Workshop to develop research, development and extension priorities for nematode control in vegetable crops

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Final Report

for

Horticulture Australia Ltd.
Project VG05026

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October 2006

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Purpose of project:

This workshop involved a gathering of vegetable nematologists and industry representatives to discuss and formulate future research, development and extension priorities in vegetable nematology.

Funding:

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Part 1 – Workshop and recommendations

Media summary

Plant-parasitic nematodes have been estimated to cause annual farm gate losses in Australian vegetable crops of $9.0 M in carrot, $3.0 M in lettuce, $12.1 M in potato and $10.6 M in tomato, with significant losses likely in other crops such as brassica. In general the vegetable industry has become reliant upon chemical fumigants and nematicides for nematode control. However, there is continual pressure on availability of these chemicals due to environmental and health concerns and due to the development of soils with capacity for enhanced biodegradation leading to poor efficacy. For example, Bayer (USA) has announced the voluntary withdrawal from production of one of the most commonly used nematicides in the vegetable industry (fenamiphos) in 2007. It is therefore timely to reassess nematode management strategies in vegetable crops.

A workshop involving 21 nematologists, agronomists and industry personnel was held on 10-11th July 2006 to discuss and formulate future research, development and extension priorities in vegetable nematology resulting in the following recommendations:

1. Prepare extension material for use by growers and consultants to improve the way nematodes are managed in the vegetable industry. *Currently there is no Australian-produced guide to help vegetable growers manage nematode problems.*
2. Demonstrate the value of rotation crops for root-knot nematode (*Meloidogyne* spp.) control in various vegetable-growing regions of Australia. *Root knot nematode is the major nematode pest of vegetable crops. Cover crops such as forage sorghum have proven effective in some vegetable growing regions due to its resistance to the common species of root-knot nematode. There is a need to demonstrate its use in other regions and identify alternative cover crop species where it is not suitable.*
3. Establish regionally-based, multi-disciplinary research groups to develop sustainable farming systems and soil management practices for local vegetable industries and ensure that there is adequate

nematological input into each research group. *Farming systems which enhance biological activity in soil through practices such as minimum tillage, crop rotation, green manuring, organic amendments and organic mulches have been demonstrated to result in soils which are suppressive to nematodes and other soil borne pathogens. The challenge is to further develop and increase the adoption of such systems.*

4. Enhance the adoption of DNA technologies for identifying and quantifying nematodes. *DNA technologies offer considerable advantages over conventional methods for identifying and quantifying nematodes. Commercial tests are available for nematodes of importance to the cereal industry. Much of the ground-work has been completed for nematodes of interest to the vegetable industry, however there is a need to further develop and commercialise such tests.*
5. Increase the number of nematologists working in the vegetable industry and ensure that programs are in place to provide the industry with nematological expertise in the long term. *Investment in vegetable nematology has been minimal over the last 15 years. Given the size of the industry and the importance of nematodes there is a need to increase R,D&E effort. In addition, nematology training has been depleted in recent years and several nematologists are due for retirement which will lead to a serious skill shortage.*
6. Support basic research that has the potential to lead to the development of innovative control strategies. *Basic research is developing a better understanding of plant-nematode interactions. Such research has the potential to lead to more targeted conventional plant resistance breeding, identify mechanisms of resistance which may be incorporated into genetically modified plants or the identification of a new generation of highly specific nematicides.*
7. Enhance Australia's biosecurity by characterising the plant-parasitic nematodes present in Australia and by developing rapid and reliable diagnostic procedures for major pests. *To prevent the introduction of new nematode pests, there is a need for reliable information on the distribution of nematode species within Australia and to be able to rapidly and reliably identify new introductions.*
8. Review progress on recommendations in 2009 and make appropriate changes where required.

Technical summary

Plant-parasitic nematodes have been estimated to cause annual farm gate losses in Australian vegetable crops of $9.0 M in carrot, $3.0 M in lettuce, $12.1 M in potato and $10.6 M in tomato, with significant losses likely in other crops. In general the vegetable industry has become reliant upon fumigants (metham-sodium, 1,3-D, 1,3-D/chloropicrin and non-fumigant nematicides (fenamiphos) for nematode control. However, there is continual pressure on availability of these chemicals due to environmental and health concerns and the development of soils with enhanced biodegradation which quickly renders the chemical ineffectual. In addition, Bayer (USA) has announced the voluntary withdrawal from production of one of the most commonly used nematicides in the vegetable industry (fenamiphos) in 2007.

A workshop involving 21 nematologists, agronomists and industry personnel was held on 10-11th July 2006 to discuss and formulate future research, development and extension priorities in vegetable nematology, resulting in the following recommendations:

Recommendation 1. Prepare extension material for use by growers and consultants to improve the way nematodes are managed in the vegetable industry.

Reasons behind the recommendation. There is no comprehensive, Australian-produced guide to help vegetable growers understand their nematode problems and improve nematode management. Much research has been done in the last 15 years but the results are found in the scientific literature or are presented in reports and publications that are often difficult to obtain. Consultants working in the vegetable industry have little knowledge of nematodes and so many potentially useful nematode management options are not being used by growers.

Tasks involved. Commission a person/group with the appropriate skills to compile a booklet and/or web-based publication containing i) basic information on the important nematode pests of vegetable crops, ii) damage thresholds and the impact of environment on crop losses due to nematodes, iii) management options (chemical and non-chemical), iv) examples of management systems that are relevant to particular crops, nematodes or regions. Use the information in the booklet to run workshops on nematode management for growers and consultants in all major vegetable-production regions

Responsibilities of HAL and Ausveg

- Commission the production of the booklet and provide appropriate funding
- Support the nematode management workshops

Timeframe. To be completed by December 2008.

Recommendation 2. Demonstrate the value of rotation crops for root-knot nematode control in various vegetable-growing regions of Australia

Reasons behind the recommendation. Forage sorghum has proved an excellent rotation crop in some vegetable-growing regions (e.g. Bundaberg) because it is easy to grow, it is resistant to all the common species of root-knot nematode and is vigorous enough to out-compete weeds capable of hosting the nematode. There is an urgent need to expand its use to other areas and to find alternatives in situations where it is not suitable.

Work required. List crops that are likely to be agronomically suitable rotation crops in various vegetable-growing areas and then use the extensive literature on root-knot nematode resistance in various crops to compile a short-list of candidates that warrant testing. Test these crops at nematode-infested field sites and in the glasshouse and then promote adoption of

nematode-resistant rotation crops that are appropriate for current farming systems.

Responsibilities of HAL and Ausveg

- Fund a three-year project on crop rotation for root-knot nematode control and ensure that several field trials are done each year in all important vegetable-growing areas.

Timeframe. Commence the project in 2007 and complete the work by 2009.

Recommendation 3. Establish regionally-based, multi-disciplinary research groups to develop sustainable farming systems and soil management practices for local vegetable industries and ensure that there is adequate nematological input into each research group.

Reasons behind the recommendation. Current vegetable farming systems often include practices such aggressive tillage, lack of crop rotation, long periods of fallow, few inputs of organic matter, routine fumigation and plastic mulching. These practices deplete soil organic matter and exhaust the soil food web. Since this food web contains the natural enemies of nematodes, the key to sustainable nematode management is to change the farming system so that it enhances the soil's biological status through practices such as minimum tillage, crop rotation, green manuring, organic amendments and organic mulches.

Work required. Multi-disciplinary research groups must be established whose primary focus is the development of farming systems that are profitable and sustainable. A good model is the one used by SRDC in the Sugar Yield Decline Joint Venture, a research program which ran from 1993 to 2006 and developed a more profitable and sustainable farming system for the Queensland sugar industry. Since different solutions will be required for different crops and regions, research groups will be formed to concentrate specifically on vegetable production in three regions where nematodes cause

major problems (i.e. northern Tasmania, the inland production areas along the River Murray in SA, Victoria and NSW, and Bundaberg in Queensland). A nematologist/soil biologist (at least 0.5 FTE) would be assigned to each group and would be responsible for monitoring nematode populations, assessing appropriate rotation and cover crops, understanding economic thresholds and checking soils for changes in suppressiveness to nematodes.

A fourth research group would be established to tackle farming systems issues specifically related to carrot production. It is envisaged that this group would be located in WA, due to the importance of carrots in that state. The reason for carrots being dealt with separately is that some tillage will always be required and economic thresholds for damage may be lower than for other crops. Because soil-borne diseases are major causes of market rejection in carrots, a research program of this nature will require strong inputs in plant pathology and nematology and will need to focus on understanding the physical and biological factors which cause problems such as forking and splitting. From a nematological perspective, it will be important to study damage thresholds and the variability of nematode populations within fields, as this will enable informed decisions to be made on the nematode management practices likely to be useful in the carrot industry.

Note: It is assumed that farming systems work in the potato industry will be done as part of the current Processing Potato R&D program.

Responsibilities of HAL and Ausveg

- Work with local industry bodies, innovative growers and state-based research organisations to establish and fund four regional, multi-disciplinary research groups and ensure adequate technical leadership of each group.

Note: Vegetable nematologists also have a responsibility to help foster the development of these research groups, and to ensure that their research is done within a sustainable farming systems framework.

Timeframe. The timeframe will depend on the resources available and the enthusiasm of regional groups. Projects do not have to be run concurrently, but the aim should be to establish groups by the end of 2008 and complete initial five-year projects by 2013.

Recommendation 4. Enhance the adoption of DNA technologies for identifying and quantifying nematodes

Reasons behind the recommendation. DNA technologies offer considerable advantages over conventional methods for identifying nematodes and quantifying nematode populations in soil. Australia leads the world in the development of these technologies, with DNA diagnostic tests for nematodes and other soil-borne pathogens being used widely in the cereal industry. Much of the ground-work has already been done for nematodes of interest to the vegetable industry but DNA diagnostic tests are still not available commercially.

Work required. Concentrate initially on root-knot nematode, because it is the most important nematode pest of vegetables and a diagnostic test has already been developed and validated. Work towards ensuring that the test is available commercially and that consultants are trained in sample collection and interpretation of results. Once that has been achieved, extend the test to the species level by including all the important *Meloidogyne* species in Australia. Continue to develop tests for other economically important nematodes and add them to the suite of vegetable tests as they are developed and validated.

Responsibilities of HAL and Ausveg

- Consult with SARDI, CSIRO and Bayer Crop Science and agree on a strategy and timeframe for commercialising their tests for *Meloidogyne*. Decide on cost-sharing arrangements that will deliver other diagnostic tests to the vegetable industry over time.

- Support emerging technologies in nematode identification and quantification that might be developed by other research organisations

Timeframe. A diagnostic test for *Meloidogyne* should be available commercially by 2008, with other tests to be delivered over the following five years.

Recommendation 5. Increase the number of nematologists working in the vegetable industry and ensure that programs are in place to provide the industry with nematological expertise in the long term

Reasons behind the recommendation. Based on the number of research and extension projects funded in Australia over the last 15 years, it is clear that investment in vegetable nematology has been minimal (less than 0.6 FTE/year). This means that several important vegetable-growing areas have had virtually no nematological input in recent years and relatively few aspects of nematode control have been investigated in detail. Given the size of the industry and the importance of nematodes, it is not unreasonable to expect that three nematologists (out of the 15-20 nematologists currently employed in Australia) should be working in the vegetable industry. Another matter of concern is that with impending retirements, current levels of research funding and the paucity of opportunities for training young people in the discipline, it is possible that the vegetable industry will not have any nematologists capable of addressing its nematode problems within 15 years. Steps must therefore be taken immediately to rectify this situation.

Work required. The problems faced by nematology are common to all specialist areas of agricultural science and cannot be solved by the vegetable industry alone. A coordinated effort from industry, research-funding bodies, tertiary institutions, co-operative research centres, biosecurity agencies and state-based employers of agricultural specialists is required to overcome the problem. A variety of solutions are available, including scholarships and

bursaries for post-graduate students, mentoring programs, improved career opportunities for graduates in agricultural science, and research funds allocated specifically for young researchers. Programs of this type will help all disciplines, but the most important issues for nematology are opportunities for post-graduate training at universities and support for experienced nematologists to run short courses and training programs for consultants, technicians and other professionals.

Responsibilities of HAL and Ausveg

- Cooperate with funding bodies such as GRDC, SRDC and RIRDC by collectively supporting at least one postgraduate student in nematology per year.
- Support the recommendations in this action plan, because this will increase in the number of nematologists working in the vegetable industry.

Timeframe. To be implemented during the period 2007-2012.

Recommendation 6. Support basic research that is likely to lead to the development of the next generation of nematicides

Reasons behind the recommendation. The chemicals currently used for nematode control are under pressure in the marketplace because they are highly toxic materials with a relatively broad spectrum of activity. The next generation of nematicides are likely to be much more acceptable because they will comprise new classes of molecules that act specifically against nematodes by targeting nematode feeding or sensory processes, or by inhibiting the development of nematode feeding sites in the plant. These nematicides will not be toxic to non-target organisms and will probably act by systemic uptake and movement in the plant.

Work required. Basic research on cellular and molecular approaches to nematode control is being done in many countries, including Australia.

Although it is difficult at present to predict which approaches are likely to be most successful, it is important that this work continues and that some of it is done in Australia.

Responsibilities of HAL and Ausveg

- Maintain a watching brief on the basic nematological research that is being done in Australia
- Provide industry support for scientists seeking funding from bodies such as ARC
- Seek out opportunities to invest in basic research projects that are likely to produce outcomes with commercial potential.

Timeframe. On-going.

Recommendation 7. Enhance Australia's biosecurity by characterising the plant-parasitic nematodes present in Australia and by developing rapid and reliable diagnostic procedures for major pests

Reasons behind the recommendation. If Australia is to prevent the introduction of new nematode pests, it must have reliable information on the distribution of nematode species within the country and be able to identify nematodes that may have been recently introduced or found in quarantine situations.

Work required. Most of the nematodes which are a threat to Australia's biosecurity are new species or races of genera that already occur in Australia. Thus to identify them, specialist taxonomic expertise is required and it must be supported by the development of appropriate DNA diagnostics. Although these issues are primarily the responsibility of Biosecurity Australia and the National Plant Biosecurity CRC, the vegetable industry must ensure that the priorities of these organisations are appropriate and that they are doing everything possible to protect the industry's interests.

Responsibilities of HAL and Ausveg

- Regularly review the biosecurity work being done in Australia and ensure that adequate resources are devoted to nematodes considered a threat to the vegetable industry.

Timeframe. On-going.

Recommendation 8. Review progress on this action plan and make appropriate changes where required

Reasons behind the recommendation. An action plan of this nature will take 7-10 years to implement. It is therefore appropriate that progress is monitored and priorities are revised as circumstances change.

Work required. Review this action plan

Responsibilities of HAL and Ausveg

- Appoint appropriate reviewers to assess the progress made in this action plan
- Decide whether the plan is still relevant to industry needs and determine whether adequate resources are being devoted to implementing the plan.

Timeframe. Undertake the review in 2009.

1. Introduction

This workshop involved a gathering of vegetable nematologists and industry representatives to discuss and formulate future research, development and extension priorities in vegetable nematology. The workshop was instituted in response to a number of factors including, i) the reliance of the vegetable industry on nematicides and fumigants for control of nematodes in vegetable crops, ii) the withdrawal from production of one of the most commonly used nematicides in Australia (fenamiphos) in the USA in 2007, iii) the limited range of alternative nematicides available to the vegetable industry, and iv) continued pressure on the availability of nematicides due to environmental and human health issues and reduced efficacy due to the development of soils with enhanced biodegredation. There is therefore a need to develop alternative strategies for control of nematodes as practical alternatives or adjuncts to nematicide use.

The workshop involved 21 participants of mainly nematologists and vegetable agronomists (Appendix 1). The meeting was structured as per appendix 2 with a separate discussion of several key strategies for control of nematodes. Participants were given a handout of potential discussion points within each strategy, based on a literature review conducted by the workshop organiser (Dr. Frank Hay, TIAR) based on a literature review (Part 2 of this report). In addition presentations on specific Issues were given by Drs. Graham Stirling (Biological Crop Protection), Jackie Nobbs (SARDI) and Kathy Ophel Keller (SARDI). At the end of the workshop a list of potential management strategies for nematodes was circulated (Appendix 3) and participants were asked to rate particular strategies for their ability to provide improvements to nematode management in vegetable crops following additional research and development or extension. As a follow up to the meeting, Industry Development Officers S. Welsh (Tasmania) and C. Feutrill (South Australia) undertook to gain further information from carrot industry groups.

2. The importance of plant parasitic nematodes to vegetable production.

While plant-parasitic nematodes are capable of causing major losses in particular crops, losses can be difficult to quantify. Feeding by some nematode species can cause obvious symptoms and signs (e.g. galling by root knot nematode, *Meloidogyne* spp.), while others may reduce yield by acting as an additional stress on the plant. The following estimates of losses in vegetable crops have been made in the USA, with similar losses likely in Australia:

- Nematodes caused 11% loss in 24 vegetable crops in USA (Feldmesser *et al*. 1971)
- Koenning *et al*. (1999) estimated losses in a range of vegetable crops in USA, generally of up to 10%.
- Root knot nematodes (*Melodogyne* spp.) were ranked 2nd out of 16 most important diseases of carrot in terms of impact on yield in USA. (Davis *et al*. 1999).

In Australia, nematodes have been estimated to cause $A300-450 M losses per annum in all crops, including carrot (6%), lettuce (4%), potato (3%) and tomato (6%) (Stirling 1992a). In terms of current farm gate values (Anon 2004) this would equate to annual losses of $9.0 M in carrot, $3.0 M in lettuce, $12.1 M in potato and $10.6 M in tomato crops in Australia (Table 1).

Table 1. Gross production of vegetable crops in Australia and percentage of production grown in different States (Anon 2004a).

	Farm gate	Gross	Production by State (%)					
	value ($ M)	production (t)	NSW	VIC	QLD	SA	WA	TAS
Asparagus	62.3	13,921	9.5	83.1	7.0	-	0.5	-
Beans (French/runner)	48.6	33,687	3.8	8.6	47.3	0.2	2.4	37.8
Beetroot	7.1	39,013	-	1.5	94.1	0.2	0.5	-
Broccoli	54.7	45,901	5.4	41.5	25.2	2.4	6.9	18.7
Brussels sprouts	8.6	5,305	-	46.3	-	42.3	2.7	11.2
Cabbages	15.7	76,093	25.0	39.9	9.5	9.5	6.8	1.6
Capsicum	48.4	41,859	0.7	0.3	95.7	1.1	2.3	<0.1
Carrot	150.6	331,130	6.2	34.2	7.8	14.2	26.6	10.8
Cauliflower	37.5	87,586	15.4	27.5	17.2	9.2	23.2	7.6
Celery	17.9	48,132	-	61.1	21.7	6.1	10.5	0.5
Chinese cabbage	5.3	11,513	25.8	8.3	53.6	0.5	11.8	-
Cucumbers	14.7	14,390	36.6	-	39.4	6.5	13.6	0.2
Garlic		300						
Leeks	15.7	6,683	10.2	60.4	-	28.1	-	1.4
Lettuce	76.2	135,015	20.1	24.4	38.9	4.6	10.5	1.4
Onions	139.6	282,517	13.9	9.2	11.2	37.4	6.0	22.3
Potato	404.8	1,333,159	11.9	22.1	8.8	25.1	5.8	26.2
Pumpkin	27.2	96,331	20.8	8.1	49.0	6.8	12.2	2.0
Spinach		4,000						
Spring onion (shallots)	12.4	5,290	12.0	33.9	45.5	4.2	2.8	1.7
Sweet corn	43.3	80,467	51.4	8.6	37.6	0.8	14.9	0.1
Tomatoes	177.1	424,950	8.2	61.8	25.8	0.7	3.3	0.2
Zucchini	19.6	15,231	15.3	9.7	68.1	1.0	5.7	0.1

Specific yield losses due to root knot nematode (*Meloidogyne* spp.) and lesion nematode (*Pratylenchus* spp.) have been measured in carrots in Australia.

- In commercial fields of carrot in Tasmania plots with low population density of *Pratylenchus* at 58 days after sowing had 12.1t/ha (year 1) and 15.9-22.3 t/ha (year 2) greater yield than plots with high numbers of *Pratylenchus* (Hay *et al*. 2004).
- In a South Australian carrot field containing *Meloidogyne*, spp., plots treated with Telone C35 or metham sodium yielded between 58.3-75.7 t/ha in comparison to only 24.4 t/ha in the non treated plots (Hay *et al*. 2004).
- In Western Australia, treatment of plots containing *Meloidogyne* spp. with Telone or Telone C-35 led to 64.1-65.2% export quality carrots (by weight) in comparison to only 35.0% in non treated plots (Hay *et al*. 2004). In a second experiment treatment of plots with Telone or Telone C-35 led to 45.3-47.6% export quality carrots (by weight) in comparison to 10.7% in non-treated plots (Hay *et al*. 2004).

Nematodes were identified as an issue at the National Vegetable Plant Pathologists Meeting, (May 2006). Nematodes were rated of high concern in root vegetables, root knot nematodes a significant problem in brassicas in NSW and WA and nematodes a significant problem in field grown tomatoes, particularly cherry tomatoes in the Sydney basin and in SA. In addition, issues of nematode management and biodegradation of chemicals were recently identified as having high economic impact pre farm gate in the Western Australian Carrot Association for Research and Development Industry Plan (2005-2010).

3. Current status of nematode control in vegetable crops in Australia.

The following generalisations can be made about management of nematodes in vegetable crops in Australia:

1. There are few vegetable varieties available with resistance to nematodes.
2. The vegetable industry has become reliant upon fumigants (metham-sodium, 1,3-D, 1,3-D/chloropicrin and non-fumigant nematicides (fenamiphos) for nematode control. However, there is continual pressure on availability of these chemicals due to environmental and health concerns and problems with the development of enhanced biodegradation with continual usage.
3. There is a lack of confidence in damage thresholds based on pre-plant counts as thresholds may vary with host susceptibility and environment (e.g. region, soil type etc.).
4. Nematicides/fumigants are often applied on an 'insurance' basis without regard for the population density of nematodes.
5. There is some use of non-host break crops or biofumigant crops to control nematodes in vegetable crops. However, the use of particular break crops may be compromised by a lack of knowledge of the particular species of nematode present and a lack of information as to the host status of particular break crops.

4. Nematodes of importance to vegetable crops in Australia.

Root knot nematode (*Meloidogyne* spp.) was considered by the workshop participants to be by far the most damaging nematode genus to vegetable production in Australia. This agrees with similar assessments overseas. Various *Meloidogyne* species have been reported on vegetable crops in Australia (Table 2). However, few surveys have been conducted to determine the prevalence of particular species, which is likely to be important in terms of management decisions, e.g. choice of rotation or break crop. Of 173 specimens of *Meloidogyne* spp. collected from carrot soils on mainland Australia and tested by J. Cobon (Queensland DPI) by PCR, 38% were *M. javanica* and 16% *M. hapla*. PCR failed to identify Meloidogyne to species in 46% of samples, either due to poor sample or due to the presence of species not tested for (e.g. *M. fallax*) (Hay *et al*. 2004) *M. fallax* was recently identified in Australia and is of concern as it is a potentially severe pathogen of potato and carrot and can survive on pasture, a common break crop used to control other *Meloidogyne* spp. However, little is known as to its distribution in vegetable growing regions, although it is likely to be more widespread than the current level of testing would suggest. In New Zealand, *M. fallax* was recently found in 19 of 92 seed potato fields using molecular techniques (Shah 2006).

Root lesion nematode (*Pratylenchus* spp.) was considered by some participants to be important in vegetable crops but clearly of secondary importance to root knot nematode. *P. penetrans* has been associated most frequently with vegetable crops in disease records (Table 3), but studies have been limited. In a survey of 77 potato fields throughout Australia, Harding and Wicks (2000) found *P. crenatus* (71% of sites), *P. neglectus* (24% of sites), *P. coffeae* (1 site), and *P. penetrans* (2 sites). *P. crenatus* can be important in vegetable crops, associated with 'carrot sickness' (Loof 1991). In a survey of 31 fields of pyrethrum in Tasmania which had previously grown vegetable crops, Hay *et al*. (2002) found *P. crenatus*, *P. penetrans*, *P. thornei* and *P.*

neglectus to occur in 27, 10, 3 and 2 fields respectively. This again highlighted the prevalent nature of *P. crenatus* and in addition suggested *P. penetrans* to be more common in at least some vegetable production areas.

Some other nematode species have been associated with damage to vegetable crops in Australia, but damage was considered by the participants to be of a sporadic nature. These included cyst nematode (*Heterodera schatchtii*) in brassica, spiral nematode (*Rotylenchus robustus*) and stunt nematode (*Tylenchorhynchus* spp.) in various crops, *Hemicycliophora saueri* and *Neodolichodorus australis* in carrot and bulb and stem nematode (*Ditylenchus dipsaci*) in onion and garlic.

There are also a number of nematode species not present in Australia which are a potential threat to the vegetable industry (Table 4). Risk assessments for plant-parasitic nematodes have been conducted by Biosecurity Australia. There is a need to ensure that DNA diagnostics are available to rapidly identify potential incursions of these nematodes or to better characterise what species are present in Australia. A specific example is the stem and bulb nematode (*Ditylenchus dipsaci*). This nematode is one of the most economically important plant-parasitic nematodes in temperate regions (Sturhan and Brzeki 1991). *D. dipsaci* exists as over 20 different races with different host ranges. At present it is believed that only the ‘oat’ and ‘lucerne’ race are present in Australia. However, limited research has been carried out to define exactly what races are present in Australia and to develop reliable and rapid diagnostics to ensure that other races present overseas are not imported into Australia.

Table 2. Reported occurrence of *Meloidogyne* spp. on vegetables in different States of Australia (N=NSW, NT=Northern Territory, S=SA, V=VIC, T=TAS, W=WA. (Adapted from Nobbs 2003)

Crop	*M. javanica*	*M. incognita*	*M. arenaria*	*M. hapla*	*M. exigua*	*M. thamesi*	*M. fallax*	*Meloidogyne* sp.
Asparagus								N
Beans (*P. vulgaris*)	N,NT,Q,S,W	N,NT,S	N	N		Q		
Beetroot	N,Q,W	N,V	N	N				W
Bean (*V. faba*)		N		N				W
Broccoli								
Brussels sprouts	N							
Cabbage	N,Q		N	N				W
Capsicum	S	N		N,Q,V				
Carrot	N,Q,V,W	N,W	N,T	N,S,T,V		N	T	
Cauliflower								N,W
Celery	N,Q,V,W	N,Q	N	N,Q,V				
Cucumbers	N,W	N,S,V						
Garlic	Q							
Leeks								N
Lettuce	N,V,W	N		N,Q,T,V				
Onion	N,S,W	Q		T,W				T
Parsnip	N,V,W	N	T	N,S,T,V				
Pea	N,Q,V,W	Q,S		N				S,T,W
Potato	N,Q,S,V,W	N,T,W	N,T,V,W	N,S,V,W	N		S,V,T	
Pumpkin	N,S,V	N,Q	N,Q					
Radish	W							N,NT
Spinach								S
Silver beet	N,Q,W	N,Q	N	N				
Spring onion								
Sweetcorn/ Maize	Q,S							
Tomatoes	N,NT,Q,S,V,W	N,NT,Q,S,T,W	N,Q,W	N				
Zucchini	Q,S	N	Q					W

Table 3. Reported occurrence of *Pratylenchus* spp. on vegetables in different States of Australia (N=NSW, NT=Northern Territory, S=SA, V=VIC, T=TAS, W=WA. (Adapted from J. Nobbs 2003)

	P. brachyurus	*P. coffeae*	*P. crenatus*	*P. jordanensis*	*P. neglectus*	*P. penetrans*	*P. pratensis*	*P. thornei*	*P. vulnus*	*P. zeae*	*Pratylenchus* spp.
Asparagus											N
Beans (*P. vulgaris*)	Q					Q,W			Q,S		W
Beetroot											
Bean (*V. faba*)			T					S			
Broccoli						Q					
Brussels sprouts											
Cabbage						Q,V					
Capsicum											
Carrot			T,V			Q,V,W	V				V
Cauliflower						Q,V		N			
Celery						Q,V,W					W
Cucumbers											
Garlic											V
Leeks											
Lettuce						Q,V,W				Q	
Onion						V					W, Radopholus - W
Parsnip											
Pea					S	Q		V			
Potato	Q	N		V		Q,V					W
Pumpkin	Q										
Radish						W					
Spinach											
Silver beet											W
Spring onion											
Sweetcorn/ Maize	Q									Q	R. similis Q
Tomatoes	Q				N,S,V						W
Zucchini											

Table 4. Some exotic nematode species posing a potential biosecurity risk to vegetable crops in Australia.

