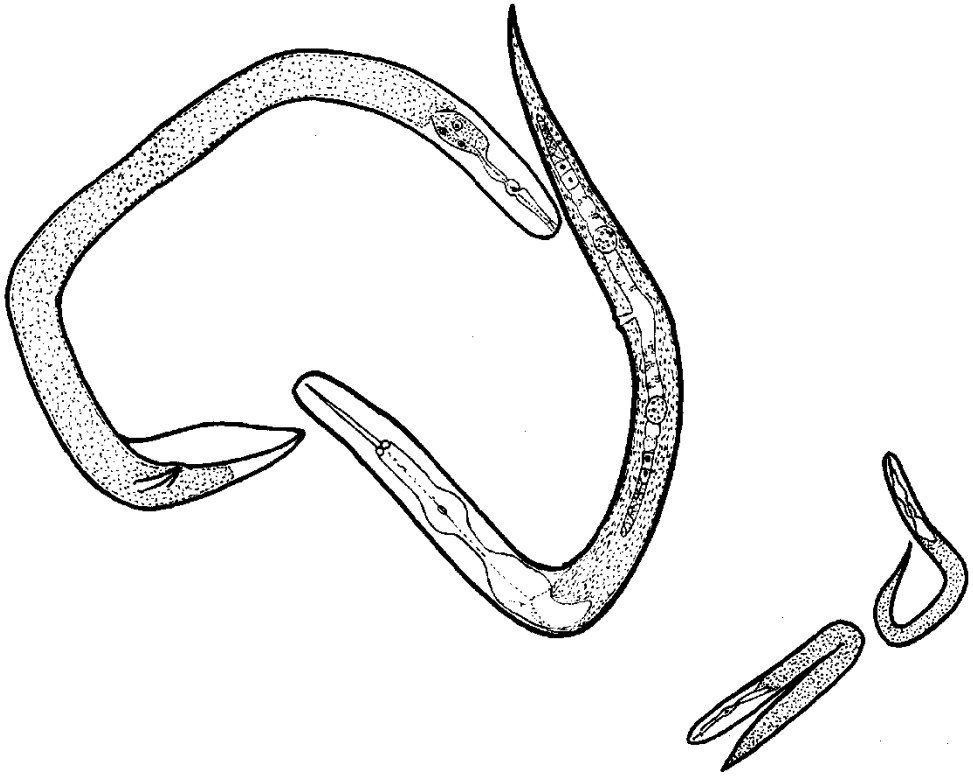
ISSN 1327-2101

AUSTRALASIAN NEMATOLOGY NEWSLETTER



Published by: **Australasian Association of Nematologists**

VOLUME 23 NO. 2 **JULY 2012**

From the Editor

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

January Issue – change of editor

The deadline for the January issue will be late December 2012. I will be overseas from late December for a couple of months, and in my absence the next newsletter will be edited by Katherine Linsell. She will notify you a month in advance so please have your material ready then.

Kerrie Davies

Contacts

Dr Mike Hodda
President, Australasian Association of Nematologists
CSIRO Ecosystem Science — Tel: (02) 6246 4371
GPO Box 1700 — Fax: (02) 6246 4000
CANBERRA ACT 2601 — Email: mike.hodda@csiro.au

Dr Sarah Collins
Secretary, Australasian Association of Nematologists
Department of Agriculture and Food — Tel: (08) 9368 3333
Locked bag 4 — Fax: (08) 9474 2840
Bentley Delivery Centre WA 6983 — Email: sarah.collins@agric.wa.gov.au

Dr Katherine Linsell
Treasurer, Australasian Association of Nematologists
SARDI Plant & Soil Health — Tel: (04) 1788 9253
Plant Research Centre, Hartley Grove — Fax: (08) 8303 9393
Urrbrae SA 5064 — Email:katherine.linsell@sa.gov.au

Dr Kerrie Davies
Editor, Australasian Nematology Newsletter
School of Agriculture Food and Wine — Tel: (08) 8303 7255
Waite Campus — Fax: (08) 8379 4095
University of Adelaide SA 5005 — Email: kerrie.davies@adelaide.edu.au

Association News

FROM THE PRESIDENT

As the AAN representative on the International Federation of Nematology Societies, I have been asked to make suggestions for session topics and chairs for the meeting. If you are thinking of going and want to help organise a session, here's your chance. I think all that they are after is names and titles. If you have any suggestions for topics you want to see, or volunteer yourself as a session organiser, please send them to me, so that I can pass on a consolidated list.

Likewise, APPS has sent a call for workshop ideas for the next biennial conference in Auckland in November next year (2013). If you have a topic, or better still, want to offer to organise it, please let me know. I don't think I'm exaggerating in saying these workshops are very much appreciated and a great chance to get together in person. This one is an extra special chance to get together with the NZ nematologists.

While on the topic of meetings, there have been several applications for the Australasian Nematology Support Fund (ANSF) this year, based on an upsurge in students in nematology. It would be very gratifying to claim that the fund is responsible for this underlying turnaround. However, it is just one small part of what will hopefully be a continuing trend.

The applications to ANSF have all been associated with attendance at the European Society of Nematologists Conference in Turkey, but the fund is open to all student members of AAN to attend any meetings or other useful travel, local or international. There is no formal application process, and applications can be sent at any time to members of the committee. As noted above, the 6th International Congress is approaching in South Africa in 2014, and prospective travellers may like to start considering their funding. It is unlikely that ANSF will be able to support all travel costs for International conferences, but it can be used as seed funding to leverage other money, which will provide good experience for the murky world of grant funding that seems an integral part of scientific careers these days.

