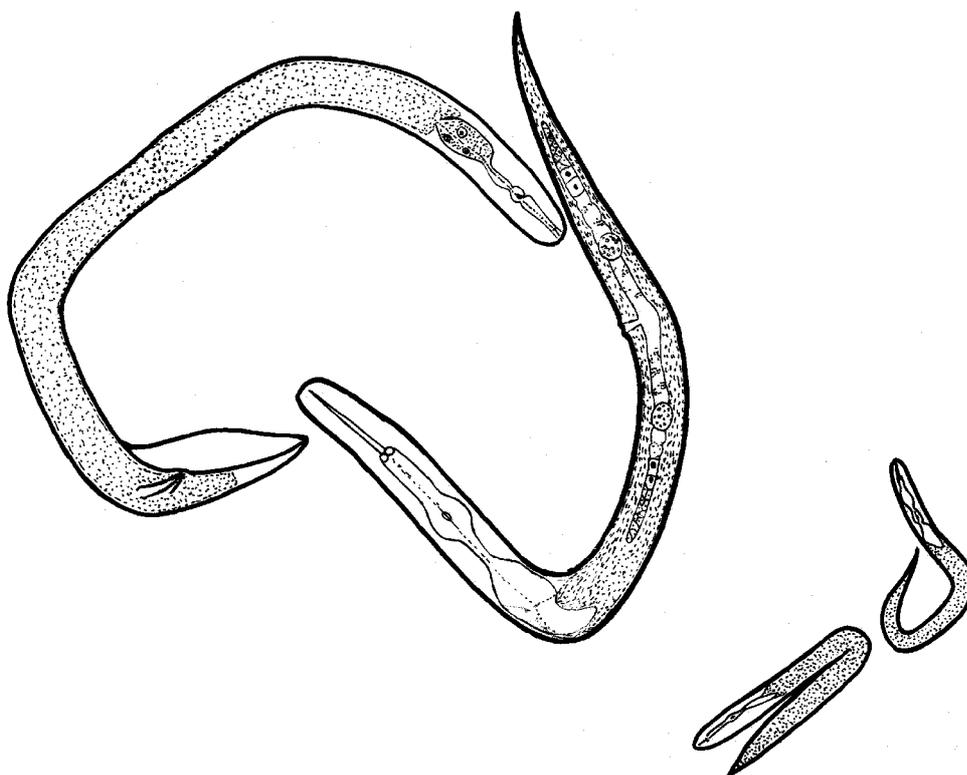


# AUSTRALASIAN NEMATODOLOGY NEWSLETTER



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# From the Editor

Thank you to all who have made contributions to this newsletter. Here in South Australia, it often feels as if nematology is a forgotten discipline, so it is most encouraging to have received so many research reports for this issue. With 5ICN approaching, let's keep it going, and make special efforts for the next few issues of this Newsletter.

## July Issue

The deadline for the July issue will be 30 June. I will notify you a month in advance so please have your material ready once again.

*Kerrie Davies*

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# Association News

## FROM THE PRESIDENT

While considering what to write, I was reminded of a joke. A novice arrives at a very strict monastery (of indeterminate religious affiliation so as not to offend anyone). At this place one is only allowed 2 words every 5 years. After 5 years the novice is allowed their 2 words, and says "more food." After another 5 years, they are allowed another 2 words and say "more blankets." After another 5 years—now 15 years after entering the order—the now-no-longer-novice says "I quit." After this, the head of the order thinks to themselves (rather uncharitably): "what a relief, that newcomer hasn't stopped complaining since arriving."

I sometimes think what I write for the newsletter must start to sound the same every time, because I always seem to be on about the international congress. Then, after reflection, I think that it is actually only every six months that you have to read this. So I hope you don't feel like the head of the order in the joke above.

The congress is only 18 months away, and this means that the issue of the scientific programme is now being discussed. The programme is developed by a committee chaired by the vice-president of the international federation—in this case Aurelio Ciancio from ESN—with representatives of each of the nematology societies that is a member of the international federation, including AAN. Unfortunately, we as the organising society do not get to just organise the scientific programme ourselves, which would be a lot simpler, but we do have a considerable say. So, within the next few months we are looking for suggestions for the following. (Remember that the conference runs from Monday to Friday with Wednesday for field trips).

### **A general format for each day of the conference**

The current suggestion (from the conference organiser we have employed) is to have a plenary session of about an hour at the start of each day to get everyone together and make announcements: this makes organisation easier. Then we have morning tea, then split into separate symposia for 3 sessions from morning tea to lunch, lunch to afternoon tea, and afternoon tea to a close. We can have informal sessions in the evening, and 2 sessions for poster viewing, probably on Tuesday and Thursday afternoons (the posters can be up for the whole time, but they will be in 2 separate rooms). The intention at the moment is to have 3 concurrent sessions with talks of 15 minutes plus 5 minutes discussion. There is an opening reception (if we can arrange sponsorship: Monday), a dinner (Thursday), and several different field trips (Sunshine Coast, Granite Belt, Darling Downs, or Gold Coast: Wednesday). Tuesday and Wednesday evenings are set aside for annual meetings of the various member societies.

### **Topics for specific symposia**

With the above schedule there are 40 symposium sessions (and 200 talks—what a feast of nematology!). An initial list of topics might include the following (taken from previous international meeting).

1. Pine Wilt Nematode
2. Molecular diagnostics
3. Precision agriculture
4. Global comparisons
5. Marine nematodes
6. Parasitism: chemical control
7. Digital Imaging
8. Root Lesion Nematode
9. Entomophilics as biocontrol agents
10. Subsistence agriculture
11. Parasitism
12. Breeding against sedentary phytoparasites
13. Systematics
14. Quarantine
15. Cereal Cyst Nematode
16. Root Knot Nematode
17. Organic agriculture
18. Mutualisms
19. Incompatible interactions
20. Diversity
21. Indicators
22. Morphology
23. Soil ecology
24. Cotton
25. Entomophilics as mutualists/other topics
26. Behaviour
27. Vertebrate parasites
28. Control methods
29. Resistance
30. Food webs
31. Soil amendments
32. Biogeography
33. Molecular taxonomy
34. Biofumigation (There will probably be an international meeting on biofumigation in the week adjacent to our conference. This has been deliberately arranged, so that it gives people in this area a much better reason to come to both conferences in Brisbane.)