Nematode species	Hosts of concern
Meloidogyne chitwoodii (races 1 & 2)	many vegetable crops including carrot (*Daucus carota*), sweet corn (*Zea mays*), garden pea (*Pisum sativum*), onion (*Allium cepa*), tomato (*Lycopersicon esculentum*) and egg plant (*Solanum melongena*)
Globodera pallida (potato cyst nematode)	Potato
Heterodera goettingiana (pea cyst nematode)	Pea/bean
Heterodera carotae (carrot cyst nematode)	Carrot
Heterodera glycines (soybean cyst nematode)	Soybean, legumes
Naccobbus aberrans (false root-knot nematode)	Many vegetable crops
Ditylenchus dipsaci (stem and bulb nematode)	'Oat' and 'lucerne' races present in Australia, but many other economically important races in overseas countries.

5. Previous research in vegetable nematology in Australia.

Several nematode related projects pertinent to the vegetable industry have been funded by Horticulture Australia Ltd. and Rural Industries Research and Development Corporation (Table 5). In addition to these, there are a number of projects funded by HAL and RIRDC which, although not directly related, have some relevance to nematodes in vegetable crops. Examples of these are development of biofumigation crops and alternatives to methyl bromide (Table 6). In addition, the Grains Research Development Corporation has funded a number of nematode-related projects in cereals, mainly on yield loss, cultivar resistance and tolerance. However nematode species associated with cereals are usually not major pests of vegetable crops, thus this research is not pertinent to the vegetable industry. In addition, there have been a few projects on novel diagnostic methods and basic research into plant/nematode interactions supported by the Australian Research Council (Table 5).

Interestingly RIRDC has supported a considerable number of nematode related research projects (Table 5). However, one disappointing aspect is that while some project reports are readily available from RIRDC, others are no longer available, other than in summary form. For example, attempts to obtain reports from RIRDC on two previous nematode workshops (AAN-1A and DAW-88A) were unsuccessful. Similarly attempts to obtain results of project DAN-98A which reviewed and catalogued plant parasitic nematodes within Australia, and project DAQ-152A in which some 240 accessions of 73 crops were screened for resistance to *Meloidogyne javanica*, *M. incognita* (races 1 and 2), *M. arenaria* (race 2) and *M. hapla* were also unsuccessful Fortunately in the latter case, results are accessible elsewhere (Stirling *et al*. 1986, Stirling 1989a), although are beginning to become difficult to access. This highlights the importance of funding bodies maintaining an easily accessible archive of research reports.

Table 5. List of some previous projects of direct relevance to vegetable nematology.

Horticulture Australia Ltd.		
VG98102	J. Nobbs (1998-2003)	Taxonomic support for diagnostics of plant-parasitic nematodes in the vegetable industry and development of a CD ROM library of nematode pests.
HG310	G. Stirling QDPI (1994-1996)	Nematode control with organic amendments and rotation crops.
VG633	G. Stirling BCP (1997-1999)	Management of soil-borne pathogens in vegetable cropping systems at Bundaberg, Queensland.
VG01087	G. Stirling BCP (2002-2004)	Suppressive soils for biological control of root-knot nematodes on vegetable crops.
VG99020	F. Hay TIAR (2000-2003)	Improved control of nematodes in carrot production.
Rural Industries Research Corporation		
DAV-103A	J. Stanton, QDPI (1990-1993)	Development of diagnostic probes to identify species and races of root-knot nematode
UQ-19A	(1992)	Biological control of root-knot nematode with soil bacteria in combination with protein or chitin soil amendments.
DAV-43A	(1990-1993)	Development of cost effective controlled release nematicides for safe use in Australian agriculture
DAN-98A	(1993)	Review and cataloguing of plant and soil nematodes in Australia.
DAQ 151-A	J. Stanton, QDPI (1993-1996)	Molecular diagnosis of root-knot nematode (*Meloidogyne* spp.)
DAQ-152A	G. Stirling, QDPI (1994-1996)	Nematode control with organic amendments and rotation crops
US26A	B. Deverall (Uni. of Sydney) (1994-1998)	Population changes and biocontrol of *Meloidogyne* on roots of woody perennials. (Ph.D training).
AAN1A	J. Curran CSIRO (1995)	Workshop on root knot and cyst nematodes: impact identification and control
AAN2A	G. Stirling (BCP) (1996-1998)	Guidelines for the operation of advisory services for nematode pests
BCP1A	G. Stirling (BCP) (1997-1999)	Development of monitoring services for nematode pests
DAW-88A	(1997)	Nematology workshop – nematode control, genes and microbes
Australian Research Council		
LP0560971	M.G. Jones, V. Vanstone (2004-2007)	Field based molecular diagnostics for identification of plant parasitic nematodes
DP0559809	M.G. Jones, D. Bird, V. Vanstone (2004-2007)	Expression profiling of giant cells induced in host plant roots by root-knot nematodes.
LP0455492	M.G. Jones, J.L. Dale (2004-2007)	A new approach to control of plant parasitic nematodes
LP0219690	M.G. Jones, S. Sharma (2002-2004)	Hidden enemies of crop plants, developing novel methods to identify plant parasitic nematodes
A00105534	M.G. Jones, R. Plowright (2001-2003)	The molecular basis of the root knot nematode-host plant interaction.

n.b. GRDC has supported a number of projects on nematodes specific to cereals.

Table 6. List of some previously funded projects with some relevance to vegetable nematology.

Horticulture Australia Ltd.		
HG98034	J. Matthiessen CSIRO (1998-2003)	Enhanced biodegradation of soil-applied pesticides – determination, risk assessment and prevention strategies
HG98051	A. Shanks Ag. Vic. (1998-2002)	Local grower trials to improve adoption of alternatives to methyl bromide soil fumigation
VX00013	J. Matthiessen CSIRO (2000-2003)	Biofumigation – optimising biotoxic Brassica rotations for soil-borne pest and disease management.
HG01005	A. Shanks Ag. Vic. (2001-2004)	Facilitating national adoption of methyl bromide alternatives.
HG98036	B. Garrett Wrightson Research (1999-2001)	Commercial development of biofumigation technology across the horticultural industries.
HG01024	P. Halley Uni Qld (2002-2004)	Development and assessment of low cost biodegradable mulch films.
Rural Industries Research Corporation		
DAV-83A	(1994-1995)	Alternatives to methyl bromide for soil fumigation.
DAV-134A	(1995-1998)	National communication of research and policy issues related to phase-out of methyl bromide for soil disinfestation.

n.b. GRDC has supported a number of nematode projects in cereals.

6. Identification of nematodes

Management of any pest (including nematodes) is based upon correct identification. Correct identification is necessary to judge what risk the soil population poses to a particular crop or to decide on suitable non-host break crops which might be used to reduce populations of particular nematodes), and for issues of trade and biosecurity. Traditionally nematodes have been identified by microscopic techniques and comparison to taxonomic keys. The advent of DNA based techniques now offers a further tool in nematode diagnostics.

Dr. Jackie Nobbs (SARDI) gave a presentation at the workshop which outlined conventional methods of nematode identification based on morphology. Using conventional techniques, identification of some nematodes to genus can be made within minutes, in comparison to 1-2 days for DNA based tests. However, other specimens may need to be processed to slides and subjected to detailed measurements with comparison to taxonomic keys. This process may take some hours or in some cases even weeks. For root knot nematode, examination of the perineal area of females under the microscope can be used to identify species, however this requires considerable experience. At present there are only two nematode taxonomists in Australia – Dr. Jackie Nobbs who works part time at SARDI and Dr. Mike Hodda (CSIRO). Horticulture Australia Ltd. has supported Dr. Jackie Nobbs (SARDI) through VG98102 'Taxonomic support for diagnostics of plant-parasitic nematodes in the vegetable industry and development of a CD ROM library of nematode pests'. This was seen by the workshop as a very valuable project. The maintenance of taxonomists present in Australia was of concern to the participants of the workshop because identification is crucial for biosecurity and trade issues as well as for assisting researchers. Even with the development of DNA based techniques taxonomists are required to ensure that any test developed is based on correct identification.

DNA based techniques have offered a major advance in terms of identification and quantification of plant pathogens, including nematodes. Kathy Ophel Keller (SARDI) presented a talk on behalf of herself and Alan McKay (SARDI) and Di Hartley (CSIRO) entitled 'DNA based quantification of nematodes'. Molecular diagnostic tools can be used for i) identification, ii) assessment of presence/absence of an organism, or iii) quantification of an organism for research purposes or as part of a risk assessment. The development of DNA diagnostics requires preliminary identification of the target organism and related taxa, DNA sequencing of individuals, design of specific DNA probes, quantitative tests in soil and calibration on samples spiked with known amounts of DNA and field validation. The most commonly used method is Real-Time PCR. In cereals, the PredictaB root disease test (Ophel Keller et al. 1999) is now available commercially to detect and quantify several pathogens from soil samples, including take-all (wheat or oat), Rhizoctonia AG8, blackspot (peas), crown rot, common root rot, Pythium and the nematodes *Pratylenchus neglectus, P. thornei, Heterodera avenae* and *Ditylenchus dipsaci*. One of the main advantages over conventional techniques is that several pathogens can be quantified from a single soil sample using the same technique and that the test is often quicker than conventional techniques. Preliminary work has shown a strong linear relationship (R^2=0.98) between the number of eggs/J2 of root knot nematode (*M. javanica, M. incognita, M. arenaria*) in spiked field soil and quantification by Real Time PCR. There was also a good linear relationship between manual counts of soil samples for *P. thornei* and quantification by Real Time PCR (R^2=0.74). DNA based tests can detect to a level of approximately 0.4 eggs/g soil, which is a similar level of sensitivity to conventional techniques. Test for nematodes which are currently available include, *Pratylenchus thornei, P. neglectus, P. penetrans, P. zeae, P. teres*-like, *Meloidogyne* spp., *Heterodera avenae, H. trifolii, Globodera rostochiensis, G. pallida* and *D. dipsaci* Current tests for *Meloidogyne* spp. based on the ITS region of the genome are able to differentiate *Meloidogyne hapla, M. fallax and M. chitwoodi* from each other and from a further group of *M. incognita, M. javanica and M. arenaria*. However, the latter three are unable to be

differentiated from each other, and further work would be required to design suitable probes to do so.

In comparison to conventional methods of quantification, DNA based techniques are quicker, able to identify nematodes to species, and can directly identify eggs and juveniles as well as adults. However, DNA based techniques will only identify those nematodes chosen for testing. In specific instances other species might be involved in crop damage which may not be tested for, but would be detected during a manual count. DNA based techniques have similar constraints to manual methods in terms of collecting a sample representative of the field, time and costs associated with sample collection and difficulties with assessing the risk which may change with host and environmental factors for a given population density of nematodes. In the cereal industry, agronomists have been trained in the interpretation of the PredictaB test and risk assessment of fields.

The only research into assessing DNA assays for their ability to predict damage due to nematodes in vegetables in Australia has been part of HAL project VG633 (Table 4). This study involved predicting risk of root knot nematode and *Fusarium oxysporum* f. sp. *lycopersici* to tomato in Queensland (Stirling et al. 2004). A two-step process was recommended in which a hazard index was calculated based on paddock history, followed by pre-plant testing by DNA assay if the hazard index was sufficiently high. The hazard index was reasonably reliable in predicting the potential damage caused by root knot nematode. The pre-plant DNA test for root-knot nematode was also found to be useful as it was sufficiently sensitive to detect nematode populations capable of causing economic damage to tomato at harvest (Stirling et al. 2004).

7. Example of successful management of nematodes through a change in the farming system.

Dr. Graham Stirling (Biological Crop Protection) presented results of a 10 year project 'Sugar Yield Decline Joint Venture' on behalf of himself, Alan Garside (QDPI), Mike Bell (QDPI) and Brian Robotham (QDPI) entitled 'Soil health benefits from a sugarcane farming system involving crop rotation, minimum tillage, controlled traffic and trash retention' (Garside et al. 2004, Garside et al. 2005, Stirling et al. 2005). The joint venture was initiated through SRDC and BSES in response to a 'productivity plateau' during 1970-1990 in which the sugar yield per harvested hectare remained static, indicating a loss in the productive capacity of sugarcane soils under long term monoculture.

In comparison to new land, sugarcane soils under long term monoculture exhibit i) increased acidity, ii) lower cation exchange capacity, iii) increased exchangeable Al and Mn, iv) reduced Cu and Zn, v) lower microbial biomass, vi) higher populations of root pathogens and plant-parasitic nematodes, vii) poor soil structure, ix) greater soil strength (compaction), x) lower infiltration rate and water holding capacity and xi) lower sugar yields.

Long term sugarcane land was considered to be degraded in terms of chemical, physical and biological properties as a result of monoculture, excessive tillage, soil compaction from harvest machinery and reduced organic matter. Soils with a continuous monoculture of sugar cane were exposed to severe compaction caused by harvest machinery which weighs some 20 tonnes. This was exacerbated in that traditional row spacings of 1.5 m continued to be used despite the move to mechanised harvesting machinery during the late 1960's/early 1970's which had wheel spacings of some 1.85 m. To remove compaction, growers employed excessive tillage practices of 10-15 machinery passes between cane cycles. However, this was only a temporary solution, with soils becoming compacted again early in the next production cycle.

A new farming system was designed involving crop rotation, minimum tillage, controlled traffic using GPS guidance and trash retention. Following the end of the cane crop, soil was tilled to remove compaction, and permanent beds were formed with the aid of controlled traffic/GPS guidance technology. Beds were established on a 1.85 m wheel spacing to match with harvest machinery and reduce compaction. The potential reduction in yield as a result of greater spacings was compensated for by planting two rows of sugarcane within each bed, 50 cm apart.

Legumes (e.g. soybean) were planted into each of the beds. Previous trials showed that break crops/bare fallow, led to increases in yield in the subsequent sugarcane crop over the industry standard practice of ploughing out and immediate replanting. In general the increase in sugar yield was greater following a break involving pasture in comparison to a break with a crop (e.g. soybean, peanut or maize),which in turn was greater than a break following a bare fallow. Breaks of 6-9 months and breaks involving a legume crop such as soybean gave a substantial increase in yield in the subsequent sugarcane crop. The response of sugarcane to a previous break with soybean was shown to be due to an improvement in soil health rather than due to an improvement in N status of the soil. For example, in comparison to continuous sugar cane, a break with a crop resulted in an increase in populations of pseudomonads, fungi, mycorrhizal fungi and free-living nematodes in the soil, a decrease in populations of the plant-pathogenic fungus *Pachymetra chaunoriza*, plant-parasitic lesion nematodes and some insect pests, and no change in populations of earthworms, bacteria and actinomycetes or in overall microbial biomass or metabolic potential.

Cane was planted with a double disc opener directly into beds without disturbing the soybean residue and grown through a full production cycle. At the end of the cycle the trash blanket was retained rather than burning off the cane, to retain high levels of total soil C and labile C, which in turn improved cation exchange capacity and aggregate stability, reduced surface crusting and increased rainfall infiltration leading to less runoff and erosion. At the end of the cane cycle, legumes were direct drilled into the stubble/trash, grown on,

and cane direct drilled into the legume stubble. Reduced tillage was found to have a beneficial impact on populations of macrofauna such as isopods, millipedes, scorpions, spiders and beetles. Such macrofauna fragment organic matter, create channels in the soil and are often predators. Reducing tillage improved water infiltration due to an improvement in macroporosity.

This system maintained sugarcane production over one cycle and led to additional income from grain, reduced fuel costs, fertiliser inputs, labour costs, and pesticide inputs and increased gross margins/ha. In addition, the move to controlled traffic and permanent beds led to reduced compaction which negated the need for tillage and hence heavier and more expensive machinery for cultivation.

Plant parasitic nematodes were shown to play a role in yield decline of sugarcane, with losses of 10% in the plant crop and 7% in ratoons leading to an industry wide loss of $80 M per annum. In long-term sugarcane soils, more than 70% of the nematode community were plant-parasites with *Pratylenchus zeae* considered of major importance. In the new farming system the legume break crop reduced cane-specific pathogens, including plant parasitic nematodes and markedly increased numbers of bactivorous and fungivorous nematodes. A single legume crop was capable of reducing populations of *P. zeae* by 80-90%. Retention of trash in the new system was shown to be important in maintaining the suppression of nematodes. Trials in which materials with a high C/N ratio (sawdust, cane trash and grass hay) were applied to soil were shown to be suppressive to plant-parasitic nematodes after 7 months in comparison to addition of materials with lower C/N ratio. Similarly addition of sugarcane trash with or without additional N or soybean break crop reduced *Pratylenchus* spp. populations in roots to 4-6% of the population in roots not treated with amendments at 5 months after planting and 11 months after adding amendments. Similarly populations of free living nematodes in soil were increased in soil treated with amendments. Factors associated with this suppression included i) increased microbial activity, ii) increased fungi and fungal-feeding nematodes, iii) more omnivorous and predatory nematodes, iv) low to moderate levels of nitrate-N

and v) an unidentified nematophagous fungus. In addition to amendments, soil disturbance was shown to encourage multiplication of plant parasitic nematodes, and conversely lack of tillage was suppressive. This indicated that effective suppression of plant-parasitic nematodes in sugarcane soils could be obtained by inputs of organic matter and reduced tillage.

The project demonstrated that a change in farming system, rather than directly targeting specific pathogens, could lead to suppression of soil borne pathogens. A factor in the success of the Sugar cane Yield Decline Joint Venture was that research was multidisciplinary and led by agronomists not nematologists or plant pathologists.

Dr. Graham Stirling presented a further talk 'Vegetable farming systems to enhance soil health and minimised losses from nematodes – can we learn something from the sugar industry?' Conventional vegetable production systems are characterised by generally poor soil health in comparison to other crops within Australia. Within current vegetable production systems there are few carbon inputs, soil is often fallowed, crops are shallow rooted, there is frequent tillage, and high inputs of pesticide and N-fertiliser. In comparison to uncropped soils, intensively cultivated capsicum soils had lower populations of fungal-feeding and omnivorous/predator nematodes, lower microbial activity and lower populations of soil fungi. Intensive vegetable production was shown to destroy the natural suppressiveness of the soil to soil borne pathogens such as plant-parasitic nematodes. Suppression of plant-parasitic nematodes occurs in systems which adopt crop rotation, green manure crops, organic amendments, appropriate fallows, reduced tillage, routine nematode monitoring and strategic use of nematicides if required.

While there is reasonably good information available on some of the components of such a system for vegetable crops e.g. break crops, there has been poor integration of various control measures into a practical system. A farming systems approach is the only medium to long-term option for the vegetable industry. Priorities would include row crops (tomato, capsicum, melons), sugarcane/vegetables in Bundaberg, systems for intensive winter

vegetable production (e.g. Bowen, Bundaberg), systems for intensive vegetable production (e.g. Victoria, Sunraysia/Riverland, Western Australia) and systems for root crops (carrot and potato).

8. Potential strategies for managing nematodes in vegetable production.

A range of issues and potential management strategies were discussed in the workshop. Background information on each is provided in this section. Each issue was then rated by participants for its potential to improve the management of nematodes in vegetable crops (1 of low potential to 5 of highest potential) in research & development and/or extension (Table 7).

8.1 Management strategies discussed at the workshop

Development of suitable break crops/rotations/ biofumigant crops

Break crops or cash crops which are either poor hosts or antagonistic to particular species of nematodes can be grown in between crop plants in the rotation to reduce nematode numbers. This is the basis of the Nematode Control Strategy instituted in the Netherlands to reduce reliance on nematicides (Molendijk and Korthals 2005). While there is some good information on suitable cover crops for growers to use for soil conservation in Australia and New Zealand (e.g. Anon 2005) this is not often integrated with knowledge of the host status to particular pests/pathogens including plant-parasitic nematodes. Similarly, while there is some information on host status of potential break crops to nematodes (Stirling et al. 1986, Stirling 1989a), this information is dated, not always readily available, and may not be pertinent to all regions of Australia. This information needs to be updated especially in light of the recent finding of *M. fallax* in Australia, which has a different host range to other *Meloidogyne* spp. There is a need to consolidate and make existing information more readily available and where required, screen and demonstrate further break crops suitable for particular localities.

The use of break crops is predicated on knowledge of what nematode species are present in particular fields. Manual counting of nematodes usually only provides information on the genus of nematode which may not be specific enough to allow recommendation of particular rotations/break crops. Stanton

and O'Donnell (1998) proposed the use of DNA based tests to identify nematode populations in the farming system and region of interest and screen potentially useful rotation crops for resistance to those nematode populations. However, there has been little further development in the vegetable industry in this area.

There may also be opportunities for industries to develop by producing and marketing seed of break crops specifically for control of nematodes and other soil borne pathogens. The selection and development of biofumigant brassicas with high levels of gluconsinilates (GSL's) is a case in point. 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, due mainly to the hydrolysis of GSL into isothiocyanates (ITC's) in the soil by the enzyme myrosinase. However, there are some problems with the use of biofumigant crops for control of nematodes and other pathogens. Careful management is required to ensure an adequate biofumigation effect. Biofumigant plants must be grown well to obtain sufficient biomass for an adequate biofumigation effect following incorporation. The crop must be adequately mulched prior to incorporation and soil must be moist to ensure good release of isothiocyanates. Subsequent irrigation is recommended to ensure a good biofumigant effect. In addition, biofumigation may be less efficient in heavier, clay soils than in sandy soils. Some biofumigant plants are good hosts of particular nematode species and unless a good biofumigation effect is obtained after incorporation can actually increase nematode populations. In some cases, biofumigant crops can be grown during cooler seasons at which time nematode multiplication rates are lower. In addition, the biofumigant effect of biofumigant crops is lessened in soils with a history of enhanced biodegradation to metham sodium. There is scope for further selection of biofumigant species which are resistant to particular nematode species.

Farming systems/integrated approach to nematode control including controlled traffic, minimum tillage

The current system of farming involves a number of aspects conducive to nematodes including soil disturbance, reduced soil carbon and high levels of nitrate-N. Research in the sugar cane industry has indicated that the development of a system based on minimum tillage, organic amendments, retention of organic matter in the surface layers and lower inputs of nitrate N can lead to suppression of soil borne diseases and nematodes. A farming systems approach to controlling soil borne diseases including nematodes would potentially be of major benefit to the vegetable industry. However, there would be considerable challenges to be overcome, e.g. machinery development. Research is being conducted in other countries to develop minimum or reduced tillage systems for vegetable production. Swanton *et al*. (2004) demonstrated that over a 4 year period a zone tillage system produced yields equal to (carrot) or better than (onion) conventional tillage. There has been limited research in Australia, including HAL projects VG90050 (Rogers et al. 2001), VG98046 (Wells 2000) and VX 01033. As part of a Ph.D project at the University of Tasmania, Shane Broad and Shaun Lisson (pers. comm.) developed machinery for a no-till system for broccoli which enabled planting of broccoli seedlings directly into beds of a cereal rye cover crop. This system substantially reduced the need for insecticides for control of diamond back moth, cabbage aphid and herbicides. Systems would need to be developed for particular industries e.g. row crops (tomato, capsicum, melons) i) sugarcane/vegetables in Bundaberg, ii) systems for intensive winter vegetable production (e.g. Bowen, Bundaberg), iii) systems for intensive vegetable production (e.g. Victoria, Sunraysia/Riverland, Western Australia), Further systems would be required for root crops (carrot and potato).

Variety trials to examine relative resistance/tolerance

There are few examples of complete resistance to particular nematode species in commercially available vegetable varieties. However, there may be differences in resistance/tolerance of commercial varieties. For example, significant differences were noted between commercial carrot varieties in galling severity caused by *M. javanica* and *M. hapla* (Hay *et al*. 2004). While

the level of resistance/tolerance is incomplete, such information would allow growers to choose varieties most suited to their particular situation as part of an integrated control strategy. Again, some information is available (e.g. Stirling et al. 1986, Stirling 1989a), which could be updated and made more readily available to growers.

Develop DNA based identification/quantification of nematodes

DNA diagnostics offers considerable advantages over conventional means of identification and quantification of nematodes. Although tests have been developed for some nematodes of concern to the vegetable industry, others need to be developed and validated. This would include the major nematodes associated with vegetables (Various *Meloidogyne* spp., *Pratylenchus penetrans*, *Pratylenchus crenatus*). Once developed, these tests could be used in a survey of vegetable soils for root knot nematode and identification to species and quantification. This work has many synergies with the current activity in the Processing Potato R&D Program, which is undertaking development of DNA based tests to quantify soil borne disease of potato, including some nematodes (e.g. *M. fallax*) which are also of interest to the vegetable industry.

While DNA based technologies are currently at the forefront of specific biochemical testing methods, other methods are under development. For example, matrix assisted laser desorption/ionization time of flight spectrometry has recently been developed in at Murdoch University, Perth, Australia to identify the nematodes *Anguina tritici*, *A. funesta* and *Meloiodgyne javanica* (Perera *et al*. 2005) and to identify races of *D. dipsaci*.

Taxonomic expertise

At present there are only two nematode taxonomists in Australia, Dr. Jackie Nobbs who works part time at SARDI and Dr. Mike Hodda (CSIRO). The lack of nematode taxonomic skills in Australasia was highlighted in a paper to Australasian Plant Pathology, 14 years ago (Stirling et al. 1992b). Taxonomists are increasingly required to provide advice for biosecurity and trade issues, as well as support for researchers, including assisting with the

development of DNA diagnostic tests. Taxonomists would also be important in teaching the next generation of quarantine personnel, researchers and agronomists in nematode identification.

Field trials to determine threshold populations or hazard indices

A better understanding of the relationship between population density of nematodes and subsequent damage to the crop in different regions would be valuable for agronomists/growers. There is some information available locally and this should be made more readily available. Replicated trials to determine thresholds would be difficult and expensive to establish and maintain. However, there may be opportunities to develop research projects in association with local growers, packers, and or processors to conduct soil tests of a large number of fields through the season and link populations of particular nematode species to yield and subsequent rejection rate at grading to develop risk models (e.g. through Bayesian statistics). This could be run in conjunction with issue surveys of nematodes to determine species associated with particular vegetable crops and losses due to nematodes.

Short courses for nematologists, agronomists and growers

There are currently no courses available to vegetable agronomists/growers and a limited number of people capable of conducting such courses. Some courses have been run for nematologists. For example, Drs. M. Hodda (CSIRO Division of Entomology) and Kerrie Davies (University of Adelaide) have run a week long course on nematodes 'Nematodes in cropping systems – identification and techniques' each year since 1999, which attracts between 10-20 participants. This course has been very valuable in increasing the skill base of nematode identification and techniques amongst research scientists.

Dr. Graham Stirling (Biological Crop Protection) has recently approached University of Sydney to conduct two courses on nematodes, i) plant parasitic nematodes and ii) nematodes in the ecosystem aimed at postgraduate students. Such courses would be a very valuable activity in ensuring a transfer of knowledge in nematology to future scientists and industry personnel.

Surveys of nematodes to determine species associated with particular vegetable crops and losses due to nematodes

Surveys of nematodes are important to identify the principal species present in particular regions. Identification of species is important to allow the identification of suitable break crops for particular regions, assist with issues of trade access and with biosecurity. In addition, surveys offer the opportunity to assess losses due to nematodes in various vegetable crops and if combined with a statistical approach, can assist in better defining damage thresholds for nematodes in particular crops. Surveys would be achieved most efficiently with the development of DNA based tests (above) to characterise the most important species of the most important genera associated with vegetables.

Development of models e.g. sampling, economic thresholds, nematode development in relation to temperature etc.

Modelling the development of insects has been used widely in IPM, but less widely used in nematology. The development of nematodes is temperature dependent and knowledge of the day degree requirements for particular nematode species could be used along with knowledge of seasonal soil temperature to better advise growers on risks associated with pre-plant nematode thresholds and planting date. For example, a delay in autumn planting in California until soil temperatures fall below 18^{o}C avoids significant root infection of carrot by *M. incognita* (Davis and Raid 2002). In Queensland, Vawdrey and Stirling (1996) recommended growing root-knot nematode susceptible crops from autumn to early spring and rotating with less susceptible crops during summer.

Biological control (organic amendments)

As discussed above in Dr. Graham Stirling's presentation (above), suppressiveness to root knot nematode in sugar cane soils was enhanced by addition of amendments with a high C/N ratio (Stirling *et al*. 2003). Suppressiveness was related to soils with low level of nitrate nitrogen, fungal dominant biology and high numbers of omnivorous nematodes. Similarly,

Tony Pattison (pers. comm.) illustrated that nematode suppression in banana soils was related to practices which increased the amount of labile C, reduced soil nitrate levels and increased the biological diversity of the soil.

High nitrogen amendments have also been demonstrated to be nematicidal. Lazarovits *et al*. (1999) obtained suppression of plant parasitic nematodes by the addition of high rates of soymeal and meat and bone meal (37 t/ha). Chitin (3-4 t/ha) has been shown to reduce populations of nematodes after incorporation into soil through an increase in chitinolytic organisms that degrade nematode egg shells and the release of ammonia during decomposition (Spiegel *et al.* 1988, Caswell and Bugg 1991). Fertilisers such as urea, ammonium sulphate and calcium cyanamide are nematicidal at high rates, again through the release of ammonia (e.g. D'addabbo et al. 1996). However, application must be timed well as ammonia release can be phytotoxic to sensitive crops. 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, 1989b). However, high N amendments may have environmental consequences. For example, rates of chicken manure over 20 t/ha may run risks of polluting water by leaching or soil erosion (Sumner *et al*., 2002).