Since the last issue, long-serving Treasurer of AAN Vivien Vanstone has retired from her position with AAN owing to ill-health. Vivien has also taken leave from all other professional duties. Vivien has done a wonderful job running the high finance of AAN since taking over from John Lewis about 5 years ago. She has also prepared spreadsheets and reformed the system to make it simpler. Best wishes to Vivien for recovery.

The new Treasurer of AAN is Katherine Linsell from SARDI. Many thanks to Katherine for taking the position at relatively short notice. Thanks also to Sarah Collins our Secretary for easing Katherine's transition to the job.

There was a completely underwhelming response to the call for comments regarding formal meetings for AAN at either ASDS or APPS. Being so spread out in Australia, our workshops and meetings provide some of the few chances we get to actually sit down and interact in person. I think there is value in having as many nematologists as possible in the same room at once, and one meeting every 2 years or so is not really excessive. The current meetings for ASDS and APPS are in far-flung reaches of the AAN area. Do we need to think about more central (and therefore cheaper for travel) locations?

Mike Hodda

Regional News

NEWS FROM THE ACT

CSIRO Ecosystem Sciences

In March this year the nematology group in Canberra welcomed Assistant Professor Dorota Porazinska from the University of Florida, who is here as a Distinguished Visiting Scientist for six months working with Mike Hodda. Dorota has worked on a number of large scale nematode diversity projects from the Costa Rica rain forests to the Antarctic dry valleys. She is working on using high-throughput DNA sequencing methods to study nematode diversity and using genomic methods to identify nematodes. There are a number of areas where high-throughput sequencing methods can be applied to including ecological monitoring studies. It has possible application in identifying quarantine organisms, including nematodes and other soil fauna. This project will build upon work already done by Mike on studying the diversity of nematodes using both traditional identification methods and genomic approaches.

The nematology group is gaining strength with two new PhD students, Natalie Banks and Kylie Crampton on board at the beginning of July. Natalie Banks is enrolled at Murdoch University with Kirsty Bayliss, but based at CSIRO Canberra. Her project is on pathways of dispersal for quarantine nematodes and their vectors. Nat has been working on a small project for the Northern Australian Quarantine Strategy (NAQS) compiling a list of nematodes in the region around Australia's north. She recently had her paper on the dispersal of potato cyst nematodes published in the journal *Phytopathology*. In the article, details are provided on the statistical analysis of the dispersal data of potato cyst nematodes (PCN's) from eight countries. The data suggest that PCN's spread a mean distance of 5.3 km/year radially from the site of first detection, and spread 212 km over ≈40 years before detection. Thus, the original site of introduction of PCN's may act as a source for subsequent spread which may occur at a relatively constant rate over time regardless of whether distance is measured by road or by a straight line (Banks *et al*., 2012).

Kylie is enrolled and located at EH Graham Centre, Charles Sturt University, Wagga Wagga with Profesor Gavin Ash, and is working on the biological control of root-lesion nematodes *Pratylenchus neglectus* and *Pratylenchus thornei*. Root lesion nematodes (RLN) are increasingly recognised as important pests affecting cereal production in Australia. Kylie aims to isolate and identify native beneficial microbes which suppress the activity of the RLN, with potential for development into a bionematicide. The formulation of a bionematicide against RLN would provide growers with a wider range of options for management of this important pest and add significant value to the grains industry.

Abdul Gafur continues his work on taxonomy and phylogenetics of *Pratylenchus*, *Radopholus* and *Achlysiella*. He has found that the relationships among these genera and their species may not be what has always been assumed. Abdul is working hard on getting

this written up for his thesis. You will have to wait to find out exactly what he has found until he gets it published.

Barry Conde, Plant Pathologist (Department of Resources, Northern Territory) has been working in collaboration with Mike and Sunil on the diagnosis of species of root-knot nematodes affecting Asian vegetables in the top end. Recently, *Meloidogyne incognita* was confirmed from diseased bitter-melon (*Momordica charantia*) root samples and the details have been reported in Australasian Plant Disease Notes (Singh *et al*., 2012). The plants were severely affected and showed symptoms of wilting and dying out. Another record of root-knot nematode from Northern Territory, this time on okra (*Abelmoschus esculentus*) is currently being investigated. Root-knot nematodes are a growing problem for vegetable growers in the Northern Territory and identification of species is a first step towards developing or determining appropriate management strategies.

Sunil has been making progress with his thesis. He has used two different approaches to analyse the biosecurity risks from 250 plant-parasitic nematode (PPN) species. The first approach required analysis of the worldwide distribution data of the selected 250 PPN using unsupervised self organizing maps. This approach is based on the assumption that species distributions are not random and that there are patterns within distribution of species. He then combined the outputs from the first approach with explicit criteria-based assessment of risks and using weighted averages, to determine the overall risks of PPN species to Australia. On the basis of the systematic risk prioritization his next step is to analyse the climatic suitability and projected distribution of selected high risk nematode species in Australia.

Publications

Banks NC, Hodda M, Singh SK & Matveeva EM (2012) Dispersal of potato cyst nematodes measured using historical and spatial statistical analyses. *Phytopathology* 102, 620-626.