There will also be 4 plenary sessions of about an hour each. Perhaps 2 invited speakers at each would need 8 speakers?

We intend also to have a speaker of general interest for the dinner as well.

So far the programme committee of IFNS has expressed a wish to have as broad a range of topics as possible covered by the symposium. Possibilities include things like:

- *Caenorhabditis elegans*—what can it tell us about other nematodes;
- Model systems—*Caenorhabditis* and *Pristionchus*;
- Other crops—coffee, tree crops, vines; and
- Tropical nematology.

Could people please email me (mike.hodda@csiro.au) with any helpful suggestions regarding the above. With the current programme, there are a few spare slots left.

*Mike Hodda*

# Regional News

## NEWS FROM QUEENSLAND

### News from Leslie Research Centre, QDPI&F, Toowoomba

Earlier this year, John Thompson, Tim Clewett and I (Kirsty Owen) completed a GRDC funded project “Cropping options to limit root-lesion nematodes” (DAQ065). Michael Osborne and Ian Dempsey helped keep things running while I was away on maternity leave (baby no. 3, a girl and definitely the last one!). One of the new findings in the project was showing the susceptibility of some grain sorghum and forage sorghum varieties to *Pratylenchus neglectus* in field trials. Four types of forage sorghum were included in the experiment with the Sweet sorghum x Sweet sorghum hybrids being most susceptible and the Grain sorghum x Sudan grass hybrids the most resistant (see table below). Grain sorghum is commonly used in rotation with wheat in the northern grain region. Its resistance to *P. thornei* makes it an ideal crop to include in crop rotations. This once again demonstrates, particularly to our grain producers, the importance of correct identification of *Pratylenchus* to species level.

We have been successful in gaining funding from GRDC for a 3 year project to continue our work on the resistance and tolerance of rotation crops in the northern grains region (DAQ107). We’ll be following up on our sorghum and *P. neglectus* work, as well as including some trials in the field and glasshouse on chickpea, wheat, barley and mungbean. The drought conditions have been somewhat of a hindrance in the first 6 months of the project, but we’ve managed to squeeze in a few field experiments. Toowoomba’s water woes (dams at 18% capacity and restrictions for water use ever increasing) haven’t prevented us from doing glasshouse experiments.

**Table 1. Populations of *Pratylenchus neglectus* after harvest of forage sorghum types.**

| Variety     | Type                          | <i>P. neglectus</i> /kg soil (0-15 cm) <sup>a, b</sup> |
|-------------|-------------------------------|--|
| Hunnigreen  | Sweet sorghum x Sweet sorghum | 9276 a   |
| Nectar      | Sweet sorghum x Sudan grass   | 8155 a   |
| Sugargraze  | Sweet sorghum x Sweet sorghum | 6119 b   |
| Lush        | Grain sorghum x Sudan grass   | 4972 bc  |
| Superdan    | Sudan grass x Sudan grass     | 4906 bc  |
| Sweet Jumbo | Grain sorghum x Sudan grass   | 4601 c   |
| Cowpow      | Grain sorghum x Sudan grass   | 4197 cd  |
| Everlush    | Grain sorghum x Sudan grass   | 2937 de  |
| BMR         | Grain sorghum x Sudan grass   | 2889 de  |
| Bettagraze  | Grain sorghum x Sudan grass   | 2620 de  |

<sup>a</sup>Preplant *P. neglectus* populations were 667/kg soil at 0-15 cm.

<sup>b</sup>Backtransformed data presented. Means with the same subscript are not significantly different at  $P = 0.05$

I have accepted the role of nematology editor for Australasian Plant Pathology in 2007. While I have some big boots to fill with Ian Riley's departure from the job, I'm looking forward to the challenge. Can I take this opportunity to encourage nematologists out there to publish in APP, especially as we approach 51CN?. It will be a great way to show the world the quality of our research and raise the profile of APP.

*Kirsty Owen*

## NEWS FROM SOUTH AUSTRALIA

### News from The University of Adelaide

Kerrie Davies trekked across to Perth in November, where she and Mike Hodda presented another short course on plant and soil nematodes at Murdoch University. From Kerrie's point of view, it went really well, with an excellent and enthusiastic group from a wide range of backgrounds. We were delighted with the help and support from Vivien Vanstone and her cohorts at DAFWA, and from Mike Jones's group at Murdoch. In addition, it was a great opportunity to catch up with nematological friends and colleagues.

Kerrie continues to search for *Fergusobia* galls – few and far between at present, probably due to the drought. She is working to finish a revision of the genus, which supports the recently-submitted phylogeny based on molecular analyses (work largely carried out by her collaborators Weimin Ye and Robin Giblin-Davis in the USA). Kerrie is also describing new species of *Schistonchus* (aphelenchids associated with pollinating agaonid wasps from *Ficus*) and the first *Parasitodiplogaster* recorded from Australia. She made another collecting trip to Cairns last August – always a pleasure in the middle of winter!

Congratulations to Zeng Qi Zhao, whose PhD thesis on aphelenchid nematodes associated with conifers has been accepted. The abstract of his thesis is included in this Newsletter. Zeng Qi has since had a stint retailing, moved to Sydney and made a trip home to China. He is currently writing papers from his thesis, and job hunting. We wish him well for his future.

*Kerrie Davies*

### News from SARDI

Sharyn Taylor has resigned as leader of the SARDI field crop nematology group and has accepted a position with Plant Health Australia in Canberra. Sharyn will be missed around the Waite, but we all wish her well in this new phase of her career.

The SARDI Diagnostic group, which includes Ian Riley on a pasture soil biology project, has been busy developing a range of quantitative real-time PCR test for fungi, oomycetes, nematodes and plants in soil. This includes six new tests for nematodes, viz. *Pratylenchus penetrans*, *Heterodera trifolii*, *Meloidogyne javanica/incognita/arenaria*, *M. hapla* and *M. fallax*, complementing the existing tests for *H. avenae*, *P. neglectus*, and *P. thornei*. Field validation and research applications will be the focus of for the work in 2007.

