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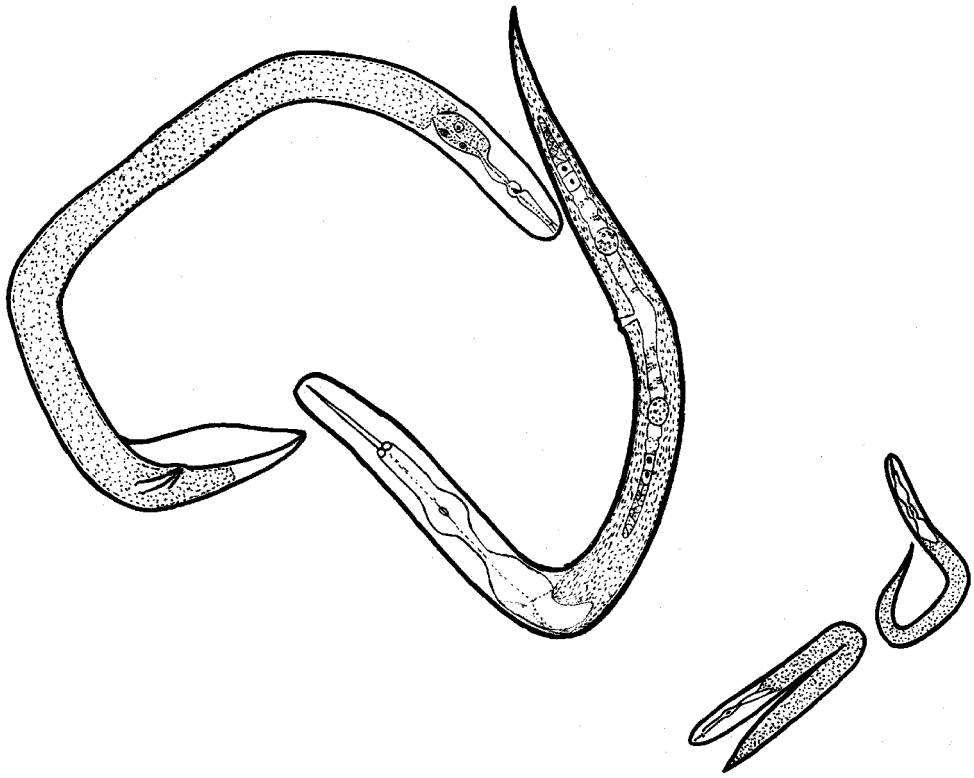
AUSTRALASIAN NEMATOLOGY NEWSLETTER



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From the Editor

Thank you to all those who made contributions to this newsletter.

January Issue

The deadline for the January issue will be December 1st. I will notify you a month in advance so please have your material ready once again.

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Association News

FROM THE PRESIDENT

I had to think a little about what to write in this column after my tome last time. Everything seems to be relatively quiet. However, among interesting things going on at the moment are Tom Powers (current President of SON) initiating a project to collate more information on the distribution of nematode species across the USA. The aim is to improve diagnosis and both internal and external quarantine. The Europeans are also interested in developing a list of all the species present in that continent. As part of my work for ABRS, I am developing lists for the Orders that I am working on in Australia. I will be pressing for the various lists to be brought together at some stage, which should be valuable for quarantine.

I was also interested when I first heard of the project using *Caenorhabditis elegans* and *C. briggsae* which is noted below under "News from Canberra". I was interested because my former graduate supervisor, Warwick Nicholas, worked in the lab. which developed the Axenic culture techniques that lead to *C. elegans* becoming a model organism for an enormous number of biological disciplines, from cellular development and differentiation to being the first multicellular animal to have its genome completely sequenced. The interest is in the huge discrepancy between the aims and aspirations of the original projects and the enormous impacts that the projects, including Warwick's, have subsequently had. The impact could just not be predicted at the time. One wonders whether such projects would be funded now. Nevertheless, despite the 50 years' studies that have intervened, and the large scientific effort which has developed from the early discoveries, it seems that *C. elegans* is still of great interest, and there is a lot still to learn about it.

I have received another few comments regarding the suggestion for holding the 5th International Congress of Nematology in Australasia, all remain in favour, with a few reservations. I will put an item on the agenda for our next general meeting, but it is still not too late to comment – or better still volunteer.

Don't forget about the AAN general meeting and Soil-Borne Disease Symposium in Adelaide in February next year.

Regional News

NEWS FROM SOUTH AUSTRALIA

News from SARDI

We seem to be busier than ever and the usual tasks are underway for the start of the grains season in the South i.e. trial site selection, field trial preparation and glasshouse and outdoor screening and experiments for cereal cyst, root lesion and stem nematode.

The only hiccups in our smooth and well-planned operation (!) are the quarantine on cereals (at the time of writing this article) for the Waite (and others) as a result of the Wheat Streak Mosaic Virus crisis, and the reasonably high drought forecast for SA. The next exciting instalment from SA will certainly have an update on whether we did or didn't get anything sown this year.

The good news comes from Jackie Nobbs, who is progressing rapidly on the completion of the CD Rom of Plant-parasitic nematodes in the grains, vegetable and sugar industries. The finished product is expected sometime in August so we'll have an official launch at the Australasian Soil-borne Disease Symposium in February 2004.

With regards to the symposium, we've been successful in gaining support from GRDC, AAN and the ASDS to fund Roger Cooke from the UK to present at both the Symposium and the Nematology Workshop. If you need further information and or wish to register, please visit the web site being set up for the symposium. The web address is:

http://www.plevin.on.net/asds2004/

Please note that registration and conference details will only be available from the web site. ***If you do not have access to the Web (or are aware of others without access who may wish to attend) please contact Ian Riley (08 83037259) or Sharyn Taylor (08 8303 9381).***
We hope to see as many Nematologists there as possible to catch up and discuss the exciting world of nematology.

Sharyn Taylor

News from The University of Adelaide

Ian Riley has been busy teaching and preparing to be a peripatetic nematologist. In May he left for Oregon and Maryland, USA, to work on Rathayibacter/*Anguina* with Dr Norm Schaad (USDA), and in June he was in Turkey, lecturing on nematodes in the root diseases course organised by Julie Nicol and funded by the Crawford Foundation.

