in carrots caused by *Meloidogyne fallax* (Melendijk and Brommer 1998).

Table 60. Temperature requirements for *Meloiodogyne hapla* from: http://plpnemweb.ucdavis.edu/nemaplex/Taxdata/G076S2.htm

Activity	Temperature (°C) Minimum	Optimum	Maximum
Hatch	-	25	-
Mobility	-	20	-
Invasion	5	15-20	35
Growth	15	20-25	30
Reproduction	20	25	-
Survival	±0	-	-

11.5 Chemicals

The last 20 years has seen a steady attrition in the number of nematicides available to growers due to their toxicity and impacts on human health and environment. More recently, Bayer Corporation has announced the voluntary cancellation of Nemacur^{R} (fenamiphos), effective May 31, 2005. This will be the last date of manufacture, distribution and sale of this product. Fenamiphos has also been under review by the Australian Pesticides and Veterinary Medicines Authority (APVMA 2003) due to potential for contamination of groundwater and potential poisoning of waterfowl. Withdrawal of this chemical will leave a large gap in the chemical arsenal for control of nematodes.

Telone and Telone C35

Telone and Telone C35 were recently registered in Australia for use in vegetable ground. Concerns have been raised over these chemicals with regard to potential groundwater contamination. It is recommended that the product is not applied in areas where soils are highly permeable and ground water is near the surface or where aquifers and sink-holes are abundant (Anon 2004a). In the USA, modification of registration has included prohibition of use in certain norther tier states based on groundwater

concerns, a 100 foot no treatment buffer around drinking water wells, prohibition of use in areas overlying karst geologies (areas where aquifers and sink holes are common (Anon 2004b). However, the Australia evaluation of 1,3-dichloropropene reported that studies in the USA indicate that it is uncommon for Telone products to be measurable in groundwater folliowing application, and that where detected they were less than 5 ppb and transient, suggesting that despite extensive use of 1,3-dichloropropene occurrences in groundwater were not expected (NRA 2001). Gan *et al*. (2000) demonstrated that atmospheric emissions of 1,3-D could be reduced considerably with the application of ammonium and potassium thiosulphate fertilisers in conjunction with Telone. Such a strategy may reduce some of the risks associated with fumigation with 1,3-D.

MIDAS

The use of the general soil fumigant methyl bromide is banned from 2005 in accordance with the Montreal Protocol as it is an ozone depeleting substance. Midas^{R} or methyl-iodide is being developed as an alternative fumigant active against weeds, nematodes, insects and soil borne pathogens by Arvesta Corporation. Midas^{R} contains iodomethane and will be available in formulations that contain chloropicrin at various concentrations. Primary use will be in pre-plant soil fumigation of high value crops. Midas^{R} has the advantage over methyl bromide that it is a liquid at room temperature rather than a gas and is therefore safer to use. Midas^{R} is rapidly broken down by sunlight before it reaches the ozone layer and therefore does not have adverse environmental effects of methyl bromide. At present Midas^{R} is slightly more expensive than methyl bromide. However, novel delivery methods are being developed to lower the cost of the product. Midas^{R} is expected to receive US EPA approval before 2005. Cost may be a prohibitive factor for carrot production.

11.6 Other strategies for control of nematodes.

A variety of biological control products are available on the market for control of nematodes. This section will highlight a few which have gained some market acceptance, but is not to be considered a comprehensive review of products available.

Ditera

This product is produced by Valent laboratories and marketed in the USA (www.valentbiosciences.com). It is a killed fermentation product of the fungus *Myrothecium verrucaria* originally isolated from soybean cyst nematode. Trials with this product have been variable, especially on turf where it is thought that the high organic matter content of the soil reduces efficacy. The product is now available as a dry flowable formulation and has been used on a wide range of crops including carrot. It has up to 6 weeks activity in soil and has been approved in the US for use on organic crops. In some cases significant yield increases have been obtained with Ditera. Yield increases are sometimes noted in the absence of any reduction in nematode numbers. Studies have shown that in addition to reducing egg hatch, exposure to Ditera can reduce the movement of nematodes, affect the ability of the nematode to find its host and reduce feeding activity. This may explain why yield increases are sometimes noted in the absence of reduced nematode numbers. There are currently no plans to distribute this product in Australia and attempts to obtain material for trials during this project were unsuccessful.