Given the rising costs of transportation and the high rates of amendments required to provide nematicidal activity, it is more economical to produce amendments on site (e.g. as a green manure) and incorporate them, than to move material from off site.

Breeding for resistance including marker assisted selection

Introducing resistance to important nematodes such as *Meloidogyne* spp. in vegetable crops by breeding would be valuable, but has proven difficult. For example, the USDA has successfully developing carrot lines with resistance to root knot nematodes, but further work is required to introduce suitable agronomic traits. The advent of DNA technology has improved the ability of

breeders to screen large numbers of progeny for particular traits such as nematode resistance, which may see future developments in this area.

Increased University teaching of nematology

The workshop participants saw the decline in teaching in nematology in Australian Universities as a major point of concern. There is little teaching of nematology within the University system in Australia at either an undergraduate or postgraduate level. Furthermore many of the current nematologists will be retiring within the next 5-10 years and there will be a consequent loss of expertise for the vegetable industry. The main centre of nematology teaching in Australia has been at the University of Adelaide, with the University co-funding Dr. Ian Riley's position with GRDC. However, Dr. Riley's position has recently been terminated and teaching of nematology considerably reduced due to budgetary constraints. Teaching of nematology at other Universities is limited. For example Agricultural Science students at the University of Tasmania may have two lectures and a laboratory in plant-parasitic nematodes during their 4-year degree. This has a flow on effect with few postgraduate students involved in nematology. Some State diagnostic laboratories now have no capacity for nematode identification. Nematology has not been able to attract sufficient funding to maintain positions and it is not seen as an attractive career option. With a lack of nematologists in Australian Universities there is reduced capacity to source funding from agencies such as Australian Research Council. The lack of nematology skills in Australasia was highlighted in a paper to Australasian Plant Pathology, some 14 years ago (Stirling et al. 1992b). Interestingly RIRDC project US-26A 'Population changes and biocontrol of *Meloidogyne* on roots of woody perennials' (1994-1998) was promoted as a means of providing training and increasing the number of nematologists in Australia (Table 4).

Improved sampling techniques

In the Netherlands, Been and Shomaker (1996) developed a computer program SAMPLE which has been used to determine the most efficient sampling intensity for detection of potato cyst nematode for regulatory purposes. Such models are valuable for improving the efficiency of sampling.

Improvements in automated sampling equipment would allow for quicker soil sampling and higher sampling intensity. This would be important for tests based on both conventional and DNA quantification of nematodes. Similarly adoption of different soil sampling equipment may increase the speed with which samples can be obtained from heavy soils (e.g. Stevens et al. 2002).

Precision agriculture for nematode control

The development of GPS guidance systems has enabled the mapping of nematode infestations in fields and site-specific treatment of areas of high population densities e.g. with nematicides. One of the disadvantages of this technique is that the cost of sampling and soil testing for nematodes usually outweighs any cost savings in control. It is unlikely that developments in diagnostics will, in the near future, lead to cost reductions substantial enough to allow routine testing of a large number of individual samples to map the spatial distribution of nematode populations within single fields. A possible method of circumventing this would be to systematically scout the field near the end of the season and assess plant roots for root knot nematode damage, and prior to the succeeding crop treat areas in which the crop was severely affected. Such a system would be more feasible in sandy soils where soil is more easily removed to allow observation of roots.

Alternative nematicides (of biological origin)

Overseas there is some ongoing development of nematicides of biological origin including i) Sinocin extracts from prickly pear (*Opuntia linheimeri*), oak (*Quercus falcata*), the sumac (*Rhus aromatica*) and the mangrove (*Rhizophora mangle*) which is registered in the USA as 'Plant Extract 620', ii) DMDP (2,5-dihydroxymethyl-3,4-dihydroxypyrrolidone) is a naturally occurring sugar analog from the tropical legume (*Lonchocarpus felipei*) which has recently been patented as a nematicide. It is of particular interest because it is mobile in phloem and when applied to tomato leaves, was able to reduce galling on roots by *M. incognita* (Chitwood 2003), iii) Ditera (Valent Laboratories USA) is available in the USA as a nematicide. It is a killed fermentation product of the fungus *Myrothecium verrucaria* originally isolated from soybean cyst nematode, iv) Omphalotin A is a cyclic dodecapeptide with

nematicidal activity isolated from the basidiomycete fungi *Omphalotus olearius* and *Lampteromyces japonicus*, v) Dragonfire-CPP (Poulenger USA Inc. Fl.) is a formulation of sesame seed oil containing aldehydes, ketones and linolenic acids) used for control of nematodes in turf in the USA, vi) Furfural (International Furan Technology, Durban, South Africa) is registered as a nematicide in South Africa and Spain and containing 2-furfuraldehyde, a derivative of pentose sugars (Chitwood 2003).

These and similar products are in various stages of development. Historically trials with such products have been unequivocal, with acceptable levels of efficacy in some trials and poor efficacy in others. In addition, cost is often prohibitive for use in vegetable crops. It is recommended that should local distributors import and market these products that they be tested for efficacy.

Biological control (development of nematophagous organisms)

During the 1970's and 1980's there was a major research effort worldwide to develop nematophagous organisms as biological control agents (e.g. Stirling 1991). Some organisms became commercially available, but there are now few on the market due to factors such as inconsistent performance and cost. One of the more promising biological control agents currently being developed is the bacterium *Pasteuria penetrans* which has been associated with soils suppressive to certain nematode species (especially root knot nematode), *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. A US company, Pasteuria Bioscience LLC, has recently developed a technique to culture *Pasteuria* (Gerber *et al*. 2002) and this may become available commercially. *Pasteuria penetrans* is present in Australia, however, there may still be issues with importation and use in Australia.

Leaflets for agronomists/growers

There is a lack of information available to agronomists/growers on management of nematodes in Australia. In particular, an updated list of

suitable cover crops available for control of particular nematodes species in particular regions would be useful for growers.

Improved conventional methods of identifying/ quantifying nematodes

Improved conventional methods of identifying/quantifying nematodes would be beneficial but difficult to achieve, other than through courses to improve the skill base of researchers/agronomists.

Improved nematode extraction techniques

Various techniques are used for extraction of different nematodes or stages of nematodes from soil and roots. Generally laboratories use the Whitehead tray technique for extraction of nematodes from soil. This technique relies upon incubation of soil wrapped in tissue over a tray of water. Over time, nematodes migrate from soil into the water with recovery by sieving. In general this technique extracts only some 50% of the nematodes present in the sample. Various laboratories employ different times of incubation (from 3-14 days) and may or may not correct for efficiency of extraction.

Trap crops

Trap crops have been used as a management tool for nematodes which hatch in response to root exudates and invade roots and form sedentary feeding sites (e.g. cyst nematode, *Heterodera* spp.). This strategy 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. In Europe, oil radish and white mustard varieties have been selected which stimulate egg hatch of *H. schachtii* but which allow little or no nematode multiplication in the roots (Muller 1991). This obviates the need for careful timing of crop destruction.

There is some potential for this strategy to be tested for control of root knot nematodes which also form sedentary feeding sites in plant roots. For example, 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

and might be developed as a trap crop for *M. hapla*. Similarly, Patel *et al.* (1991) demonstrated that pink and white periwinkle (*Catharanthus roseus*) were effective trap plants for *Meloidogyne incognita* and *M. javanica*.

Conventional nematicides, including development of new active ingredients or registration of active ingredients available overseas

Few new nematicides have become available over the last 20 years. Factors which contribute to the efficacy of a nematicide such as water solubility, long lasting activity and ability to move through the soil profile, also contribute to the potential to contaminate the environment and enter ground water. It is therefore often difficult to reconcile efficacy with environmental requirements. Most currently available nematicides are either general soil fumigants or organophosphate compounds for which there is continued concern from a human health and environment perspective and also reduced efficacy due to enhanced biodegradation. Nematicides are also only a short term solution, with nematode populations able to increase following the loss of activity in the soil. If new nematicides become available it is likely that local distributors will market them in Australia.

Genetically engineered transgenic crops

There is an ongoing effort worldwide to understand the mechanisms of resistance at a molecular level to various plant pathogens including nematodes and to transfer this resistance into plant varieties in combination with suitable agronomic characteristics (e.g. Heinrich *et al*. 1998). In addition to technical difficulties, issues of legislation and consumer acceptance currently limit the usefulness of this strategy. However, continued research in this area is important as such research generates a fundamental understanding of the host/nematode relationship and may lead to further novel control methods, e.g. selection of less toxic, nematode specific chemicals which inhibit the development of nematode feeding sites or disrupt nematode sensory processes.

Solarisation

Solarisation involves placing clear plastic sheet over soil to raise soil temperatures to levels which kill nematodes and other plant pathogens. The sheet can then be sprayed with white paint to reduce soil temperatures for the ensuing crop, and the crop transplanted through the sheet. The technique has limitations in many parts of Australia due to cloud cover and an inability to achieve the temperatures required for a long enough period to be effective. In some cases the solarisation is effective only in the top 20 cm, allowing nematodes deeper in the soil profile to migrate into the root zone once the crop is planted. In addition, plastic sheeting is expensive and limited to a few high value crops. Plastic also constitutes a considerable waste issue. However, new biodegradable plastics may offer some advantages.

8.2 Ranking of management strategies.

At the end of the workshop discussions each of the management issues above were ranked by the workshop participants in order of importance on a 1-5 scale in terms of their potential to improve the management of nematodes in vegetable crops (1 of low potential to 5 of highest potential) in Research & Development and/or Extension. Following the workshop, responses were summed and expressed as a percentage of the total possible score and ranked in order (Table 7).

Table 7. Rating and ranking of nematode management strategies for their potential to improve the management of nematodes in vegetable crops through further research, development and/or extension.

Issue:	**Rating**[1]	**Rank**
Development of suitable break crops, rotations and biofumigant crops.	86.9%	1
Farming systems/integrated approach to nematode control including controlled traffic	85.4%	2
Variety trials to examine relative resistance/tolerance	75.4%	3
Develop DNA based identification/quantification of nematodes	73.8%	4=
Taxonomic expertise	73.8%	4=
Field trials to determine threshold populations or hazard indices.	72.3%	6
Short courses for nematologists/ agronomists/growers	70.0%	7
Surveys of crops to determine species and losses due to nematodes	69.2%	8
Development of models e.g. sampling, economic thresholds, nematode development in relation to temperature etc.	67.7%	9=
Biological control (organic amendments)	67.7%	9=
Breeding for resistance including marker assisted selection	66.7%	11=
Increased University teaching of nematology	66.7%	11=
Improved sampling techniques	63.1%	13=
Precision agriculture for nematode control	63.1%	13=
Alternative nematicides (of biological origin)	61.5%	15=
Biological control (development of nematophagous organisms)	61.5%	15=
Leaflets for agronomists/growers	61.3%	17
Improved conventional methods of identification/quantification of nematodes	60.0%	18
Improved nematode extraction techniques	55.0%	19
Trap crops	52.3%	20
Conventional nematicides (development of new active ingredients or registration of active ingredients available overseas)	47.7%	21
Genetically engineered transgenic resistance	43.1%	22
Solarisation	30.0%	23

[1] Calculated as the total score allocated to the issue by workshop participants as a percentage of the total possible score for that issue.

In addition to the above, individual participants listed the following as potential priorities:

- Induced resistance
- Participatory trials on management systems for nematode control on grower's properties.
- Long term soil systems trials

In addition to the workshop discussions, this ranking was used to develop workshop recommendations.

9. Options for the management of nematodes in the Australian vegetable industry

The workshop participants noted that management of nematodes must ultimately be integrated with management of other pathogens and with the agronomy of the crop. In his presentations, Dr. Stirling (Biological Crop Protection) outlined three options which effectively summarised the future possibilities for the vegetable industry:

1. Accept the status quo

- Assume that fumigants and nematicides will always be the basis of nematode control in vegetables.
- Assume that such chemicals will always be available and acceptable to consumers.
- Assume that enhanced biodegradation will not eventually compromise efficacy.
- Assume that the environmental consequences of today's intensive farming practices can be ignored.

The use of fumigant and non-fumigant nematicides in agriculture is under continued pressure due to their toxicity and potential for environmental harm (e.g. Gooch et al. 1998). Bayer Corporation has announced the voluntary withdrawal of Nemacur (fenamiphos). Production of Nemacur in the USA is to be phased out by May 2007, and distribution to cease by May 2008. Currently (as of 2006) the USA EPA is evaluating several soil fumigants (chloropicrin, dazomet, metam sodium, methyl bromide and iodomethane) as part of the EPA's program to ensure that all pesticides meet current health and safety standards (Anon 2004b). A similar assessment of 1,3-D (Telone), which has recently been registered in Australia (Anon 2001), was completed in the USA in 1998 (Anon 1998). Use of 1,3-D was suspended in California in 1990 for several years due to its detection in air distant from sites of application. There have been a number of measures taken to reduce risks of

use in the USA (Anon 1998), including the requirement for a 300 foot buffers from residential areas and caps on the amounts that can be used around townships in California (Chitwood 2003), which can lead to shortages (Becker et al. 1997). Other restrictions include that recommendation that 1,3-D 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 to mitigate ground water contamination (Anon 2004c). The potential for environmental impact, continual restrictions on usage, and the continual pressure from consumers for reduced pesticides in agricultural production would indicate that reliance of the vegetable industry on nematicides is not a long term strategy for the vegetable industry.

2. ***Keep trying to make integrated pest management strategies work with current farming systems which are conducive to plant parasitic nematodes.***
   - Improve nematode monitoring programs.
   - Overcome obstacles limiting the use of DNA based tests.
   - Apply knowledge of economic thresholds.
   - Encourage the use of well-researched practices such as fallowing, crop rotation, biofumigation and solarisation.
   - Nematicides recommended as a last resort.
   - Keep testing new nematicides and biological control agents.

   This option is a continuation of the current R,D&E effort. As a strategy it continues to treat the symptom (nematodes) rather than the underlying problem (farming system) and while capable of providing improvements in nematode management to the vegetable industry, such improvements are likely to continue to be of a limited nature.

3. ***Redesign the farming system so that it is less conducive to nematode and other soil related problems.***
   - Institute permanent beds, appropriate rotations, minimum tillage and organic mulches.

- This would require further research and development in the areas of:
    - Optimum mulches and rotations.
    - Equipment to plant seedlings and mulch crops through trash.
    - Effects on soil temperature for winter plantings.
    - Management and re-use of trickle tubing.
    - Management of nutrients to optimise mineralisation and minimise leaching.
    - Pest, disease and weed management issues.
    - Productivity and economics.

A farming systems approach was seen by the workshop participants as the only medium to long-term option for the vegetable industry. It would potentially provide improvements in many aspects of the farm business including control of soil borne diseases, weeds and insect pests, reduced pesticide inputs, reduced fertiliser inputs, reduced machinery costs, improved soil structure and by adopting more environmentally sound practices lead potentially to improved marketability and consumer acceptance of products.

Previous farming systems research in the vegetable industry

Three previous projects supported by Horticulture Australia Ltd. (VG90050, VG98046 and VX 01033) have investigated the ability to integrate controlled traffic systems into the vegetable industry. However, there appears to have been no plant pathological input into these projects which, if benefits were demonstrated, would add considerable impetus to their adoption. Similarly, research has been conducted in Tasmania over the last 3 years by Shane Broad and Shaun Lisson (TIAR, University of Tasmania) developing a no-till system for broccoli which uses cover crops and controlled traffic beds. In this system a cereal rye cover crop is sown into beds in September and dessicated with glyphosate in December. A machine has been designed which rolls the cover crop and transplants seedlings directly through the trash. This has been demonstrated to be feasible system and to substantially reduce the need for pesticides by reducing numbers of diamond back moth, cabbage aphid and weeds.

The workshop recommended a similar approach be adopted by the vegetable industry and tailored to the requirements of particular production systems within States e.g. row crops (tomato, lettuce, cucurbit, brassica) and root crops (carrot, onion, potato). While a minimum tillage system is likely to be more difficult for root crops, there are opportunities for zone tillage systems for these crops in which only a portion of the bed is cultivated immediately prior to sowing. Such systems are being developed for vegetable production in north east USA and elsewhere and may still accrue the benefits of soil suppressiveness to pathogens as seen in the sugar cane and banana industries (discussed above). For example, Swanton et al. (2004) reported no significant difference between conventional versus zone tillage in total or marketable yield of carrot over 4 years, while total yield and marketable yield of onion was significantly greater under zone tillage than conventional. In addition, problems with establishing small seeded crops might be overcome with seed coating or fluid drilling technologies.

10. Workshop recommendations.

Recommendation 1. Prepare extension material for use by growers and consultants to improve the way nematodes are managed in the vegetable industry.

Reasons behind the recommendation. There is no comprehensive, Australian-produced guide to help vegetable growers understand their nematode problems and improve nematode management. Much research has been done in the last 15 years but the results are found in the scientific literature or are presented in reports and publications that are often difficult to obtain. Consultants working in the vegetable industry have little knowledge of nematodes and so many potentially useful nematode management options are not being used by growers.

Tasks involved. Commission a person/group with the appropriate skills to compile a booklet and/or web-based publication containing i) basic information on the important nematode pests of vegetable crops, ii) damage thresholds and the impact of environment on crop losses due to nematodes, iii) management options (chemical and non-chemical), iv) examples of management systems that are relevant to particular crops, nematodes or regions. Use the information in the booklet to run workshops on nematode management for growers and consultants in all major vegetable-production regions

Responsibilities of HAL and Ausveg

- Commission the production of the booklet and provide appropriate funding
- Support the nematode management workshops

Timeframe. To be completed by December 2008.

Recommendation 2. Demonstrate the value of rotation crops for root-knot nematode control in various vegetable-growing regions of Australia

Reasons behind the recommendation. Forage sorghum has proved an excellent rotation crop in some vegetable-growing regions (e.g. Bundaberg) because it is easy to grow, it is resistant to all the common species of root-knot nematode and is vigorous enough to out-compete weeds capable of hosting the nematode. There is an urgent need to expand its use to other areas and to find alternatives in situations where it is not suitable.

Work required. List crops that are likely to be agronomically suitable rotation crops in various vegetable-growing areas and then use the extensive literature on root-knot nematode resistance in various crops to compile a short-list of candidates that warrant testing. Test these crops at nematode-infested field sites and in the glasshouse and then promote adoption of nematode-resistant rotation crops that are appropriate for current farming systems.

Responsibilities of HAL and Ausveg

- Fund a three-year project on crop rotation for root-knot nematode control and ensure that several field trials are done each year in all important vegetable-growing areas.

Timeframe. Commence the project in 2007 and complete the work by 2009.

Recommendation 3. Establish regionally-based, multi-disciplinary research groups to develop sustainable farming systems and soil management practices for local vegetable industries and ensure that there is adequate nematological input into each research group.

Reasons behind the recommendation. Current vegetable farming systems (which often include practices such aggressive tillage, lack of crop rotation, long periods of fallow, few inputs of organic matter, routine fumigation and plastic mulching) deplete soil organic matter and exhaust the soil food web.

Since this food web contains the natural enemies of nematodes, the key to sustainable nematode management is to change the farming system so that it enhances the soil's biological status through practices such as minimum tillage, crop rotation, green manuring, organic amendments and organic mulches.

Work required. Multi-disciplinary research groups must be established whose primary focus is the development of farming systems that are profitable and sustainable. A good model is the one used by SRDC in the Sugar Yield Decline Joint Venture, a research program which ran from 1993 to 2006 and developed a more profitable and sustainable farming system for the Queensland sugar industry. Since different solutions will be required for different crops and regions, research groups will be formed to concentrate specifically on vegetable production in three regions where nematodes cause major problems (i.e. northern Tasmania, the inland production areas along the River Murray in SA, Victoria and NSW, and Bundaberg in Queensland). A nematologist/soil biologist (at least 0.5 FTE) would be assigned to each group and would be responsible for monitoring nematode populations, assessing appropriate rotation and cover crops, understanding economic thresholds and checking soils for changes in suppressiveness to nematodes.

A fourth research group would be established to tackle farming systems issues specifically related to carrot production. It is envisaged that this group would be located in WA, due to the importance of carrots in that state. The reason for carrots being dealt with separately is that some tillage will always be required and economic thresholds for damage may be lower than for other crops. Because soil-borne diseases are major causes of market rejection in carrots, a research program of this nature will require strong inputs in plant pathology and nematology and will need to focus on understanding the physical and biological factors which cause problems such as forking and splitting. From a nematological perspective, it will be important to study damage thresholds and the variability of nematode populations within fields, as this will enable informed decisions to be made on the nematode management practices likely to be useful in the carrot industry.

Development and demonstration of farming system based on controlled traffic/minimum tillage would require further work in:

- Monitoring soil pathogens, nematodes, beneficial organisms, soil nutrients, soil structure.
- Developing optimum mulches, cover crops and rotations. Screen further break crops against a range of root knot nematode species including *M. fallax*.
- Selecting suitable varieties.
- Developing equipment to plant seedlings and mulch crops through trash,
- Addressing any effects on soil temperature for winter plantings.
- Developing optimum irrigation regimes
- Management of nutrients to optimise mineralisation and minimise leaching,
- Addressing pest, disease and weed management issues
- Assessing productivity and economics.

Central to the success of such a program would be to build a multidisciplinary team including agronomists, plant pathologists/nematologists/entomologists, plant nutritionists, soil scientists, agricultural engineers and key growers.

Note: It is assumed that farming systems work in the potato industry will be conducted as part of the current Processing Potato R&D program.

Responsibilities of HAL and Ausveg

- Work with local industry bodies, innovative growers and state-based research organisations to establish and fund four regional, multi-disciplinary research groups and ensure adequate technical leadership of each group.

Note: Vegetable nematologists also have a responsibility to help foster the development of these research groups, and to ensure that their research is done within a sustainable farming systems framework.

Timeframe. The timeframe will depend on the resources available and the enthusiasm of regional groups. Projects do not have to be run concurrently, but the aim should be to establish groups by the end of 2008 and complete initial five-year projects by 2013.

Recommendation 4. Enhance the adoption of DNA technologies for identifying and quantifying nematodes

Reasons behind the recommendation. DNA technologies offer considerable advantages over conventional methods for identifying nematodes and quantifying nematode populations in soil. Australia leads the world in the development of these technologies, with DNA diagnostic tests for nematodes and other soil-borne pathogens being used widely in the cereal industry. Much of the ground-work has already been done for nematodes of interest to the vegetable industry but DNA diagnostic tests are still not available commercially.

Work required. The work would concentrate initially on root-knot nematode, because it is the most important nematode pest of vegetables, and a diagnostic test has already been developed and validated. Work towards ensuring that the test is available commercially and that consultants are trained in sample collection and interpretation of results. Once that has been achieved, extend the test to the species level by including all the important *Meloidogyne* species in Australia. Continue to develop tests for other economically important nematodes and add them to the suite of vegetable tests as they are developed and validated.

Responsibilities of HAL and Ausveg

- Consult with SARDI, CSIRO and Bayer Crop Science and agree on a strategy and timeframe for commercialising their tests for

Meloidogyne. Decide on cost-sharing arrangements that will deliver other diagnostic tests to the vegetable industry over time.

- Support emerging technologies in nematode identification and quantification that might be developed by other research organisations

Timeframe. A diagnostic test for *Meloidogyne* should be available commercially by 2008, with other tests to be delivered over the following five years.

Recommendation 5. Increase the number of nematologists working in the vegetable industry and ensure that programs are in place to provide the industry with nematological expertise in the long term

Reasons behind the recommendation. Based on the number of research and extension projects funded in Australia over the last 15 years, it is clear that investment in vegetable nematology has been minimal (less than 0.6 FTE/year). This means that several important vegetable-growing areas have had virtually no nematological input in recent years and relatively few aspects of nematode control have been investigated in detail. Given the size of the industry and the importance of nematodes, it is not unreasonable to expect that three nematologists (out of the 15-20 nematologists currently employed in Australia) should be working in the vegetable industry. Another matter of concern is that with impending retirements, current levels of research funding and the paucity of opportunities for training young people in the discipline, it is possible that the vegetable industry will not have any nematologists capable of addressing its nematode problems within 15 years. Steps must therefore be taken immediately to rectify this situation.

Work required. The problems faced by nematology are common to all specialist areas of agricultural science and cannot be solved by the vegetable industry alone. A coordinated effort from industry, research-funding bodies, tertiary institutions, co-operative research centres, biosecurity agencies and state-based employers of agricultural specialists is required to overcome the

problem. A variety of solutions are available, including scholarships and bursaries for post-graduate students, mentoring programs, improved career opportunities for graduates in agricultural science, and research funds allocated specifically for young researchers. Programs of this type will help all disciplines, but the most important issues for nematology are opportunities for post-graduate training at universities and support for experienced nematologists to run short courses and training programs for consultants, technicians and other professionals.

Responsibilities of HAL and Ausveg

- Cooperate with funding bodies such as GRDC, SRDC and RIRDC by collectively supporting at least one postgraduate student in nematology per year.
- Support the recommendations in this action plan, because this will increase in the number of nematologists working in the vegetable industry.

Timeframe. To be implemented during the period 2007-2012.

Recommendation 6. Support basic research that is likely to lead to the development of the next generation of nematicides

Reasons behind the recommendation. The chemicals currently used for nematode control are under pressure in the marketplace because they are highly toxic materials with a relatively broad spectrum of activity. The next generation of nematicides are likely to be much more acceptable because they will comprise new classes of molecules that act specifically against nematodes by targeting nematode feeding or sensory processes, or by inhibiting the development of nematode feeding sites in the plant. These nematicides will not be toxic to non-target organisms and will probably act by systemic uptake and movement in the plant.

Work required. Basic research on cellular and molecular approaches to nematode control is being done in many countries, including Australia. Although it is difficult at present to predict which approaches are likely to be most successful, it is important that this work continues and that some of it is done in Australia.

Responsibilities of HAL and Ausveg

- Maintain a watching brief on the basic nematological research that is being done in Australia
- Provide industry support for scientists seeking funding from bodies such as ARC
- Seek out opportunities to invest in basic research projects that are likely to produce outcomes with commercial potential.

Timeframe. On-going.

Recommendation 7. Enhance Australia's biosecurity by characterising the plant-parasitic nematodes present in Australia and by developing rapid and reliable diagnostic procedures for major pests

Reasons behind the recommendation. If Australia is to prevent the introduction of new nematode pests, it must have reliable information on the distribution of nematode species within the country and be able to identify nematodes that may have been recently introduced or found in quarantine situations.

Work required. Most of the nematodes which are a threat to Australia's biosecurity are new species or races of genera that already occur in Australia (Table 4). Thus to identify them, specialist taxonomic expertise is required and it must be supported by the development of appropriate DNA diagnostics. Although these issues are primarily the responsibility of Biosecurity Australia and the National Plant Biosecurity CRC, the vegetable industry must ensure

that the priorities of these organisations are appropriate and that they are doing everything possible to protect the industry's interests.

Responsibilities of HAL and Ausveg

- Regularly review the biosecurity work being done in Australia and ensure that adequate resources are devoted to nematodes considered a threat to the vegetable industry.

Timeframe. On-going.

Recommendation 8. Review progress on this action plan and make appropriate changes where required

Reasons behind the recommendation. An action plan of this nature will take 7-10 years to implement. It is therefore appropriate that progress is monitored and priorities are revised as circumstances change.

Work required. Review this action plan

Responsibilities of HAL and Ausveg

- Appoint appropriate reviewers to assess the progress made in this action plan
- Decide whether the plan is still relevant to industry needs and determine whether adequate resources are being devoted to implementing the plan.

Timeframe. Undertake the review in 2009.

11. Conclusions

Plant parasitic nematodes are a significant issue for the Australian vegetable industry and can cause considerable losses per annum. The industry has become reliant upon the use of a few fumigants and nematicides which are under increasing pressure from an environmental, human health and market acceptance point of view, in addition to issues of poor efficacy due to enhanced biodegradation.

The workshop developed a series of recommendations which if implemented would be expected to considerably reduce the impact of plant-parasitic nematodes on vegetable production in Australia. These recommendations included: i) Prepare extension material for use by growers and consultants on nematode management, ii) Demonstrate the value of rotation crops for root-knot nematode (*Meloidogyne* spp.) control, iii) Establish regionally-based, multi-disciplinary research groups to develop sustainable farming systems and soil management practices for local vegetable industries, iv) Enhance the adoption of DNA technologies for identifying and quantifying nematodes, v) Increase the number of nematologists working in the vegetable industry vi) Support basic research that has the potential to lead to the development of innovative control strategies, vii) Enhance Australia's biosecurity by characterising the plant-parasitic nematodes present in Australia and by developing rapid and reliable diagnostic procedures for major pests, viii) Conduct a review on progress on recommendations in 2009 and make appropriate changes where required.