With Mike Hodda, Kerrie Davies has been making plans for the next short course in Nematology, to be held in Adelaide this year from 1st to the 5th of December. We look forward to meeting enthusiastic nematologists at the course. Kerrie and Val Kempster have also been developing techniques to do SEM of *Fergusobia* (why did no-one warn them of the problems preparing nematodes only 300µm long for SEM?). To keep sane, they have data-based the Waite collection of *Fergusonina* and associated insects. *Fergusobia* is on the way. Kerrie will be in parts north in June and July, catching up with Adrian Evans in the U.K., with Suzanne Charwat, Astrid Schmitz and Andreas Hensel in Germany, and Julie Nicol in Turkey. Astrid is now studying for her Ph. D. at Bonn University, and her supervisor is Professor Richard Sikora. Andreas is working in pharmacological research.

Zhao Zheng Qi has commenced studies for his Ph. D. He will be looking at *Bursaphelenchus* and related genera of nematodes in Australian conifers, and the project is funded by Forest and Wood Products research and Development Corporation.

Imelda Soriano attended the ICPP in Christchurch which was made possible by GRDC and SA-APPS funding. She also visited the lab of Dr David Wharton (the University of Otago - Zoology Dept.) to learn techniques in ultrastructure microscopy. They also discussed the biology and behaviour of nematodes in response to biochemical stress from plants.

Congratulations to Motiul Quader on the successful acceptance of his Ph. D. thesis on grapevine nematodes. Motiul is now in Sydney, working for the NSW Department of Agriculture.

Max Dewdney has been working on interactions between species of the genus *Pratylenchus*. Currently he is conducting a series of experiments investigating biological attributes that may affect the relative fitness of individual species.

Elise Head is continuing her part-time PhD investigations of the *Fergusonina* fly/ *Fergusobia* nematode gall association on *Eucalpytus* host species. She recently completed 12 months of field observations in the Urrbrae Wetlands of Mitcham City Council. Aspects of tree phenology and gall growth were recorded monthly, and will be compared with climatic data. Elise will also be making histochemical analyses of gall material, and is attempting to grow nematodes and fly larvae on eucalypt callus tissue cultures.

Entrepreneur Mark Potter is busy making and teaching others to make cheeses, under the name of “Blessed Cheeses”. He is writing the papers arising from his work on the relationships between canola and *Pratylenchus*.

We enjoyed flying visits from Vivien Vanstone (WA Department of Agriculture) and Janine Paynter (nee Lloyd) (Darwin). Janine introduced us to her beautiful 4 month-old daughter, Jennifer. Quentin Paynter has accepted a position with Land Care in New Zealand, and they will be moving to Auckland in July/August.

NEWS FROM CANBERRA

Undoubtedly the major news from my part of CSIRO is the release of the update of the nematode interactive key, which went live on the 5th of June at:

http://www.ento.csiro.au/science/nematode.html

(Thanks once again to the supporters, the Australian Biological Resources Study, and indirectly to GRDC, who support the collection on which part of the key is based.) The key now includes:

- all life stages of freshwater aquatic nematodes from the Murray-Darling Basin and south-eastern Australia;
- all species of *Pratylenchus* known from Australia;
- all species of the families Dorylaimidae, Aporcelaimidae, Qudsianematidae, Nordiidae, Actinolaimidae, Trachypleurosidae, Carcharolaimidae which have been found in Australia;
- some species and genera from the other Dorylaimid families (work continuing next year); and
- some genera and species of Tylenchid plant parasites (world fauna).

To get just the taxa which have been found in Australia, select the character **distribution: (continents)** and then choose **Australia**. However, there is no guarantee that the list is comprehensive. The amount of collecting and number of specimens are just too small to place much reliance on the records from Australia. I recommend starting with the world list at the moment. If you find anything which does not seem to be present please send some specimens to me, and hopefully the species can be added to the next edition (due in a year's time).

I was invited to the International Plant Protection Congress in Beijing in July, but the congress has been postponed for a year because of SARS. What is it about Plant Pathologists? Collapsing airlines, major health scares. Anyone would think the science is not interesting enough.

My past PhD student at UWS Emma Broos headed off to a one year Posdoctoraal fellowship with Diana Wall at Colorado State University in February. From all reports, she is finding it a very stimulating environment. Present student Sosamma Pazhavarical has just been successfully through her first review, and is currently setting up cultures of nematodes and plants for her model systems.

In John Curran's lab., work is continuing on sequencing a number of nematodes in collaboration with Jackie Nobbs and Kathy Ophel-Keller at SARDI and Vivien Vanstone at AgWA. Wolfgang Wanjura and Di Hartley (former Treasurer of AAN) continue to perfect techniques for obtaining DNA and RNA from ever more difficult nematodes with ever greater efficiency.

In addition to the work in John Curran's and my lab, there is also some interesting work involving *Caenorhabditis elegans* in the lab of one of our Entomology colleagues Steve Whyard. He has a PhD student, Jeremy Brownlie, who recently received his Bachelors degree at the ANU. A significant part of his studies involved the molecular characterisation of a novel transposon found in the nematode *Caenorhabditis elegans*, and a closely related species, *C. briggsae*. Transposons are mobile pieces of DNA, capable of moving from one chromosomal location to another. As you might expect, they are a natural source of genetic variation due to their random movements. It appears that this novel nematode transposon, which we have called maT, entered the worm's genome relatively recently, presumably by horizontal transfer from another species. How this occurred is uncertain, but since its entry into *C. elegans*, it has been slowly proliferating throughout the worm's genome. For a typical transposon, after a proliferation phase in a new host (after many generations), the multiple copies gradually accumulate mutations and eventually are no longer capable of movement. We have found that maT is still active in both species of worms, and we're now considering using maT as a genetic transformation tool, to carry DNA into a new host's chromosomes.

NEWS FROM WESTERN AUSTRALIA

Investigation of Western Australian Vineyard Nematodes by Professor Franco Lamberti

Vivien Vanstone, Department of Agriculture WA **(**vavanstone@agric.wa.gov.au**)**

From mid-February, Professor Franco Lamberti (Instituto di Nematologia Agraria, Bari, Italy) spent one month at the Department of Agriculture WA (DAWA). His visit was made possible by a GWRDC Regional Innovation and Technology Adoption grant to the Wine Industry Association of WA.