Pasteuria penetrans

A range of fungi which parasitise nematodes or nematode eggs have been commercialised in the last 30 years as 'biological nematicides', e.g. *Paeciolomyces lilacinus*. However, the degree of nematode control achieved with such products has been very variable and often they have been withdrawn from the market. Recently there has been a resurgence in developing the bacterium *Pasteuria penetrans* as a biological control agent. *Pasteuria penetrans* has been associated with soils suppressive to certain nematode species (especially root knot nematode), indicating that it has the

capacity to be a very effective biological control agent in cropping situations. *Pasteuria* produces long-lived spores that adhere to and penetrate the cuticle of nematodes and grow and multiply within them. A single spore can kill a nematode and millions of spores can be produced within an infected nematode. This organism has long been regarded as one of the better potential biological control agents of nematodes. However the development of *Pasteuria penetrans* as a biological control agent has been hampered by an inability to culture it *in vitro*. A US company, Pasteuria Bioscience LLC, has recently developed a technique to culture large quantities of *Pasteuria* (Gerber *et al*. 2002). This is a major step forward and may offer an alternative method for control of nematodes for at least some species of nematodes in the not too distant future.

Other biological products for control of nematodes

There are a wide variety of 'biological' products available overseas for the control of nematodes. Noling and Gilreath (1999) tested a range of these for control of root knot nematode in tomato (Table 61). Results from three studies indicated that they provided little or only very weak nematicidal activity, with none producing a significant reduction in harvest root gall severity compared to the untreated control. Further information on such products and other methods of nematode control can be obtained from Dufour *et al*. (2003). Walker and Morey (1999) also tested several 'biological' products against the nematode *Tylenchulus semipenetrans* in citrus, including Prosper Nema (a formulation of nematophagous fungi), Nutri-life 3/20 (formulation of bacteria) and Tri-D25 (a formulation of *Trichoderma* spp.). None of these provided control of nematodes in this trial.

Addition of organic matter to soil can often lead to a suppression of nematodes (e.g. Rodriguez-Kabana 1986, Caswell and Bugg 1991). Experiments in this project (section 9.3) suggested that Johnson's pure lucerne fertiliser at above 15t/ha and poultry manure could reduce nematode damage in carrot. Nematode suppression following incorporation of organic matter has been attributed to the build up of populations of nematophagous fungi in the soil and to the release of nematicidal compounds such as

ammonia. However, the enhancement of nematophagous fungi by organic matter does not typically exert a strong effect on nematode population densities and the effect is short-lived (Kerry 1987). Amendments or N containing fertilisers that release ammonia have been shown to be nematicidal. Lazarovits *et al*. (1999) obtained suppression of plant parasitic

Table 61. List of biological products for control of nematodes tested by Noling and Gilreath (1999).

Product	Composition
-Actinovate Plus	*Streptomyces lydicus*
-Agri-50	Stablilised colloid mixture
-Champion Insect Control Concentrate	Mixture of pepper, mustard and citrus oils
-Deny	0.6% *Burkholderia cepacia*
-DiTera WDG	toxin derived from *Myrothecium verrucaria*
-Fumafert	rapeseed meal and neem oilseed meal
-Nemastop	Plant extracts and fatty acids
-Neotrol	Ground sesame plant
-Prosper Nema	Mycorrhizal spores
-Safety Green	Secondary alcohols
-SuperNeem	humic acid, seaweed extract, neem