Singh SK, Conde B & Hodda M (2012) Root-knot nematode (*Meloidogyne incognita*) on bitter melon (*Momordica charantia*) near Darwin, Australia. *Australasian Plant Disease Notes*, 1-4. DOI: 10.1007/s13314-012-0052-z

Sunil Singh

NEWS FROM SOUTH AUSTRALIA

The University of Adelaide

Kerrie Davies has been busy finalising various manuscripts for publication. She and 'Fred' Bartholomaeus completed descriptions of five new species of *Schistonchus* from *Ficus* in eastern Australia. Sadly, the ABRS funding which supported Fred has finished, and she is missed.

In January, Kerrie spent two happy weeks in Auckland at the invitation of Landcare, working with Zeng Qi Zhao. They described a new anguinid nematode from local *Pittosporum* and a new species of *Diploscapter* from ants (see report this newsletter). A new neotylenchid was also collected from the ants, but cannot be described until the free-living males and females are collected. Hopefully, this will give her another excuse to visit beautiful New Zealand.

Kerrie recently spent a couple of days at CSIRO in Canberra, catching up with Mike Hodda and Dorota Porazinska.

Kerrie Davies

NEWS FROM WESTERN AUSTRALIA

Murdoch University

The Plant Nematologists at Murdoch University are part of the Plant Biotechnology Research Group based in the labs of the WA State Agricultural Biotechnology Centre (SABC) at Murdoch University. The overall focus of the work is (i) to increase our knowledge of the molecular basis of nematode-plant interactions, and (ii) to make use of that knowledge to develop new forms of host resistance. The work also includes nematode diagnostics, and developing new tools for biosecurity and field applications.

Overall, this has been a very busy and successful year for the Murdoch Plant Nematology Group.

Highlights

- Publication of the book chapter on root knot nematodes in the COST ACTION 872 book (Michael G K Jones, and Derek B. Goto (2011). Root-knot nematodes and giant cells, in *Genomics and Molecular Genetics of Plant Nematode Interactions*, Eds John Jones, Godlieve Gheysen and Carmen Fenoll, Springer; Chapter 5 pp 83-100).
- Publication of the first transcriptome (all the expressed genes) of *Pratylenchus thornei* (Paul Nicol, Reetinder Gill, John Fosu-Nyarko and Michael G. K. Jones, 2012; *de novo* analysis and functional classification of the transcriptome of the root lesion nematode, *Pratylenchus thornei*, after 454 GS FLX sequencing. *International Journal for Parasitology* **42**, 225-237).
- An additional 5 papers (at least) in preparation on the transcriptomes of *P. zeae* and *Heterodera schachtii*, and on different aspects of transgenic host resistance – actually its hard to find the time to write them all up.
- Matthew Tan was awarded one of two APPS student scholarships to go to Japan to learn about diagnostics of pine wilt and related nematodes. He has also attended all the meetings of the CRC for National Plant Biosecurity and presented different aspects of his work at all the Science Exchanges.
- Matthew Tan has also written up his thesis and submitted it for examination (in less than 3 years), and now has a job with the quarantine service in Singapore. He has three papers to write from his thesis work (abstract provided below).
- One PCT patent and a second preliminary patent have been submitted – these have slowed submission of a series of some of the additional publications now in the pipeline.
- A joint Australia India Research Fund project with Dr Uma Rao, from the Indian Agricultural Research Institute (IARI), New Delhi, has been in progress over the past 3 years. The Murdoch group is working on root lesion nematodes, with the IARI group working on cereal cyst nematode (*H. avenae*). Uma will be visiting Perth in July.

- Mike Jones participated in the final EU COST Action 872 meeting in Ghent, where he presented work on the *P. thornei* transcriptome, and visited Godlieve Gheysen's lab in Ghent, SESvanderHave in Tienen and Rosanne Curtis at Rothamsted Research. He has since submitted an ARC Discovery grant application with John Jones (formerly SCRI, now James Hutton Institute, in Scotland), who was Convenor of the COST Action 872.
- Mike has also expended links with A/Prof. Derek Goto at Hokkaido University, Sapporo, in Japan – Mike was a keynote presenter for a meeting in January 2012 in Hokkaido organised by Derek, and also managed to get in a couple of days skiing (the snow was 4 metres high in Sapporo). Derek has been recognised by Murdoch University as a Sir Walter Murdoch Distinguished Collaborator, and this award provides Derek with funds to visit Murdoch and undertake collaborative research for the next 3 years. We are planning another joint Australia - Japan scientific meeting to be held at Murdoch University at the end of 2012.
- A number of new PhD students have joined the group (see below).

The Murdoch group has also continued to interact with the DAFWA group, and were sorry to see Vivien Vanstone needing to take time off. We look forward to seeing her back in action.

The following researchers are in the Plant Nematology group:

Research staff

Prof Mike Jones – group leader
Dr John Fosu-Nyarko – molecular biology, synthetic resistance

PhD Students
Paul Nicol – Application of bioinformatics to plant parasitic nematodes
Joanne Tan – Characterising the secretome of root lesion nematodes and establishing a protocol for its use as a model for RNA interference in plant parasitic nematodes
Sadia Iqbal - Genes involved in the RNAi pathway of root knot nematodes
Harshini Herath - Comparative and functional analysis of transpliceosome units of cyst and root lesion nematodes
Malathy Rathinasamy - RNA processing in plant parasitic nematodes
Vinneta Bilgi - Using RNAi to confer host resistance to pests.

Staff recently moved on

The following plant nematology research staff have moved on recently:

Matthew Tan, Dr Motiul Quader, Dr Doug Chamberlain, Dr Reetinder Gill, Dr Perry Francisco, Ms Jyoti Rana, Ms Jamie Tan, Ms Shui Hi Tan (Honours).