The aim of Franco's study was to sample as many vineyards as possible, across all the major vine-growing regions, in search of *Xiphinema* and *Meloidogyne*. Thanks to Neil Lantzke (DAWA Wine Grape Project) and numerous regional Horticulture staff, Franco was able to sample 70 vineyards plus 64 fruit and other trees. Franco often sought out figs and olives, as these are known to host his favoured genus, *Xiphinema*.

BACKGROUND

A combination of factors predispose WA vineyards to nematodes: light, sandy soils; warm climate; cultivation of many "own rooted" vines; and use of land previously planted to vegetable crops such as potato.

Meloidogyne has been associated with vine decline in the South West (Peter Wood, DAWA, unpublished). Gray (1998) also detected RKN at several sites on "own rooted" vines, with highest populations after potato. Identity of species from these studies, however, was not confirmed. It was also recognised that a wider investigation of vine-growing areas was required.

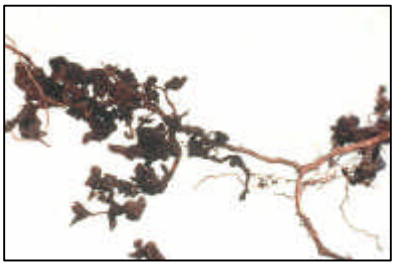
Sampling for *Xiphinema* was included in Franco's study as these transmit nepoviruses, and the nematodes damage roots. Franco was keen to obtain specimens from Australia.

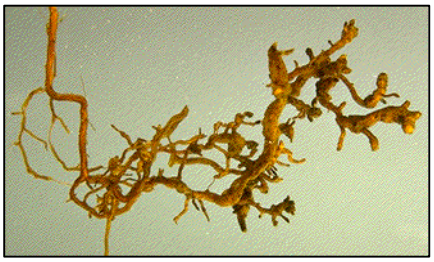
Accurate, specific identification of *Xiphinema* and *Meloidogyne* by morphological and/or morphometric methods alone is difficult. DNA based identifications are considered the most reliable.

THE USUAL SUSPECTS

Meloidogyne: Four RKN species have been found in major vine-growing areas of Australia: *M. javanica, M. hapla, M. arenaria, M. incognita*. It is not uncommon for plants to be infected by more than one species.

Xiphinema: One or more species are present in grape-growing areas worldwide, and at least 28 species have been reported on vines. These nematodes are common in Australian vineyard soils, but no detailed study of species has been made.





Xiphinema *symptoms on roots*

X. index is of particular concern, since it transmits the devastating nepovirus Grapevine Fan Leaf (GFLV). Fortunately, the only record of *X. index* from Australia is from a limited area around Rutherglen, Victoria, where it has been contained. Other species of *Xiphinema* are often associated with vines, but their economic impact is largely unknown. Species other than *X. index* can transmit economically important viruses to a wide range of crops.

There is substantial morphometric and morphological variation between *Xiphinema* populations, and *X. americanum* alone has been defined by Lamberti *et al.* (2000, 2002) as a complex ("the *X. americanum*-group") comprising 51 species. This has been arrived at by principal component and hierarchical cluster analysis of up to 17 morphometric characters, with identifications confirmed by isozyme and DNA analyses. Identifications are further confounded by deviations from the type species populations due to environmental and/or geographic influences, possibly leading to incorrect identifications if based solely on phenotypic morphometric data. Franco has accumulated morphometric and molecular data for *Xiphinema* from over 50 countries. The specimens from WA will represent the first inclusion of Australian data to his comprehensive studies.

Pratylenchus: RLN usually occur in low populations on vines, but are widespread and potentially more damaging than RKN if in high numbers. Several species are possibly associated with vines in Australia: *P. vulnus, P. scribneri, P. brachyurus, P. neglectus, P. pratensis*.

CATCHING THE CRITTERS

Rhizosphere soil (~ 5kg per sample) was collected from 70 vineyards and from 64 fruit and other trees during Feb/March. Biosecurity procedures were observed between and during property visits (i.e. fashionable blue booties, and a rusty spade courtesy of all the bleach and metho used!).

Soil was wet sieved (710µm followed by 63µm) and the resulting slurry placed in water overnight at room temperature in 90µm sieves in Petri dishes to obtain "clean" extracts. Extractions were qualitative not quantitative.

Helen, Clare and Sean were responsible for soil processing, while Franco and various horticulture staff embarked on the numerous sampling expeditions. My important task was to make sure the European soccer results were downloaded for Franco first thing every Monday morning!

Between sampling trips, Franco investigated each extract, and picked out *Xiphinema* into 1M NaCl for transport to Italy. These specimens will be subjected to isozyme and DNA studies.

Remaining extract was fixed in 4% formalin to obtain *Xiphinema* specimens for detailed morphometric and morphological study. Juvenile stages will be observed to determine if there are 3 or 4. *Pratylenchus* from these extracts will be identified by Nicola Vovlas in Bari.

Galled roots contained in the soil samples were removed prior to sieving. Molecular studies and identification of the RKN species will be done in conjunction with Franco's collaborators in Portugal.

OBSERVATIONS

Representative samples were collected from all major vine-growing areas of the State. *Xiphinema* and/or RKN were found in all regions investigated.

Fourteen vineyards with obvious symptoms of decline were sampled, and all were infested with RKN. Roots displayed moderate to severe galling. Many of these were "young" vines (~ 5 years old), and *Xiphinema* did not seem to be associated with this decline. However, both *Xiphinema* and RKN were found in samples from 20 and 30 year old vines. Galled roots were also obtained from vines with no obvious decline.

Of the vine samples, *Xiphinema* were associated with 14 vineyards (20%) and RKN with 22 (31%). All observations are outlined in the Table.

Of course, *Pratylenchus* were also observed in extracts from most of the soil samples!!

Decline in vines due to Root Knot

Franco was surprised at the lack of species diversity in the *Xiphinema*. It seemed, from his initial investigation, that there were only 2 species (both of the *X. americanum*-group), and most were probably *X. rivesi*. However, identifications remain to be confirmed with the more detailed work being conducted in Italy.

X. rivesi was originally described from France, and has since been found in Bulgaria, Germany, Spain, Portugal, North and Central America, South America and Pakistan. In North America, *X. rivesi* is the most widespread species of the *X. americanum*-group. This species transmits at least 3 nepoviruses. It is parthenogenetic, and among one of the 5 *X. americanum*-group that has only 3 juvenile stages.