nematodes by the addition of high rates of soymeal and meat and bone meal (37 t/ha). Chitin has been shown to reduce populations of nematodes after incorporation into soil. Chitin is a component of crustacean shells and a waste product of the seafood industry. Chitin is also a component of nematode eggshells. Nematode suppression is through the stimulation of chitinolytic organisms (bacteria and actinomycetes) that degrade nematode egg shells and the release of ammonia during decomposition (Spiegel et al. 1986, 1987, 1988). However, some 3-4 tons are required per acre to achieve a significant effect on nematode populations, making the cost of chitin prohibitive for many crops (Caswell and Bugg 1991). In addition ammonia release can by phytotoxic to sensitive crops. Fertilisers such as Urea, ammonium sulphate and calcium cyanamide have been shown to be nematicidal at high rates, through the release of ammonia (e.g. D'addabbo *et al*. 1996). However, this strategy is not often used because of potential

phytotoxicity to crop plants and the potential for run-off of nitrogen into streams and groundwater.

Trap crops

Trap crops are crops sown prior the main crop, which are used to 'trap' nematodes in the roots and destroy them prior to growing the main crop. Trap crops are usually used against cyst nematodes that hatch in response to host exudates and form sedentary feeding sites. There is potential for this strategy to be used for control of root knot nematodes, as *Meloidogyne* also form sedentary feeding sites in plant roots. This strategy involves involves i) planting a trap crop that is a host for the nematode, ii) allowing time for nematodes to migrate into the roots of the developing seedlings and form sedentary feeding sites, iii) destroying the crop prior to nematodes producing eggs, iv) planting the main crop. If insufficient time is allowed for the nematodes to form sedentary feeding sites, then nematodes will merely migrate out of the roots of the trap crop and into the soil from which then can invade the roots of the subsequent crop. Conversely, if too much time is allowed, then nematodes in the trap crop may be able to reproduce and increase the population density prior to the crop being planted. The correct timing of trap crops is often difficult to achieve and the added costs and effort of cultivating, planting and killing off the trap crop are added disincentives to growers adopting this strategy. This strategy could be improved by research to determine the number of degree-days necessary for a particular root knot nematode species to develop through its life stages. Monitoring soil temperatures and destroying the trap crop after the number of degree days necessary for nematodes to invade and form sedentary feeding positions, but before egg production, could then be an effective and less-risk strategy. Belair and Benoit (1996) reported that the weed *Senecio vulgaris* formed galls as a result of *M. hapla* feeding, but eggs or J2 were not produced. This suggested that *M. hapla* invaded and produced a feeding site, thereby stimulating galling, but did not develop further. Belair and Benoit (1996) suggested therefore that *Senecio vulgaris* might be developed as a trap crop for *M. hapla*. As a trap crop, *S. vulgaris* would have the added advantage that timing of the destruction of the crop would not be so critical compared to a

plant species that allowed nematodes to develop to the egg stage. However to our knowledge, little further research has been attempted in this area.

12. Recommendations for growers

- **Adopt a strategy of nematode monitoring rather than merely conducting pre-plant counts prior to susceptible crops**. Monitoring nematode numbers through the rotation (even for those crops which are not susceptible) would provide valuable information on whether nematode numbers are increasing or decreasing in a particular field. The nematode count at the end of the previous crop in the rotation may be more of a reflection of the potential hazard to the subsequent susceptible crop than a pre-plant count conducted after a fallow period in winter when nematode numbers have fallen to below detectable limits.
- **Increase the intensity of sampling**. To achieve reasonably accurate estimates of nematode populations, there is a need for a bulk sample consisting of 40-50 samples per hectare taken to a depth of 20-30 cm. This is then gently crumbled by hand, mixed well and a subsample of 400-500 (grams or millilitres) is sent to the laboratory for analysis. The accuracy of any nematode count or other soil test is dependent upon providing a representative sample to the laboratory. Keep the soil sample at a moderate temperature (10-20^{o}C) after collection. Extremes of heat or cold may kill nematodes and lead to an inaccurate nematode count. Many laboratory extraction techniques rely on nematode movement to separate nematodes from soil prior to counting. There may be potential for the grower to intensively sample areas of the field in which there has been a recognised nematode problem in previous years. Monitoring these areas would give an indication of whether nematode numbers have been increasing or decreasing in the rotation after the previous susceptible crop and give the greatest chance of detecting nematodes. There may also be opportunity to economise on nematicide by treating only those areas of

the field known to have had previous nematode damage and their surrounds.