Acknowledgements.

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Appendices

Appendix 1: List of workshop participants

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Appendix 2. Workshop programme

VG 05026 Workshop to develop research, development and extension priorities for nematode control in vegetable crops.

Venue: Tasmanian Institute of Agricultural Research, University of Tasmania Cradle Coast Campus, 16-20 Mooreville Road, P.O. Box 3523, Burnie, TAS 7320.

Contact: Dr. Frank Hay
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Phone: 03 6430 4907, mobile: 0418 331 060

Date: Monday 10th & Tuesday 11th July 2006

Purpose: This workshop involved a gathering of vegetable nematologists and industry representatives to discuss and formulate future research, development and extension priorities in vegetable nematology.

Agenda

Monday 10th July

08.30-08.45	Welcome and introductions
08.45-09.00	Current status of nematode control in vegetable crops
09.00-09.20	List nematode/crop associations of economic and quarantine importance.
09.20-10.05	Talk: *Soil health benefits from a sugarcane farming system involving crop rotation, minimum tillage, controlled traffic and trash retention* (G. Stirling)
10.05-10.30	Discussion
10.30-11.00	Morning tea
11.00-11.30	Talk: *Vegetable farming systems to maximise soil health and minimise losses from nematodes – lessons from the sugar industry* (G. Stirling)
11.30-12.00	Discussion
12.00-1.00	Improving nematode identification and quantification (Talks by J. Nobbs and K. Ophel Keller)
1.00-1.45	Lunch
1.45-3.00	Sampling/modelling, biological control/organic amendments
3.00-3.30	Afternoon tea
3.30-4.30	Nematicides and 'biological nematicides'
6.45-	Group dinner

Tuesday 11th July

08.30-9.30	Education/training – nematologists, advisors, growers
09.30-10.00	Other strategies:crop rotations/biofumigant plants fallow, trap crops, solarisation, resistance
10.00-10.30	Morning tea
10.30-12.00	Identification and development of priorities
12.00-12.45	Lunch
12.45-3.00	Recommendations
3.00-3.30	Afternoon tea and end of workshop

This project facilitated by Horticulture Australia Ltd., in partnership with AUSVEG and funded by the National Vegetable Levy with matching funding from the Australian Government.

Appendix 3: Questionnaire rating potential advances in vegetable nematology

Please rate the following on a 1-5 scale in terms of their potential to improve the management of nematodes in vegetable crops (1 of low potential to 5 of highest potential) in Research & Development and/or Extension.

Issue:	**Research & Development**	**Extension**
Surveys of crops to determine species and losses due to nematodes		
Field trials to determine threshold populations or hazard indices.		
Improved sampling techniques		
Improved nematode extraction techniques		
Develop DNA based identification/quantification of nematodes		
Improved conventional methods of identification/quantification of nematodes		
Development of models e.g. sampling, economic thresholds, nematode development in relation to temperature etc.		
Alternative nematicides (of biological origin)		
Conventional nematicides (development of new active ingredients or registration of active ingredients available overseas)		
Precision agriculture for nematode control		
Biological control (development of nematophagous organisms)		
Biological control (organic amendments)		
Breeding for resistance including marker assisted selection		
Genetically engineered transgenic resistance		
Development of suitable break crops/rotations/biofumigant crops		
Variety trials to examine relative resistance/tolerance		
Solarisation		
Trap crops		
Farming systems/integrated approach to nematode control including controlled traffic		
Increased University teaching of nematology		
Short courses for nematologists/ agronomists/growers		
Leaflets for agronomists/growers		
Taxonomic expertise		
Others:		

Final report for
Horticulture Australia Ltd.
Project VG 05026

Workshop to develop research, development and extension priorities for nematode control in vegetable crops

Part 2 - Literature review:
Management of nematodes in vegetable production.

1. Introduction

Part 2 of this report constitutes a literature review of plant-parasitic nematodes in vegetable production and their management. This literature review was conducted as part of Horticulture Australia Ltd. project VG 05026. A summary of the review was provided to participants of the workshop for discussion purposes. Note that this project is supported by the Vegetable Growers Levy. As the Potato Levy is separate, the review discusses nematodes of importance to the potato industry only where relevant to other vegetables. Issues such as potato cyst nematode in Australia are not discussed in detail.

2. The importance of plant-parasitic nematodes to vegetable production.

Plant-parasitic nematodes are an important constraint to yield and quality of many vegetable crops worldwide. Plant-parasitic nematodes have been estimated to cause approximately 11% losses, equating to 3.8 million metric tons, in 24 vegetable crops in the USA (Feldmesser 1971, Johnson 1992). Koenning *et al.* (1999) estimated losses in a range of crops in USA. In vegetables, losses ranged from 0-20% and were generally between 5-20%. In some crops nematodes are rated amongst the most damaging of pests and diseases. For example, Davis *et al*. (1999) ranked nematodes (mainly root knot nematode - *Meloidogyne* spp.) as the second most economically important carrot disease in the USA. Stirling *et al.* (1992a) estimated that nematodes caused losses of $300-450 million per annum to Australian agriculture including carrot (6%), lettuce (4%), potato (3%), and tomato (6%) (Table 1). In terms of current farm gate values (Table 2), these losses would amount to carrot ($9.0 M), lettuce ($3.0 M), potato ($12.1 M) and tomato ($10.6 M). Stirling *et al.* (1992a) noted that vegetable crops tended to experience severe losses from nematodes, particularly when grown in regions with a warm climate. Root knot nematode was considered the most common problem In Australian vegetable production (Table 1), with susceptible crops such as tomato, potato, sweet potato, carrot, egg plant, ginger and lettuce routinely treated with nematicides. Root knot nematode also caused sporadic problems in other crops such as celery, bean, capsicum, beetroot and cucurbits (Stirling *et al.* 1992a). However, quantification of losses from nematodes is very difficult to estimate.

Table 1. Estimation of losses in vegetable production in Australia due to nematodes (adapted from Stirling et al. 1992a)

Crop	Gross value of production (A$M)	Crop losses with current control: %	A$M	Crops utilising nematode control: Chemical	Other	Main nematode species
Bean	23	-	-	-	-	*Meloidogyne* spp.
Crucifers	65	-	-	-	-	*Meloidogyne* spp.
Carrot	50	6	3	+	-	*Meloidogyne* spp.
Lettuce	45	4	2	+	-	*Meloidogyne* spp.
Onion	65	-	-	-	-	*Ditylenchus dipsaci*
Pea	14	-	-	-	-	-
Potato	270	3	8	+	-	*Meloidogyne* spp.
Tomato	124	6	8	+	-	*Meloidogyne* spp. *Rotylenchulus reniformis*
Cucurbits	-	-	-	+	-	*Meloidogyne* spp.

Although root knot nematode predominate (Table 3), other nematodes cause economic damage to particular vegetables in Australia (Table 3). These include *Ditylenchus dipsaci* (onion and garlic), *Paratrichodorus minor* (onion), *Pratylenchus penetrans* (crucifers and celery), *Rotylenchulus reniformis* (tomato), *Rotylenchus robustus* (range of crops) (Stirling *et al.* 1992a). Other nematodes present in Australia have been associated with yield losses in vegetable crops overseas but have not been associated with yield losses in vegetables in Australia (Table 3).

Table 2. Gross production of vegetable crops in Australia and percentage of production grown in different States (Australian Horticulture Statistics Handbook 2004).

	Farm gate value ($ M)	Gross production (t)	Production by State (%) NSW	VIC	QLD	SA	WA	TAS
Asparagus	62.3	13,921	9.5	83.1	7.0	-	0.5	-
Beans (French/runner)	48.6	33,687	3.8	8.6	47.3	0.2	2.4	37.8
Beetroot	7.1	39,013	-	1.5	94.1	0.2	0.5	-
Broccoli	54.7	45,901	5.4	41.5	25.2	2.4	6.9	18.7
Brussels sprouts	8.6	5,305	-	46.3	-	42.3	2.7	11.2
Cabbages	15.7	76,093	25.0	39.9	9.5	9.5	6.8	1.6
Capsicum	48.4	41,859	0.7	0.3	95.7	1.1	2.3	<0.1
Carrot	150.6	331,130	6.2	34.2	7.8	14.2	26.6	10.8
Cauliflower	37.5	87,586	15.4	27.5	17.2	9.2	23.2	7.6
Celery	17.9	48,132	-	61.1	21.7	6.1	10.5	0.5
Chinese cabbage	5.3	11,513	25.8	8.3	53.6	0.5	11.8	-
Cucumbers	14.7	14,390	36.6	-	39.4	6.5	13.6	0.2
Garlic		300						
Leeks	15.7	6,683	10.2	60.4	-	28.1	-	1.4
Lettuce	76.2	135,015	20.1	24.4	38.9	4.6	10.5	1.4
Onions	139.6	282,517	13.9	9.2	11.2	37.4	6.0	22.3
Potato	404.8	1,333,159	11.9	22.1	8.8	25.1	5.8	26.2
Pumpkin	27.2	96,331	20.8	8.1	49.0	6.8	12.2	2.0
Spinach		4,000						
Spring onion (shallots)	12.4	5,290	12.0	33.9	45.5	4.2	2.8	1.7
Sweet corn	43.3	80,467	51.4	8.6	37.6	0.8	14.9	0.1
Tomatoes	177.1	424,950	8.2	61.8	25.8	0.7	3.3	0.2
Zucchini	19.6	15,231	15.3	9.7	68.1	1.0	5.7	0.1

Table 3. Nematodes species present in Australia of importance or potential importance to vegetable production.

Nematodes associated with losses in vegetables in Australia:	
Root knot nematode	Various Meloidogyne spp.
Root lesion nematode	*Pratylenchus penetrans, P. crenatus*
Cyst nematode	*Heterodera schatchtii* (sugar beet cyst nematode)
Bulb and stem nematode	*Ditylenchus dipsaci*
Nematodes associated with sporadic losses in vegetables in Australia:	
Hemicycliophora saueri	Carrot
Neodolichodorus australis	Carrot
Spiral nematode	*Rotylenchus robustus*
Stunt nematode	*Tylenchorhynchus* spp.
Stubby root nematode	*Paratrichodorus minor*
Nematodes of potential importance to vegetable production, but not associated with losses in Australia:	
Needle nematode	*Longidorus* present in Australia but not associated with vegetables
Pin nematode	*Paratylenchus hamatus* important overseas, but not Australia
Sting nematode	*Belonolaiumus longicaudatus* important overseas, but not Australia

3. Nematodes of importance to vegetable production

Johnson (1998) provided a summary of nematode parasites of vegetable crops worldwide. A description of the major nematode parasites of vegetable crops follows.

3.1 Root knot nematodes

Species of importance to vegetable production.

Root knot nematodes are generally considered the most important nematode parasites of vegetable crops worldwide, being geographically widespread and capable of almost complete crop loss in susceptible crops. There are over 90 recognised species of Meloidogyne worldwide (Eisenback and Triantaphyllou 1991). Traditionally the North Carolina differential host test was devised to identify the four most common species (*Meloidogyne javanica, M. hapla, M. arenaria* and *M. incognita* and to differentiate host races of the latter two species (Hartman and Sasser 1985). Taylor and Sasser (1978) collected and characterised over 1000 living populations of Meloidogyne from 75 countries, mostly from crops growing in fields and many from vegetable crops. *M. incognita*, *M. javanica*, *M. arenaria*, *M. hapla* and other species made up 52%, 30%, 8%, 8% and 2% of those collected respectively. Taylor and Sasser (1978) characterised species by means of differential host tests and found *M. incognita* and *M. arenaria* to consist of 4 and 2 races respectively, with *M. javanica* and *M. hapla* showing no clear evidence of host races. However, *M. hapla* and *M. arenaria* were shown to have two cytological races (Eisenback and Triantaphyllou 1991).

Meloidogyne incognita, *M. javanica*, *M. arenaria*, *M. hapla* and *M. chitwoodi* are considered the most important species associated with vegetable crops (Johnson 1998). Of these species, all but *M. chitwoodi* are present in Australia (Table 4). *M. javanica*, *M. incognita*, *M. arenaria* and *M. hapla* predominate in records associated with vegetables within Australia (Table 5).

Stanton and O'Donnell (1998) assessed 41 Australian populations of *Meloidogyne* spp. by the North Carolina differential host test. While the test provided clear identification of most, the test was not considered adequately reliable for species/race identification when used alone.

A further root knot nematode species, *Meloidogyne fallax*, was recently recognised as a separate species and capable of damaging a number of crops including vegetables such as carrot and potato (Karssen 1996, Beek *et al*. 1997). *M. fallax* was originally considered a race of *M. chitwoodi*. *M. fallax* has been reported in South Australia and Tasmania (Nobbs *et al*. 2001, 2003), and it is possible that its distribution may be more widespread in Australia than currently recognised.

Table 4. Species of root knot nematode reported on vegetable crops in Australia (Nobbs 2003).

	State					
Species	**Qld[1]**	**NSW**	**Vic**	**Tas**	**SA**	**WA**	**NT**
M. arenaria	+	+	+	+	+	+	
M. exigua		+					
M. fallax			+	+	+		
M. hapla	+	+	+	+	+	+	
M. incognita	+	+	+	+	+	+	+
M. javanica	+	+	+		+	+	+
M. thamesi	+	+	+				

[1]Qld (Queensland), NSW (New South Wales), Vic (Victoria), Tas (Tasmania), SA (South Australia), WA (Western Australia), NT (Northern Territory)

In a survey of carrot soils on mainland Australia, Hay *et al*. (2004) reported that *M. javanica* and *M. hapla* predominated, with 38% and 16% of individuals identified as *M. javanica* and *M. hapla* respectively by DNA based techniques (Table 6). Interestingly a total of 46% of samples did not amplify either due to problems during DNA extraction and PCR, or potentially due to the presence of species for which primers were not tested (i.e. *M. fallax*). In the latter case, this might suggest that *M. fallax* is more common in Australia than originally thought. Similarly in New Zealand, *Meloidogyne fallax* was recently

identified in 19 of 92 seed potato fields using molecular techniques (Shah 2006).

***Root knot nematodes of importance overseas but not reported in Australia* (*M. chitwoodi*)**

Subsequent to the study of Taylor and Sasser (1978), *M. chitwoodi* was characterised and reported as an important pest, attacking many vegetable crops including carrot (*Daucus carota*), sweet corn (*Zea mays*), garden pea (*Pisum sativum*), onion (*Allium cepa*), tomato (*Lycopersicon esculentum*) and egg plant (*Solanum melongena*) (O'Bannon *et al*. 1982). However, *M. chitwoodi* has not been reported in Australia. 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 7). The suitability of a plant as a nematode host is often described by the reproductive factor (Rf) which is calculated as Pf (final population density)/Pi (initial population density) after a set time period. Mojtahedi *et al*. (1988) demonstrated that isolates of both races of *M. chitwoodi* from Oregon, Washington and Idaho varied in Rf from <0.01 to 10.7, with Rf > 2 considered a suitable host (Table 7). 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 5. Reported occurrence of *Meloidogyne* spp. on vegetable crops in different States of Australia (N=NSW, NT=Northern Territory, S=SA, V=VIC, T=TAS, W=WA. (Adapted from Nobbs 2003)

Crop	*M. javanica*	*M. incognita*	*M. arenaria*	*M. hapla*	*M. exigua*	*M. thamesi*	*M. fallax*	*Meloidogyne* sp.
Asparagus								N
Beans (*P. vulgaris*)	N,NT,Q,S,W	N,NT,S	N	N		Q		
Beetroot	N,Q,W	N,V	N	N				W
Bean (*V. faba*)		N		N				W
Broccoli								
Brussels sprouts	N							
Cabbage	N,Q		N	N				W
Capsicum	S	N		N,Q,V				
Carrot	N,Q,V,W	N,W	N,T	N,S,T,V		N	T	
Cauliflower								N,W
Celery	N,Q,V,W	N,Q	N	N,Q,V				
Cucumbers	N,W	N,S,V						
Garlic	Q							
Leeks								N
Lettuce	N,V,W	N		N,Q,T,V				
Onion	N,S,W	Q		T,W				T
Parsnip	N,V,W	N	T	N,S,T,V				
Pea	N,Q,V,W	Q,S		N				S,T,W
Potato	N,Q,S,V,W	N,T,W	N,T,V,W	N,S,V,W	N		S,V,T	
Pumpkin	N,S,V	N,Q	N,Q					
Radish	W							N,NT
Spinach								S
Silver beet	N,Q,W	N,Q	N	N				
Spring onion								
Sweetcorn/ Maize	Q,S							
Tomatoes	N,NT,Q,S,V,W	N,NT,Q,S,T,W	N,Q,W	N				
Zucchini	Q,S	N	Q					W

Table 6. Identification of root-knot nematode (*Meloidogyne* spp.) females collected from carrot fields within Australia by PCR-based diagnostic test. (From Hay *et al*. 2004).

	Total no. samples	*M. javanica*	*M. hapla*	No amplification
Western Australia	150	56	22	72
South Australia	8	5	0	3
Victoria	14	4	5	5
Queensland	1	1	0	0
Total	173	65 (38%)	27 (16%)	80 (46%)

Table 7. Host status of different varieties of carrot to different races of root knot nematode, *Meloidogyne chitwoodi* as measured by reproductive factor[1].

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 (Pf)/initial population density (Pi).
- not assessed, * non-host, ** poor host, *** moderate host, **** suitable host

Examples of damage by root knot nematode in vegetable crops

Root knot nematode can be particularly damaging to susceptible crops such as carrot. Slinger and Bird (1978) showed that only 58% of 'Spartan Premium' carrots grown in soil with *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 and 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 and Baker 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*.

Meloidogyne spp. also form disease complexes with other fungal and bacterial pathogens on many vegetable crops, which can exacerbate crop damage (see Johnson 1998).

The pre-plant economic threshold density (action threshold) is often used in nematology as a management tool and will be discussed later in this review. The economic threshold can be defined as the initial population density of nematodes at which the predicted damage to the crop is equal to the cost of controlling the nematode population. Establishing the economic threshold population density for root knot nematode in susceptible crops such as 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.

Lifecycle of root knot nematodes

The development of most nematodes generally follows a progression from egg through four juvenile stages (J1 – J4) to the adult, with moulting occurring between each of the juvenile stages. Root knot nematode undergoes a moult within the egg and hatches 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. Approximately 14 days after assuming a sedentary position the juvenile moults and the feeding apparatus consisting of stylet and oesophageal bulb disappear. A further three moults take place up until approximately day 18. No feeding takes place until the fourth and final moult at which time the stylet and oesophageal bulb regenerate, the female reproductive system is formed and a perineal pattern is visible (Eisenback and Triantaphyllou 1991). The female matures into a pear-shape and 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 and can reproduce by parthenogenesis (Eisenback and Triantaphyllou 1991). 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.

Pinkerton *et al*. (1991) studied the population dynamics of *M. chitwoodi* on potato in the USA in relation to degree-day accumulation. Population densities of J2 were highest at harvest (mid autumn), declined through the winter and were lowest in early summer. Overwintering nematodes were demonstrated to produce egg masses on roots by 600-800 degree days (DD) (base 5^{o}C) after planting. Second and third generation eggs hatched at 950-

1100 and 1500-1600 DD respectively. In one year a fourth generation was observed at 2150 DD. Differences between potato production regions in the amount of damage due to *M. chitwoodi* could be explained by degree day accumulation (Pinkerton *et al*. 1991). Mercer (1990) and Tzortzakakis and Trudgill (1996) studied the day degree requirements of root knot nematode associated with white clover and *M. javanica* respectively. The development of *M. chitwoodi* on potato in relation to day degree accumulation has been used in potato crops in the USA to time the application of the nematicide Vydate (oxamyl) for control of *M. chitwoodi* (Ingham *et al*. 2003, Anon 2006).

3.2 Cyst nematode (*Heterodera* spp.)

Cyst nematodes of importance to vegetable production

Only two of the approximately 57 species of Heterodera spp. described are considered important parasites of vegetable crops (Baldwin and Mundo-Ocampo 1991). *Heterodera schachtii* (sugar beet nematode) and *H. cruciferae* (brassica cyst nematode) both parasitise cruciferous crops. *H. cruciferae* has been reported from cabbage (*Brassica oleracea* var *capitata*) in South Australia, while *H. schachtii* species have been reported on various vegetables including Brassica spp., *Beta vulgaris* (table beet) and *Raphanus sativus* (radish) and occurs in Queensland, South Australia, Victoria, Western Australia and New South Wales (Table 8) (Nobbs 2003). In Brassica seedlings, feeding by *H. cruciferae* caused production of extra lateral roots with small gall-like structures, stunted shoots, leaves which were reddened or with interveinal chlorisis and dying of plants, with areas of low plant density and stunted plants with chlorotic leaves as the crop aged (McCann 1981).

Life cycle of cyst nematodes

The life-cycle of cyst nematodes is similar to root knot nematode. Eggs within the dead swollen body of the adult female (cyst) remain viable for several years. The juvenile nematode (J1) undergoes one moult within the egg to

form a J2. Egg-hatch is often stimulated in response to exudates from roots of particular plant species and the J2 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. The adult female may lay several hundred eggs. Following egg-laying the adult female dies and the outer cuticle forms a protective cyst around the eggs.

There is little information of the impact of cyst nematodes on vegetable crops in Australia. However, *H. schachtii* can reportedly cause considerable yield loss to cruciferous crops (cabbage, Chinese cabbage, cauliflower, Brussels sprouts, broccoli, turnip, radish and Swede) as well as to beets (red and silver), rhubarb and spinach (Vanstone 2006). In New York State *H. schachtii* has been reported to cause significant yield losses to cabbage and table beet, with initial population densities of 9-64 eggs and juveniles per gram of soil causing losses in marketable yield of 23-54% in these crops (Abawi and Mai 1980). Similar yield losses were reported in Canada (Olthof *et al*. 1974).

Cyst nematodes of importance overseas but not reported in Australia

- 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). 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).

- *Heterodera goettingiana* and *Heterodera glycines*

Heterodera goettingiana (pea cyst nematode) is an important parasite of bean (*Vicia faba*) and pea (*Pisum sativum*) in many temperate regions (Johnson 1998), but has not been reported in Australia (Nobbs 2003).

Heterodera glycines (soybean cyst nematode) is an important parasite of soybean in many countries, and has been reported to cause isolated problems in leguminous crops including snapbean and pea (Baldwin and Mundo-Ocampo 1991). *H. glycines* has not been reported in Australia (Nobbs 2003).

- *Heterodera betae*

Recently Wouts *et al.* (2001) described the yellow beet cyst nematode *Heterodera betae* (previously *H. trifolii* f. sp. betae or beet race of *H. trifolii*). *H. betae* infects sugar beets, peas and beans and is found in several European countries.

Table 8. Reported occurrence of selected other nematode spp. on vegetable crops in different States of Australia (N=NSW, NT=Northern Territory, S=SA, V=VIC, T=TAS, W=WA. (Adapted from Nobbs 2003).

	Heterodera schachtii	*Heterodera crucifera*	*Heterodera* sp.	*Paratrichodorus lobatus*	*Paratrichodorus minor*	*Paratrichodorus* sp.	*Ditylenchus dipsaci*	*Ditylenchus* sp.
Asparagus					N			
Beans (*P. vulgaris*)								
Beetroot								
Bean (*V. faba*)							N,S	
Broccoli								
Brussels sprouts								
Cabbage	N,Q,S,V,W	S			S			
Capsicum								
Carrot							S,W	
Cauliflower	S,V,W							
Celery			W		V	V,W		W
Cucumbers								
Garlic							S,V	
Leeks					V			
Lettuce				N,W	Q	V,W		
Onion					S,V	W	N,S,T,V	
Parsnip								
Pea								
Potato							W	
Pumpkin					Q	S		
Radish	N,W							
Spinach	V							
Silver beet	N,W		W					
Spring onion								
Sweetcorn/ Maize				S	Q			
Tomatoes							N	Q
Zucchini								

3.3 Lesion nematode (*Pratylenchus* spp.)

Lesion nematodes of importance to vegetable production

There are approximately 70 described species of Pratylenchus, that parasitise over 400 host plant species. Potter and Olthof (1993) considered Pratylenchus to be the second most economically important group of nematodes to vegetable production in temperate agriculture, after Meloidogyne. *P. penetrans* is considered to be the most economically important plant-pathogenic nematode in north-eastern USA (Johnson 1998). Netscher and Sikora (1990) reported some 10 species associated with vegetable crops. Species most commonly associated with damage to vegetable crops include *P. brachyurus*, *P. coffeae*, *P. penetrans*, *P. vulnus*, and *P. scribneri* (Jensen 1972), of which all but the latter are present in Australia (Table 9). The most commonly recorded species of Pratylenchus on vegetables in Australia is *P. penetrans* (Table 10). Harding and Wicks (2000) surveyed 77 potato fields throughout Australia for prevalence of *Pratylenchus* spp. The following species were recorded; *P. crenatus* (71% of sites), *P. neglectus* (24% of sites), *P. coffeae* (1 site) and *P. penetrans* (2 sites).

Table 9. Distribution of *Pratylenchus* spp. associated with vegetable crops within Australia (Nobbs, 2003).

	State						
Species	**Qld[1]**	**NSW**	**Vic**	**Tas**	**SA**	**WA**	**NT**
P. brachyurus	+	+				+	+
P. coffeae	+	+	+		+	+	
P. penetrans	+	+	+	+		+	
P. vulnus	+	+	+		+	+	

[1]Qld (Queensland), NSW (New South Wales), Vic (Victoria), Tas (Tasmania), SA (South Australia), WA (Western Australia), NT (Northern Territory)

Table 10. Reported occurrence of *Pratylenchus* spp. on vegetable crops in different States of Australia (N=NSW, NT=Northern Territory, S=SA, V=VIC, T=TAS, W=WA. (Adapted from J. Nobbs 2003)

	P. brachyurus	*P. coffeae*	*P. crenatus*	*P. jordanensis*	*P. neglectus*	*P. penetrans*	*P. pratensis*	*P. thornei*	*P. vulnus*	*P. zeae*	*Pratylenchus* spp.
Asparagus											N
Beans (*P. vulgaris*)	Q					Q,W			Q,S		W
Beetroot											
Bean (*V. faba*)			T					S			
Broccoli						Q					
Brussels sprouts											
Cabbage						Q,V					
Capsicum											
Carrot			T,V			Q,V,W	V				V
Cauliflower						Q,V		N			
Celery						Q,V,W					W
Cucumbers											
Garlic											V
Leeks											
Lettuce						Q,V,W				Q	
Onion						V					W, Radopholus - W
Parsnip											
Pea					S	Q		V			
Potato	Q	N		V		Q,V					W
Pumpkin	Q										
Radish						W					
Spinach											
Silver beet											W
Spring onion											
Sweetcorn/ Maize	Q									Q	R. similis Q
Tomatoes	Q				N,S,V						W
Zucchini											

Hay *et al.* (2002) surveyed pyrethrum crops in northern Tasmania in soils which had previously been used for intensive vegetable cropping. *P. crenatus*, *P. penetrans*, *P. thornei* and *P. neglectus* occurred in 27, 10, 3 and 2 of 31 crops respectively.

Damage to vegetable crops caused by lesion nematode

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 *P. crenatus* to carrots was 600 individuals/L soil as the initial population density. Carrot is also a host of *P. neglectus* (Siddiqui *et al*. 1973) and of *P. 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 species of Pratylenchus can be cultured on carrot taproot disks *in vitro*, (e.g. Verdejo-Lucas and Pinochet 1992) but may not be particularly pathogenic in the field. For example, *P. 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). Reported crop losses due to *P. penetrans* are summarised (Table 11)

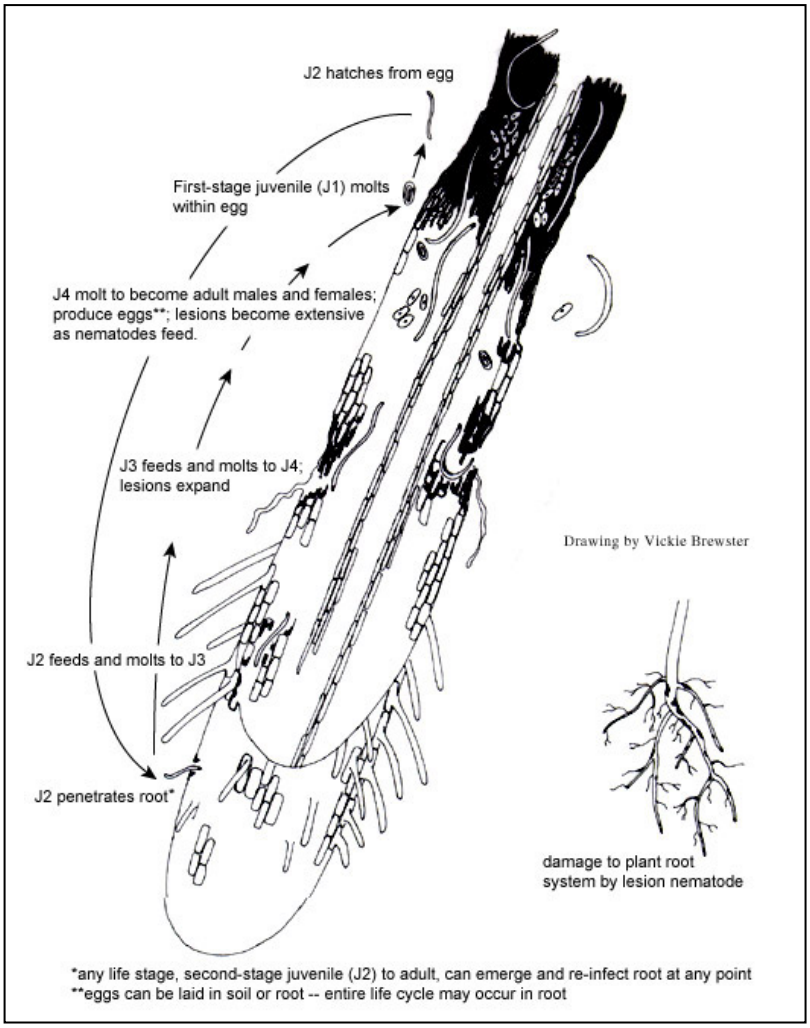
Lifecycle of lesion nematodes

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.