The distinctive *X. index* was not detected in any of the samples.

OTHER ACTIVITIES

Franco examined and re-confirmed as *X. index* specimens from Rutherglen. For us, it was our first encounter with *X. index*. Thankfully, there were no problems with quarantine, as the nematodes had been dead since 1963!

At a DAWA seminar, Franco summarised some of his 40 years worth of nematology experience (during which time he has published 600 papers, edited 5 books, and founded and edited *Nematologia Mediterranea*). He addressed a grower meeting, was interviewed by ABC radio, and appeared in the *Wanneroo Community* newspaper!

The conclusion to a hectic and rewarding month with the enthusiastic and delightful Franco was "*Xiphinema* School", attended by staff and students from DAWA, Murdoch and UWA. This involved a comparison of specimens extracted and demonstration of the features that make a *Xiphinema*.

Nematodes detected in vine and tree samples, WA, 2003

	No. of samples containing:				Total no. of samples examined
	Xiphinema	***Meloidogyne***	***Heterodera***	***Belonolaimus* ?**	
Grape	14 (20%)	22 (31%)	1		70
Fig	2				10
Peach	1				2
Apricot	1				5
Pear	1				1
Apple	5				10
Olive	1	1			4
Avocado					2
Plum					3
Cherry					1
Nectarine					1
Persimmon					1
Walnut					1
Mulberry					1
Poplar					3
Maple					1
Xanthorrhoea				1	1
Eucalyptus	1			3	9
Geraldton Wax					1
Melaleuca					1
Pine					2

INFORMATION FROM

Gray M 1998 - Controlling nematodes of grapevines in the South West of Western Australia. Honours thesis, University of WA.

Lamberti F 1989 - Nematode parasites of grapevine and their control. Pp. 163-172 in Plant-protection problems and prospects of integrated control in viticulture (ed. R Cavalloro). Proceedings of the CEC/IOBC international symposium, Lisboa-Vila Real, Portugal, June 6-9 1988.

Lamberti F, S Molinari, M Moens, DJF Brown 2000 - The *Xiphinema americanum*-group. I. Putative species, their geographical occurrence and distribution, and regional polytomous identification keys for the group. *Russian Journal of Nematology* **8(1):** 65-84.

Lamberti F, S Molinari, M Moens, DJF Brown 2002 - The *Xiphinema americanum*-group. II. Morphometric relationships. *Russian Journal of Nematology* **10(2):** 99-112.

Nicol JM, GR Stirling, BJ Rose, P May, R Van Heeswijck 1999 - Impact of nematodes on grapevine growth and productivity: current knowledge and future directions, with

special reference to Australian viticulture. *Australian Journal of Grape and Wine Research* **5(3):** 109-127.

IF ANYONE HAS *Xiphs*, OR KNOWS WHERE TO GET SOME, PLEASE E-mail ME. FRANCO WOULD LOVE TO SOURCE MORE AUSTRALIAN SPECIMENS!!!!

News from the Plant Nematology Group, Plant Biotechnology Research Group, WA State Agricultural Biotechnology Centre (SABC), Murdoch University, WA

Mike Jones, Zhaohui Wang, Angela Hollams and Modika Perera

- **A novel approach to identification of plant parasitic nematodes**

Modika Perera has recently joined the plant nematology group working with Mike Jones (SABC) and Vivien Vanstone (DAWA). Modika is an entomologist who has just finished a 3 year GRDC postdoctoral fellowship. She has begun a new ARC Linkage research project entitled " Hidden enemies of crop plants: developing novel methods to identify plant parasitic nematodes". The overall aim of the project is to develop simple and reliable methods to identify plant parasitic nematodes. The method being developed is based on rapid generation and analysis of specific protein profiles.

So far we have developed rapid protein extraction and purification methods and used these to generate protein profiles of nematodes using MALDI-TOF mass spectrometry for protein analysis. Diagnostic protein profiles have been found for several species. The work is being extended to study a range of economically important nematodes, different life stages of each target species, and also different populations of nematodes, to determine the resolution of the approach.

In parallel, we are comparing results from this novel approach with established techniques, such as isozyme patterns and PCR diagnostics. The aim is to develop routine species profiles for each target species. This approach may provide a rapid, cost-effective way to identify nematodes, and so be useful to assist advisers and growers on methods to use to control these pathogens.

To extend this project, we would very much appreciate if anyone could supply us with authenticated RKN species to test the accuracy and to validate the method. Please contact Modika Perera on (08) 9360 6335 or e-mail (mperera@central.murdoch.edu.au).

Gene expression in giant cells

Zhaohui Wang and Mike Jones have had the following paper accepted for publication in the journal *Molecular Plant Pathology:* **Differential display analysis of gene expression in the cytoplasm of giant cells induced in host plants by *Meloidogyne javanica.***

If you are interested in this work, an e-copy may be available from the publisher's web site soon, or you can contact us for more details.

- **Professor David Bird appointed as Adjunct Professor at Murdoch University**

A/ Professor David Bird (North Carolina State University, Raleigh, NC, USA) has been made an Adjunct Professor at Murdoch University, as a part of an international

collaboration with the new **Centre for Biology of Nematode Parasitism**. Murdoch University, SABC, has been invited to be one of only a few International Affiliates to this centre, which combines research on both plant and animal parasites. We are planning to run a nematology workshop with David Bird in Perth later in 2003 or in early 2004.

Investigation of possible biotic disease factors & allometric relationships in the decline of Wandoo (*Eucalyptus wandoo*) in the Shires of York & Corrigin, WA

Ryan Hooper, University of Western Australia **(**rye_81@hotmail.com**)**

Foliage death and crown decline in an extensive area of *Eucalyptus wandoo* woodland was noticed in Talbot forest block (31-53 S, 0 116-30 E) from York Road in early 1999 and a preliminary study was conducted by CALM into possible factors (Wills *et al.*, 1999) although no systematic surveys were performed. York locals say the condition was widespread by the mid to late 1990's and many residents showed concern when trees on their properties showed symptoms of decline. Symptoms are typical of eucalypt decline in many regional areas throughout Australia generally referred to as "dieback" or "rural dieback". The disease is most severe on *E. wandoo*, although it also effects powderbark wandoo (*E. accedens)* and inland wandoo (*E. capillosa)* to varying degrees. The condition is active in the current season and many "flags" (branches showing recent foliage death) can be seen on the Lakes Rd and on the Brookton Hwy. The condition starts with foliage and twig death on the upper and outer limbs, followed by a cycle of regeneration by epicormic shoots that subsequently die resulting in progressive crown decline.