Mike Jones

NEWS FROM QUEENSLAND

BSES

Lea Meagher has accepted an Erasmus Mundus Scholarship to complete a Master of Science in Nematology at Ghent University in Belgium. Over the next 2 years she will be

studying a range of subjects covering nematology from all angles including behaviour and physiology, taxonomy, molecular biology, biocontrol and management and many more specialised topics. As the Masters course will be co-ordinated through several European universities, including Bielefeld University in Germany, University of Evora in Portugal and University of Jaen in Spain, this will give her an international perspective on all aspects of nematology from the highly qualified and respected staff of these universities. Lea is looking forward to immersing herself in a new culture and to meeting fellow classmates from various nations who will also participate in this course of study. Following completion of the Masters degree Lea intends to return to Australia to apply this knowledge in the agricultural industry.

The course website is (http://www.eumaine.ugent.be). It contains a substantial amount of information that could be useful/interesting.

On arrival in Ghent, Lea will be presenting a lecture about the state of nematology in Australia. She has been requested to supply a 'who's who' of Australian Nematologists and nematology laboratory details in this speech along with possible thesis topics that would be relevant to Australian Nematology.

If anyone has any information that could be helpful please contact Lea. Her mobile number is 0408 666 624 and email address is lea.meagher@gmail.com

Lea Meagher

ESP, Boggo Rd., DAFF

It has been eighteen months since I have written so it's time again to put pen to paper. The tropical and sub-tropical nematology team has had another busy time and I think the Brisbane staff finally feel settled into our new premises – Ecosciences Precinct (ESP), Boggo Rd. (we have been here since Oct 2010, so it is about time). All phone numbers and fax lines have changed from our Indooroopilly days, so please email us if you want to update those contact details. Please come and visit us.

View of the old façade of Boggo Rd Gaol on the left with the new Ecosciences Precinct behind and to the right.

The recent state election saw a change of government in Queensland and the creation of a new stand alone department known as the Department of Agriculture, Fisheries and Forestry (DAFF). Nematology staff members throughout the state who were previously employed in the mega department of DEEDI are now employed in DAFF QLD. Email access has changed in line with the new departmental name (firstname.lastname@daff.qld.gov.au).

Our project work continues in bananas, vegetables and potatoes and other crops such as strawberry and sweet potato in the areas of plant-parasitic nematodes and soil health. As well as nematology projects, Wayne O'Neill and Tony Pattison have again travelled to Indonesia on an ACIAR project which focuses on soil health in relation to *Fusarium* wilt of bananas.

Tony Pattison travelled to the University of Bonn to defend his PhD which he has now been awarded. Congratulations to Dr Pattison.

The trip also had the following objectives and outcomes as written by Tony in his trip report.

The purpose of this travel was:

- To attend the 43rd reunion Organisation of Nematologists of Tropical America (ONTA) in Coimbra, Portugal 5-9 September 2011,
- To deliver an oral presentation on the use of soil nematodes as indicators of soil health in vegetable production systems at the ONTA meeting,
- To view vegetable production in Spain relevant to soil health, and
- To review the soil health research at the University of Bonn.

The outcomes from this travel were:

From attendance at the ONTA conference in Portugal

- An increased awareness of how nematodes play a central role to soil and plant health. Particularly how plant-parasitic nematodes are able to interfere with plant defence mechanisms by altering hormone balances within the plant, allowing other soil borne pathogens to establish themselves and cause economic crop losses. Therefore, management of nematode problems may result in better management of other soil borne diseases.
- Development of new horticultural production systems is being investigated to reduce the need for pesticides and to retain economic production in a number of crops.
- Methods for determining the role of nematode antagonists mostly fungi in the soil were discussed.
- My presentation on our work was viewed as relevant, innovative and important in advancing global production of horticulture and reducing impact on the environment.
- Increased international network of nematologists working on suppression and cropping systems.

From viewing horticulture production in Spain:

- A greater awareness of the intensive horticulture production in Spain and on the heavy reliance on inputs to maintain production. There is current research to demonstrate the impact these practices are having on soil ecosystems and to

develop alternative systems to manage soil borne problems particularly in glasshouse export vegetable production.

- There is a heavy reliance on small family gardens to supply vegetables for the domestic market in Spain and the establishment of small local markets complete the supply chain in most regional centres. Many of the practices used appear detrimental to good soil health, but due to the small scale of production, rotations and the use of inputs are able to compensate for a decline in soil health.

From visiting the University of Bonn in Germany:

- New techniques of soil health research and cooperative links have been established to increase the research potential in Australia. Discussions were held with Professors in soil science, plant health, nematology, and organic agriculture. There is potential to increase the linkages between projects allowing greater research outcomes. There is also the potential to interchange students increasing skills development. A decline in students doing agricultural studies has meant that the University of Bonn has changed focus from applied to a more molecular focus.
- A field trip to the Julius Kühn Institute at Elsdorf showed how long term trials were being used to manage beet cyst nematodes and determine factors associated with changes in the nematode's impact on sugar beet production.

Tony and Tegan Kukulies – both at South Johnstone – have recently travelled to Samoa. Tony has spent more time in Kiribati while I travelled again to Fiji – all on our ACIAR project focusing on soil health in taro crops in the south Pacific. Tony and I expect to do further trips to Kiribati and Fiji later this year.

The Boggo Rd nematology team conducted a training course "Plant-parasitic nematodes – survey, extraction, identification and control" for Miss Phetsamone Songvilay (Noi) in the latter months of 2011.