Table 11. Reported yield losses due to *P. penetrans* in vegetable crops.

Crop	Yield loss	Reference
Onion	Loss in marketable yield ranging from 14% (67 *P. penetrans*/100 g soil) to 71% (1800 *P. penetrans*/100 g soil)	Olthof and Potter (1973)
Celery	Fresh weight of celery in pots was reduced by 31, 48 and 83% at 61, 313 and 1565 *P. penetrans*/100 cm^3 soil respectively.	Townshend (1962) in Merrifield (2000).
Table beet	Yield reduced by 27% at 1800 *P. penetrans*/100 cm^3 soil.	Potter and Olthof (1974)
Cauliflower	Loss in marketable yield ranged from 19% at 67 *P. penetrans*/100 cm^3 soil to 59% at 1800 *P. penetrans*/100 cm^3 soil)	Olthof and Potter (1973)
Lettuce	Loss in marketable yield ranged from 18% at 67 *P. penetrans*/100 g soil to 33% at 1800 *P. penetrans*/100 g soil.	Olthof and Potter (1973)
	1800 *P. penetrans*/100 g soil reduced yield by 43%.	Potter and Olthof (1974)
Tomato	In pots Pi of 201, 458 and 1436/100 g soil reduced fruit production by 38% and weight by 44%.	Potter and Olthof (1977)
	In pots Pi of 45/100 g soil resulted in height reductions of 15-54% height reduction in seedlings of 8 varieties.	Miller (1978)
Sweet corn	Losses in marketable yield ranged from 30% at 67 *P. penetrans*/100 g soil to 49% at 1800 *P. penetrans*/100 g soil.	Olthof and Potter (1973)

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



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 invade and 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 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.

Disease complexes between species of Pratylenchus and other pathogens such as fungi have been described in vegetable crops. For example, several species of Pratylenchus (e.g. *P. penetrans*, *P. scribneri* and *P. thornei*) interact with the fungus *Verticillium dahliae* to cause potato early dying disease (Stevenson *et al*. 2001).

3.4 Bulb and stem nematode (*Ditylenchus*)

Species of importance to vegetable production

The most important species of bulb and stem nematode associated with damage to vegetable crops is *Ditylenchus dipsaci*. This nematode is one of the most economically important plant-parasitic nematodes in temperate regions (Sturhan and Brzeki 1991). It has a wide host range (over 450 plant species), but is composed of over 20 recognised races which are differentiated by host range. In addition there are diploid and tetraploid (giant) forms. *D. dipsaci* can cause significant losses in a range of crops including onion, garlic, leeks, shallots, lucerne, red and white clover, ornamental bulbs, oats, peas, beans, parsnips, carrots and potatoes.

D. dipsaci has been reported in most states within Australia (Table 8). Only two races (oat and lucerne) of *D. dipsaci* have been reported in Australia (McLeod 1980, Nobbs 2003). However, research has been limited and there may be other races present. The host range of the oat race includes *Allium cepa*, *Vicia faba*, *Phaseolus vulgaris*, *Pisum sativum*, *Beta vulgaris*, *Fragaria* sp., *Avena fatua* and many weed species, but not wheat and barley or red clover and lucerne (Sturhan and Brzeski 1991). The host range of the lucerne race includes *Medicago* spp., *Melilotus* spp., *Trifolium hybridum*, *Onobrychis viciaefolia*, *Phaseolus* beans, Phlox and several weeds and wild plants, but not onion. *D. dipsaci* has been reported in New South Wales, South Australia,

Tasmania, Victoria, and Western Australia from a range of vegetables (*Allium cepa*, *Allium sativum*, *Daucus carota*, *Lycopersicum esculentum*, *Pastinaca sativa*, *Phaseolus vulgaris*, *Pisum sativum*, *Solanum tuberosum* and *Vicia faba* (Nobbs 2003). There are several native species of Ditylenchus which may be confused with *D. dipsaci* (Nobbs 2003).

Some states within Australia such as Tasmania keep a register of known infestations of stem and bulb nematode. Despite sporadic outbreaks, distribution in Tasmania appears limited. In 1992-93, 250 onion paddocks with a total area of more than 1,150 ha were surveyed for symptoms of *D. dipsaci* (L. Ransom pers. comm.). Plants with possible symptoms of nematode infestation were collected from 142 paddocks. *D. dipsaci* was detected in only three paddocks from two properties in which the nematode had been recorded previously (L. Ransom pers. comm.). Surveys of onion crops for export have been conducted in more recent years to ensure exports meet phytosanitary certification requirements.

Damage to vegetable crops caused by D. dipsaci

The economic threshold for *D. dipsaci* is reportedly low, with densities of 10 nematodes/500 g of soil capable of causing serious damage to onion and carrot (Sturhan and Brzeski 1991). Similarly Decker (1969) reported an economic threshold level of 2 *D. dipsaci*/100 g of soil on onion, celery and carrot. *D. dipsaci* has been reported to cause severe damage to carrot and celery in Italy (Greco 1993) and in Sicily (Schiliro *et al*. 1995) including stunted growth, foliar discoloration and wilting, giving rise to withering of the interior leaves and decay. *D. dipsaci* can invade young seedlings, causing poor emergence and heavy infestations complete crop loss. In onion, infested plants are stunted, with short thickened, chlorotic leaves which may exhibit wilting. The onion bulb becomes soft and spongy leading to distortion and 'bloating'. When bulbs are cut open scales may appear browned in concentric circles. The number of split or double onions may increase in the field and if conditions are moist, secondary rots set in. In garlic, the nematode causes leaf yellowing and death but does not cause the same deformation or

swellings as in onions. Infested garlic is easily invaded by fungal and bacterial rots.

Lifecycle of D. dipsaci

D. dipsaci is a migratory endoparasite. As with many other nematodes there are four juvenile stages (the first occurring in the egg). All stages of *D. dipsaci* can infect plants, however the pre-adult stage (J4) is considered most important as they can form an anhydrobiotic aggregation ('wool') of nematodes which can survive for months or years. In this form they can be distributed in dry seeds, plant debris or dry soil. When there is adequate soil moisture nematodes can be revived and penetrate and enter plant tissue, feeding on the parenchymatous tissue of stems and bulbs. The lifecycle on onion is completed in 19-23 days at 15^{o}C. (Yuksel 1960). Mating is required for reproduction and females may survive for some 70 days, with each female producing 200-500 eggs (Johnson 1998). Populations can build up rapidly under optimum conditions.

Stem nematodes reportedly survive best in clay soils, but rapidly decline in sandy soils (Seinhorst 1956). Some populations have been shown to survive in the soil for at least 8-10 years in the absence of host plants. Stem nematodes may invade and feed within non-hosts and heavy infestation may kill such plants, however, reproduction does not occur (Sturhan and Brzeki 1991)

3.5 Spiral and related nematodes.

Rotylenchulus reniformis is considered the only species of the genus to cause major economic damage to agricultural crops (Jatala 1991), parasitising over 100 plant species, and causing economic loss in many vegetable crops (Sikora and Greco 1990, Heald and Robinson 1990). *R. reniformis* has also been reported on carrot in China and nematicide treatment increased carrot yield by 10-24% compared to untreated (Liao *et al*. 1999). *R. reniformis* has

been reported in Queensland, Northern Territory and Western Australia (Nobbs 2003).

3.6 Stubby root nematode

Paratrichodorus minor (syn. *Trichodorus christei*) causes severe crop losses to many vegetable crops, including onion, tomato, pepper, eggplant, beet, broccoli, brussels sprout, cabbage, cauliflower, chinese cabbage, radish rutabaga, turnip, endive, lettuce and spinach (Johnson 1998). It has been reported in Queensland, New South Wales, South Australia and Victoria (Nobbs 2003) (Table 8). Stubby root nematodes are ectoparasites, feeding mainly at root tips, leading to cessation of growth and typical 'stubby root' symptoms. Species of Trichodorus and Paratrichodorus are also vectors of pea early browning virus, infecting pea and lucerne in Western Europe (Decraemer 1991) and tobacco rattle virus, which infects a wide range of species but causes the economically important disease 'spraing' in potato and colour break in ornamental bulbs. In S. America *P. minor* has also been reported to transmit pepper ringspot virus (Decraemer 1991).

3.7 Needle nematode (*Longidorus* spp.)

Several species of the ectoparasitic nematode *Longidorus* have been reported on vegetable crops in other countries and while they can be economically important, they normally cause localised problems. *L. africanus* occurs in Israel, the United States and Zimbabwe and is a pathogen of many vegetable crops in the Imperial Valley of California (Radewald *et al*. 1969), including carrot (Davis and Raid 2002). Huang and Ploeg (2001) reported that growth of lettuce and carrot was severely affected by *L. africanus*. Ploeg (1999) demonstrated that multiplication on tomato was highest at 29^{o}C. Egg development required a base temperature of 14.3^{o}C with a heat sum of 94.1 DD above this temperature (Ploeg 1999). 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*. *L. elongatus* occurs in Europe and was reported by Hooper (1973) to cause severe damage to carrot. Species of *Longidorus* occur in Australia, including one record of *L. elongatus* from *Lolium* sp. in South Australia (Nobbs 2003). However, Longidorus have not been associated with damage to vegetable crops in Australia (Nobbs 2003).

3.8 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 vegetables, including 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 there have been few reports (Nobbs 2003).

3.9 Sting nematode (*Belonolaimus* spp.)

The main species of sting nematode associated with damage to vegetable crops is *Belonolaimus longicaudatus*. This species can cause major damage to carrot, bean, cabbage and other crucifers, celery, sweet corn, cucumber, pumpkin, okra, onion, pea and pepper (Swart and Nguyen 1991). The threshold for crop damage may be a single nematode in a soil sample (Johnson 1998), and where high population densities are present there may cause complete loss of susceptible crops (Rhoades 1971).

B. longicaudatus has been reported 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). 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, but not on vegetable crops (Nobbs 2003).

3.10 Spiral nematodes

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 lettuce and parsnip in Tasmania and carrot in Tasmania and Victoria (Nobbs 2003). It is not considered common in Tasmania, but was found recently in 2003 in a pyrethrum paddock in the North West Coast of Tasmania (Hay pers. comm.), probably associated with weed hosts. *R. robustus* can cause yield losses in lettuce, pea and carrot (Johnson 1998).

R. uniformis was reported to affect some 15% of carrot production in Holland with damage severe in some cases but overall less than 1% reduction in yield (Seinhorst and Kuniyasu 1969). Carrots had a tolerance limit of 30/5g of soil at 17^{o}C and 2/5g soil at 5-10oC. Populations increased 3.5 times on carrot after 10 weeks in sand culture with significant effects on plant growth at 30-60 nematodes/5 g soil (Seinhaust and Kuniyasu 1969).

3.11 Stunt nematodes

Johnson (1988) noted that many *Tylenchorhynchus* spp. have been associated with vegetable crops (Netscher and Sikora 1990, Anderson and Potter (1991). *T. brassicae* is the only species recognised as a significant

economic parasite of vegetable crops, with high population densities associated with areas of poor growth in cabbage and cauliflower fields (Khan 1969). Nobbs (2003) listed a few reports of species of Tylenchorhynchus within Australia, from Queensland, Western Australia, New South Wales, Southern Australia and Tasmania. However, *T. brassicae* was not reported.

3.12 Other nematodes

Many other species of nematodes may cause localised and sporadic damage to vegetable crops while not considered of wide scale economic importance. High population densities of *Dolichodorus heterocephalus* are highly pathogenic to tomato and celery (Johnson and Fassuliotis 1984), however there have been no reports of this nematode in vegetables in Australia. *Neodolichodorus australis* (Hodda and Nambiar 2005) and *Hemicycliophora saueri* (Walker 2004) were recently associated with damage to carrot in Australia. Similarly, the false root-knot nematode (*Naccobbus aberrans*) is an important parasite of several vegetable crops in other countries, including bean, carrot, cucumber, eggplant, lettuce, garden pea, pumpkin and tomato (Johnson 1998), but has not been reported in Australia.

4. Quantifying nematode populations

Sampling for nematodes may be undertaken for a variety of reasons i) to determine presence/absence in a number of fields (prevalence) e.g during a survey for exotic incursion, ii) to determine population density within fields (incidence), e.g. for prediction of yield loss or assessment of population change due to a specific treatment, or iii) to determine spatial pattern, e.g. for precision management. The sampling intensity required within the sampling unit (e.g. field) increases from i) to iii). The probability with which a nematode species is detected when it is present in a field is influenced by many factors including soil type, vertical distribution in the soil profile, time of year, number of soil samples collected, the size samples, the amount of soil that is processed and the method of nematode extraction and identification (Davis and Venette 2004).

Obtaining accurate estimates of the population density of nematodes or other soil borne pathogens is difficult given the small amount of soil which can be processed as a proportion of that in the field. Evans *et al.* (2003) noted that there is *ca.* $2.5x10^9$ g soil to a depth of 20 cm per hectare which means that if the detection threshold for a particular nematode is 1 per gram of soil, there could be $2.5x10^9$ nematodes present in a hectare even though none are detected.

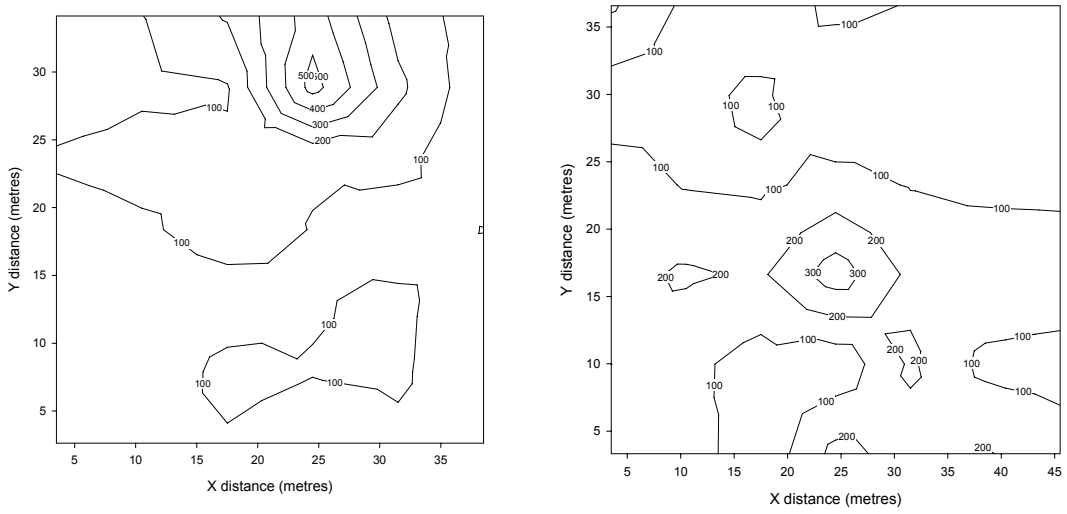
4.1 Spatial distribution of nematodes in relation to sampling.

Sampling for nematodes is problematic as nematodes are usually spatially aggregated within a field rather than being uniformly distributed (Figure 2). For nematodes, frequency distributions have often been described by the negative binomial distribution which is composed of the mean and the exponent *k* which is a measure of the amount of aggregation (Anscombe 1950, Southwood 1978, Seinhorst 1982). McSorley (1987) reported *k* values for nematode distributions varied between 0.02-60, but that most were less than 1, indicating aggregation. However, Allsop (1990) found that *k* was not

constant relative to the mean and considered the negative binomial function was inappropriate for describing nematode populations in sugarcane fields in Australia. Taylors power law can also be used to describe the frequency distribution of nematodes (Boag and Topham 1984). It relates variance to mean density by $s^2=ax^b$, where a is a sampling factor, and b is an index of aggregation which is constant for a species in a certain habitat, ranging from

Figure 2. *Pratylenchus*/200 ml soil in soil in two carrot crops in Tasmania at 58 days after sowing (a) Crop 1, Feburary 2001 and b) Crop 2, November 2001 (carrots in rows running along X axis) (from Hay *et al*. 2004)

a) b)



b<1 for uniform distributions, b=1 for random distributions and b>1 for aggregated distributions. Taylors power law has been used to devise appropriate sampling strategies for nematodes (e.g. McSorley and Dickson 1991). Allsop (1990) used Taylors power law to develop a sequential soil sampling plan in Australian sugar cane fields which incorporated the use of stop lines for a given level of precision. This allowed cumulative numbers of nematodes to be plotted successively against the corresponding number of samples until the stop line of the required precision was crossed. This could reduce the number of samples which needed to be counted (Allsop 1990).

4.2 Sampling equipment and depth

Soil samples for vegetable crops are normally taken with a corer 2.5 cm diameter, or similar tool, to the approximate depth of the rootzone (20-30 cm). However, the choice of sampling equipment is often not optimal. In wet soils of high clay content it may be difficult to remove the soil sample from core type equipment such as an Oakfield sampler. Stevens *et al.* (2002) tested various types of probe for obtaining soil samples for nutrient analysis. Sampling time was reduced in sandy loam and clay soils with a cone probe compared to a straight tube or foot pedal probe. The cone design reduced clogging and allowed 8-10 cores to be collected before soil samples needed to be emptied from the probe (Stevens *et al.* 2002). Augers can also be used for soil sampling, but may cause damage to nematodes. However, in general, Hollaway *et al*. (2003) found no significant difference between sampling with an auger or corer in the number of root lesion nematode detected by Whitehead tray technique or DNA based techniques (discussed later). At one site there was significantly lower numbers obtained from dry soil by Whitehead tray using the auger compared to the corer, which was attributed the former sampling tool causing damage to nematodes which may have been in an anhdrobiotic state (Hollaway *et al*. 2003). Furthermore there was generally better recovery of root lesion nematode by Whitehead tray from clay soils sampled by auger than with corer. This was attributed to the latter causing some compaction of the clay soil during sampling and either killing nematodes or inhibiting extraction (Hollaway *et al*. 2003). Turner (1993) found no significant difference in detection efficiency of potato cyst nematode (PCN) between augers of 3, 10 or 12 ml volume, when the number of samples was adjusted to give a standard volume of 360 ml.

The depth of sample taken should normally encompass the root zone of the crop to be grown. For carrot, 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). However, it may be difficult to obtain a sample of sufficient

depth in soils which are compacted and the ability to extract and detect nematodes is often less from dry soils, or soils which have been cultivated.

To account for the often aggregated distribution of nematodes across the field, it is important that core samples are collected from representative points within the sampling unit. This increases the chances of detecting foci of high nematode population density. For this reason it is often recommended that pre-plant samples should be collected along a zig-zag or 'W' pattern within the sampling unit (Evans *et al.* 2000). Turner (1993) investigated the effect of sampling pattern on the ability to detect PCN in 45 fields. He found no significant differences in the ability to detect PCN from 60 samples taken from each field in each of four different sampling patterns, i) a zig zag pattern, ii) a joined cross-diagonal arrangement, iii) an 'M' shaped pattern or iv) samples collected from the perimeter of the field approximately 10 m in from the headland. A perimeter method could minimise costs associated with sampling (Turner 1993). However, a perimeter method of sampling pattern might be expected to provide a non-representative quantitative estimate of average population density over the field in comparison to other methods which involve collection of samples from across the field. Evans *et al.* (2000) reported a perimeter sampling to provide poor results in comparison to a 'W' pattern or regular grid. There is scope for other studies to investigate the effect of sampling pattern on population estimation of different nematode species for advisory purposes, as a means of maximising accuracy and minimising cost.

Sampling is usually undertaken at a time which maximises the possibility of detection (e.g. at highest population density) and/or allows management decisions to be made (e.g. preplanting or following a treatment to judge the efficacy of that treatment).

4.3 Sample processing and nematode extraction

From each sampling unit, the 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 (10-20^{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. Hollaway et. al. (2003) reported that storage of undried samples at room temperature or at 4^{o}C did not have a significant effect on quantification of root lesion nematodes by Whitehead tray or DNA assay. A subsample of the soil sample may be extracted which further reduces the likelihood of detection. Davis and Venette (2004) showed that the likelihood of detecting a nematode from extracting a 300 ml subsample of a 1 L sample remains less than 90% until population densities reach *ca.* 11-75 nematodes per litre of soil.

There are a wide range of techniques for extracting nematodes from soil and roots (Southey 1978, Barker 1985, McSorley et al. 1984). The efficiency of extraction from soil samples is greatest when 100 g (*ca*. 70 ml) to 450 g (*ca*. 300 ml) of soil are processed (Ingham and Santo 1994b). However, extraction efficiency can be low. For Meloidogyne, extraction efficiencies of 13-45% were noted (Barker 1985, Ingham and Santo 1994a). Three common extraction techniques are listed below.

Extraction of vermiform nematodes from soil: Whitehead tray method (Whitehead and Hemming (1965).

The Whitehead tray method is used extensively for nematode extraction from soil, often with slight modification (e.g. Vanstone *et al*. 1998). Soil (generally 200-400 g or ml) is crumbled to a fine tilth to a depth of approximately 2 cm over the surface of tissue paper suspended in a tray by means of a mesh basket. The tray is filled with water to a level approximately half way up the level of the soil. Trays are incubated at room temperature for a period (generally 72-96 hours) to allow nematodes to migrate from the soil, through the tissue into the water. Nematodes are recovered by passing the suspension through a 20-30 µm sieve and washing nematodes from the surface of the sieve into a collection vial. As nematodes are of smaller diameter than the sieves used there is a possibility that some may pass

vertically through the sieve. This is minimised by maintaining the sieve at a 45^{o} angle and resieving water which passes through the sieve a second time to recover a proportion of nematodes which may have passed through the first time.

Taylor and Evans (1998) found less recovery of root lesion nematode from dry soils in comparison to wet soils by Whitehead tray technique and suggested that mechanical disturbance during sampling of dry soils may have killed nematodes leading to poor recovery. Reduced recovery from dry soils may also be related to the relative proportion of eggs to vermiform stages at the time of sampling (Hollaway *et al*. 2003). Some authors have circumvented this by increasing the incubation time (e.g. Castillo *et al*. 1996).

The way in which extraction techniques are conducted may also alter the results. For example, with the Whitehead tray technique extraction periods of 3 to 4 days are often quoted in the literature. However, in some situations much longer periods of extraction are necessary. E. Davison and A. McKay (AgWA) (in Hay *et al*. 2004) showed that numbers of Meloidogyne J2 extracted by Whitehead tray over a period of 3 days from soil samples taken immediately prior to planting carrot were poorly correlated with the subsequent level of seedling infection as assessed by staining seedling roots ($r = 0.27$). Further work identified that significantly more Meloidogyne J2 were recovered following longer periods of extraction of up to 3 weeks (Table 12). On the basis of this, nematode extractions conducted at the AgWest laboratories were subsequently increased to 14 days, with collection of nematodes from Whitehead trays at 7 and 14 days.

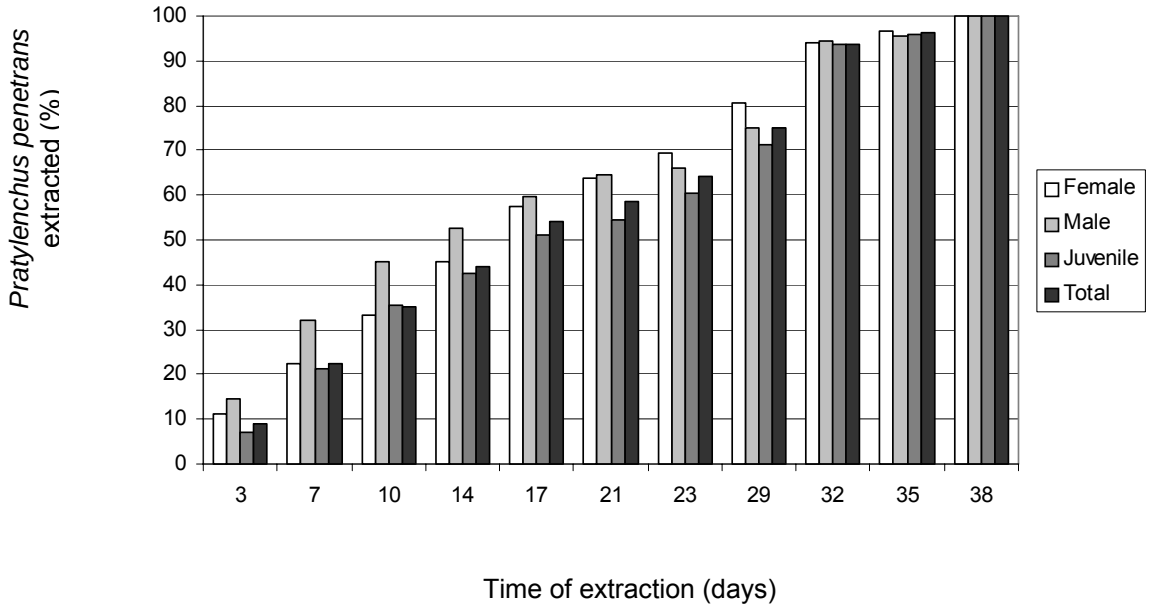
Mishra and Gaur (1987) showed increased recovery of Meloidogyne and Rotylenchulus following storage, presumably due to egg hatch. Gnanapragasam and Sivapalan (1991) noted increased recovery of *Pratylenchus loosi* from samples stored for 7 days compared to those processed on the day of collection, which was attributed to migration of nematodes from root fragments during storage.

Table 12. Number of *Meloidogyne* J2 recovered from 200 g soil after different times of extraction by the Whitehead tray technique (Hay *et al*. 2004).

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

A study of the ability of Whitehead tray technique to extract *Pratylenchus* spp. from soil and roots of pyrethrum (*Tanacetum cinerarifolium*) was conducted in which 60 soil samples, comprising 400 ml of soil and chopped root material, were placed on Whitehead trays with (Hay unpubl. data). Extractions were made periodically at intervals of 3-5 days over a period of 38 days, with replacement of water in the Whitehead tray to prevent microbial growth in the trays which may have affected the viability of the nematodes. The total number of Pratylenchus, adult females, males and juveniles extracted over 38 days was 328,762, 126,045, 14,128, and 180,891 respectively. Extraction periods of 17 days achieved between 50-60% of the number extracted at 38 days, while an extraction period of 32 days was required to achieve over 90% extraction of the total number extracted at 38 days (Figure 3). It is presumed that the long duration over which Pratylenchus were extracted in this experiment was due to the requirement for nematodes to migrate out of roots and the hatching of juveniles from eggs within roots.

Figure 3. The number of *Pratylenchus penetrans* extracted from soil and roots of pyrethrum at different times by Whitehead tray technique as a percentage of the total number extracted after 38 days (Hay unpubl. data).



Extraction of vermiform nematodes from roots: Mist technique (Southey 1986).

Roots are chopped into short lengths (approx. 1 cm), weighed and placed in a mesh basket and placed in the top third of a glass funnel. The funnel is placed inside a tall glass test tube and the stem is fitted with a plastic hose so that it discharges near the base of the test tube. The root sample is subjected to a fine, intermittent mist of warm water (e.g. 30 seconds every 5 minutes). Nematodes which migrate from the roots are washed into the base of the test tube, while excess water overflows over the sides of the test tube and is drained away. Nematodes are extracted for a period of 3-5 days and then are recovered by sieving as for the Whitehead tray technique.