Several factors have been implicated in the decline of *E. wandoo* including abiotic factors such as drought, fire and salinity and biotic factors such as insect damage and aerial canker fungi, but as yet no evidence suggests which (if any) of these factors are interacting to cause decline. CALM has suggested the condition is stabilising and a net thinning of Wandoo stand density will be the result when climatic conditions return to normal. It is likely decline symptoms are related to several stress events of varying age and severity (Mercer, in press) but the idea of allowing the condition to run its course without any concrete evidence to suggest it is a cyclic phenomenon is a concern, especially considering the importance of the Wandoo-belt for managing water-tables and water supply for regional areas in South-west WA.

My project is run through the honours program (B.ENVSC.HONS) at UWA and is jointly supervised by Peter Wood (AgWA) and Siva (UWA) with input from many professional personnel in various government departments. Essentially it will investigate possible causative organisms and will provide (hopefully) the catalyst for a more thorough investigation into the decline. Borer damage, aerial canker fungi and nematodes will be targeted by comparing external symptoms and population numbers in healthy (mainly around Corrigin) and unhealthy stands (at York). To investigate the contribution of plant-parasitic nematodes to the current decline of *E. wandoo,* soil will be sampled using a modified corer to take an intact core from 5 to 55 cm depth. A systematic sampling design will be utilised to take four samples per tree from around the

"drip zone" for 8 trees within a 50 by 50 m quadrat. Each quadrat has a known average crown rating (after Mercer, in press) based on the system of Grimes (1987) that uses visual estimates of crown density, dead branches and epicormic growth to give a rating out of 23 (healthiest). Each of the four samples will be given a North, South, East, West coordinate and each of the coordinates will be bulked from the 8 trees to give 4 bulk replicates for each quadrat. Approximately one quarter of the sample (~ 600 g) will be extracted using large extraction trays and the remaining sample will be used in a baiting experiment using 1 month old *E. wandoo* seedlings grown up in pasteurised soil. Roots and soil will be extracted upon completion of the baiting experiment and numbers of plant-parasitic nematodes will be estimated, identified at the genus level and compared for different health categories. Preliminary investigations showed very low numbers of parasitic and saprophytic nematodes but the sampling design has been modified to account for the spatial heterogeneity of nematode populations in native soil. The project is due for completion in November 2003.

Research

CAN BRASSICAS BE USED TO MANAGE ROOT-KNOT NEMATODE IN TROPICAL VEGETABLE PRODUCTION?

Tony Pattison[1], Tanya Martin[1], Steve Akiew[2], Joanna Arthy[2], Caroline Versteeg[1] and John Kirkegaard[3],

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2. *QDPI, PO Box 1054, Mareeba, Queensland, 4880, Australia*
3. *CSIRO Plant Industry, Canberra (02 6246 5080)*

Root-knot nematodes (*Meloidogyne* spp.) are a major constraint to the production of vegetables in tropical locations around the world. One method of nematode management has been rotations with crops that are poor host to nematodes. *Brassica* species are moderate hosts to nematodes and contain glucosinolates within the leaf (and root) of the plant. The glucosinolates hydrolyse to form products including isothiocyanates (ITCs) that are reputed to control nematodes. The aim of the trials detailed below was to assess the feasibility of using isothiocyanate-liberating Brassica biofumigant rotation crops as part of an integrated root-knot nematode management strategy in tropical vegetable crops.

Brassica resistance

Commercial Brassica varieties were tested for resistance to root-knot nematode in a pot trial. Brassicas were found to be only partially resistant to *Meloidogyne javanica*. Therefore, Brassicas have the potential to carry over nematodes to the following crop (Figure 1). The most resistant Brassica variety was the radish (*Raphanus sativus*) cultivar Weedcheck. However, no Brassica was as resistant as sorghum to nematode multiplication.

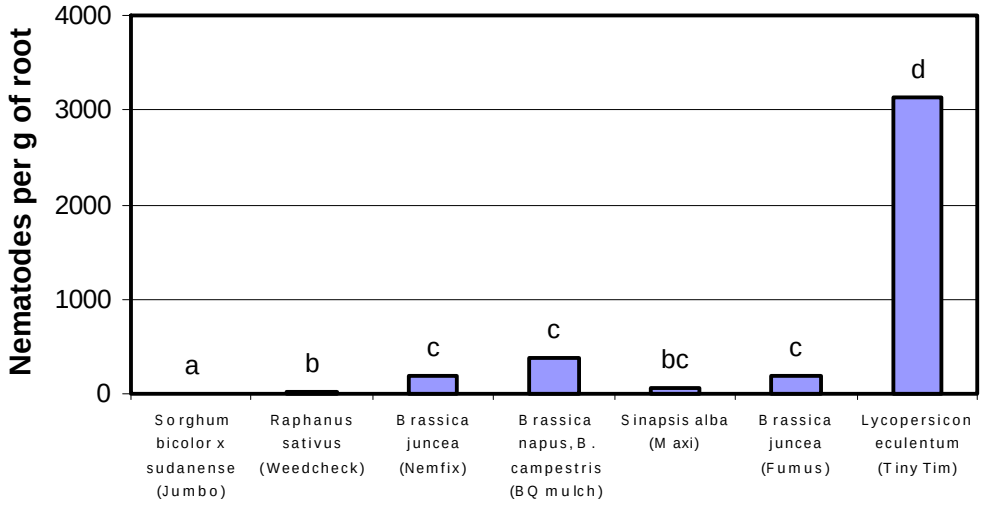

Figure 1. Resistance of commercial Brassica varieties to root-knot nematodes (*M. javanica*) relative to tomatoes. (Bars with the same letter above are not significantly different from one another (*P*<0.05).)