Noi is a plant-pathologist in her own country of Laos who received funding from the Crawford Foundation to study plant-parasitic nematodes with us. Lester Burgess was the driving force behind Noi's successful application as he had recognised her dedication and drive to expand her knowledge in plant pathology. Noi was an exceptional student during her time with us and rapidly learnt the characteristics important in the identification of most of the plant-parasitic nematodes we encounter in QLD. This included, but was not limited to, *Radopholus similis*, *Pratylenchus goodeyi*, *Pratylenchus* spp., *Helicotylenchus multicinctus*, *H. dihystera*, *Rotylenchus brevicaudatus*, *Rotylenchulus reniformis*, *R. parvus*, *Meloidogyne* spp., *Tylenchorhynchus* spp., *Paratylenchus* spp., *Xiphinema* spp., and *Tylenchulus semipenetrans*. As you can see, we made sure she was very busy in the lab.

We also enjoyed the opportunity to do field work each week during her stay. This gave Noi the chance to see many of our crops growing while eating fresh produce straight from the field (strawberries and avocado were her favourites), growing practices adopted here, control of nematodes from the practical point of view, and to see some of the Queensland countryside.

And her weekends were full. She saw whales off Caloundra, kangaroos in the wild, all our native animals at Lone Pine Sanctuary, the surf and long beaches of the Sunshine Coast, the sites of Brisbane often and her favourite (but my absolute terror) a day at Dreamworld.

We wish her every success with her future career. We miss her now she has returned to Lao, but hope we will always remain in contact.

Noi helps us find some Aussie "bush tucker" for lunch - wild raspberries growing near our trial site. Delicious!

Noi with the Boggo Rd team L to R, Tim Shuey, Noi, Jenny Cobon and Wayne O'Neill.

I hear with pleasure that Matthew Tan is nearing completion of his PhD with Mike Jones. Matthew was an Honours student with us in Brisbane before he ventured west. Best wishes Matthew!

Tony is representing our team at ASDS in Fremantle this year where I will have a couple of posters although I'm unable to attend – I hope it is a huge success and I'll miss being there.

Jennifer Cobon

NEWS FROM NEW ZEALAND

Landcare Research, Auckland

From 19 May to 2 June 2012, Zeng Qi Zhao visited nematology laboratories in the USA. This trip was supported by Landcare Research. The aim of the trip was to seek potential collaboration with the nematologists in the United States. He visited Professor Robin Giblin-Davis, of the University of Florida, Fort Lauderdale; Dr Wemin Ye, Department of Agriculture, Raleigh, North Carolina; Dr Zafar Handoo, of the USDA Nematode Collection in Beltsville; and Professor Dr David Fitch, at New York University. In addition, he met Dr David Chitwood, Dr Lynn Carta, and Dr Karin Kiontke.

At each research group, Zeng Qi gave a presentation on his research work at Landcare Research. He was able to get an overview of the research activities of each group visited. In addition, he toured their laboratories and had discussions with the staff and students. This visit established initial contacts between nematology in Auckland and several research groups in America. It is hoped that collaborations will follow to strengthen NZ research capabilities and extend research areas.

Zeng Qi Zhao

RESEARCH REPORTS

CEREAL RESISTANCE AND TOLERANCE TO PEST NEMATODES IN THE SOUTHERN REGION

R.S. Davey[A], B. Gogel[C], S. Casonato[B], G.J. Holloway[B] and A.C. McKay[A]

[A] SARDI, Waite Campus, Urrbrae, SA.

[B] DPI Victoria, Horsham

Abstract Information on tolerance and resistance classifications for cereal cultivars to economically-important nematodes such as cereal cyst nematode (*Heterodera avenae*) and root lesion nematodes *Pratylenchus thornei* and *P. neglectus*) is important for grain growers in planning cropping programs to minimise risk of yield loss in current and subsequent season's crops. In the southern region a new field trial program has been established to assess tolerance using a split plot design in which low and high nematode populations are produced in the preceding season by growing resistant and susceptible hosts. The plots are then over sown in the following season and the test cultivars and tolerance assessed by comparing yields on the split plots. These trials also provide an opportunity to assess cultivar effects on low and high nematode populations. While analysis of the data is progressing, three broad resistance groups are already apparent. These include 1) cultivars that reduce low and high populations, 2) those that increase low but reduce high populations and 3) those that increase low and maintain/increase high. Intolerant cultivars eg Estoc, were able to support high nematode populations.

Introduction

Resistance is usually assessed under controlled conditions using pot assays (1). Such assays do not predict cultivar affects on different nematode densities under field conditions. This paper explores following changes in nematode levels in field trials established to assess tolerance, and to assess resistance under field conditions. This will potentially improve information for growers.

Materials and methods

Tolerance trials for *P. thornei* were established in 2010 using field pea and narbon beans to create low and high nematode levels in a split plot design. These plots were over sown in 2011 with named cultivars (5 replicates) to assess tolerance to the nematode. The initial and final nematode populations in the low and high split plots were assessed prior to seeding and post harvest respectively using PreDicta B (2). BLUP analysis of the plot yields for the split plots was used to assess tolerance of each cultivar.

Results

Average initial and final *P thornei* numbers/g soil for the low and high nematode density plots, sorted by the low final levels, are presented in Figure 1. Cultivars vary in the impact they have on the low and high initial nematode densities. The most resistant cultivars reduced both the low and high nematode populations, others increased the low and reduced the high, and a third group increased the low and maintained/increased the high. Additional categories may become evident when analysis of the data is finished.