Extraction of immobile stages of nematodes (e.g. cysts)

Several methods can be used for isolating cysts from debris (Turner 1998), based on flotation (e.g. Fenwick can) or elutriation. In the latter method water enters at the base of a conical column at a constant rate (minimum of 0.6 L/min.) Soil is added into the column using a funnel fitted with a baffle to prevent soil particles sinking to the base of the column too quickly. Soil particles fall to the base of the column while the less dense cysts are carried by the overflow water where they are retrieved on sieves (53µm diameter) (Anon 2004)

The efficiency of the particular 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 is often assumed to have 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 2000). Therefore numbers per volume soil are an acceptable approximation of numbers per weight of soil (corrected for dry weight) for making management decisions (Merrifield 2000).

4.4 Sampling strategies

The intensity of sampling undertaken is limited by cost/benefit and practicality. For detecting presence/absence it is cost effective to reduce sample intensity or sample size taken from within fields and survey a large number of fields, even though the likelihood of finding a particular species in an individual field

is low (Davis and Venette 2004). For quarantine nematodes which are known to occur in a country (e.g. PCN) then a large number of samples must be collected from individual fields to certify with a high level of confidence that the probability of a nematode species being present in a particular field is low (Davis and Venette 2004). A review of sampling with special relevance to potato cyst nematode was recently published (Been and Schomaker 2006).

Sampling to assess population density

Soil sampling is undertaken at points across the field, samples are bulked and a subsample counted to obtain an average population density over the field. Samples are bulked to reduce the cost of extraction and enumerating nematodes. However there is a loss of valuable information on the spatial distribution of nematodes across the field, because nematode populations are usually aggregated,

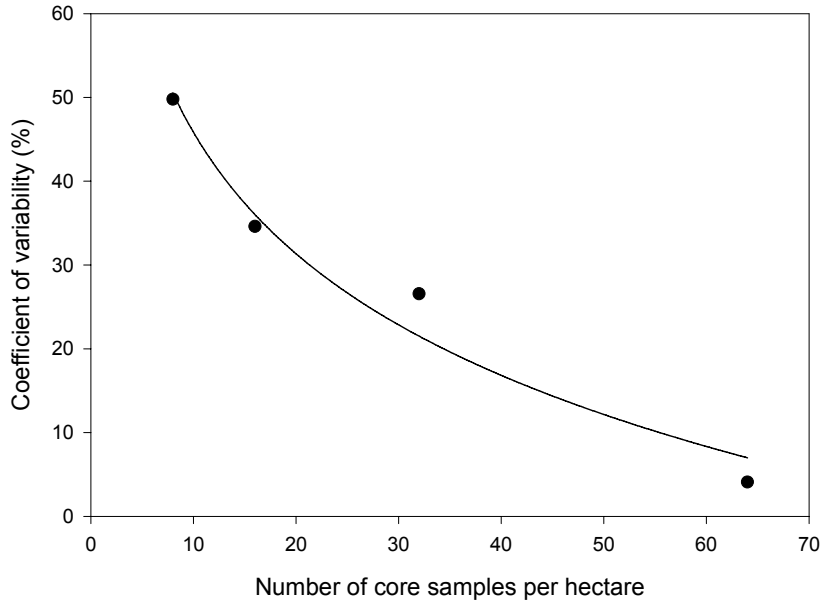
Two important decisions with regard to sampling are the size of the sampling unit and the number of core samples taken per sample unit (Stirling *et al.* 1999). A sampling unit is the unit land area of choice for sampling, with several samples obtained from each sampling unit and bulked to provide a single sample per sampling unit. A sampling unit may comprise the entire field. However, where the field is large or composed of different soil types or areas of different cropping history in which there may be different population density of nematodes, several sampling units 'strata' within the field may be chosen. Stratification of the field sampled minimises variance within each stratum, making the estimate of the mean in each stratum more representative of the true population density and allows some determination of spatial distribution of nematodes.

For estimation of thresholds, Stirling *et al*. (1999) noted that the size of the sampling unit and intensity of sampling is dependent upon the value of the crop. For example, for crops valued at approximately $5000 per hectare, the sampling unit could constitute a size of 1 ha (Stirling *et al*. 1999).

The coefficient of variation around the mean number of nematodes counted is reduced in a curvilinear fashion with increased intensity of soil sampling (Stirling *et al.* 1999, Evans *et al.* 2000). Sample sizes large enough to measure population density to within 25-50% of the true mean are often considered sufficient for diagnostic purposes (McSorley 1982). A composite sample of 40-50 soil cores per hectare was required to reduce variability to less than 20% (Figure 4) (Hay *et al*. 2004). This is in agreement with Stirling *et al*. (1999), who 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. Procter and Marks (1974) estimated that at least 7 hours of field sampling and laboratory analysis was required to obtain an estimate of the mean population density of *Pratylenchus penetrans* in a plot of 0.01ha with a coefficient of variation around the mean below 20% and 95% level of confidence. This demonstrates that a high density of sampling is required to ensure a sufficiently accurate estimate of the true population upon which to base management decisions.

Any management decision for nematodes is dependent upon identifying and quantifying the nematodes present. 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). However, this pattern of monitoring was far more intensive than is usually employed. 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.

Figure 4. Relationship between number of core samples taken per hectare and the coefficient of variability around the mean number of total soil nematodes (Hay *et al*. 2004).



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 Pi and Pf and thereby assess 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 highly susceptible vegetable crops 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 reach their highest population density. This would 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. Staining root tissue can also improve detection (e.g. Sharma and Mohiuddin 1993, Thies *et al*. 2002). E. Davison and A. McKay (AgWA) (in Hay *et al*. 2004) assessed invasion of carrot seedlings approximately 1 month after planting using the trypan blue staining method (Sharma and Modiuddin 1993). They found a good correlation (r=0.65) between the number of carrot seedlings invaded by *Meloidogyne* spp. in the current season in comparison to that in the previous. Patches of nematode infestation could be monitored through the rotation and/or be selectively treated with nematicide when another susceptible crop is to be established.

However, despite the apparent importance of time of sampling on assessing risk to subsequent crops, there have been few other studies conducted with other nematode species in vegetable crops to define the best time of sampling. Sampling plants from different parts of the field later in the season has further advantage in that it would also allow the grower to locate patches of nematode infestation.

Bioassays are an alternative to extracting and counting nematodes from soil samples. Bioassays involve estimating nematode populations by planting susceptible plants in soil samples or directly in the field and assessing subsequent damage. A bioassay involving counting the number of galls developing on tomato cv. Rutgers was 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 in microplots between marketable carrot yield in the current year and bioassay gall counts from onion grown in the previous year (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.

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) determined 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 Boivin (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 Boivin (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. However, 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 might be to determine i) the proportion of carrots at grading rendered unmarketable by nematode damage which was equal 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.

Roberts and Matthews (1988) developed a similar system for prediction of *Meloidogyne incognita* damage in cotton crops. Root infection ratings based on weighted galling indices made in the field at the end of the first crop year were found to provide a reliable quantitative prediction of yield loss to a succeeding cotton crop in the second crop year for a given set of conditions (e.g. soil and cultivar). The Weighted Nematode Rating (WNR) system is currently promoted by the University of California Davis (www.ipm.ucdavis.edu). Fields are split into representative blocks and 15-20

root samples are taken from each block. Soil is shaken or washed from roots and roots compared to diagrams and given a weighted rating dependent upon the percent of each root system with galls: 0 (0%), 1 (1-25%), 3 (25-50%), 5 (51-75%), 7 (76-100%). The number of roots sampled in each rating category is multiplied by their respective weighted ratings and summed to give a total WNR, which is expressed as a percentage of the total WNR possible (i.e. Total number of roots sampled x maximum rating possible – 7). The recommendation was not to plant susceptible cotton varieties into a field with a percent WNR greater than a threshold of 10% unless the nematode population was managed first.

Sampling for presence/absence

For regional surveys of nematodes Prot and Ferris (1992) recommended a composite sample of 10 cores per sampling unit (field), with each core taken approximately 55 m apart throughout the entire field, from fields which have contained one or more hosts of the particular nematode in the rotation. Davis and Venette (2004) demonstrated that a 10 core composite sample was efficient at detecting nematodes which are frequent and abundant. For nematodes which were aggregated, with a *k*-value from the negative bionomial of k=0.5 to more uniformly distributed over the field (k=2.0) there was an over 80% probability of detecting at least one nematode in a sample unit with this sampling intensity when the mean number of nematodes per sample unit was *ca.* 10 or above. Davis and Venette (2004) demonstrated that the number of fields to be sampled was a function of the anticipated frequency of infested fields and the probability of detecting nematodes within a field. For example, if 1 in 100 fields were infested then 600-6000 fields would need to be sampled (depending upon the likelihood of detecting nematodes in an individual field) to have 95% confidence of finding an infestation within a region (Davis and Venette 2004).

Sampling for regulatory purposes.

A higher intensity of sampling per sampling unit is required where there is a necessity to certify with a high level of confidence that the probability of a nematode species occurring in an individual field is very low. A number of sampling strategies have been developed for Potato Cyst Nematode in many countries. Haydock and Evans (1993) gave an example of regulatory sampling for PCN in Europe. Approved methods of soil-sampling and nematode extraction for detection purposes were described (Anon 1991), with a recommendation of 100 soil cores (ea. 4-5 ml) taken from the top 5 cm of soil, providing a total soil sample of some 500 g for extraction. In this case there was interest in detecting small, unevenly distributed populations of PCN, with a qualitative outcome, i.e. presence or absence. Been and Schomaker (1996) developed a simple exponential model which described the general shape of a focus based on 39 infestation foci mapped in square metre units throughout the Netherlands. Within this model the expected population density at any location in a focus could be calculated relative to a given central population density. The model was incorporated into a computer program SAMPLE to automate developing and testing of sampling methods for detection of infestation foci. Been and Shomaker (1996) tested sampling methods for detection of infestation foci using a computer program SAMPLE, assuming a central population density of 50 cysts/kg of soil with a detection probability of 90%. Results suggested an optimal sampling grid dimension near 5 x 5 m with a core size of 52 g and a sample size of 6.9 kg/0.33 ha. This sampling intensity provided the best compromise between minimising sample size and variance of detection probability, while minimising time needed to collect and process samples. This sampling regime was compared with the statutory soil sampling procedure currently used in the Netherlands involving a 7.5 x 7.5 m grid and core size of 3.3 g with a sample size of 200g/0.33 ha and an average detection probability of 90%. The new sampling procedure detected foci with central population densities 100 times smaller at a cost of 3-4 times as much. The requirement for a more intensive sampling procedure could be solved by using automated sampling devices mounted on a jeep and elutriators for the extraction of cysts from soil samples of up to 3

kg. During the period 1984-1988 some 21.3 million kg a.i. of nematide was used in The Netherlands, equivalent to an average of 20 kg a.i. of nematicide on each hectare of arable land per year (Molendijk and Korthals 2003). With this new sampling procedure, it has been possible to reduce by 77% the use of soil fumigants in seed and ware potato-growing areas in the Netherlands by 1995 (Been and Shomaker 2000). Further work (Schomaker and Been 1999) verified that the exponential model was valid over a range of localities. Been and Shomaker (2000) demonstrated that SAMPLE could be used to design sampling protocols which were more efficient than statuatory protocols adopted by several sampling agencies. This involved a sample unit of 50 cores (2.5 cm x 15 cm) collected on a 5 x 6 m grid. This resulted in 2 kg of soil per sample unit with a sample size of 6-7 units/ha. This intensity of sampling provided a ≥ 90% probability of detecting nematode foci with ≥ 200 cysts/kg soil. With some modification the SAMPLE program could be used to design optimum sampling techniques for other nematode species (Been and Schomaker 2000). Been *et al* (2003) reported on the adaptation of SAMPLE for *M. chitwoodii* and *M. fallax*, with preliminary work showing Meloidogyne to be highly aggregated.

Intensive sampling coupled with Global Positioning Systems (GPS), Geographic Information Systems (GIS) and Precision Agriculture has led to the possibility of reducing nematicide use and control costs by applying nematicides only to those parts of the field in which the nematode population density is above a damage threshold (e.g Baird *et al.* 2001, Evans *et al.* 2003). Wrather *et al*. (2002) mapped root knot nematode population density across a field of cotton in Missouri by collecting soil samples on a 0.25 acre grid and extracting and enumerating nematodes within each sample. The nematicide aldicarb (Temik 15G) was applied at 0 lb, 3.5 lb, 5.0 lb and 7.0 lb Temik/acre to parts of the field with root knot nematode densities of 0-200, 200-500, 500-1000, or >1000 J2/600 ml respectively and compared with a uniform rate (3.5 lb/acre) across the field. There was no significant difference in cotton yield between the variable rate and uniform rate of nematicide, while both had significantly higher yield than areas with no nematicide. The variable rate treatment used 46% and 61% less chemical than the standard treatement

in each of two years. However, the cost of the variable rate treatment was calculated at US$100/acre which was much higher than the uniform rate at US$5/acre. The higher cost of the former was due to the cost of developing the nematode density distribution map, which included costs of collecting soil samples at a density of 4 samples per acre at US$5/sample (US$20/acre), and the costs of extraction and enumeration of nematodes from each of the 4 samples per acre (US$80/acre). For the uniform rate the cost of collecting and analysing was estimated at US$25/5 acres, which included collection of 1 sample per 5 acres (US$5/sample) and nematode analysis (US$20). Less Temik was used in areas treated with a nematode density specific rate than areas treated with the uniform rate. However, the authors did not account for savings in nematicide in their economic analysis. An attempt to reduce costs of mapping was made by redrawing the distribution map based on 0.5 acre grid, however this sample density was not sufficient to provide a sufficient estimation of nematode distribution. Baird *et al*. (2001) demonstrated that variable rate application of Telone II to cotton in Georgia produced either similar or higher yields than uniform application. Furthermore in this study, the cost of soil sampling and assay needed to develop maps of nematode density for the variable application was offset by the savings in the cost of the nematicide Telone II for each of the two years of the study (Rich *et al.* 2003).

Further savings may be made if the spatial distribution of nematodes remained similar between years and the distribution map remained representative over a number of succeeding years. This would be reliant upon the spatial distribution of nematodes remaining similar with time. Using geostatistical analysis, Avedano *et al*. (2003) demonstrated that over a two year period there were areas within fields at which SCN was not detected or remained at low density between years and areas of high or low density which remained approximately at the same locations between years. Gavassoni *et al* (2001) demonstrated that in soybean fields with initially aggregated populations of *H. glycines* that no tillage or ridge tillage led to further aggregation while conventional and reduced tillage systems resulted in a less aggregated spatial pattern. This suggested that reduced tillage systems in which the spatial distribution of nematodes remains similar between years

may be better suited to precision management of nematodes than those systems undergoing tillage.

The cost of soil sampling and manual counting of nematodes is often too expensive to enable the intensive sampling necessary for the construction of sufficiently accurate maps of nematode infestation. Some authors have attempted to use data other than soil counts to determine spatial pattern, which may be less costly. Rich *et al*. (2003) suggested that root gall ratings on infected hosts could be used for Meloidogyne, estimation of cyst number on roots of host plants for Heterodera or symptoms of Ditylenchus infestation of susceptible crops such as onion might be used. For Meloidogyne, collecting samples at intervals within a susceptible crop such as carrot and using an assessment of galling as a means of mapping the spatial distribution of populations might be used as a means of defining areas for variable rate nematicide application in a succeeding susceptible crop. In Quebec, identification of foci of root knot nematode damage in carrot fields led to the widespread adoption of spot treatments with nematicides and subdivision of fields for cropping to non hosts crops (Duncan 1991). Belair and Boivin (1998) were able to relate an average gall index in one carrot crop to a gall index and potential yield loss in a succeeding carrot crop, which suggests that such a method of mapping would be practicable. Attempts to reduce the cost of mapping nematode infestations for site-specific purposes by measuring soil attributes (e.g. soil organic matter and texture) which may be correlated with nematode distributions, as a surrogate for nematode counts have been largely unsuccessful (e.g. Wyse-Pester 2002). Spectral reflectance from the crop canopy measured by satellite imagery, aerial imagery or from hand-held multispectral radiometers is often a good indicator of plant stresses including that from nematode feeding (e.g. Heath *et al.* 2000, Hay *et al.* 2002, Nutter *et al.* 2002). Such an approach has potential to identify areas of nematode infestation in a crop and allow site specific prophylactic treatment prior to a succeeding crop.

4.5 Identification of nematodes

Once extracted, nematodes must be identified and counted. This process normally involves placing a suspension of nematodes in a counting dish and examining with a microscope at 50-100 x magnification. In some cases an aliquot from the total nematode extract is counted, leading to potential for further errors. In other cases further cleaning of the nematode suspension may be required, (e.g. sucrose density gradient centrifugation), followed by sieving. Such processing results in a compromise between the potential for further loss of nematodes versus increased ability to visualise nematodes in the sample.

A substantial amount of training is required for the operator to be able to identify most of the commonly occurring plant parasitic nematodes to genus level with a high degree of confidence. For some nematodes the difference between genera may be subtle and require careful and time consuming slide preparation and examination of morphological features at high magnifications (400-1000 x) as is required for species identifications (e.g. Nickle 1991). Conversely, in some cases nematodes may be identified to species at low magnifications during the counting procedure. Where extensive studies have shown only a limited number of species of a particular genus to be present in a particular geographic area on a particular host, this latter technique might be used with some confidence. In some cases the presence or absence of male nematodes provides further evidence of the species. Some Pratylenchus species may be differentiated on basis of the vulval position in relation to the length of the body during counting. However, in reality the vulval position of particular species of Pratylenchus is variable and may overlap with other species, leading to the possibility of misidentifications.

Identifications to species based on morphology normally require specimens to be mounted on slides and examined at high power under the light microscope. Measurements of various features can then be compared with keys or detailed descriptions of nematodes to identify to species (e.g. Nickle 1991). In the case of root knot nematode, the pattern on the perineal region of the

adult female can be used to identify species (e.g. Eisenback and Triantaphyllou 1991). Visual identifications to species are normally based on morphometrics of the adult female only. Where juvenile nematodes are present, they are assumed to be of the same species as the adult females present in the sample. However, where adult females of more than one species of a particular genus are present in a sample it is difficult to identify the juveniles to species with confidence. Because of the difficulty in identifying juvenile nematodes to species and because of the time consuming nature of slide preparation of adult females and keying to species, most counts based on visual appraisal can only provide identifications of nematodes to genus level. Where there are differences in host range or pathogenicity of particular nematode species within a genus to a particular crop the inability to identify nematodes to species makes management decisions difficult. For example, several root knot nematode species (*Meloidogyne* spp.) within Australia are not hosted by grass species and can be managed by planting a cereal or grass crop in rotation. However, *M. fallax* is hosted by grass species and was recently identified in South Australia and Tasmania (Nobbs 2003). Therefore identification to species is necessary to determine which break crop to employ.

4.6 Damage threshold

Once nematodes have been identified and enumerated, the information is often used to determine whether a resident population is a threat to a following crop. This requires knowledge of the relationship between nematode population density and yield loss. This relationship has been described by various mathematical functions including quadratic, inverse logistic, exponential decay and inverse linear (McSorley and Duncan, 1995). However, Seinhorst (1965) described a generalised relationship between plant parasitic nematode densities at planting and relative yield of a susceptible host which is often used in nematology.

$Y=m+(1-m)z^{(P-T)}$ for $P>T$ and $Y= 1.0$ for $P<T$

Where Y=yield at a density of P nematodes/yield in the absence of nematodes, m = minimum yield at high densities of P, z= the proportion of undamaged root at P=1.0, P=nematode density, T= the highest value of P below which yield is unaffected.

Ferris (1978) combined this equation along with information on economics of control to allow estimation of the economic threshold population densities of plant-parasitic nematodes. At low population densities, increasing the number of nematodes has no observable impact upon plant growth/yield. However, as population densities increase further, a point is reached at which there is an observable reduction in plant growth/yield ('damage threshold'). As nematode density is increased further there is a sharp reduction in plant growth/yield until a point is reached at which the economic losses due to nematode damage are equal to the cost of control ('economic threshold'). As nematode densities increase further, the rapid decline in plant growth/yield tends to plateau.

The relationship between initial population density of nematodes (Pi) and crop yield losses is usually derived from greenhouse experiments or small plot trials in the field either involving i) introduction of nematodes, ii) manipulation of endogenous populations with nematicides or growth of hosts of differing susceptibility or iii) intensive field sampling to locate areas of differing nematode population density (McSorley and Duncan 1995). Viaene *et al*. (1997) developed the computer program 'SeinFit' to calculate the Seinhorst equation which best fit such experimental data describing the relationship between preplant nematode densities and plant growth. This information has been extrapolated into guidelines for the field. For example Dickerson *et al*. (2000) provided nematode guidelines for a range of field crops, commercial vegetable, home garden, turfgrass species, fruit and ornamental plants in South Carolina USA. For each crop, the dominant nematode parasites were listed along with the pre-plant nematode population density which constituted an 'action threshold' in either i) sand to sandy loam, ii) clay loam to clay soils or soil which had been ploughed/disced prior to planting. Similarly Barker and Olthof (1976) summarised thresholds for various host/nematode combinations

derived from a range of studies and Stirling et al. (1986) provided information for a range of crops in Australia. Ferris (1978) extended the concept of the economic threshold to introduce the concept of the optimisation threshold, i.e. that population density (following management) at which the difference in the predicted value of the crop and the management cost to attain that density are maximised. Damage thresholds have been provided for a range of different crops (e.g. Barker and Olthof 1976, Ferris 1986). However, McSorley and Duncan (1995) noted that it is currently difficult to obtain valid estimates of economic thresholds because of insufficient precision in quantifying nematodes and defining the nematode-crop damage function.

More accurate methods of assessing nematode population densities in soil and roots are required to improve the accuracy of thresholds. Ways in which improvements in sampling could be brought about included i) improved understanding of sampling requirements (Schmitt *et al*. 1990, Duncan *et al*. 1994), mechanisation of sampling equipment (Ferris *et al*. 1990), improvements in processing efficiency (McSorley 1987) and new methods of quantifying nematodes in samples including antibody based techniques (Schots *et al*. 1992) and more recently, DNA based techiques (Ophel-Keller *et al.* 1999). Haydock and Evans (1994) listed several potential improvements to soil sampling.

Various attempts have been made to automate sampling to allow a greater sample intensity from the sampling unit than is possible with current labour intensive methods. Anon (1972) developed vacuum sampling technique to disturb the surface of the soil and suck up less dense material including cysts into a sample bag. The California beet cyst nematode sampler was developed to be fitted to an ATV motorcycle and was shown to reduce sampling time by 67% in comparison to sampling by hand (Cooke *et al*. 1979, Roberts and Thomason 1981). Brodie *et al*. (1993) described the USDA wheel sampler which attached to the rear linkage of a tractor and took 1 g soil samples to a depth of approximately 2 cm at intervals of 25-200 cm. Schomaker and Been (1992) described an automated sampler used by the Dutch Plant Protection Service.

However, a further complicating factor in providing a valid estimate of economic threshold is that the host-parasite relationship and thus the nematode-crop damage function can be altered by many factors (McSorley 1992). For example *M. incognita* caused severe damage to tomato in microplots in the coastal plains of North Carolina, USA, but caused only slight losses in mountain regions where conditions such as heavier soil, higher rainfall and lower temperature were more favourable for plant growth (Barker *et al.* 1976). The amount of yield loss is a function of the plant disease concept, the 'disease triangle' and is dependent upon a combination of host, nematode and environmental factors in any given situation. Model variability can be reduced by identifying important intrinsic factors such as soil texture and crop variety and adjusting the model accordingly (Dickerson *et al*. 2000, Schmitt and Barker 1981), or allowing for the difference in host-parasite response between broad classes of extrinsic factors (e.g. wet and dry years) and using historic data to estimate probabilities associated with such conditions (McSorley 1992). Pinkerton *et al.* (1991) noted differences between regions in the amount of damage to potato crops which occurred due to root knot nematode, and related this to differences in degree day accumulation. This suggests that a historical knowledge of day degree accumulation or monitoring of day degrees in different regions could be used to develop more accurate thresholds.

4.7 Biochemical and DNA based diagnostics

Various biochemical methods of identifying nematodes have been developed, including electrophoretic profiles of proteins or isozymes and serologically-based methods using polycolonal or monoclonal antibodies (Barker and Davis 1996). Schots *et al.* (1992) reported the use of monoclonal antibodies which differentiated *G. rostochiensis* from *G. pallida* and used in an enzyme linked immunosorbent assay to quantify PCN in soil samples. Serological techniques have been used in The Netherlands for diagnosis and regulation of PCN (Schomaker and Been 1992).

DNA techniques has revolutionised many aspects of plant pathology and nematology (Barker and Davis 1996). DNA based techniques are now available for the identification of a range of nematodes species including cyst nematodes (e.g. Bulman and Marshall 1997, Fleming *et al*. 1998, Amiri *et al.* 2002, Maafi *et al* 2003), root lesion nematodes (e.g. Uehara *et al* 1999, Waeyenberge *et al* 2000) and root knot nematodes (e.g Peterson and Vrain 1996, Stanton *et al*. 1997, Zijlstra 2000, Zijlstra *et al*. 1997, Zijlstra *et al.* 2000). Some work has been conducted in Australia into identification of root knot nematode species of importance to vegetables (e.g. Stanton et al 1997, Stanton *et al.* 1998).

DNA techniques have also been useful for taxonomic studies aimed at investigating the degree of relatedness between and within genera (e.g. Powers *et al* 1997, Al-Banna *et al* 1997, Carta *et al*. 2001).

In Australia, SARDI and CSIRO entomology have developed the Predicta-B soil test which is commercially available for cereal growers. This test is based upon Real Time PCR and detects several cereal pathogens including *Pratylenchus neglectus* and *P. thornei* in DNA extractions from soil (Ophel-Keller *et al.* 1999). There is much interest in developing this technology for other crops and nematodes (e.g Stirling *et al.* 2004). However, there have been relatively few studies in Australia into the use of DNA based techniques for quantificiation of nematodes and soil borne pathogens associated with vegetable crops. In one such study, Stirling *et al.* (2004) developed a two-step process for assessing the risk of loss from root knot nematode and Fusarium wilt in tomato. A hazard index was calculated based on cropping history, disease history, soil texture and expected soil temperature during the growing season. If the index suggested a level of risk then soil samples were collected and inoculum densities of root knot nematode and Fusarium were determined using DNA techniques. The hazard index was found to provide a reasonable level of prediction of potential risk, although accurate predictions require access to reliable historical information. Furthermore the DNA based

test was able to detect root-knot nematode populations capable of causing economic damage to tomato crops (Stirling et. al. 2004).

For crops sensitive to nematodes e.g. carrot, 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, without regard to the population density. 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 are becoming commercial, such techniques will also suffer from the inherent inaccuracy of soil sampling and establishment of meaningful thresholds. At present DNA based techniques provide a similar level of sensitivity of detection as conventional techniques. However, 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. Stanton and O'Donnell (1998) proposed the use of molecular techniques to identify nematodes in the farming system or region in combinations with the screening of potentially useful rotation crops for resistance to those nematode populations only. However, there has been little further development of molecular tests for nematodes of interest to vegetable production in Australia since this time. Similarly the host status of potential rotation crops to particular nematode species is not always known or information not readily available.

DNA based methods have been shown to have other advantages over conventional techniques. For example DNA detection of root lesion nematode was less affected by soil water content, method of sampling and sample drying and gave a more uniform result in comparison to the Whitehead tray

method (Hollaway *et al*. 2003). If the cost of DNA based techniques could be reduced further, then there may be opportunities for mapping nematode populations and using variable management strategies. However, obtaining samples will still constitute a major part of the costs.

Although current DNA based diagnostic techniques have been based mainly on Real Time PCR techniques, other technologies have been developed which may prove more suitable for identification and quantification. For example Lievens and Thomma (2005) discussed the use of DNA array technology for the detection and quantification of multiple plant pathogens in a single assay. This technology is now used in several laboratories worldwide to provide a commercial service for diagnosis of plant pathogens. While DNA based technologies are currently at the forefront of specific biochemical testing methods, other methods are under development. For example, matrix assisted laser desorption/ionization time of flight spectrometry has recently been developed in at Murdoch University, Perth, Australia to identify the nematodes *Anguina tritici*, *A. funesta* and *Meloiodgyne javanica* (Perera *et al*. 2005) and to identify races of *D. dipsaci*.

5. Modelling nematode population dynamics

McSorley and Duncan (1995) summarised the use of models for management of nematodes. These fall into three broad categories, modelling either 1) the relationship between nematode population density and crop damage (discussed previously), 2) nematode population change and 3) efficacy of control.