Fumigation effect

The fumigation effect of Brassica leaves was tested in a laboratory petri dish assay, by adding 1, 000 nematode eggs to 30 g of soil and placing either 0.75, 1.5 or 2.25 g (equivalent to 2.5, 5.0 or 7.5 % leaf to soil) of frozen leaf material on the soil surface. Leaves were frozen to ensure tissue disruption and ITC release. *B. juncea* cultivars Fumus and Nemafix were as effective in reducing the development of eggs to juvenile root-knot nematode as methyl ITC (Table 1). Both Fumus and Nemfix had relatively high concentrations of 2-propenyl glucosinolate (36.0 and 33.6 µmoles.g leaf $^{-1}$) in the leaf tissue compared to the other varieties. 2-propenyl glucosinolate is considered to be more volatile than forms of glucosinolate in the other varieties. The higher rates of Brassica leaf material added to the soil increased the mortality of nematodes, except for the addition of sorghum. This suggested that nematode mortality in the laboratory assay was affected by the volatile compounds within the leaf and not by the addition of organic matter.

Table 1. Effects of Brassica leaf material added at three rates on the recovery of root-knot nematode from 30 g of soil in a petri dish assay.

Treatment	Weight of leaf tissue (g.g soil^{-1})	Nematodes per g soil	
Untreated	0	2.30	h
Methyl ITC	10 µg.g soil^{-1}	0.13	bcde
BQ Mulch	0.02	1.77	gh
B. napus & *B. campestris*	0.05	0.90	fgh
	0.07	0.40	cdefg
Fumus	0.02	0.07	abc
B. juncea	0.05	0.00	a
	0.07	0.00	a
Maxi	0.02	2.47	h
Sinapsis alba	0.05	1.00	gh
	0.07	0.43	defg
Nemfix	0.02	0.10	abcd
B. juncea	0.05	0.03	ab
	0.07	0.03	ab
Sorghum	0.02	2.40	h
Sorghum bicolour x sudanense	0.05	1.03	gh
	0.07	1.50	gh
Weedcheck	0.02	1.53	gh
Raphanus sativus	0.05	0.63	efgh
	0.07	0.20	bcde

Numbers with the same letter following are not significantly different from one another ($P<0.05$)

Incorporation

The incorporation of leaf material into the soil reduced nematode numbers recovered from the roots of tomato plants (Figure 2). Brassica tissue was ineffective at rates less than 0.02 g per g of soil, while sorghum was effective at all rates. The results suggested a general impact of organic matter addition as well as impacts from chemicals in the leaf. Several components effect root-knot nematode when Brassicas are added into the soil. As well as the production of volatile compounds water soluble compounds may also be produced when Brassicas are added to the soil. There could also be stimulation in parasites of nematodes as the organic matter decomposes. The effect of these different components must be separated to determine their relative importance in nematode control.

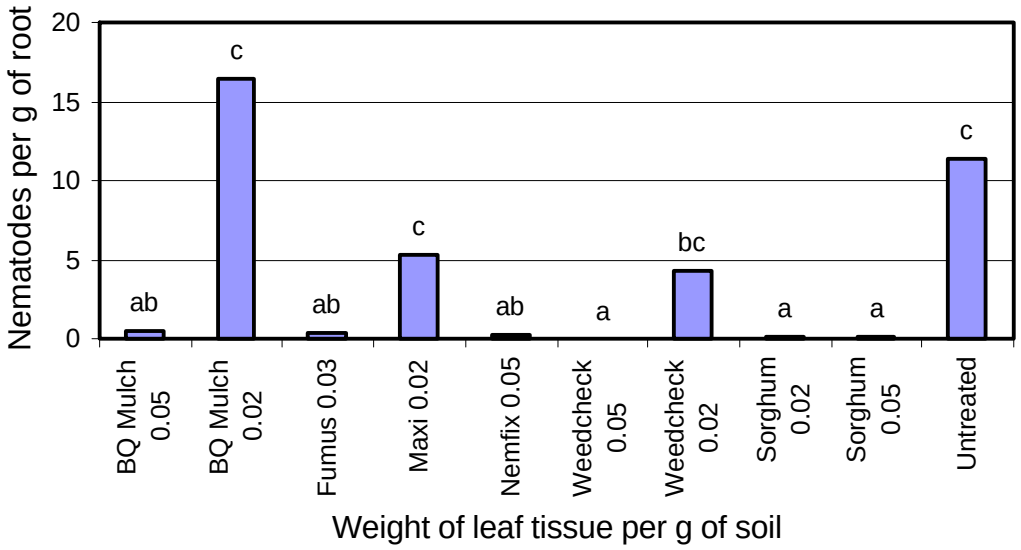

Figure 2. Root-knot nematode (*M. javanica*) recovered from the roots of tomato plants grown after the incorporation of leaf material into soil infected with root-knot nematode. (Bars with the same letter above are not significantly different from one another (P<0.05))

Conclusion.

- Brassicas are partially resistant to *M. javanica*. The radish cultivar Weedcheck had the best resistance, although it was not as resistant as forage sorghum.
- Brassicas are able to develop volatile, nematicidal compounds within their leaves that are released when their cells are disrupted.
- Incorporation of Brassica leaf material affects nematodes in at least two ways: by organic matter and by toxic compounds from within the leaf.
- Some Brassicas exhibiting both high resistance and high toxicity are currently being tested in the field.
- New *R. sativus* varieties are currently being tested to determine if they have improved resistance to root-knot nematode.

NEMATICIDE BIODEGRADATION IN VICTORIAN CARROT SOILS

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Introduction

In 1999 the Australian carrot (*Dacus carrota*) industry produced 267,000 tonnes of carrots from approximately 7,500 hectares to be the third most important vegetable crop in Australia. Victoria is the largest carrot growing state in Australia, producing 38% of the countries carrots in the 1997 season with yields of approximately 40 tonnes/hectare. A constraint to carrot production and a concern for importing countries is plant parasitic nematodes, which cause significant losses in carrot quality and yields. There are 15 common plant parasitic nematode species that affect carrot production including *Radopholus similis*, the burrowing nematode (Potter and Olthof 1993). The damage resulting from carrot feeding nematodes causes malformations with the taproot becoming forked, fasciculation, galling and stubbing of the roots reducing the crops marketability.