Greg Walker has been working on a collaborative project with Trevor Wicks and colleagues on an onion project, examining the cause of 'Mallee onion stunt syndrome'. One case involved *Paratrichodorus* sp. as the probable main factor, and high densities of *Pratylenchus*

spp. have been found at some farms. However, other factors, particularly fungal pathogens including *Rhizoctonia solani*, *Pyrenochaeta terrestris*, *Pythium* spp. and *Fusarium* spp. were implicated at other farms. It seems that multiple causes are involved in this syndrome (or group of diseases). Greg has been testing soil and root samples as part of a survey of Mallee farms, and has set up pot experiments examining the effects of cover crops, organic matter amendments and nematicides on disease caused by fungal pathogens and nematodes in naturally-infested soils. He has also been establishing *Meloidogyne* populations for development of DNA-based identification/quantification systems, and has promoted plant nematology to the public with an interactive display at the recent Waite Festival, and a radio interview on 891 Adelaide with Jon Lamb. He hopes that a few of the children who looked with wonder at nematodes under the microscope may be stimulated to become nematologists, and keep AAN afloat. Other current research activities include evaluation of organic amendments in tomatoes.

*Ian Riley & Greg Walker*

## **NEWS FROM WESTERN AUSTRALIA**

### **News from Department of Agriculture & Food Western Australia (DAFWA)**

#### ***PCN “area freedom” project***

Sarah Collins and Dyane Jardine continue sampling WA potato growing areas and extracting organic matter with the aid of our giant Fenwick can “*Big Bertha*”, who is able to deal with 5kg batches of soil.

One third of the planned sampling is completed, consisting of 29,600 50-g soil cores from 74 ha, resulting in 1.5 t of soil to be processed. All potato fields included in the survey are being sampled on a 5 m x 5 m grid, collecting 400 sub-samples (approx. 20 kg) per hectare. Total organic matter is extracted from the entire bulk sample.

Dyane completed organic matter extraction from the first 1.5 t of soil in early December. Sarah and Dyane will commence sampling additional potato-growing areas in late December/January.

PCR detection methods are being refined with the aid of PCN DNA from NZ and WA CCN DNA, and CCN cysts are being used to test extraction protocols from soil organic matter prior to testing the actual potato field samples for presence of PCN.

Dr John Marshall spent 2 weeks at DAFWA in Nov/Dec 2006 working with Sarah to perfect the art of extracting DNA from soil organic matter extracts. John has recently retired from New Zealand Crop & Food Research, but maintains his collaboration with the WA PCN project.

Sarah plans to travel to New Zealand again in 2007 to be able to practice with the aid of actual PCN, which we are not able to work with in WA due to the strict quarantine against this nematode.

### ***Nematode course in Perth***

The short course “*Nematodes in cropping systems - identification and techniques*” was conducted by Drs Mike Hodda (CSIRO Canberra) and Kerrie Davies (University of Adelaide) in Perth, 20-24 November 2006. Professor Mike Jones and Dr Modika Perera facilitated the conduct of the course at Murdoch University, in association with the State Agricultural Biotechnology Centre WA and DAFWA Nematology.

Mike and Kerrie were understandably nervous about teaching the course “away from home”, but it was a huge success, and they did a fantastic job as always.

Nematodes are obviously still a subject of interest (or confusion?) to many, and there were 13 participants from diverse areas.



***L-R:*** Motiul Quader (DPI Vic), Helen Hunter (DAFWA Nematology), Oliver Knox (Cotton CRC Narrabri NSW), Jurgen Otto (AQIS Sydney), Alex Chandra (Singapore), Sandy Mack (DAFWA Diagnostics), Sarah Collins (DAFWA Nematology), Ali Bhatti (DAFWA Nematology), Kerrie Davies, Andrew Wherret (DAFWA/UWA), Lila Nambiar (DPI Vic), Aaron Maxwell (AQIS Perth), Shuie Liu (Murdoch Uni), Ming Pei You (DAFWA Nematology).



Certificates of completion were presented to each participant by Professor Jones.

### ***Pastures and weeds***

In July 2006, additional funding was gained from GRDC to enable investigation of pasture species and cultivars to determine their role in hosting Root Lesion Nematodes in cereal cropping rotations.

Drs Ali Bhatti and Ming Pei You joined DAFWA Nematology to fulfil this task. Ming Pei (DAFWA Pasture Pathologist) brings to us her considerable expertise in the growth of all pasture species.

Ali and Ming Pei are currently screening cultivars of sub, arrow leaf, crimson, purple, gland, balansa, Persian, bladder, eastern star and rose clover, yellow and French serradella, burr and barrel medic, lucerne, biserrula and sulla.

Ali is also screening common WA grass and broad-leaf weeds of crops. Although this has already been done in SA<sup>#</sup>, we considered it important to further screen WA weeds against WA nematodes.

<sup>#</sup> Vanstone, VA and MH Russ 2001. Ability of weeds to host root lesion nematodes (*Pratylenchus neglectus* and *P. thornei*). Part 1: Grass weeds. *Australasian Plant Pathology* 30(3):245-250.

Vanstone, VA and MH Russ 2001. Ability of weeds to host root lesion nematodes (*Pratylenchus neglectus* and *P. thornei*). Part 2: Broad-leaf weeds. *Australasian Plant Pathology* 30(3):251-258.

### ***New projects***

A HAL funded workshop was convened by Dr Frank Hay at the University of Tasmania in July to discuss research, development and extension priorities for nematode control in vegetable crops. HAL subsequently issued three priority areas for nematode research:

- Workshop to develop more sustainable farming systems for the vegetable industry
- Sustainable farming systems for the Australian vegetable industry
- Managing Root Knot Nematode (RKN) in carrots.

Frank has led the submission of projects with involvement from nematologists Australia-wide.

RKN is a constraint to carrot yield and quality in all States, and there is a need to reduce reliance on fumigants and nematicides. If successful, DAFWA Nematology will be involved with screening a range of agronomically suitable potential break-crops for management of RKN species which will be applicable to all vegetable industries. Glasshouse screening will be conducted in WA, SA and Qld. Complementary field trials will follow in WA, SA, Qld, Vic and Tas.

In response to the draft Vegetable Industry Biosecurity Plan prepared by Plant Health Australia and the National Vegetable Industry, Dr Satendra Kumar (DAFWA Quarantine Plant Pathologist) has submitted a proposal to HAL to develop a Pest Specific Incursion Management Plan (PSIMP) and Pest Risk Analysis (PRA) for Carrot Cyst Nematode. Project outcomes will provide the national industry with a clear outline of protocols to follow should this Emergency Plant Pest enter Australia.

The PRA will provide information on ways to minimise the chances of incursion through consolidation of phytosanitary measures to minimise the probability of entry, establishment and spread. The PSIMP will give clear guidelines on the early detection and incident

response should an incursion occur, outlining detection, containment and eradication measures. A pest specific contingency plan and diagnostic protocols will be developed.