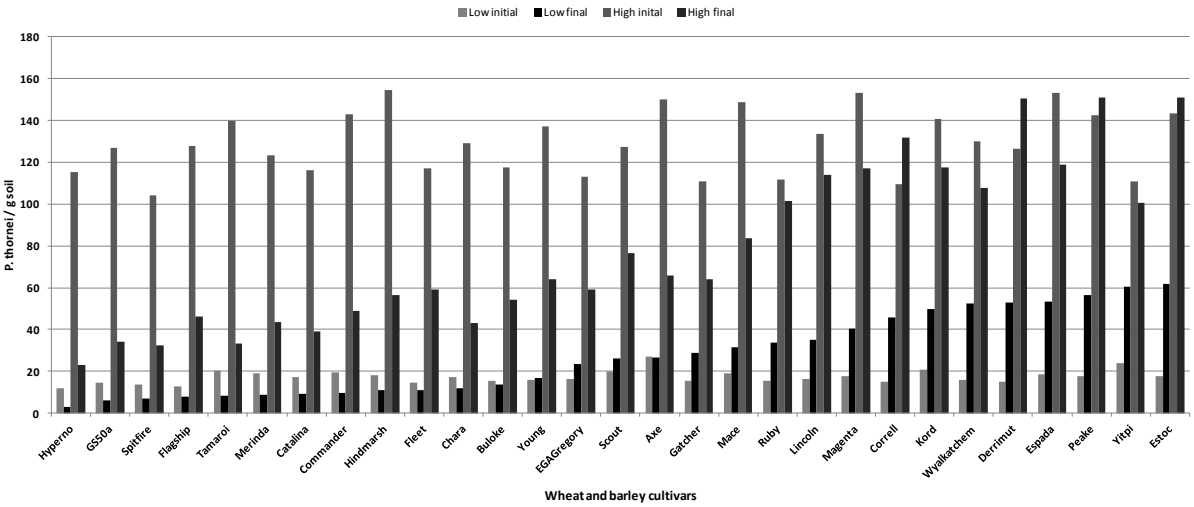


Figure 1. Average initial and final numbers of *Pratylenchus thornei*/g in the low and high split plots of a field trial established at Minnipa SA.

Discussion

Assessing changes in nematode levels in field trials established to assess nematode tolerance of new cultivars, has potential to provide grain producers with more information on how to use specific cultivars to manage nematode populations. Replicating these trials across regions and seasons should also provide useful information on variation in nematode multiplication between regions and seasons. Using tolerance trials to classify nematode resistance of current cultivars also frees capacity of the pot assays to support breeding programs.

Acknowledgements

The southern nematology program is funded by GRDC. Amanda Cook's and Wade Sheppard's role in managing the field trial and Ian Rileys' advice and support are gratefully acknowledged.

References

1. Cook, R. and Noel, G.R. (2002). In 'Plant Resistance to Parasitic Nematodes' eds. J.L. Starr, R. Cook and J. Bridge, pp71-105. (CAB International)
2. Ophel-Keller, K., McKay, A., Hartley, D., Herdina, and Curran, J. (2008). Development of a routine DNA-based testing service for soil-borne diseases in Australia. Aust. Pl. Path. 37: 243-253.

NEW NEMATODES FROM NEW ZEALAND

Zeng Qi Zhao

Landcare Research Ltd, Private Bag 92170, Auckland New Zealand

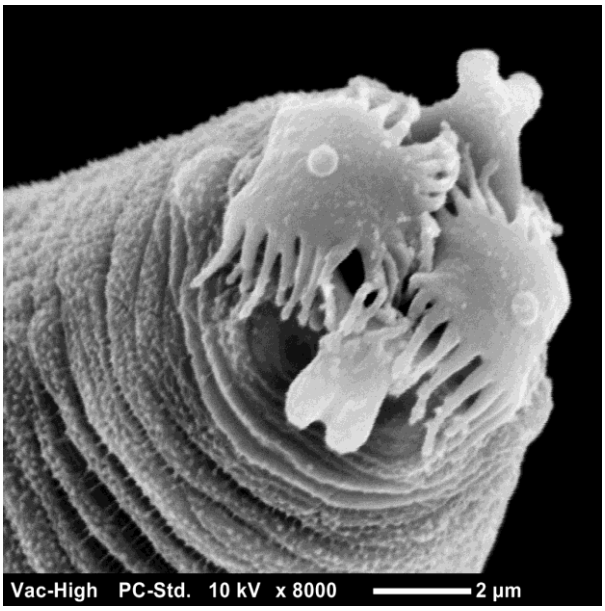
In January 2012, Dr Kerrie Davies was invited by Landcare Research New Zealand to work on nematode taxonomy. During about two weeks in New Zealand, she worked with Zeng Qi Zhao on three nematodes: one from the plant *Pittosporum tenuifolium,* and two from the native ant *Prolasius advenus*.