Changes in nematode populations are often expressed as end of season population density (Pf) related to pre plant density (Pi) (e.g. Ferris 1985), although in some cases multiple function simulation models have been developed (e.g. Ferris 1976, McSorley and Ferris 1979). The ratio Pf/Pi is termed the reproductive factor (Rf) and is often used in nematology as an indication of the relative host status of a particular plant species to a particular nematode species. Rf values >10, 1-10, 1 and 0-1 indicate an excellent host, good host, maintenance host and poor or non host respectively (Ferris *et al*. 1993). Population decline in the absence of hosts, due to nematophagous microorganisms or nematicides has also been modelled with density dependent functions (Ferris 1985, Jaffee *et al*. 1992) and density independent functions (Kinloch 1982, Schmitt *et al*. 1987, Noe *et al*. 1991). For example, decline of *Ditylenchus dipsaci* in Utah was predicted accurately when season, soil temperature and moisture were taken into account (Teng *et al.* 1968). Location can play a major factor in survival. In Belgium, *Meloidogyne naasi* had one generation during the growing season and more than 90% of eggs overwintered and hatched the following spring when soil temperatures increased to above 10^{o}C (Barker and Olthof 1976). However in the southeastern USA, *Meloidogyne arenaria* has several generations during the growing season but less than 5% survive the winter (Barker and Olthof 1976)

McSorley and Duncan (1995) discussed a range of models developed to examine the long term effects of nematodes and various management strategies including crop rotation, use of resistant varieties and chemical and biological control on yields of annual and perennial crops (e.g. Noling and Ferris 1987, Ferris and Greco 1990, Noe *et al*. 1991). Such models have been used to identify potentially useful sequences of control options (Ferris

and Greco 1990, Noe *et al*. 1991). Burt and Ferris (1996) provided a model for determining the optimum length of a non-host rotation (see http://www.plpnemweb.ucdavis.edu/nemaplex/). However, McSorley and Duncan (1995) noted that insufficient research had been undertaken to validate these models in the field. Elliott *et al.* (2004) developed a computer program describing the relation between population sizes of *Globodera pallida* at planting and harvest and yield of potato. The program was used by UK potato growers to examine the effect of changing management strategies such as varietal tolerance/resistance, the length of rotations and the efficacy of chemical treatment.

6. Resistant varieties

The development of resistant varieties offers a potentially major economic benefit for vegetable growers. For example Bradley and Duffey (1982) estimated the economic benefit of the soybean cultivar 'Forrest' which is resistant to *M. incognita* and *H. glycines* at US $400 million at a cost of US$1 million to breed. However, the list of crop species with effective resistance to nematodes from conventional breeding is limited (Roberts 1992). Furthermore in some cases, the available host resistance has limitations. For example, resistance to *Meloidogyne* spp. in bean and tomato is heat-sensitive and may fail in warm climates (Roberts 1992). A recent summary of resistance in crops to nematodes was provided by Cook (2004). Belair (1987) noted that the carrot cv. Spartan Classic showed field tolerance to *M. hapla* as compared to the susceptible cv. Gold Pak 28. Breeding work overseas is showing promise in developing carrot varieties resistant to root knot nematode (Simon *et al.* 2000). 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 in Australia has suggested some differences in susceptibility to root knot nematode (Hay *et al.* 2004). Further testing would be necessary to determine whether some of the currently available varieties have a useful level of resistance.

Some information on host status of particular crops/break crops is available for growers (e.g. Stirling *et al.* 1986, Stirling 1989b) but such information needs to be updated to incorporate new varieties and made more readily available to growers. In addition, existing information often does not extend across a range of nematode species e.g. *Meloidogyne fallax*. This is further complicated in that varieties within a species of potential break crop may vary in their ability to host particular nematode species. For example, a break of pasture was often recommended for control of *Meloidogyne hapla*, however *Meloidogyne fallax* is hosted by pasture species. There is therefore a need to examine the host range of potential break crops which may be grown in

different localities. In the Netherlands, the Nematode Control Strategy (NCS) was implemented to reduce reliance on nematicides (Molendijk and Korthals 2005). This strategy involved the identification of the main nematode species associated with damage to crops followed by greenhouse and field trials to identify crops and or green manure species which could be used in rotation. The NCS identified that the growing of perennial ryegrass (*Lolium perenne*) and black salsify (*Scorzonera hispanica*) were important causes of problems with *Meloidogyne fallax.* Fodder radish (*Raphanus sativus*) could be used as a green manure to control *M. fallax* and that bean (*Phaseolus vulgaris*), barley, fallow and peas could be used to reduce populations of *M. fallax* and *M. chitwoodi*. The NCS also assessed the relative susceptibility of potato varieties to Meloidogyne spp. and identified less susceptible varieties which could be grown under situations of low nematode pressure (Molendijk and Korthals 2005).

Varieties of lima bean, snap bean, sweet potato, bell pepper, hot pepper, tomato and cherry tomato are resistant to some (but not all) *Meloidogyne* spp (e.g. www.agr.state.nc.us/AGRONOMI/nnote12.htm). However information is lacking for Australia growers.

Trudgill (1997) recently reviewed root knot nematode, including resistance mechanisms. Atkinson *et al*. (2003) discussed current research and strategies for the development of genetically engineered resistance in crop plants.

7. 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.

7.1 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 a,b). Less damage to carrot was possible when crops were sown later in the spring. 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. In Tasmania, *P. crenatus* was shown to survive in a weedy fallow in fields following carrot with little change in population density over a period of 6 months (Hay *et al*. 2004), presumably because nematodes survived on weed hosts.

Overwintering models for determining damage threshold densities for the subsequent crop have been proposed (Barker and Imbriani 1984, Ferris 1981, Starr and Jeger 1985). Jeger *et al* (1993) provided a theoretical model of winter survival dynamics of Meloidogyne spp. which incorporated loss of egg

viability and population losses due to predation or microbial decomposition. Such models would be useful for determining damage threshold levels for subsequent crops, but have yet to be developed sufficiently for predictive purposes.

7.2 Break crops

For economic reasons and for soil conservation it is more desirable to grow a non-host break or cover crop between cash crop cycles rather than maintain a bare fallow. Break crops are either those which are poor or non hosts of particular nematodes or those which are antagonistic. There are several mechanisms by which some plant species are antagonistic to nematodes (Table 13).

Several studies have shown that some Sudan grass (*Sorghum sudanense*), sorghum-Sudan grass hybrids (*S. bicolor* X *S. sudanense*) and Sudan grass hybrids (*S. sudanense* X *S. sudanense*) are effective in suppressing infection and damage of vegetables by *Meloidogyne* spp. when incorporated into soil as green manure (e.g. Mojtahedi et al. 1993, Viaene and Abawi 1998, Widmer and Abawi 2000). The nematicidal properties of some Sudan grass cultivars are related to a cyanoglucoside compound (dhurrin) present in the leaf tissue, which is converted to the biocidal compound hydrogen cyanide by the enzyme β-glucosidase when the tissue is damaged or begins to decompose (Widmer and Abawi 2000). Widmer and Abawi (2002) noted that the cyanide ion level in leaf tissue of 14 hybrids of sudan grass varied between 0.04 to 1.84 ppm. Incorporation of leaf tissue into soil in pots to which *M. hapla* was added led to a 54% reduction in root gall severity in lettuce with a correlation between the amount of free cyanide in soil and reduction in galling. Incorporation of white clover, another cyanogenic plant, similarly led to a 45% reduction in root gall severity (Widmer and Abawi 2002). Kratochvil et al. (2004) demonstrated that sorhum-sudan grass grown annually as a green manure crop following nematode-susceptible crops (potato or cucumber) gave control of *M. incognita* comparable to a nematicide application 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.

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. However, 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.

Table 13. Mechanisms of antagonism to nematodes by plants.

Polythienyls	Tagetes
Gluconsinilates→isothiocyanates	Brassica
Glycoside dhurrin→cyanide	Sorghum (sudangrass) Cassava roots
Polyacetylenes	Astereaceae e.g. thiarubrine C from black-eyed Susan (*Rudbeckia hirta*)
Alkaloids	Calabar bean
Terponoids	Basil, peppermint, Myrtaceae
Sesquiterponoids	e.g. production of phytoalexin solavetivone in potato linked to resistance to PCN (*G. rostochiensis*) and rishitin nematoxic to *Ditylenchus*.
Quassinoids, steroids, triterponoids	*Quassia amara* *Azadirachta indica* (Neem) Cucumber with less cucurbatinin roots attracted less RKN J2
Phenolics	Many toxic to nematodes Salicylic acid toxic at 50 ug/ml (also mediator of systemic acquired resistance)
Sulphur containing compounds	Asparagusic acid (asparagus) Allicin (garlic)
Lectins	Concanavalin A from jackbean (*Canavalia ensiformis*).
Proteins	Cystatins (protease inhibitors)

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*. Belair (1992) studied cropping sequences on population densities of *M. hapla* and carrot yield. Spinach, radish, barley, oat and wheat were poor or non hosts of *M. hapla*, while populations were maintained or increased on cabbage, celery, lettuce, leek, marigold and potato (Belair 1992). Total root weight and marketable percentage of carrot was greater following spinach, oat, radish and fallow-onion than following two crops of onion or carrot. A rotation of carrot-onion-oat-carrot decreased population densities of *M. hapla* and increased marketable yield by 282% compared to carrot monoculture. Two consecutive years of onion increased *M. hapla* and led to severe root galling and a 50% yield loss in the succeeding crop of carrot. Belair (1992) recommended that carrots could be grown economically for two years following radish, spinach and oat, but not following onion and carrot without the use of nematicides.

It is important to recognise that weed species may also act as hosts of nematodes (e.g. Tedford and Fortnum 1988). 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 (Rf=16) on 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 14). 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 15). 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 16). Less is known about the host range of *M. fallax*. Brommer (1996 a,b) reported that Italian ryegrass, potato and carrot were good hosts, while maize and other cereals were poor hosts.

Table 14. 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 15. 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*
Jointvetch (*Aeschynomene americana*)	*M. arenaria, M. incognita*[1]
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 16. Break crops suppressive to Meloidogyne spp. (Hagan *et al*. 1998)

Suppressive crop	Root knot nematode species: *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 14, 15, 16), 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 15, 16). 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 15) 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, a lectin 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 17), again highlighting that many of the species described above were effective break crops for *M. arenaria*, *M. incognita* and *M. javanica*.

Table 17. 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 management of species of Pratylenchus is difficult due to their wide host ranges and number of species. There has been considerable effort in the Australian cereal industry to develop varieties with resistance to *Pratylenchus thornei* and *P. neglectus* (Hodda 2002). However, cereal varieties with resistance to these nematodes are not immediately useful as break crops for the vegetable industry as different species of Pratylenchus with different host ranges are important in vegetable production (e.g. *P. penetrans*). Further screening of these cereal varieties for species of Pratylenchus associated with vegetables would be required. 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*.

Host range studies on *P. penetrans* in Tasmania suggested in general leguminous species were better hosts than grass species (Hay *et al.* 2002). 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*. (1991) 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.

In South Australia, 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 (Hay *et al*. 2004). 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. 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 was not recommended as a green manure crop in soils infested with this nematode as it resulted in reduced yield in a subsequent carrot crop. *B. napus* cultivar and sorghum cv. Jumbo, did not reduce 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.

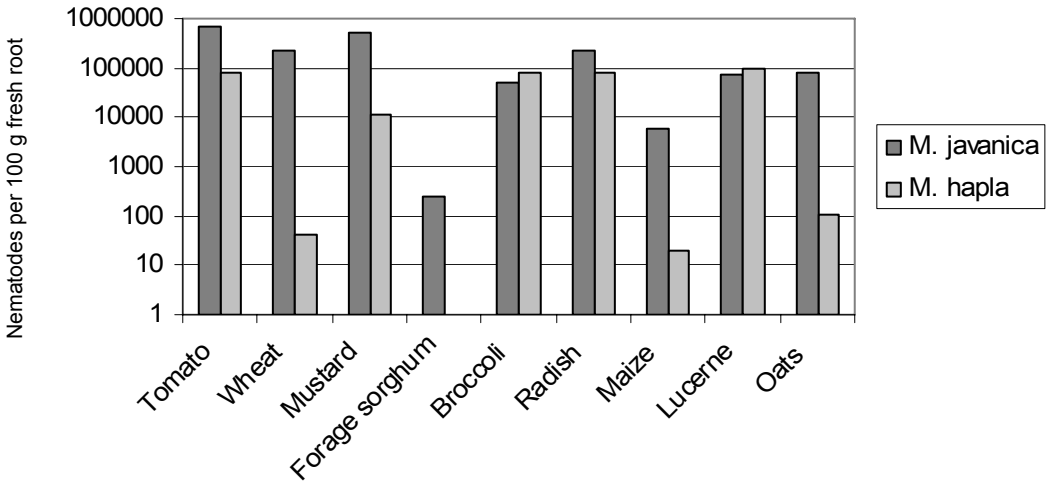
Therefore soghum cv. Jumbo may have some potential for use as a green manure especially if it can be grown for a reduced period not allowing significant nematode multiplication. However, when used as a green manure crop, cv. Jumbo does not control *Pratylenchus jordanensis* as it hosts this nematode (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 (Hay *et al*. 2004).

Lucerne is often used as a green manure crop in Australia, but is susceptible to *M. javanica*, although some cultivars have resistance to some *Meloidogyne* populations (Griffin and Gray, 1995). In pot experiments, Lucerne 'Rippa' was shown to be an intermediate host of *M. javanica*, *M. hapla*, *P. thornei* and *P. neglectus* (Hay *et al*. 2004) Similarly, lucerne as a green manure was not effective at lowering populations of *P. penetrans*, as it hosted this nematode (Abawi and Widmer, 2000).

The host suitability of several potential green manure crops to *M. javanica* and *M. hapla* in Queensland, Australia was assessed in pot experiments tomato (*Lycopersion esculentum* 'Tiny Tim'), wheat (*Triticum aestivum* 'Baxter'), Mustard (*Brassica napus* 'BQ Mulch'), Forage sorghum (*Sorghum halepense* x *sudanense* 'Jumbo'), Broccoli '*Brassica oleracea* 'Shogun') Radish (*Raphanus sativa* 'Weedcheck'), Maize (*Zea mays* 'DK689'), lucerne (*Medicago sativa* 'Rippa') and Oat (*Avena sativa* 'Taipan') (Hay *et al*. 2004).

No rotation cultivar was resistant to *M. javanica* and *M. hapla* (Figure 5). 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 in Queensland. Lucerne was found to be an intermediate host for both *Meloidogyne* spp. and Broccoli was an intermediate host to *Pratylenchus* spp. and *M. javanica* and a good host to *M. hapla* (Hay

Figure 5. Recovery of *M. javanica* and *M. hapla* from different host species following 7 weeks after inoculation of 10,000 eggs per pot at 2 weeks after sowing. (From Hay et al. 2004).



et al. 2004). Forage sorghum was found to be the most resistant of the crops tested to *M. javanica* and *M. hapla*.

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.

7.3 Trap crops

Trap crops or ‘catch crops’ are usually used against cyst nematodes that hatch in response to host exudates and form sedentary feeding sites. 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 can be improved by determining the number of degree-days necessary for a particular cyst 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-risky strategy. The technique has been further improved In Germany and the Netherlands, where oil radish and white mustard varieties have been selected which stimulate egg hatch of *H. schachtii* but which allow little or no nematode multiplication in the roots (Muller 1991). This eliminates the need for critical timing of trap crop destruction. Growing these varieties can decrease populations of *H. schachtii* by 70-90% (Muller 1991). In Wyoming, USA, Held *et al.* (2000) reported that growing radish (*Raphanus sativus*) as a second crop following barley for the control of *H. schachtii* in sugar beet cost $223/ha less than the cost of nematicide and increased net return as a percent of land value from 3.9% to 5.9%, and to 9.3% if lambs were grazed on the radish crop.

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. 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. Patel *et al.* (1991) also demonstrated that pink and white periwinkle (*Catharanthus roseus*) were effective trap plants for *Meloidogyne incognita* and *M. javanica*.

7.4 Biofumigant plants.

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. 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, Pattison *et al*. 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 to metham sodium, 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.

Metham sodium has been shown to suffer from enhanced microbial breakdown following repeated use which renders it ineffective (Warton *et al*. 1999, Matthiessen and Warton 2000, Warton *et al*. 2001). 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. Further work (Matthiessen and Warton 2002, Warton et al. 2003) showed that other isothiocyanates were biodegraded at an accelerated rate in soils known to biodegrade MITC. This demonstrated that the effectiveness of biofumigation using biofumigant plants could be diminished in soils exhibiting enhanced biodegradation of MITC. 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 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 (Pung *et al.* 2004). This indicates that biofumigation can be an effective strategy in cooler regions.

8. Planting or harvest 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 (Roberts 1993, 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 (Belair 1987). This is related to the temperature dependent nature of the lifecycle of nematodes. (Table 18). Postponement of sowing by one month has also been shown to reduce quality damage in carrots caused by *Meloidogyne fallax* (Molendijk and Brommer 1998). Delayed planting may also contribute to greater nematode attrition in the absence of a host, leading to lower population densities at planting. Similarly Huang and Ploeg (2001a) recommended sowing of carrot or lettuce at soil temperatures equal to or below 17^{o}C to significantly reduce damage caused by *Longidorus africanus*.

In Queensland, Australia Vawdrey and Stirling (1996) showed that serious economic losses in tomato due to root knot nematode occurred with crops that were harvested between November to January (summer), in comparison to generally minimal losses in crops maturing at other times of the year. This was attributed to environmental stresses at this time which exacerbated the effect of nematode feeding. They recommended growing root-knot nematode susceptible crops such as tomato from autumn to early spring and rotating with less susceptible crops (beans, sweet-corn, capsicum, chinese cabbage, squash, broccoli and zucchini) during the summer (Vawdrey and Stirling 1996).

Some crops can vary in the time required to reach physiological maturity. Early maturing varieties may be used to suppress final nematode population density.

Table 18. 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	-	-

9. Nematicides

9.1 Nematicide use

Nematicides have been one of the most widely used methods of controlling nematodes in vegetable production. Advantages of nematicides include that they are a relatively quick and easy approach to nematode control, and can often be cost-effective. Disadvantages of nematicides include the need for specialised application equipment, generally high toxicity, short-lived activity, deleterious impact on the environment and potential for development of enhanced biodegredation in which continual use leads to the build up of soil microflora capable of rapidly degrading the chemical and thus rendering it ineffective. In addition, the cost of nematicide adds greatly to the variable cost of growing crops. The costs of nematicide application for control of potato cyst nematode in the U.K. were estimated at £360-£550/ha (Evans *et al.* 2003), adding more than 50% to the cost of potato production (Harkett 1996).

Within the USA, some 46 M kg of active ingredient of nematicide/fumigant is used annually on a total of 43,000 farms, which includes metam sodium (26-28 M kg a.i.) methyl bromide (9-11 M kg a.i.), dichloropropene (9-11 M kg a.i.) and chloropicrin (2-4 M kg a.i.) (Kiely 2004). Fumigants are used to control a range of soil-borne pathogens, including nematodes. A breakdown of fumigant use by crop in the USA is given (Table 19). In some crops, including brussels sprout, carrot, cucumber, pepper, potato, sweet potato and tomato, a significant proportion (>10%) of the total crop is treated with fumigant (Table 19). In Australia, figures for nematicide use are not readily available, however sales of nematicides in Australia totaled A$ 4.2 M in 2001 (Anon 2002).

Table 19. Fumigant use (kg a.i.) in vegetable production in the USA (Becker et al. 2005).

	Chloropicrin	Metam sodium	Methyl bromide	1,3-Dichloropropene	Total
Artichokes	0	22,500 (5%)[1]	0	0	22,500
Beans (green)	18,000 (<1%)	45,000 (<1%)	90,000 (<1%)	90,000 (<1%)	243,000
Broccoli	0	315,000 (<1%)	0	18,000 (<1%)	333,000
Brussels sprout	0	27,000 (30%)	0	27,000 (45%)	54,000
Cabbage	0	27,000 (<1%)	0	90,000 (5%)	117,000
Carrot	31,500 (<1%)	4,050,000 (40%)	31,500 (<1%)	675,000 (10%)	4,788,000
Cauliflower	0	27,000 (<1%)	0	36,000 (<1%)	63,000
Celery	0	90,000 (10%)	0	0	90,000
Cucumber	45,000 (5%)	135,000 (<1%)	585,000 (5%)	405,000 (10%)	1,170,000
Eggplant	0	2700 (<1%)	90,000 (45%)	0	92,700
Garlic	0	90,000 (<1%)	0	13,500 (<1%)	103,500
Lettuce[2]	18,000 (<1%)	225,000 (<1%)	90,000 (<1%)	31,500 (<1%)	364,500
Onion	90,000 (<1%))	765,000 (5%)	90,000 (<1%)	405,000 (5%)	1,350,000
Peas (green)	0	1350 (<1%)	0	0	1350
Pepper	315,000 (10%)	315,000 (5%)	1,665,000 (20%)	90,000 (5%)	2,385,000
Potato	90,000 (<1%)	14,265,000 (20%)	90,000 (<1%)	4,455,000 (5%)	18,900,000
Pumpkin	0	4500 (<1%)	0	0	4500
Spinach	0	90,000 (<1%)	135,000 (<1%)	0	225,000
Squash	36,000 (<1%)	45,000 (<1%)	180,000 (5%)	90,000 (5%)	351,000
Sweet corn	0	13,500 (<1%)	0	36,000 (<1%)	49,500
Sweet potato	45,000 (6%)	135,000 (1%)	360,000 (5%)	360,000 (20%)	900,000
Tomato	765,000 (10%)	3,150,000 (15%)	4,770,000 (20%)	135,000 (<1%)	8,820,000
Total	1,453,500	23,818,050	8,176,500	6,979,500	40,427,550

[1] Percentage of crop treated.

[2] In addition, 585,000 kg a.i. of metam potassium is used in lettuce production.

9.2 Types of nematicides

In general nematicides are grouped either as 'fumigants' which have a general biocidal effect on soil biota including nematodes and 'non-fumigant nematicides' which are nematicidal/insecticidal.

Fumigants for control of nematodes and other pests have to be applied prior to planting, with a delay between application and planting of the crop. Fumigants which are registered in Australia for control of nematodes in various crops include metham sodium, Telone (1,3 dichloropropene) and Telone C35 (1,3 dichloropropene and chloropicrin). Following application to soil, metham sodium is converted to methyl isothiocyanate which has a general biocidal nature. Metham sodium is commonly used in vegetable production in Australia. Telone and Telone C35 were recently registered in Australia for use in vegetable production, following evaluation (Anon 2001). 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). In addition, 'township caps' limit the total amount of 1,3-D that can be used in a given area in California (Chitwood 2003). 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 following 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 in the future be applied to reduce some of the risks associated with fumigation with 1,3-D.

In the case of Telone there have been a number of measures taken to reduce risks of use in the USA (Anon 1998b):

- Measures to mitigate risks to workers include, lowered maximum rates (30-65% depending on crop), closed loading requirements, technology to minimise spillage during handling, stipulation of clothing and respirator requirements, restricted entry for 5 days after application, soil moisture and sealing requirements and modified application techniques.
- Measures to mitigate residential inhalation include, a 300 foot no-treatment buffer around residential areas (100 feet for drip irrigation), lowered application rates, loading requirements, technology to minimise spillage during application, soil moisture and sealing requirements and modified application techniques. Use of 1,3-D was suspended in California in 1990 for several years due to its detection in air distant from sites of application, which necessitated the above buffers and caps in total use around townships in California (Chitwood 2003)
- Measures to mitigate risks to residential drinking water include, ground water advisory, prohibition of use in some northern tier states with shallow ground water and vulnerable soils, 100 foot buffer between drinking water wells and treated fields and prohibition of use in areas overlying karst geology.

Non-fumigant nematicides are generally either carbamates or organophosphates. Those currently registered in Australia include terbufos and carbofuran (Furadan) for control of cereal cyst nematode, fenamiphos (Nemacur) for control of nematodes in a range of crops including many vegetables, oxamyl (Vydate) in banana, Rugby (cadusafos) in some perennial crops and tomato, and ethoprophos. Specific registration details are given at

the APVMA website (www.apvma.gov.au) and on the product label. Nemacur (fenamiphos) is one of the more commonly used non-fumigant nematicides in vegetable production within Australia. However recently, Bayer Corporation has announced the voluntary withdrawl of Nemacur. The production of Nemacur in the USA has been reduced progressively since this announcement and Nemacur will be sold and distributed in the USA through to May 31, 2007, with distributors allowed to sell and distribute until May 31, 2008. 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.

9.3 Efficacy of nematicides

Numerous trials have been published on the efficacy of nematicides in vegetable production. 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).

Anon (1998a) 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.

The results of several nematicide trials on yield of carrot in Australia were reported by Hay *et al.* (2004). In South Australia at a site at which *M. javanica* was present, treatment of soil with Telone C35 at 520 L/ha or metham sodium at 300-525 L/ha led to carrot yields of 58.3-75.7 t/ha, significantly above that of the non treated (24.4 t/ha). In Western Australia, treatment of soil containing *M. hapla* with Telone 130 kg/ha or Telone C35 at 185 or 270 kg/ha gave yields of export quality carrot ranging from 45.3 – 47.6 t/ha, significantly higher than the non treated (10.7 t/ha) (Hay *et al*. 2004). In a second trial in Western Australia at which *M. javanica* was present, treatment of soil with Telone at 135 kg/ha or Telone C35 at 270 kg/ha gave yields of export quality carrots of 56.5 and 58.6 t/ha respectively. This was significantly higher than that from fenamiphos at 24 L/ha and non treated (24.7 and 29.2 t/ha respectively). The poor performance of fenamiphos in this trial may have been due to enhanced biodegradation (Hay *et al*. 2004).

The effect of nematicides is generally not long lived. Non-fumigant nematicides are generally nemstatic in that they do not kill nematodes but paralyse them (Bunt 1987). Populations may recover as the chemical dissipates in the soil. 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. With nematicides which are nemastatic in action, the population density of nematodes may not be reduced significantly following treatment. However, delaying nematode feedling for some weeks after planting may be sufficient to reduce damage to the crop. Huang and Ploeg (2001b) noted that inoculating Longidorus africanus simultaneously with lettuce or carrot seed severely reduced plant growth, but adding nematodes 10 days significantly increased yields. In some cases placement of non-fumigant nematicides with seed in the furrow is used to provide sufficient efficacy and reduce application rates in comparison to broadcast application.

9.4 Enhanced biodegradation of nematicides

Repeated applications of fumigants (e.g. metham sodium) or non fumigant (e.g. fenamiphos) nematicides to the same soil can lead to enhanced biodegradation, in which there is build-up of populations of micro-organisms in the soil which are capable of rapidly breaking down the nematicide into non-nematicidal byproducts (e.g. Stirling et al. 1992, Smelt et al. 1996, Matthiessen and Warton 2000). Enhanced biodegradation of many other nematicides has been documented around the world including aldicarb, ethoprop, oxamyl and 1,3-D (Chitwood 2003). In Australia, Matthiessen and Warton (2000) reported that addition of metham sodium to a soil which had no previous history of use led to 93% conversion to methyl isothiocyanate which was detectable for 17 days. In contrast addition of metham sodium to a soil with a history of use led to only 43% methyl isothiocyanate production which reduced to non detectable levels 7 hours later. Sterilisation of this soil led to 88% production of methyl isothiocyanate and detectable levels up to 18 days after application (Matthiessen and Warton 2000). Matthiessen and Warton (2002) noted that risk of the development of enhanced biodegradation to metham sodium 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.

Stirling *et al.* (1992b) demonstrated enhanced biodegradation of fenamiphos in tomato soils in Australia. Pattison *et al.* (2000) devised a bioassay for identifying banana soils with enhanced biodegradation of nematicides, including fenamiphos, in Queensland Australia. This technique was used in a survey of carrot soils in Victoria Australia (Hay *et al.* 2004). Of 13 soils tested, five sites were found to have enhanced biodegradation of fenamiphos, with greater than 75 % nematode recovery in non sterile soil treated with fenamiphos relative to untreated soil (Hay *et al*. 2004). A further five sites had advanced biodegradation of fenamiphos, with between 20 and 75% nematode recovery in the unsterile, fenamiphos treated soil relative to untreated soil. While some reduction in nematode numbers could be expected in these soils, fenamiphos applied to these sites would be expected to have reduced efficacy

(Hay *et al*. 2004). Only three sites exhibited a high level of efficacy of fenamiphos, with less than 20% of nematodes recovered in unsterile, fenamiphos treated soil relative to untreated soil. This suggested there was no biodegradation of fenamiphos at these sites and that the efficacy of the chemical was preserved. Fenamiphos applied to these three sites would be expected to give good control of plant-parasitic nematodes (Hay *et al*. 2004). Sterilisation of soils exhibiting enhanced biodegredation improved the efficacy of fenamiphos at 10 sites, which suggested that the degradation of fenamiphos was due to a biological cause and not due to chemical degradation.