We are currently in negotiation with GRDC for submission of a funding proposal in Feb 2007 to continue broadacre Nematology work beyond July. The new proposal will focus on intensive assessment of hosting abilities of crops to develop nematode species-specific rotational recommendations for management.

We hope to also be able to define host commonalities for nematode species to aid in management of mixed or unidentified populations. This should also aid in circumventing “population shifts” in response to rotations. This was demonstrated on one property in 2006, where a wheat/narrow-leafed lupin/field pea rotation was implemented to manage *Pratylenchus neglectus*. Narrow-leafed lupin and field pea are resistant to *P. neglectus*, but not to *P. penetrans*. Eight years later, *P. penetrans* had reached enormous populations (up to 900,000 nematodes/g root) in the field pea crop, causing extensive damage and yield loss.

Plants will be inoculated with Root Lesion (*P. neglectus*, *P. teres*, *P. penetrans* and *P. thornei*) and Burrowing Nematode (*Radopholus nativus* and an as-yet-unidentified *Radopholus* species) in the glasshouse, followed by field validation. *P. teres* and *Radopholus* are being generated in pot culture, accompanied by further attempts to establish these species in carrot culture. *Pratylenchus neglectus*, *P. penetrans* and *P. thornei* have been established and maintained in carrot culture.

### ***Meloidogyne fallax***

*Meloidogyne fallax* was identified for the first time in WA in 2006 when a potato grower suffered virtually 100% (around \$800,000 in this case) crop loss due to this nematode. Tubers were excessively “warted” and not suitable for processing due to extensive internal “flecking”. These observations rang alarm bells, so samples were sent to Dr Jackie Nobbs (Nematode Taxonomist SARDI Adelaide) who confirmed the identity of *M. fallax*.

The field had not previously been used for potato cultivation, and it is thought that the nematodes were present on clover, grasses and weeds previously growing on the area.

Further attempts to investigate the host range and distribution of this nematode in WA have fallen on deaf ears. Potato growers regularly apply nematicide for control of RKN, so it is not currently seen as an issue. However, we plan to include *M. fallax* in the host studies for the HAL RKN carrot project, and a captive population is being maintained in the glasshouse on potato, carrot and tomato plants.

*Vivien Vanstone*

### **News from the WA State Agricultural Biotechnology Center (SABC), Murdoch University**

Dr Zhaohui Wang travelled to Cairns in August, attending the Tropical Crop Biotechnology Conference (TCBC 2006). He met Prof. Steve Briggs (UC San Diego) who’s working on proteome profiling of rice and *Arabidopsis* infected with root-knot nematode *M. incognita*. Zhaohui then travelled to Adelaide for the 8<sup>th</sup> International Congress of Plant Molecular Biology (ISPMB 2006), together with Prof. Mike Jones. In November, he had a meeting with the ARC linkage project collaborators, Prof. James Dale and Dr Ben Dugdale (QUT), in Perth when they came over for the 7<sup>th</sup> Australasian Plant Virology Workshop. They discussed the current ARC linkage project, and confirmed their continuing input for the next phase of

the ARC linkage project to generate transgenic tobacco plant resistant against root-knot nematodes.

Dr John Fosu-Nyarko continues work on an ARC Discovery Project aimed at studying transcriptional events and changes in protein expression that occur in developing giant cells at early stages of infection. This involves constructing and characterising a giant cell-specific cDNA library using Laser Microdissection and Catapulting (LMC) to specifically obtain cytoplasmic contents from giant cells induced by *Meloidogyne javanica* on tomato roots 4-10 days after infection. In the last quarter of 2006 he focussed on modifications to the fixation and embedding protocols to obtain high quality total RNA from which cDNA clones have been developed from amplified mRNA. These are currently being characterised for compilation into a library of all genes expressed in giant cells induced in tomato roots. From the libraries, novel genes expressed at the early stages of infection will also be identified.

Shuie Liu joined the plant nematology group as a PhD student co-supervised by Mike, Zhaohui and A. Prof. Bernard Dell (Murdoch University). Her research is part of the current ARC linkage project which involves generating transgenic tobacco with modified double transformation strategy, and testing different plant promoters in response to root-knot nematode infection. She is also verifying the reporter gene expression pattern in the transgenic plants with Zhaohui. In addition, Shuie Liu attended the fifth biennial “*Nematodes in cropping systems, identification and techniques*” course at Murdoch University in November.

Wan-Hon Kon finished her ISC project in the past semester (from July to November). She analysed the transgenic tobacco and *Arabidopsis* lines produced by a former Honours student Angelina Ho. Those transgenic plants contain a GUS reporter gene driven by two different *Arabidopsis* transcription factor gene promoters. Some transgenic tobacco lines have been identified with GUS expression restricted in giant cells but not in any other part of the gall tissue. Wan-Hon and Shuie also tried to clone some truncated versions of the promoter showing activities in giant cells, to further confirm the specificity of its expression.

From September to December, 2006, we were visited by a PhD student, Juan Emilio Palomares Rius of the Department of Crop Protection, Institute of Sustainable Agriculture, C.S.I.C, Cordoba, Spain. He worked closely with Dr Fosu-Nyarko on a joint project aimed at studying the mechanism of resistance of chickpea to *Fusarium oxysporium* F sp cicer in the presence of *Meloidogyne arteliellia* using the LMC technology and proteomics approach. With modifications to the fixation and embedding protocols used for tomato tissues, high quality total RNA was obtained from giant cells induced in chickpea roots 20-30 days after infection with J2 juveniles of *M. arteliellia*. Work is underway to study the gene expression levels of some defence-related genes using amplified RNA obtained from the cytoplasmic contents of the giant cells. The results will provide insight into the genetic basis for the loss and maintenance of resistance of two cultivars of chickpea to *Fusarium oxysporium* F sp cicer in the presence of *M. arteliellia* infection.

Another visitor, Dr Reddy Kankanala from India, spent three months with the plant nematology group from September to November. He has tissue culture background, and worked with Zhaohui and Shuie to generate some of the double transformation tobacco lines. Dr Kankanala also participated in PCR analysis of the transgenic plants.