Nematicides have been used to help control the damage caused by plant-parasitic nematodes on carrots. However, by repeatedly applying the same nematicide to the same area, the nematicides become more susceptible to enhanced biodegradation. Nematicides are generally active in the soil for six to eight weeks, however if enhanced biodegradation of the nematicide occurs, it will only be active for less than two weeks. Two weeks is not long enough for non-volatile nematicides to reduce nematode populations, as nematicides don't directly kill nematodes. Instead, nematicides need to persist in the soil long enough to cause the nematodes to starve whilst also affecting the nematodes nervous system and ability to complete its lifecycle from the hatching to reproductive stage (Bunt 1987).

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

Materials and methods.

Soil was sampled from 13 carrot producing fields in Victoria (supplied by Deborah Keating, NRE Victoria). Each soil sample was divided into 5 sub samples so that they could have different treatments applied and follow a bioassay protocol for testing for enhanced biodegradation (Pattison *et al.* 2000). The treatments applied to the soil were:

1. Untreated soil (UTC)
2. Sterilised ($121^{o}C$ for 15 minutes) + Nemacur (SN)
3. Unsterilised + Nemacur (USN)

4. Sterilised (121°C for 15 minutes) + Telone (ST)
5. Unsterilised + Telone (UST).

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

All samples were then stored in plastic takeaway containers inside polystyrene boxes with water in the bottom at room temperature (20-35°C). 35g samples were taken from each of these sub samples at 0, 2, 4 and 6 weeks after nematicide application. 3 mung bean (*Vigna mungo*) seeds were planted into the 35g of soil from each sub sample. Sieved river sand (5 g) was then placed on the top of each vial to allow for a better seed germination and watered with 5mL before being placed in the glasshouse.

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

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

- Enhanced biodegradation occurs when nematode recovery in the unsterile Nemacur® (USN) treated soil was greater than or equal to 75% of UTC.
- Advanced biodegrading was occurring when nematode recovery in the USN was less than 75% but greater than 20% of UTC.
- No biodegradation occurred when nematode recovery in the USN less than or equal to 20% of UTC.

Results and discussion

Five carrot producing soils were found to have enhanced biodegradation of Nemacur 400®, GWSBR, PTP, GSP, LP25 and BC4, using the criteria of enhanced biodegradation occurring if nematodes recovery of the unsterile soil was 75% or greater of the untreated control (Table 1). The five soils that were determined to have enhanced biodegradation had low recovery of nematodes when the soil was sterilised and the Nemacur 400® added to the soil (Table 1). This confirms that loss of nematicide efficacy was due to a biological cause. Nemacur 400® would not be expected to be efficacious at reducing nematode numbers at these five sites due to enhanced biodegradation. The decomposing

of the nematicidal products of Nemacur 400® would be expected to have occurred before the products could reduce nematode numbers in carrot production.

Five carrot producing sites, LP21, DBS, CBP, GBH and PBP, were found to have greater than 20% nematode recovery relative to the untreaed soil, when nematodes were added to Nemacur 400® treated, unsterile soil (Table 1). This was considered to be advanced biodegradation of Nemacur 400®, as the product would not be expressing its full efficacy in these soils to control plant parasitic nematodes. Again, the recovery of nematodes in the sterile Nemacur 400® treated soil was less than 10.4% of the untreated soil, suggesting that the cause of the reduced control of nematodes was due to biological degradation of the chemical. Some reduction in nematode numbers in the field could be expected in these soils. However, Nemacur 400® applied to these sites would not be expected to have maximum efficacy to manage plant-parasitic nematodes in carrots.

Nemacur 400® applied to soil from three sites, BB3, GSH and CLP was found to have similar efficacy in both the sterile and unsterile soil (Table 1). This suggested that there was no biodegradation of Nemacur 400® at these sites and that the efficacy of the chemical was preserved. Nemacur 400® applied to these three sites would be expected to give good control of plant parasitic nematodes.

Telone C-35® lost its effectiveness from week 0 in the majority of soils (data not shown). However, this was expected, as Telone® is a soil fumigant and therefore only has a short active life in the soil. Smelt *et al.* (1996) reported that there was a very fast degradation of Telone® despite infrequent applications of this soil fumigant in the past. In their study they also measured the rapid decline of Telone® within 3-6 days of application in soils treated with the fumigant once or twice before and in soils that have previously been untreated (Smelt *et al.* 1996). Because of the short persistence of Telone® in the soil, it was difficult to distinguish between chemical and biological degradation of the active ingredients of Telone C-35®. This short persistence in soil led to inconsistent recovery of nematodes from sterile and unsterile soil relative to the untreated soil (Table 1).

Telone C-35® causes nematodes to die through contact rather than starvation and therefore has a different mode of action to Nemacur 400®. Telone C-35® does not need to persist in the soil like organophosphate nematicides, such as Nemacur® to reduce nematode populations. This short persistence, a metabolic half-life of 1.5 hours (Tomlin 1997) in the soil was why the bioassay method (Pattison *et al.* 2000) was not effective in determining the degradation rate of Telone C-35®.

Table 2. Percentage nematode recovery averaged over a 6 week period relative to the untreated control (UTC) for 13 carrot growing soils with sterile and un-sterile soil treated with Nemacur 400® (sterile Nemacur® SN, un-sterile Nemacur® USN) and Telone C-35® (sterile Telone® ST, un-sterile Telone® UST).

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

Conclusion

Five sites growing carrots were found to have enhanced biodegradation of Nemacur 400®, with grater than 75 % nematode recovery in unsterile, Nemaur 400® treated soil relative to untreated soil. A further five sites had advanced biodegradation of Nemacur 400® with between 20 and 75% nematode recovery in the unsterile, Nemaur 400® treated soil relative to untreated soil. Only three soils from the 13 carrot producing sites in Victoria had what would be expected to be "optimal" efficacy of Nemacur 400®, with less than 20% of nematodes recovered in unsterile, Nemaur 400® treated soil relative to untreated soil. The sterilisation of the soil improved the efficacy of Nemacur 400®, which suggested that the degradation of Nemacur 400 was due to a biological cause. The bioassay was not a useful technique for determining the amount of enhanced biodegradation of Telone C-35®, because of its short persistence in the soil and being unable to distinguish between biological and chemical degradation of the soil fumigant.