- **Know the nematode history of the field and link this to the crop rotation**. For example if there has been a root knot nematode problem in carrot previously in the rotation then a knowledge of what crops have been grown subsequently and a knowledge of the host range of the nematode will give an indication of whether populations of nematodes are likely to have increased or decreased.
- **Use fallow periods or break crops**. Bare fallowing or planting break crops is a useful strategy for reducing nematode populations prior to planting a susceptible crop. Bare fallow has been shown to reduce *Meloidogyne* populations by 75% after 1-2 months, with less than 10% survival after 3-4 months. However while significant, such reductions may not be sufficient to ensure that the crop does not suffer economic damage. If a bare fallow is to be instituted then control of weed hosts is of great importance. Using break crops which are non-hosts or suppressive to nematodes is an alternative to bare fallow and has advantages of soil conservation and adding organic matter to the soil. However, this relies upon knowing which species of nematode is present and its particular host range. Mixed species of *M. hapla* and *M. javanica* were found in Victoria and Western Australia in carrot crops. However, *M. hapla* was the dominant plant-parasitic nematode in Victoria, whereas *M. javanica* was the dominant root-knot nematode species in South Australia and Western Australia. Forage sorghum cv. Jumbo was identified as a poor host to non-host of *M. javanica* and *M. hapla* and therefore a good break crop for both species. However, forage sorghum cv. Supergraze was a good host of *M. javanica* and cannot be recommended for control of this nematode. Rapeseed (*Brassica napus* cv. Dwarf Essex) was also a good host of *M. javanica*. Radish 'Weedcheck', mustard 'BQ Mulch' and lucerne 'Rippa' were identified as good hosts of *M. javanica* and *M. hapla*. However, wheat 'Baxter', Oats 'Taipan' and maize 'DK689' were identified as poor hosts of *M. hapla*, but good hosts of *M. javanica*. Biofumigant species may

be useful but note that some are good hosts of nematodes. Biofumigant species should be grown in cooler months of the year to reduce nematode build up on roots, and incorporated well to achieve the maximum biofumigation effect. However, as these crops release isothiocyanates similar to metham sodium, they may not be effective on ground that has suffered from enhanced biodegredation of metham sodium. Other break crops mentioned in section 11.3 could also be used if available.

- **Use nematicides wisely**. Follow the label recommendations for rate and application method. Do not use one active ingredient continually on the same ground to prevent the development of enhanced biodegredation. Telone (nematicide only) and Telone C35 (nematicide and general soil fumigant) are recently registered potential alternatives to currently used chemicals such as fenamiphos (Nemacur) and metham sodium. However, care is required with their use on areas with sandy soils and high rainfall/irrigation, to prevent leaching into the ground water. Nematicides are costly and are amongst the most hazardous of agrichemicals used on the farm. Nematicides should always be combined with monitoring of nematode populations in the field to ensure that they are only applied when necessary. This will minimise impacts on the environment, prevent the onset of enhanced biodegredation and ultimately prolong the effective life of the few chemicals currently available.
- **Adjust planting date**. Adjustment of planting date has been shown overseas to be an effective management strategy for root knot nematode on carrot, by planting when soil temperatures are sufficiently low that nematode juveniles are inactive in soil. For *M. incognita*, a species that is adapted to warmer climates, a delay in autumn planting in California until soil temperatures fall below 18^{o}C avoids significant root infection. A similar strategy could be adopted for *M. javanica*. For *M. hapla*, a species adapated to cooler climates, early Spring plantings in May in Quebec Canada, when soil temperatures range between 6-

8°C increased marketable yield by 20-50% in soils infested in comparison to mid-June plantings when soil temperatures have risen to 15°C.

- **Future developments**. Future improvements to nematode control in carrots may arise from the development of DNA testing for nematodes, resistant carrot varieties, biofumigant Brassica species which are resistant to nematodes and the commercial development of the bacterium *Pasteuria penetrans* as a biological control agent.