*Zhaohui Wang*

## NEWS FROM NEW ZEALAND

Two of New Zealand's long-serving nematologists have retired. **John Marshall** retired in June 2006, having begun work in the potato cyst nematode era. He completed his PhD in 1984. In 1987 John published a pioneering paper on the use of molecular tools for distinguishing populations of PCN and more recently was applying molecular tools to other plant pathogens. His skills will be sorely missed in New Zealand and overseas.

**Richard Watson** retired in November. An entomologist by training, he had been active in nematology since the RH25 series of trials on the effects of nematodes on white clover was commenced in the Waikato in 1982. He had continual involvement in the programme aimed at incorporating nematode resistance in new lines of white clover.

**Karen Knight** will be known to members for her detailed work on host records, especially her very valuable 1997 review "Plant-parasitic nematodes of New Zealand recorded by host association" in *Journal of Nematology*. In this post-modern age her position was dis-established in mid 2006. We wish her well with a new life outside science.

**Jim Starr**, from Texas A&M University, has been working with Chris Mercer since August 2006. His sabbatical work concerns mainly isozyme and PCR-based identification of root-knot nematodes in pasture.

In October, Chris, Jim and Gregor Yeates made contributions to the NZ Society for Parasitology conference, held this year at Massey University.

*Gregor Yeates & Chris Mercer*

# Research

## **THE SUGAR YIELD DECLINE JOINT VENTURE: A GOOD EXAMPLE OF COLLABORATIVE R & D**

**Graham Stirling**

Biological Crop Protection Pty Ltd, 3601 Moggill Road, Moggill Qld 4070

The Sugar Yield Decline Joint Venture (SYDJV) was established by the Sugar Research and Development Corporation (SRDC) in 1993 to address the issue of a productivity plateau in the Australian sugar industry. Sugarcane yields increased by an average of 0.7 t/ha/year between 1900 and 1970 but did not change in the next 20 years.

Yield decline (or the loss in productive capacity of sugarcane growing soils under long-term monoculture) was thought to be one of the main reasons that yields were no longer increasing and the role of the SYDJV was to find out what was causing the problem and develop solutions. The original term of the joint venture was six years, but following a favourable review in 1996, it was extended for a second six year phase and concluded in June 2005.

I joined the SYDJV team in 1995 and it proved to be the most challenging, motivating and rewarding assignment of my career. The most enjoyable part was working in a multi-disciplinary team that contained two agronomists, a soil physicist, a soil chemist, an engineer, a plant pathologist, a soil biologist and an extension specialist. We came from different research agencies and did not always agree, particularly in the early stages, but in the process of setting objectives and planning experiments we learnt a lot from each other. By the time the second phase commenced, we had an enthusiastic group who were committed to working together to achieve outcomes for industry.

The main achievement of the SYDJV was the development of a completely new way of growing sugarcane. In the past, sugarcane was grown on beds 1.5 m apart, which meant that much of the field was compacted by harvest machinery (which weighed 20-30 tonnes and had wheel spacings of 1.8 m). Soil carbon levels were not as high as they could have been because crop residues were often burnt rather than retained. After a plant and 2-4 ratoon crops, fields were ripped and cultivated to alleviate compaction and sugarcane was replanted into soil that was laden with plant-parasitic nematodes and other soil-borne pathogens. Soils were clearly degraded from a physical and chemical perspective, but 20-40% yield increases following fumigation indicated that biological constraints were also a major factor. An important finding of the SYDJV was that responses similar to those obtained with fumigation could be achieved by breaking the sugarcane monoculture with a legume crop.

The farming system devised by the SYDJV separated the traffic and cropping zones by establishing permanent beds with the same row spacing as the wheels of the harvest machinery. A new planter was designed to plant sugarcane into these beds using minimum tillage techniques. Crop residues were retained as mulch on the soil surface after each harvest and soybeans were direct drilled through the trash blanket at the end of the cane cycle. Because the legume fallow increased yields and supplied all the nitrogen needed for the plant crop, minimum tillage reduced fuel and labour costs and soybeans provided an additional source of income, the new farming system has been received enthusiastically by

growers and is being adopted throughout the industry. By 2004, an estimated 21,000 ha of sugarcane land was being farmed using at least one component of the new farming system.

From a nematological perspective, the new farming system has many advantages over the previous system. Populations of *Pratylenchus zae* and other plant-parasitic nematodes are substantially reduced by the soybean fallow, improvements in the soil physical and chemical status increase the number of nematodes required to cause economic damage, while the reduction in tillage and increased retention of organic matter enhances the biological suppressiveness of soil to nematodes.

One feature of the SYDJV was the commitment of the project team to ensure that any changes to the sugar farming system were underpinned by good science. More than 100 research papers were published during the life of the joint venture, or an average of more than eight publications for each year of the project. For those interested in the detail some of the more important references in the areas of nematology and soil biology are listed below.

Although the SYDJV finished in June 2005, most of the project team are still working together because SRDC funded a three-year follow-on project that is allowing us to fine-tune our new farming system. One of its main objectives is to better match nutrient availability from crop residues (both sugarcane and soybean) with the nutrient requirements of the sugarcane crop. Initial results suggest that monitoring free-living nematodes (particularly the rhabditids) will help us understand the dynamics of nutrient immobilisation and mineralisation because nematode populations increase markedly when nitrogen is being mineralised within the trash layer or soil.

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# RESISTANCE TESTING OF WHEAT BY SIMULTANEOUS INOCULATION WITH TWO ROOT-LESION NEMATODE SPECIES

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## Introduction

Root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) are widely distributed in the Australian wheat belt and cause substantial grain yield losses. *P. thornei* is particularly a problem in southern Queensland and northern New South Wales, while *P. neglectus*, is also found in the northern grain region, but is most common in southern and western grain regions (Taylor *et al.*, 2001).

Wheat varieties usually differ in their tolerance and resistance to *P. thornei* and *P. neglectus*. At the Leslie Research Centre, Queensland Department of Primary Industries & Fisheries (QDPI&F), assessment of resistance of wheat varieties to *P. thornei* and *P. neglectus* is currently carried out in separate glasshouse experiments using inoculation with one nematode species. We tested whether this assessment could be done using inoculation of single plants with both species of nematode simultaneously. Such a method would have obvious advantage in selecting single plants in segregating breeding populations that carry effective resistances to both nematode species.