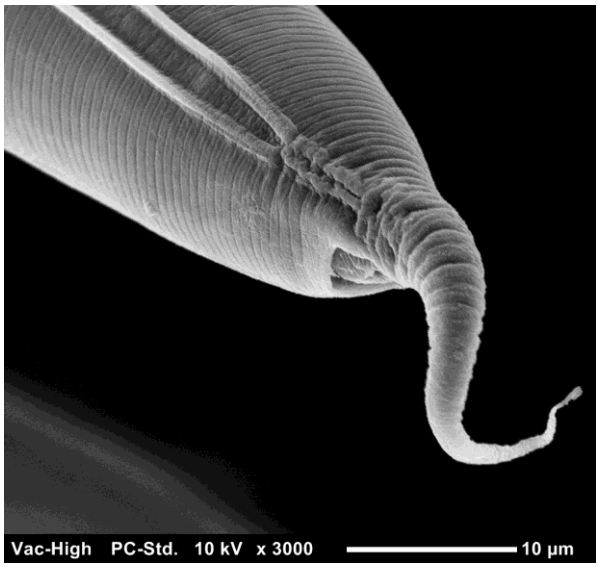
The nematode from *Pittosporum tenuifolium* was described as a new genus and species of anguinid, *Zeatylenchus pittosporum* gen. n., sp. n. It was recovered from leaves of *Pittosporum tenuifolium* from Hahei, Coromandel Region, North Island, New Zealand. The genus is characterised by having slender males and females, excretory pore opening level with the knobs of the retracted stylet, pharynx with a weak non-muscular median bulb, pharyngeal glands in two bulbs with one overlapping the intestine, females with a single gonad with a quadricolumella and post-vulval uterine sac; and males with slender arcuate spicules and the bursa arising <1 anal body diam. anterior to the cloacal aperture and extending *ca* 30% of distance to the tail tip. Its feeding does not induce galls, only foliar chlorosis. The species has particular characters including a short, robust stylet with conus forming *ca* 40% of stylet length and small rounded compact knobs, and tail offset dorsally with a pointed tip. Molecular phylogeny of near full length small subunit, D2/D3 expansion segments of the large subunit and internal transcribed spacer rRNA genes supports the description of *Zeatylenchus pittosporum* gen. n., sp. n. as a new genus and species.

Two nematodes were collected from the native ant *Prolasius advenus*. However, so far only one has been described and the other is still in preparation. *Diploscapter formicidae* sp. n. was collected from the ant *Prolasius advenus* and its nests in native beech forests of the South Island, New Zealand. This is a new host record for the nematode genus and the first report of an ant associate from the southern hemisphere. *Diploscapter formicidae* sp. n. appears to be native to New Zealand. No males were found from collections from 16 nests, in agreement with previously published data on the other members of this genus suggesting that males are absent or very rare. The adult females have bilateral symmetry of the head, characteristic dorsal and ventral projections of the putative cheilostom with paired hook-like structures or hamuli, expansive membranous lateral lip flaps or laciniae; gymnostom and stegostom with parallel walls; a swollen procorpus, large terminal bulb with a strong valve; paired ovaries with medial vulva; and a short conoid tail with slender pointed or spicate tip. Scanning electron micrographs (see photos below) of the structure of the head confirmed that the lateral laciniae with finger-like tines or filopodia are moveable (alternately covering and exposing the mouth). These lateral lip flaps arise posterior to the stoma, but anterior to the pore-like amphidial openings. The anterior margin of the cheilostom possesses apomorphic lateral bell-shaped projections and the hamuli are broader and less pointed than other species that have been examined. Molecular phylogeny of near full length small subunit, D2/D3 expansion segments of the large subunit rRNA gene, and heat shock protein 90 (Hsp90) gene showed that *D. formicidae* sp. n. is monophyletic

with the *Diploscapter* species and isolates available in GenBank, but is on an independent trajectory supporting separate species status.

Descriptions of both nematode species have been accepted for publication in *Nematology*.





Head and tail of *Diploscapter formicidae*.

Thesis Abstracts

MOLECULAR APPROACHES TO DIAGNOSTICS FOR PLANT PARASITIC NEMATODES OF BIOSECURITY CONCERN

Matthew Tan

WA State Agricultural Biotechnology Centre, Loneragan Building, Murdoch University
Perth, Western Australia 6150

Summary of thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy, 2012.

Plant parasitic nematodes are one of the most destructive plant pests: worldwide the annual loss to agriculture they cause is estimated at about USD$157 billion. The focus of this thesis was the development and application of new, practical, molecular approaches to detect and characterise plant parasitic nematodes.

An ITS-based PCR diagnostic approach was used to identify three *Pratylenchus* (RLN) species. Within–species sequence differences were small, but between-species differences were large. For example, the ITS sequence of *P. neglectus* shared only 61.4% identity with that of *P. thornei*. Similar results were obtained for cyst nematodes.

A protein-based diagnostic approach was also tested. Nematode proteins were analysed using MALDI-ToF MS to generate distinct protein profiles. Species-specific profiles were generated for eight species. Two dimensional protein gel electrophoresis was assessed as an alternative method to develop diagnostic biomarkers for nematode identification. Of the 58 distinct protein spots for *Pratylenchus* spp. and 89 spots for *Heterodera* spp., 13 and 9 spots respectively were further analysed as diagnostic biomarkers. Individual proteins were excised and sequenced after trypsin digestion and the identites of 16 proteins was confirmed.

An additional diagnostic method investigated was termed 'Multiplex anti-primer denaturation PCR' (MAD PCR). Here anti-primer technology was combined with 'auto-sticky' PCR, and qPCR with fluorescent labels. The 'anti-primer' concept was used with different fluorescence labels to tag different primers specific to different RLN species. The results obtained successfully differentiated three RLN species in a multiplex system.