9.5 Future of nematicides

During the 1940's and 1950's the discovery of the nematicidal properties of a number of fumigant chemicals such as DD, 1,3 dichloropropene, 1,2 dichloroporpane and related C3 hydrocarbons led to a major shift in nematode management practices. Soil fumigants were relatively cheap and effective and their use became standard practice in many cases. During the 1960's a number of organophosphate and carbamate contact nematicides were also introduced providing control of insects and nematode pests. Since then there has seen a steady attrition in the number of nematicides available to growers due to their toxicity and impacts on human health and environment. DBCP was found to cause male sterility and be carcinogenic, and following its discovery at unsafe levels in groundwater was phased out during the 1970's. Subsequent studies in the USA demonstrated EDB, 1,2 dichloropropane component of DD, aldicarb, carbofuran and ethoprop in groundwater leading to removal of many of these chemicals from the market. Methyl bromide, which has also been used for its nematicidal activity in high-valued crops is also being phased out in line with the Montreal Protocol due to its ozone depletion potential (Rosskopf *et al.* 2005). There is also continued public concern and pressure on registration of non-fumigant organophosphates and carbamates (Gooch *et al.* 1998).

The loss of methyl bromide has prompted considerable investigations around the world into alternative fumigants (e.g. Rosskopf *et al.* 2005). One of the most promising alternatives is Midas which is being developed by Arvesta Corporation as an alternative fumigant active against weeds, nematodes, insects and soil borne pathogens. Midas contains methyl iodide (iodomethane) and 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 is also rapidly broken down by sunlight before it reaches the ozone layer and therefore does not have adverse environmental effects of methyl bromide. Midas 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. At present Midas is slightly more expensive than methyl bromide, so cost may be a prohibitive factor for vegetable production. However, novel delivery methods are being developed to lower the cost of the product. Hutchinson *et al*. (1999) reported on trials to control *Meloidogyne incognita* in carrot production in California with methyl iodide. Methyl iodide at various rates (112-336 kg/ha) and methyl bromide at 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. Midas was expected to receive US EPA approval before 2005 however has been subject to delays. Currently (as of 2006) the USA EPA is evaluating several soil fumigants (chloropicrin, dazomet, metam sodium, methyl bromide and iodomethane) as part of the EPA's program to ensure that all pesticides meet current health and safety standards (Anon 2004c). A similar assessment of 1,3-D (Telone) was completed in 1998 (Anon 1998b). The incorporation of iodomethane in this review has meant that consideration of the registration of Midas has been carried over to 2007.

Chitwood (2003) listed a range of chemicals which have been used as nematicides in other countries but are not currently registered in Australia including Enzone (sodium tetrathiocarbonate) which releases biocidal carbon disulphide gas and the recently released organophosphate fosthiazate (Nemathorin). Other chemicals with nematicidal properties which are currently being developed or evaluated overseas were listed by Chitwood (2003) and Rosskopf (2005) and include:

- Sodium azide (American Pacific Corp.)
  Sodium azide was registered as a pesticide in the USA for a period during the 1970's. Sodium azide has a wide range of activity against nematodes, fungi and weeds and is currently under evaluation by US EPA under the formulation SEP™100.

- Propargyl bromide
  Originally patented as a broad-spectrum fumigant in 1957 by Dow Chemical Company, but taken off market due to high volatility. This chemical has been reformulated and has undergone evaluation in USA but not registered as at June 2005.

- Propylene oxide (Aberco Inc.)
  Propylene oxide has been used for many years as a stored-product treatment and is currently under development as a soil treatment in the USA as Propozone. It is effective against nematodes and fungal plant pathogens, and at higher rates against weeds.

- Dimethyl disulphide (DMDS) Cerexagri, AFOTINA Chemicals Phil.
  This compound is one of the volatile fungicidal/nematicidal compounds produced when soil is amended with cabbage and solarised. It has been effective against pathogenic fungi and nematodes in trials and is currently under development.

- AJMC-330 (Ajay N. America)
  Broad spectrum activity against fungal plant pathogens, weeds and root-knot nematode.

- Avicta
  Avicta 500 FS was recently registered in the USA by Syngenta as a nematicidal seed treatment for control of nematodes in cotton. The active ingredient is abamectin, which is used as a mammalian anthelmintic. In the past, seed treatment with nematicides has not been successful due to the inability to apply sufficient quantity to the seed coat to provide control beyond the seedling stage (Chitwood 2003). However, protection of the seedling may be sufficient in some crops to considerably reduce damage at harvest. It is not known whether Syngenta will extend the use of this product as a seed treatment to other crops.

- Systemic acquired resistance chemicals
  In recent years there have been many studies on a range of chemicals for their ability to stimulate a systemic acquired resistance response in plants to a variety of pathogens (e.g. Gozzo 2003). Results have often been variable, especially in relation to nematode control (e.g. Vavrina *et al.* 2004).

Some of these chemicals may in the future offer alternatives to current nematicides. However, there is no guarantee that they will achieve registration in vegetables or be cost-effective in vegetable production.

The use of fumigant and non-fumigant nematicides in agriculture is under continued pressure due to the toxicity and potential for environmental harm from these pesticides (e.g. Gooch *et al*. 1998). Davis *et al*. (1999) outlined the amount of fumigant/nematicide applied to carrot fields in various parts of USA (Table 20) and showed the impact of substituting these chemicals with various

alternative control methods (Table 21). The loss of all fumigants from carrot production in the USA was estimated to result in an annual loss of 17.9% (312,144 tons).

Table 20. Fumigants and area treated for control of nematodes in carrot production in USA (Adapted from Davis et. al. 1999).

Fumigant	State	Total acres in production	Target pests	% of total acreage on which applied
1,3-D/chloropicrin	CA	66580	Nematodes	7
	CO	3700	Nematodes	24
	TX	9400	Nematodes	8
	WA	8360	Nematodes, Wireworms	70
Metham sodium	CA	66580	Nematodes, weeds, pathogens	70
	CO	3700	Nematodes	5
	MI	7120	Nematodes, weeds, pathogens	70
	WA	8360	Nematodes, weeds, pathogens	10
	WI	3660	Nematodes, weeds	12
Oxamyl	TX	9400	Weevil, leafhopper, nematodes	15

Table 21. Impact of substituting fumigants with alternative control methods in carrot production in USA (Adapted from Davis et. al. 1999)

State	Acres	Current area treated (%)	Alternatives and % use	Effect on yield (%) of substitution
CA	66580	84%	Crop rotation (100%)	-25%
CO	3700	29%	Crop rotation (100%), Cultivation (75%), nematode Reducing crops (5%)	-10%
MI	7120	70%	Crop rotation (90%)	-4%
TX	9400	8%	Crop rotation (50%)	-25%
WA	8360	80%	Crop rotation (80%), field selection (100%), cover crops (25%)	-40%
WI	3660	12%	3-4 year rotation (12%)	-20%

10. Biological control

There have been many studies in biological control of nematodes over the last 50 years (Stirling 1991). The finding of soils naturally suppressive to cereal cyst nematode in Europe and which could maintain nematode numbers below a threshold during a continuous monoculture lead to considerable research interest in this area. Suppressiveness to CCN was attributed to two nematophagous fungi (*Verticillium chlamydosporium* and *Nematophthora gynophila*). Since then there have been many attempts to isolate nematophagous organisms from the soil, develop means of culturing and packaging them as a product which might be applied to the soil to control nematodes as an 'innundative' strategy (Stirling 1991). Others have sought to add organic amendments to the soil which might stimulate resident populations of nematophagous organisms as an 'augmentative' strategy or which release nematicidal compounds as they decompose (Stirling 1991). A further strategy is the isolation of nematicidal phytochemicals from extracts of biological origin e.g. plants, fungi bacteria (Chitwood 2003).

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 it should not to be considered a comprehensive review of products available.

Ditera

This product is produced by Valent laboratories (http://www.valentbiosciences.com) and marketed in the USA (Chitwood 2003). 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.

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 an 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.

Sinocin

Sinocin is registered in the USA as 'Plant Extract 620' for the control of nematodes. It consists of extracts from prickly pear (*Opuntia linheimeri*), oak (*Quercus falcata*), the sumac (*Rhus aromatica*) and the mangrove

(*Rhizophora mangle*). It has provided good control of nematodes in some cases (Chitwood 2003).

Dragonfire-CPP (Poulenger USA Inc. Fl.)

Formulation of sesame seed oil containing aldehydes, ketones and linolenic acids). Used in the USA on turf.

Furfural (International Furan Technology, Durban, Sth. Africa)

Registered as a nematicide in South Africa and Spain in 2001 and containing 2-furfuraldehyde, a derivative of pentose sugars (Chitwood 2003).

DMDP

DMDP (2,5-dihydroxymethyl-3,4-dihydroxypyrrolidone) is a naturally occurring sugar analog from the tropical legume (*Lonchocarpus felipei*) which has rercently been patented as a nematicide (Chitwood 2003). It has been shown to inhibit hatching and movement of Globodera spp.. This compound is of particular interest because it is mobile in phloem and when applied to tomato leaves, was able to reduce galling on roots by *M. incognita* (Chitwood 2003).

Omphalotin A

A cyclic dodecapeptide with nematicidal activity isolated from the basidiomycete fungi *Omphalotus olearius* and *Lampteromyces japonicus* (Mayer *et al.* 1999). The LD_{50} for *M. incognita*, *H. schachtii* and *P. penetrans* of 2.0, 30 and 25 mg/L respectively (Mayer *et al.* 1999). Commercial development has been hindered by low yield of this compound.

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 22). 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.

Table 22. 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

Organic amendments

Addition of organic matter to soil can often lead to a suppression of nematodes (e.g. Mankau 1968, Mankau and Minteer 1962, Mian and Rodriguez-Kabana 1982, Rodriguez-Kabana 1986, Caswell and Bugg 1991). Hay *et al*. (2004) reported 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 and nitrous acid. Amendments or N containing fertilisers that release ammonia have been shown to be nematicidal. Lazarovits *et al*. (1999) obtained suppression of plant parasitic nematodes by the addition of high rates of soymeal and meat and bone meal (37 t/ha). Chitin (Clandosan) 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). Calcium cyanamide (CaNCN) has been used for many years both as an N-fertiliser and for biocidal action against organisms in the soil. In pot trials, Rodriguez-Kabana *et al.* (2003) showed cyanamide (H_2NCN) to be more nematicidal than calcium cyanamide (CaNCN), with the former providing good control of reinform nematode in soybean at over 250 mg/kg soil. This rate approximated to 500 kg/ha which on a bulk price for cyanamide equated to US$1100-2200, a potentially economic proposition for high valued crops (Rodriguez-Kabana *et al*. 2003). Care must be taken because of potential phytotoxicity to crop plants and the potential for run-off of nitrogen into streams and groundwater. Band application of calcium cyanamide was used by Donald *et al.* (2004) for control of clubroot in Australia, which reduced the cost of application from A$1400/ha to A$467/ha in brassicas. Interestingly Hairy Vetch (*Vicia villosa*) was shown to contain cyanamide in leaves and stems which was linked to its allelochemical properties (Kamo *et al*. 2003), although while growing it is susceptible to root knot nematode species.

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 1989a). 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.

The nematicidal effects of high-N amendments are generally short-lived as nematicidal compounds such as ammonia and nitrous acid remain at high levels for only a limited time (Stirling *et al.* 2005). In addition to the release of nematicidal compounds during decomposition, organic matter may also contribute to an increase in nematophagous activity in the soil. Following the addition and subsequent decomposition of organic matter in soil, free-living nematodes multiply and in turn, support higher populations of a succession of nematophagous fungi, predacious nematodes and mites (Linford *et al.* 1938). Recent research in sugarcane soils (Stirling *et al.* 2003, Pankhurst *et al.* 2005) has shown that amendments with high carbon to nitrogen ratio (sawdust, grass hay and sugarcane trash) induced suppressiveness to *Meloidogyne javanica* and *Pratylenchus zeae* 4 and 7 months after addition to the soil. However, additions of amedments with lower C/N ratio (lucerne hay, feedlot and poultry manure, chitin and mill mud) did not suppress nematodes. Suppression of nematodes by amendments with high C/N ratio was associated with low levels of nitrate-N in soil, a fungal dominant soil biota and high numbers of omnivorous nematodes (Stirling *et al.* 2003). Field experiments demonstrated that at 47 weeks following addition of sugarcane trash (10 t C/ha) and 23 weeks after planting sugarcane, populations of *Pratylenchus zeae* were 95% lower in sugarcane roots in amended versus unamended soil (Stirling *et al.* 2005).

11. Farming systems suppressive to plant-parasitic nematodes.

In recent years the sugar cane industry in Queensland has experienced issues of yield decline. This led to the development of a new farming system based upon a combination of controlled traffic, minimum tillage and legume breaks (Garside *et al.* 2004, Garside *et al.* 2005). In the new farming system, soil was tilled to remove compaction following the end of the cane crop, and permanent beds were formed with the aid of controlled traffic/GPS guidance technology. Beds were established on a 1.85 m wheel spacing to match with harvest machinery and thus reduce compaction. The potential reduction in yield as a result of greater spacings was compensated for by planting two rows of sugarcane within each bed, 50 cm apart.

Legumes (e.g. soybean) were planted into each of the beds, based on previous trials which showed that break crops/bare fallow, led to increases in yield in the subsequent sugarcane crop over the industry standard practice of ploughing out and immediate replanting. In general the increase in sugar yield was greater following a break involving pasture in comparison to a break with a crop (e.g. soybean, peanut or maize),which in turn was greater than a break following a bare fallow. Breaks of 6-9 months and breaks involving a legume crop such as soybean gave a substantial increase in yield in the subsequent sugarcane crop. The response of sugarcane to a previous break with soybean was shown to be due to an improvement in soil health rather than due to an improvement in N status of the soil. For example, in comparison to continuous sugar cane, a break with a crop resulted in an increase in populations of pseudomonads, fungi, mycorrhizal fungi and free-living nematodes in the soil, a decrease in populations of the plant-pathogenic fungus *Pachymetra chaunoriza*, plant-parasitic lesion nematodes and some insect pests, and no change in populations of earthworms, bacteria and actinomycetes or in overall microbial biomass or metabolic potential.

Cane was planted with a double disc opener directly into beds without disturbing the soybean residue and grown through a full production cycle. At the end of the cycle the trash blanket was retained rather than burning off the cane as this retained higher levels of total soil C and labile C, which in turn improved cation exchange capacity, improved aggregate stability, reduced surface crusting and increased rainfall infiltration leading to less runoff and erosion. At the end of the cane cycle, legumes were direct drilled into the stubble/trash, grown on and cane direct drilled into the legume stubble. Reduced tillage was found to have a beneficial impact on populations of macrofauna such as isopods, millipedes, scorpions, spiders and beetles, which fragment organic matter, create channels in the soil and are often predators. Reducing tillage improved water infiltration due to an improvement in macroporosity.

This system maintained sugarcane production over one cycle and led to additional income from grain, reduced fuel costs, reduced fertiliser inputs due to N from legumes, less labour, fewer pesticide inputs and increased gross margins/ha. In addition the move to controlled traffic and permanent beds led to reduced compaction which negated the need for tillage and hence heavier and more expensive machinery for cultivation.

Plant parasitic nematodes were shown to play a role in yield decline of sugarcane, with losses of 10% in the plant crop and 7% in ratoons leading to an industry wide loss of $80 M per annum. In long-term sugarcane soils, more than 70% of the nematode community were plant-parasites with *Pratylenchus zeae* considered of major importance. In the new farming system the legume break crop reduced cane-specific pathogens, including plant parasitic nematodes and markedly increased numbers of bactivorous and fungivorous nematodes. A single legume crop was capable of reducing populations of *P. zeae* by 80-90%. Retention of trash in the new system was shown to be important in maintaining the suppression of nematodes. Trials in which materials with a high C/N ratio (sawdust, cane trash and grass hay) were applied to soil were shown to be suppressive to plant-parasitic nematodes after 7 months in comparison to addition of materials with lower

C/N ratio. Similarly addition of sugarcane trash with or without additional N or soybean break crop reduced *Pratylenchus* spp. populations in roots to 4-6% of the population in roots not treated with amendments at 5 months after planting and 11 months after adding amendments. Similarly populations of free living nematodes in soil were increased in soil treated with amendments. Factors associated with this suppression included i) increased microbial activity, ii) increased fungi and fungal-feeding nematodes, iii) more omnivorous and predatory nematodes, iv) low to moderate levels of nitrate-N and v) an unknown nematophagous fungus. In addition to amendments, soil disturbance was shown to encourage multiplication of plant parasitic nematodes, and conversely lack of tillage was suppressive. This indicated that effective suppression of plant parasitic nematodes in sugarcane soils could be obtained by inputs of organic matter and reduced tillage (Pankhurst *et al.* 2003, Stirling *et al.* 2005, GR Stirling Biological Crop Protection pers. comm.).

Similarly in the banana industry in Queensland, practices which increased the amount of labile carbon and reduced the soil nitrate levels led to an increase in biological diversity, which were all correlated with increased suppression of plant-parasitic nematodes in the roots of banana (T. Pattison DPI Queensland pers. comm.).

While controlled traffic and minimum tillage practices have been adopted widely by some cropping industries (e.g. cereals), there has been less adoption in intensive vegetable production. However, there is ongoing research to develop such farming systems for vegetables (Morse 1999). No till production requires i) production of dense, uniformly distributed cover crops, ii) skilful management of cover crops before transplanting/seeding to leave a heavy, uniformly distributed killed mulch cover on the soil surface, iii) establishment of transplants/seedlings into cover crops with minimum disturbance of residues and surface soil and iv) adoption of integrated year-round weed control strategies (Morse 1999). Such systems offer advantages of soil and water conservation, improved soil chemical properties, reduction in irrigation requirements, reduced labour requirements and greater nutrient

cycling (Hoyt *et al.* 1994). However potential disadvantages include lower soil temperature which can affect maturity date, higher chemical input of herbicide (in some cases), potential pest/pathogen carryover on residues (Hoyt *et al.* 1994). Hocking and Murison (1989) compared conventional tillage, plastic mulch, weed matting and minimum tillage of raised beds on a rotation of cucumber, tomato, lettuce, tomato, broccoli, melon and silver beet in New South Wales. They demonstrated no difference in yield and quality between cultivation practices. Interestingly, the trial was terminated when unspecified nematode infestations resulted in poor growth and yield of the final silver beet crop, with the highest infestations occurring in the conventional tillage and least in the minimum tillage plot (Hocking and Murison 1989). Bergerson and Ferris (1996) also reported greater *Pratylenchus* populations in maize plots with more intense cultivation. However, the effect of tillage practices on nematode densities has often been inconsistent in the literature (McSorley and Gallagher 1993).

No tillage techniques may be difficult to adopt for some crops including root vegetables where a friable growing zone is required to ensure suitable tap root or tuber development. However, zone or strip tillage techniques have been developed in which only a portion of the bed is cultivated immediately prior to sowing which may be appropriate for these vegetables. For example, Swanton *et al*. (2004) reported no significant difference between conventional versus zone tillage in total or marketable yield of carrot over 4 years. Total yield and marketable yield of onion was significantly greater under zone tillage than conventional (Swanton *et al.* 2004). Similarly yield and quality of tomato was similar under conventional tillage versus zone tillage (Thomas *et al.* 2001). McKeown *et al.* (1998) compared strip tillage in which 45 cm wide strips were tilled through a killed cover crop to 15 cm depth using a rotovator to conventional tillage with no surface residue. Yield of tomato was lower in strip tillage in comparison to conventional tillage during a 6 year experiment. They concluded that while strip tillage was a feasible alternative to conventional tillage for processing tomato, choice of cover crop was important to reduce populations of local nematode species and issues of potential allelopathy due to the cover crop needed to be considered. For example,

some authors (e.g. McKeown *et al*. 1998) have reported tomato yield to be lower following perennial ryegrass, possibly due to allelopathy. Allelopathy is more important when residue is left on the surface than when incorporated (Smith and Martin 1994). However, it is often desirable to leave residues on the surface to retain moisture, inhibit weeds and contribute to soil C. McKeown *et al.* (1998) demonstrated that at some times of the year populations of *Pratylenchus* and *Meloidogyne* were higher under strip tillage than conventional tillage, while at other times the reverse occurred. It is therefore unknown whether zone or strip minimum tillage systems might still accrue the benefits of developing soil suppressiveness to pathogens as seen in the sugar cane and banana industries (discussed above).

Two projects supported by Horticulture Australia Ltd. (VG90050 and VX 01033) have investigated the ability to integrate minimum tillage systems into the vegetable industry. However, there appears to have been no plant pathological input into these projects which, if benefits were demonstrated, would add considerable impetus to their adoption. Similarly research has been conducted in Tasmania over the last 3 years by Shane Broad and Shaun Lisson (TIAR, University of Tasmania) developing a no-till system for broccoli which uses cover crops and controlled traffic beds. In this system a cereal rye cover crop is sown into beds in September and dessicated with glyphosate in December. A machine has been designed which rolls the cover crop and transplants seedlings directly through the trash. This has been demonstrated to be feasible system and to substantially reduce the need for pesticides by reducing numbers of diamond back moth, cabbage aphid and weeds.

12. Gaps in knowledge/technology

With the potential loss in availablility of nematicides there is a need to move back to a cropping system approach to nematode management. Davis *et al.* (1999) summarised the alternative methods currently used in carrot production in USA and rated them for their effectiveness (Table 23). Crop rotation, economic thresholds and field selection were the most commonly used techniques for nematodes, ranging from very good control to poor control.

Table 23. Non chemical control methods used for nematode control in carrot production in USA (Adapted from Davis *et. al.* 1999).

Control method	State	Total acres in production	Target pests	% of total acreage on which practiced
Cover crops	WA	8360	Nem (3)[1]	15
Crop rotation	CA	66580	Pyth, Nem, Alt (1)	100
	CO	3700	Alt (3), Cer (3) Nem (2)	100
	FL	6780	Pyth(3), Alt(3), Nem(3), Scl(3)	50
	MI	7120	Alt(3), Cer(3), Nem(2)	100
	TX	9400	Nem and foliar pathogens	100
	WA	8360	Pyth,Alt,Rhiz,Scler,Nem (3)	90
Economic thresholds	CA	66580	Nem(2)	80
	TX	9400	Nem(2)	25
	WA	8360	Nem(2)	75
Field selection	CA	66580	Pyth and Nem (3)	25
	CO	3700	Nem(2)	60
	WA	8360	Pyth and Nem (4)	80
Flooding when fallow	FL	6780	Nem(1), Scler(1), Scl(3)	50
Resistant vars			Not used for nematode control	

[1]Efficacy of control practice based on 1=very good control to 5=very poor control.
Nem=nematodes, Pyth=Pythium, Alt=Alternaria, Cer=Cercospora, Scl = Sclerotium, Rhiz=Rhizoctonia, Scler=Sclerotinia,

However, a cropping sytems approach 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. Priority areas for further research are:

1. Identification of nematodes

More rapid, sensitive and cheaper means of detecting, identifying and quantifying nematodes in soil and/or plant tissue to species and/or race are likely to greatly improve the ability to manage nematodes. The most likely improvements in this area are through DNA based techniques, although it is vitally important that specialists be trained in morphological means of identification (Stirling *et al.* 1992c). Stanton and O'Donnell (1998) suggested that the advent of molecular tests to identify nematode species in the farming system and region could be combined with screening of potentially useful break crops for resistance to those nematode species, an approach that had been employed in the Queensland vegetable industry (Stirling *et al.* 1986). However, there have been few attempts to link DNA diagnostics of vegetable crops in Australia to management decisions (e.g. Stirling *et al.* 2004).

2. Break crops/resistant varieties

A better understanding of the host range of local populations of nematode species will aid in the design of suitable crop rotations and break crops to help manage nematode populations. However, to take full advantage of this will require a species specific soil test to enable growers to know which species of nematode is present (1 above). Some information on suitable resistant/tolerant break crops and varieties is available (e.g. Stirling *et al.* 1986). However, there is a need to update this information, adapt it to other growing regions and provide it in a readily accessible

form. Some good general information on potential cover crops to prevent soil erosion, improve soil organic matter levels and increase soil-N, and their agronomic requirements is available to growers (e.g. Anon 2005). However this is not usually integrated with a knowledge of susceptibility to nematodes or other soil borne pathogens.

While biofumigation with mustard and brassica spp. has become more commonplace in Australian vegetable production the use of other species with known antagonistic effects to nematodes has not. Research is required to determine the cost/benefits of integrating these species into the rotation in particular localities and perhaps to establish industries which provide sufficient seed at a suitable cost for growers to use.

Although there are few examples of commercially available vegetable varieties which are resistant to nematodes, there is scope to screen commercial varieties of vegetables for their relative susceptibility to particular nematode species, so that growers may have the option of planting a relatively more resistant or tolerant variety as part of an integrated management strategy. The development of transgenic plants offers a means of developing resistance in crop plants, but is some way from commercialisation or consumer acceptance. Irrespective of the development of transgenic plants, the advent of DNA techniques offers a means of identifying gene markers for resistance in particular crops to enable more rapid progress in conventional breeding.

3. Economic threshold/hazard indices

Deriving meaningful pre-plant thresholds or hazard indices for a range of nematode species on a range of crops would greatly enhance management decisions. Thresholds for particular nematode/crop combinations have been published but are of limited use due to the effect of local conditions on the relationship between nematode population density and yield/quality and risk assessments based on edaphic/historical factors (e.g Stirling *et al.* 2004). The development of more accurate thresholds will require more accurate and cheaper means quantifying

specific nematodes in soil (1 above) and cheaper methods of sampling soil (e.g. automation) so that more intensive soil sampling can be undertaken. Modelling of nematode-crop damage dynamics, may potentially improve the accuracy of thresholds. For example, linking pre-plant nematode counts with a knowledge of day degree accumulation (soil temperature) for a geographic area after a particular planting date and how it relates to plant development and rate of development of nematode populations and subsequent crop damage. Studies such as that of Stirling *et al.* (2004) which validated molecular techniques in the field and which combined a knowledge of site specific risk factors and nematode quantification in assessing potential risk to Queensland tomato would be useful in other crops.

4. Nematicides

There has been a continual loss of nematicides from the market due to health and environmental concerns and issues with enhanced biodegradation. Although alternatives are under developed in other countries, it remains to be seen whether these will obtain registration and will be cost-effective in vegetable production. From a grower's perspective nematicides are often cost-effective but are a relatively expensive addition to variable costs. Cheaper, cost-effective alternatives would be a welcome addition. Improvements in detection and sampling which lead to the ability to map nematode infestations in the paddock would lead to the ability to adopt variable rate applications of nematicides. This would lead to lower cost and less environmental hazard from application. However, soil sampling constitutes a major proportion of the costs of nematode testing. Therefore it is unlikely that soil counts will ever be sufficiently economical to provide the intensive maps of nematode distribution required for variable rate application. However, other methods may be cheaper, such as mapping of the distribution of root knot damage in one crop to allow variable rate application of nematicide to sites of most damage, prior to the next susceptible crop.

5. Biological control and soil amendments

There have been many studies in biological control, both in terms of innundative strategies where nematophagous organisms are added to the soil, or augmentative strategies where a soil ameliorant is added to improve the nematophagous nature of the soil (e.g. chitin or organic amendments). The existence of suppressive soils gives some indication that with a greater understanding of soil biology this approach might become a practical reality (see 7 below). However although some success has been achieved, biological control has not become a widespread reality. In general, amendments are prohibitively costly due to the high rates at which they need to be applied to achieve control. Similarly, biological control agents have not shown sufficient efficacy to be successfully adopted. The recent ability to culture the nematophagous organism *Pasteuria pentrans* represents a potentially important break through in biological control as this organism has many of the attributes required of a biological control agent (e.g. long lived propagules which are resistant to environmental degredation).

6. Modelling

A greater understanding of nematode population dynamics and nematode development in relation to environment (e.g. day degrees) is necessary to develop models which i) predict nematode decline over winter, ii) predict crop damage from pre-plant nematode population density and planting date, iii) predict nematode populations build up and decline with different crops and during particular rotations. Predictive tools would be of benefit in aiding the management of nematode populations during the rotation, especially if linked to economic information.

7. Development of farming systems for suppression of plant-parastic nematodes and other soil borne pathogens.

Considerable agronomic, economic and environmental advantages have been demonstrated in the sugarcane industry with the adoption of a system which incorporates elements of controlled traffic, minimum tillage, legume breaks and trash retention (Garside *et al.* 2005), including suppression of plant-parasitic nematodes. Such a system combines and integrates many of the elements discussed in this literature review. There are considerable challenges to be overcome in developing such a system for vegetable production. However, research in this area may provide a major advance in managing soil borne diseases and in providing more sustainable production systems for the vegetable industry, less reliant on chemical inputs.

13. Conclusions

Nematodes are a major constraint to vegetable production worldwide. For many years nematicides have been the mainstay of control. However, the continual loss of nematicides due to environmental and health concerns and the development of enhanced biodegradation suggest the need for different management strategies. This literature review comprises background information for Horticulture Australia Project VG 05026 which supported a meeting of vegetable nematogists and industry representatives to workshop research, development and extension requirements for the vegetable industry into the future.

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