## Materials and Methods

Twenty-five wheat lines that differed in resistance/susceptibility to *P. thornei* and *P. neglectus* individually were subjected to four inoculation treatments (i) *P. thornei* alone (10,000/kg soil) (ii) *P. neglectus* alone (10,000/kg soil) (iii) *P. thornei* and *P. neglectus* (each at 5,000/kg soil) and (iv) *P. thornei* and *P. neglectus* (each at 10,000/kg soil). The plants were grown in the glasshouse in native tube pots and on a bottom watering, capillary matting system. There were three replicates per treatment. After 16 weeks, nematodes were extracted from the roots and soil by the Whitehead tray method. Nematode populations were assessed under the microscope and also by species specific DNA analysis (Root Disease Testing Service, SARDI).

## Results and Discussion

Good relationships were obtained between the counts for the nematode species individually and their counts in mixed cultures (see Table 1). Discrimination between varieties was maintained when plants were inoculated with the lower rate of nematodes (5000/kg soil of each species). Counting mixed nematode samples under the microscope did require greater patience and discipline compared to counting single nematodes. There were also excellent correlations between the nematode counts under the microscope and the nematode counts from the DNA method (for *P. thornei* microscope counts in all four inoculation regimes and *P. thornei* DNA counts  $R^2 = 0.8084^{***}$ ;  $n=300$ ; and for *P. neglectus* microscope counts in all four inoculation regimes and *P. neglectus* DNA counts  $R^2 = 0.7684^{***}$ ,  $n=300$ ).

Inoculation with both nematode species of wheat varieties did not alter the expected susceptibility/resistance response of varieties tested against each nematode species individually. In the field, where *P. thornei* and *P. neglectus* are both present, *P. thornei* is considered to dominate due to its higher multiplication rate (Taylor *et al.*, 2001).

Discrimination of resistance and the order of ranking of wheat varieties was maintained using dual species inoculation in one glasshouse experiment.

The project has been very successful in the development of a resistance test for two species of nematodes in wheat and offers significant savings in glasshouse space and materials. Further research, using this mixed inoculation method for phenotyping single wheat plants was presented at APPS conference in 2005 (Huang *et al.*, 2005) and experiments with sorghum and mungbean varieties are currently underway in the GRDC funded project DAQ107.

**Table 1. Correlation coefficients between nematode counts for a range of wheat varieties tested for resistance to *Pratylenchus thornei* and *P. neglectus* singly or in mixed inoculum. Results based on log (x+c) transformed nematode counts.**

| Inoculation                               | Mixed <i>P. thornei</i> & <i>P. neglectus</i> inoculum<br>(5000 of each/kg soil) | Mixed <i>P. thornei</i> & <i>P. neglectus</i> inoculum<br>(10000 of each/kg soil) |
|---|--|---|
| <i>P. thornei</i> alone (10000/kg soil)   | 0.8414***  | 0.8406***   |
| <i>P. neglectus</i> alone (10000/kg soil) | 0.7059***  | 0.5585***   |

\*\*\*= statistically significant at  $P < 0.001$ , n=25

### Acknowledgement

We thank Dr Alan McKay for DNA results provided through the Root Disease Testing Service (RDTS), SARDI.

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## THE SCOT, THE COTTON INDUSTRY AND THE NEMATODES

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When Kerrie asked me to put down a few words about my nematode work I sat for a long while just trying to get my head around what to write. The main reason for this is that I do not consider myself a nematologist; but nematodes fascinate me within the soil biological system.

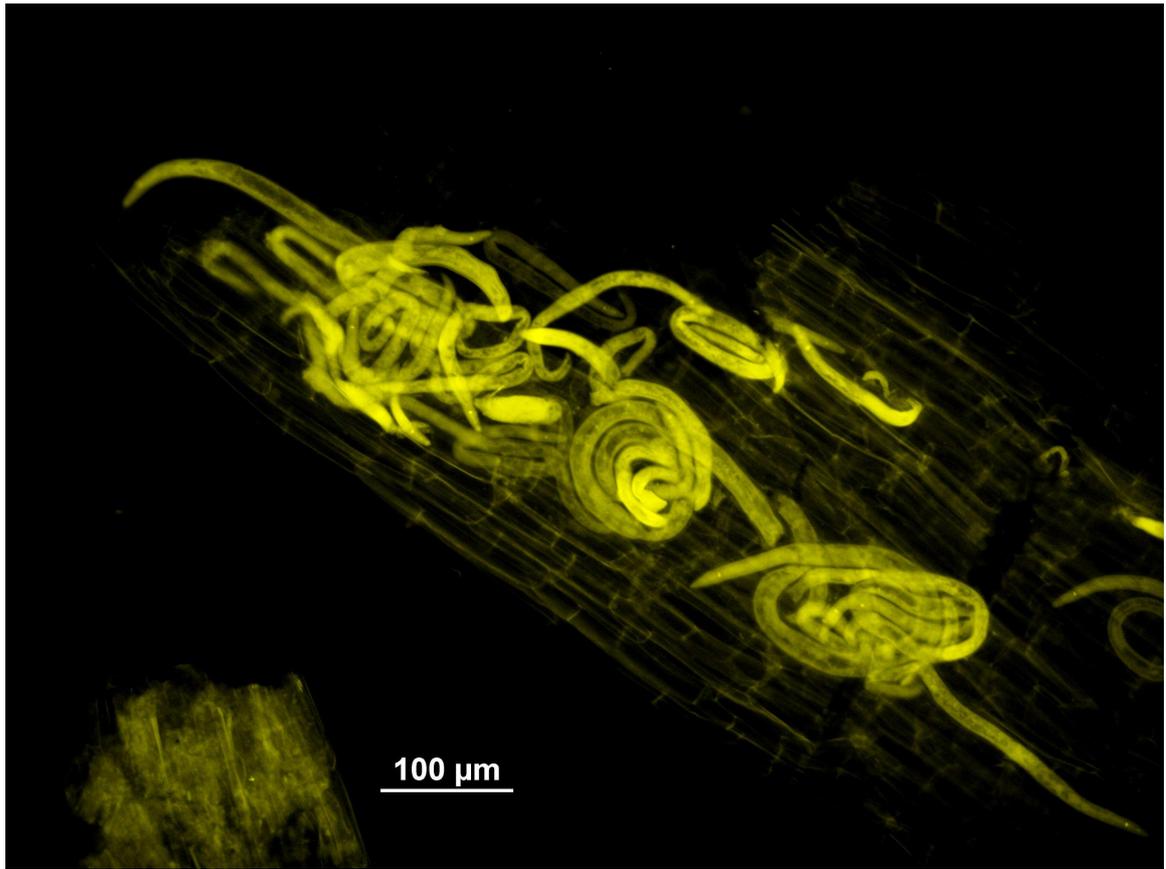
This is reflected in the work I have done involving nematodes over the last six years.