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Review

DNA BASED SAMPLING, DETECTION, QUANTIFICATION AND DAMAGE THRESHOLD OF *MELOIDOGYNE* SPP. IN GRAPEVINE

By

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Summary of a PhD thesis

Summary

Studies were conducted to develop soil sampling, detection and quantification techniques for *Meloidogyne* spp. (root-knot nematodes, RKN) in vineyards. A survey was conducted in vineyards of South Australia (SA) to validate a DNA based quantification method for RKN and to determine the population structure of RKN across the grapevine growing areas of SA. The effect of different RKN densities on vine growth during their establishment was also determined.

The distribution pattern of RKN was studied in five vineyards in three locations in SA. Nematodes were extracted from soil samples at two depths (0-300 and 300-600 mm) for each of five positions; three along the vine row, one under cover crops and one adjacent to cover crops in the inter-row. RKN were found to be aggregated along the vine rows. The highest RKN population was found in samples taken close to vines, especially those at about 100 mm from the base of vine, and the second highest was in the row between two vines. No significant difference was found between RKN population levels at the two soil depths. RKN populations under cover crops were significantly lower than in the vine rows. It was concluded that core samples for quantification of RKN population in vineyards should only be collected along the rows. To standardise the collection procedure for RKN, it is recommended that soil samples should be collected about 100 mm from the vine to a depth of 300 mm. The effect of cover crops on RKN populations in vineyards is discussed.

The sampling method developed for RKN was tested for its suitability for ectoparasitic *Xiphinema* spp. and migratory endoparasitic *Pratylenchus* spp., nematodes that also affect grapevines. Using the same methodology, the horizontal, vertical and seasonal distribution of dagger nematodes (*Xiphinema* spp.) and root lesion nematodes (*Pratylenchus* spp.) were monitored monthly for 12 months in a Barossa Valley vineyard of SA. Nematode densities were determined at five different horizontal positions from the vines, including rows and inter-rows, at two depths 0-300 and 300-600 mm. The

dagger nematodes occurred mainly along the rows and at higher density at 300-600 mm. Whereas, root lesion nematodes were at similar densities in rows and inter-rows, but occurred at greater density at 0-300 mm. The population densities of both nematodes were greater in October and November and lowest in February (late summer). Based on these data and other reports, sampling near the vine to a depth of 600 mm in late spring is considered to be the best option for *Xiphinema* and *Pratylenchus* in SA vineyards.

Identification methods based on the North Carolina differential host test and DNA methods were assessed for their ability to distinguish a collection of SA populations of RKN from vineyards. The NC differential host test differentiated *M. incognita* but not *M. arenaria* race 2 from *M. javanica*. A combination of the NC host test and PCR amplification of mtDNA could differentiate between *M. arenaria, M. incognita* and *M. javanica*. A mtDNA based method was successfully used to differentiate *M. arenaria* from *M. incognita* and *M. javanica* by PCR amplification. However, subsequent RFLP analysis of PCR-mtDNA product did not differentiate between *M. incognita* and *M. javanica*. The PCR amplifications of D3 expansion region of 28S rRNA gene and intergenic sequences of ribosomal DNA (IGS-rDNA) were also made to distinguish *M. arenaria, M. incognita* and *M. javanica.* The identification of these species with D3 expansion region of 28S rRNA gene was not possible. The sequences of this region are highly conserved among the species, limiting the possibility of their identification based on this D3 expansion region alone. PCR amplification of IGS-rDNA of genomic DNA from a single female of each species produced distinct banding patterns that can differentiate the species from each other. These species-specific banding patterns were reproducible across a range of individual nematodes of each species collected from different geographical locations of Australia. The method may also be applied to the examination of intraspecific variation of *Meloidogyne*.

A DNA based quantification method was evaluated under controlled condition on species of RKN from grapevines. A clear relationship was found when the DNA assay was applied to soil samples with addition of known numbers of RKN juveniles. A strong relationship was also found between the DNA assay and addition of nematodes for both *M. incognita* and *M. javanica*. The relationship between the DNA assay and number of nematodes added remained robust in both sand and clay soil types. In these experiments, the DNA assay could detect levels as low as 40 juveniles per 400 g soil. The DNA assay appears not only to be adequately sensitive but is consistent for the accurate estimation of both important species (*M. incognita* and *M. javanica*) in both clay and sandy soils, so it is likely that the method could be successfully applied to a range of soils occurring in Australian vineyards.

The sampling and identification methods developed were used to validate the DNA quantification method under vineyards condition and to survey vineyards of SA. A comparative study, based on extraction, bioassay and DNA methods, was performed for the quantification of *Meloidogyne* spp. in vineyards of SA. DNA based species identity and differences in the sequences of internal transcribed spacers (ITS-1 and ITS-2) of rRNA genes in individuals of *Meloidogyne* were also determined. The DNA method was consistently better than commonly used methods for quantification of RKN in various vineyard soils. Four species, *M. javanica, M. incognita, M. arenaria* and *M. hapla,* were recorded in vineyards of SA. The former three species were predominantly found in warmer and *M. hapla* in cooler regions. The DNA sequences in ITS regions of rRNA

genes were highly conserved (<2% divergence) among the individuals of the main species in SA vineyards. Variability in rRNA genes and its relation to the DNA based method for quantification of RKN is discussed.

A microplot experiment was conducted over two years (2000-2002) to determine the effect of *Meloidogyne incognita* population densities on the growth of grapevine during establishment. Four RKN population densities, 25, 154, 960 and 2400 per 1000 ml soil, were assessed on a susceptible cultivar Colombard and a moderately resistant cultivar Sultana. At the first assessment, there was a direct relationship between inoculum density and root gall number in Colombard, but no galls were found in Sultana roots. In the first growing season, RKN did not reduce the growth of either cultivar. However, in the second season, RKN population densities greater than 25 per 1000 ml soil significantly reduced the pruned weight of Colombard but increased pruned weight in Sultana. Therefore, the damage threshold of RKN for grapevines will vary between cultivars. However, for an apparently intolerant cultivar, such as Colombard, the damage threshold for RKN would be about 1 to 25 per 1000 ml soil at establishment. The damage threshold density was found to be 1.5 *M. incognita* per 1000 ml soil by the Seinhorst crop-loss model. This damage threshold for *M. incognita* on grapevines and its implication to the decision making process for the establishment of a vineyard is discussed.