13. Conclusions and further research needs

Management of nematodes in carrots in Australia is heavily reliant upon the use of nematicides such as metham sodium and fenamiphos (Nemacur). Production of Nemacur in the USA is to cease in 2005. This, and the development of enhanced biodegredation in soils regularly treated with fenamiphos or metham sodium, suggests that alternative strategies will be required for nematode control in the future. The strong market demand for reduced pesticide in food production will also put pressure on the use of nematicides. While Telone and Telone C35 have been identified as alternative chemicals in this project, care must be exercised to ensure that these chemicals (and other nematicides) are used in an environmentally responsible manner, especially with respect to preventing ground water contamination. An integrated strategy for control of nematodes is advocated involving pre-plant nematode counts to determine the need for nematicides and suitable crop rotations or break crops to reduce nematode populations in soil prior to carrot. This will reduce the need for nematicides and thereby reduce the likelihood of enhanced biodegredation occurring. Other methods of control described in this report that may be useful in particular cases include manipulation of planting date.

With potential loss in availablility of nematicides there is a need to move back to a cropping system approach to nematode management. However, this requires detailed biological information on a) identification of the nematode species, isolate, races, pathotypes, b) population density present, c) relationship between nematode population density and yield/quality, d) nematode biology, host range and population dynamics, e) effects and economics of control treatments (Wang and McSorley 2001). Wang and McSorley (2001) point out that many of these factors are site specific and that research is needed on rotation crops for different regions.

To achieve a cropping system approach to nematode control in vegetable production there will be a requirement to:

- **Develop more sensitive tests for nematodes and tests that quantify individual species.** The advent of DNA based soil tests will enhance ability to identify and quantify individual nematode species in the soil.
- **Identify suitable break crops and suppressive crops for particular regions.** Host range studies of suppressive crops (marigold, resistant biofumigant varieties) and potential break crop species would allow the grower more choice in types of break crop. Such studies would need to include an assessment of the host status of varieties within plant species as these may vary in their host response to particular nematode species.
- **Establish damage thresholds.** This will require the determination of the relationship between nematode tests and nematode damage to carrot crops. Accuracy could be improved by determining this relationship in particular regions and perhaps for particular times of year, given that the rate of nematode development is temperature dependent.
- **Assess resistance/tolerance.** Assessment of the resistance/tolerance of commercially available carrot varieties and development of resistant carrot varieties acceptable to the market place.
- **Investigate manipulation of planting date.** Adjustment of planting date to avoid nematode damage is a useful strategy employed overseas. A better understanding of the relationship between temperature and development of particular nematode species would aid in such decisions and in establishing thresholds.
- **Investigate currently non-registered chemicals.** Some older chemicals such as carbofuran and oxamyl were tested in this project. These have been used as nematicides in vegetable production in the past but are no longer registered for this use. The registration of such materials in vegetables could be pursued as a short term measure to

replace chemicals that are being removed from the market or for use in situations where the efficacy of current chemicals has been reduced by enhanced biodegredation.

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Appendix 1. Extension activities

Davison E (2003) Improved control of nematodes in carrot production. Oral Presentation at the Carrot Field Day, Medina Research Station 20th March 2003.

Davison E, McKay A, Hay F (2002) Nematodes – galling problem for carrot growers. The Western Australian Grower Vol. 34 (3) 14 –15.

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Additional activities:

Tony Pattison submitted a report on the efficacy of Rugby 200L® to the manufacturers.

Deborah Keating gave a presentation to several Victorian growers during the sampling of soil for the bio-degradation of nematicide testing in 2002. A sampling kit was sent to each participating grower which consisted of information on sampling correctly for accurate results and two free vouchers for nematode testing through the Victorian plant diagnostic service, Crop Health Services to further encourage the adoption of testing as part of the growers' paddock preparation.

Frank Hay participated in preparation and delivery of a short course to 13 field officers and growers as part of the University of Tasmania School of Agricultural Science, Agricultural Professional Development Course in Plant Pathology during 2002. This course included lectures on plant-parasitic nematodes and their management with particular reference to pyrethrum and carrot production.