My initial interest was sparked by Dr Mike Wilson (Uni. of Aberdeen) who introduced me to bacterial feeders. We carried out a series of controlled experiments that clearly indicated a beneficial affect on rhizosphere colonisation by seed applied bacteria in the presence of these nematodes in soil. This work resulted in my first large scale exposure to nematologists when I presented at the Linnean Society's offered papers on nematology in December of 2002. I was the only person not talking about entomophilic or plant parasitic nematodes! Undeterred, and encouraged by all the entomophilic work, we continued our studies, but using non-feeding entomophilics as one of the controls. This work has recently wound down, but by then I was in Australia.

In 2003 I relocated from Aberdeen to Australia, and from microcosm grown wheat to irrigated cotton fields. After a year finding my feet, one of the station pathologists approached me about looking at nematodes as a potential contributor to incidence of *Verticillium* wilt of cotton. Now here was a challenge, because before coming to Australia I'd never experienced anything like the self mulching grey vertisols common to many cotton producing areas and I could find very little published material on nematodes in Australian cotton. What else could I do, but accept.

Over the last couple of seasons a group of us at the ACRI have conducted nematode recoveries from soil and cotton roots in fields where *Verticillium* is known to occur. I have also, in collaboration with my supervisor Dr Gupta Vadakattu (CSIRO Entomology, Adelaide), looked at nematodes under GM and non-GM cotton. This has broadened further my knowledge of nematology and has resulted in observations of *Helicotylenchus dihystera*, bacterial and hyphal feeding nematodes within cotton roots, and the presence of *Tylenchorhynchus ewingi* in soil supporting cotton. We have seen no discernible differences between GM and non-GM crops, and noted that nematode numbers are generally low (less than 1 per g soil) in the vertisols, but are prone to rapid expansion (25 per g of soil) following defoliation of the cotton.

These experiments have, as so many do, further highlighted the limited knowledge that we have of the soil biota. I hope to continue to include nematodes in my future studies of cotton and its vertisols by exploring their biology, contribution to nutrient cycling, and developing pot assays to assess potential pathogens.



*Helicotylenchus dihystra* in a cotton root sampled from the Breeza plains.

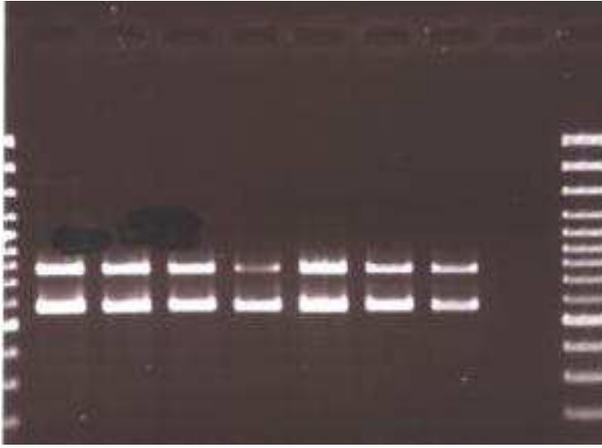
**MELOIDOGYNE FALLAX DOESN'T CUT IT: PCR-RFLP OF MTDNA AS A DIAGNOSTIC TEST FOR ROOT-KNOT NEMATODES (*MELOIDOGYNE* SPP.)**

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A multiplexed PCR based diagnostic test that simultaneously amplifies two small regions of the mitochondrial genome, is used as the target for restriction haplotyping with restriction enzymes *Hinf* I (h) and *Mnl* I (m) to identify species of *Meloidogyne*. The resulting DNA banding patterns, separated on an agarose gel, are interpreted depending on the length/position of the DNA bands per restriction enzyme digest, to determine *Meloidogyne* haplotypes.

The diagnostic test identifies haplotypes that correspond to *M. arenaria*, *M. incognita*, *M. javanica*, *M. hispanica*, *M. hapla* and *M. chitwoodi*.

| DNA amplification   |   |   |   |   |   |   |     |   |   |  |
|---|---|---|---|---|---|---|-----|---|---|--|
| A   | B | C | D | E | F | G | -ve | L | <p>DNA was amplified from unknown samples A, B, C, D, E, F, G and a negative control (no template control) using the multiplexed PCR.</p> <p>To visualise the DNA bands, produced by the PCR amplification or the digest with enzymes, products were always separated on a 2% agarose gel, stained with ethidium bromide and viewed under UV light.</p> <p>Two DNA bands can be viewed for all samples.</p> |  |
|  |   |   |   |   |   |   |     |   |   |  |

| Digest of product with restriction enzymes |   |   |   |   |   |   |   |  |
|--|---|---|---|---|---|---|---|--|
| L  | A | A | B | B | G | G | L | The DNA product from samples was digested or cut with the restriction enzymes <i>Hinf</i> I (h) and <i>Mnl</i> I (m).  |
|  | h | m | h | m | h | m |   |  |
|  |   |   |   |   |   |   |   | <p>The banding patterns, produced by <i>Hinf</i> I (h) and <i>Mnl</i> I (m), identify Sample A and B as <i>M. incognita</i>.</p> <p>Sample G, with different banding patterns produced by <i>Hinf</i> I (h) and <i>Mnl</i> I (m) identifies as <i>M. javanica</i>.</p> |

After recently receiving a *M. fallax* sample from Dr Ian Riley, DNA was amplified using the multiplexed PCR based diagnostic.

| DNA amplification |   |   |   |   |   |   |     |   |   |
|-------------------|---|---|---|---|---|---|-----|---|---|
| L                 | 1 | 2 | 3 | 4 | 5 | 6 | -ve | L | DNA was amplified from samples 1, 2, 3, 4, 5, 6 and a negative control (no template control) using the multiplexed PCR.   |
|                   |   |   |   |   |   |   |     |   |   |
|                   |   |   |   |   |   |   |     |   | <p>To visualise the DNA, the PCR product was run on a 2% agarose gel, stained with ethidium bromide and viewed under UV light.</p> <p>Sample 1 (<i>M. fallax</i>) is the only sample that produced a single DNA band.</p> |

| Digest of product with restriction enzymes |   |   |   |   |   |   |   |   |   |   |   |
|--|---|---|---|---|---|---|---|---|---|---|---|
| L  | 1 | 1 | 2 | 2 | 3 | 3 | 4 | 4 | 5 | 5 | L |
|  | h | m | h | m | h | m | h | m | h | m |   |

*M. fallax* (Sample 1) did not cut with either enzyme.

Samples 2, 4 and 5 have banding patterns, produced by digesting with *Hinf* I (h) and *Mnl* I (m), that correspond to *M. javanica*.

Sample 3 has banding patterns, produced by digesting with *Hinf* I (h) and *Mnl* I (m), that correspond to *M. arenaria* A.

The *M. fallax* was not digested by the restriction enzymes and therefore cannot be misidentified as *M. arenaria*, *M. incognita*, *M. javanica*, *M. hispanica*, *M. hapla* or *M. chitwoodi* with this diagnostic procedure.

Conversely, with the above procedure, *M. arenaria*, *M. incognita*, *M. javanica*, *M. hispanica*, *M. hapla* and *M. chitwoodi* cannot be misidentified as *M. fallax*.

We need to further investigate these results by sequencing the mtDNA of *M. fallax* to establish its DNA sequence and subsequently identify restriction enzymes to use to produce unique fragment banding patterns to distinguish it from other species.

# Workshop Report

## WORKSHOP TO DEVELOP RESEARCH, DEVELOPMENT AND EXTENSION PRIORITIES FOR NEMATODE CONTROL IN VEGETABLE CROPS

Frank Hay

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Dr Frank Hay of the Tasmanian Institute of Agricultural Research (TIAR), University of Tasmania, co-ordinated a workshop as part of Horticulture Australia Ltd. project VG05026 'Workshop to develop research, development and extension priorities for nematode control in vegetable crops'.

The workshop was held on 10-11 July 2006 at TIAR, University of Tasmania, Cradle Coast Campus Burnie and involved 21 participants including nematologists, agronomists and industry personnel. Participants with nematological interests included, Graham Stirling, John Marshall, Greg Walker, Kathy Ophel Keller, Vivien Vanstone, Jackie Nobbs, Tony Pattison, Lila Nambiar, Motiul Quader, Hoong Pung, and Dale Griffin.

The purpose of the workshop was to discuss and formulate future research, development and extension priorities in vegetable nematology. While providing a great opportunity for old friends and colleagues to catch up, the workshop also resulted 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.

A final report on the workshop was submitted to Horticulture Australia Ltd. in October 2006. Frank Hay presented recommendations to members of Horticulture Australia Ltd. and the Vegetable Industry Advisory Committees in a meeting in Sydney, and it was gratifying to see the recommendations form the basis for a number of priorities in the recent Horticulture Australia funding round. All the workshop participants are to be congratulated for their input into the workshop. However, Frank would like to particularly thank Graham Stirling for his assistance in formulating the recommendations.

# Thesis Abstract

## OCCURRENCE, TAXONOMY, BIOLOGY AND PATHOGENICITY OF APHELENCHID NEMATODES ASSOCIATED WITH CONIFERS IN SOUTH-EASTERN AUSTRALIA

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Summary of thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy, The University of Adelaide, 2006

Australia has large plantations of exotic *Pinus radiata* conifers. This species is highly susceptible to *Bursaphelenchus xylophilus*, the pine wilt nematode, which is not found in Australia. Potentially pathogenic nematodes were isolated from several dead *Pinus* trees in Williamstown, Heidelberg and Knoxfield, suburbs of Melbourne, Victoria in 2000-2002. A survey of the above-ground nematode fauna of *Pinus* and other conifers in south-eastern Australia was undertaken. Stands of *Pinus* were surveyed in the Kuinto Forest and the South-East Region of SA; the south-west and the Gippsland region of Victoria; and the Hume region in NSW; and native *Callitris preissii* was sampled in the Murray Mallee. A total of 1140 samples from *P. radiata*, 50 from *P. pinaster* and 40 from *C. preissii* were examined. No nematodes were found in wood or young shoots of conifers except in the wood samples from diseased trees at Knoxfield and Heidelberg in Victoria. In contrast, nematodes were common in the bark samples of healthy trees.

Morphologically, extracted nematodes were classified into five trophic groups, including: aphelenchida (plant, fungal and lichen feeders), rhabditids and areolaimids (bacterial feeding), *Macrolaimus* spp. (saprophagus), tylenchids (plant feeding), and dorylaimids (bacterial and algal feeders). Aphelenchids were the most commonly found trophic group. Three genera and twelve morphospecies of aphelenchids were identified. Eight species of *Laimaphelenchus* and one putative species of *Acugutturus* appear to be new records for Australia. Descriptions of two new species, *L. preissii* and *L. australis* have been published. Three species of *Aphelenchoides* were also found. No *Bursaphelenchus* spp. were found.

Molecular studies included sequencing of the ITS region of *Laimaphelenchus preissii*, morphospecies Aphelenchid K1, and Aphelenchid H1; D2D3 fragments of 28S and 18S of *L. preissii*, morphospecies Aphelenchid K1, Aphelenchid K2, and Aphelenchid H1, *L. australis*, and *Laimaphelenchus* Heidelberg; and COI of three aphelenchid morphospecies *L. preissii*, *Laimaphelenchus* Heidelberg and Aphelenchid K1. Phylogenetic analyses confirmed that the *Laimaphelenchus* isolates are new species and that the unknown aphelenchids are close to *Aphelenchoides*. None of the six isolates studied from Australia was close to *Bursaphelenchus*.

Population growth and mean doubling time of *L. preissii*, Aphelenchid K1 and Aphelenchid H1 were studied at different temperatures and on different food resources. The different species

had markedly different population growth rates, which were significantly affected by temperature and food.

A study on desiccation was carried out with *L. preissii* and morphospecies Aphelenchid K1, Aphelenchid K2, Aphelenchid H1 and *Laimaphelenchus* Heidelberg. Ability to survive desiccation varied between species, and the recovery rate of the different species was significantly different.

A pathogenicity study was performed using young *P. radiata* trees in a shadehouse. No symptoms were observed following inoculation with Aphelenchid K1, Aphelenchid K2, Aphelenchid H1 and *Laimaphelenchus* Heidelberg isolated from diseased *P. radiata* in Victoria, or *L. preissii* from native *Callitris* in South Australia.