We combined rapid extraction of nematode DNA from soil samples with molecular identification in a method we called 'DNA Isolation Rapid Technique from soil'- 'DIRTs'. This involves extraction of DNA from soil samples using a blender (using a novel method that takes only a few minutes) followed by qPCR analysis. The complete procedure takes 4 hr. Using the DIRTs technique and 'anti-primer' technology, three different RLN species were identified successfully in multiplex reactions.

The results are discussed in relation to current techniques used for nematode diagnostics. It is suggested that both protein-based and PCR-based technologies can provide robust approaches for detection and characterization of plant parasitic nematodes.

COLD TOLERANCE MECHANISMS OF ENTOMOPATHOGENIC NEMATODES

Farman Ali

Department of Zoology, University of Otago, P.O. Box 56, Dunedin, New Zealand

Email: drfarman@gmail.com

Summary of thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy, 2012.

Entomopathogenic nematodes (EPN), despite their great biological control potential, cannot currently compete with conventional chemical pesticides. Among other reasons, their limited shelf-life is the major impediment; which makes their production costs high, and thus limits their application to use against selective insect pests of high value crops only. Attempts to base a storage technology on partial desiccation and anhydrobiosis have met with limited success. This thesis investigates the cold tolerance abilities of two EPN species, *Steinernema feltiae* and *Heterorhabditis bacteriophora*, and the underlying mechanisms involved in the hope that a storage technology based on freezing may be possible.

Steinernema feltiae and *H. bacteriophora* have modest levels of cold tolerance; with 50% survival temperatures (S_{50}) of −12.6 °C and −11.8 °C respectively. This level of survival is achieved when freezing of the nematodes is nucleated by ice and they are left overnight at −1 °C after cooling at 0.5 °C min^{-1}. Acclimation (two weeks at 5 °C) significantly enhances the survival of *S. feltiae* (S_{50} = −13.16 °C), but not of *H. bacteriophora*. This effect is in addition to that of the overnight freezing effect (at −1 °C). There was no response to rapid cold-hardening or cold shock under the conditions tested.

Infective juveniles of *S. feltiae* avoid inoculative freezing at −1 °C as observed on a microscope cold stage, suggesting the occurrence of partial cryoprotective dehydration. However, at temperatures −2 °C and below, the nematodes freeze completely confirming that the species survives predominantly through a freezing tolerance strategy.

The pattern of ice crystal formation and location was further visualised using freeze substitution and transmission electron microscopy. It was found that *S. feltiae* can survive intracellular ice formation, providing the second example of intracellular freezing survival amongst animals (after the Antarctic nematode, *Panagrolaimus davidi*). The pattern of ice crystal formation was not as controlled as in *P. davidi*. Both small and large ice crystals were found in individual nematodes but small ice crystals were more common in nematodes frozen at high sub-zero temperatures than at lower temperatures. Snap-frozen nematodes in liquid nitrogen had only small ice crystals, but did not survive.

Low molecular weight cryoprotectants were involved in the freezing tolerance of *S. feltiae*. The nematodes accumulate large quantities of trehalose and glycerol in response to cold acclimation (2 weeks at 5 °C) and the freezing process *per se*, which appear to protect the nematodes from freezing injury by reducing the formation of ice and replacing the lost water.

A high molecular weight cryoprotectant was found to be inhibiting recrystallization in the frozen nematodes. This presumed protein is heat-stable, but has no thermal hysteresis or ice nucleation activity, and is thus termed a recrystallization inhibiting protein. The recrystallization inhibition activity was moderate, but could enable a freeze tolerant animal to control the size, shape and location of ice crystals after freezing.

The modest freeze tolerant abilities of these nematodes in part reflect their response to the environment in which they live in. A survey of cold tolerant EPN in Otago revealed that the distribution of EPN was limited to low and medium altitude sites (<700 m a.s.l.). Two species, *S. feltiae* and *S. kraussei* were recovered; the latter being the first record of this species from the Southern Hemisphere. This study also reports the isolation of a virulent strain of a nematophagous/entomopathogenic fungus (*Pochonia bulbillosa*) from the Rock and Pillar Range of Otago, New Zealand.

This study suggests that the natural capacity of EPN for freezing tolerance could be exploited as a first step towards developing a method for their long-term storage.

Other News

SHORT COURSE

NEMATODES IN CROPPING SYSTEMS - IDENTIFICATION AND TECHNIQUES

The next course is expected to be held in Perth in April 2013. If you are interested, please submit a non-binding expression of interest to Mike Hodda at CSIRO Ecosystem Sciences or Kerrie Davies at The University of Adelaide (addresses below).

As in previous presentations of this course, Kerrie Davies and Mike Hodda, the co-convenors, will try to tailor the course to suit the needs of participants. We envisage the following.

The workshop will suit researchers and professionals working in agriculture, quarantine, green keeping, and soil biology, who need to understand the principles and practice of handling soil, plant and insect nematodes. It will provide hands-on experience in sampling, extraction, specimen preparation, culturing, diagnosis, and identification (including molecular techniques). There will be opportunity for interaction with experts in the field.

Participants should have a degree which includes biology, agriculture, or soil science or have appropriate work experience to undertake the workshop. Less experienced participants can be supplied with recommended reading material prior to the workshop.

Nematodes to be considered include:

Meloidogyne Tylenchulus Heterodera Tylenchorhynchus

Pratylenchus Morulaimus Ditylenchus Radopholus

Anguina Bursaphelenchus Scutellonema Hemicycliophora

Paratrichodorus Filenchus Xiphinema Tylodorus

Aphelenchoides Heterorhabditis Helicotylenchus Steinernema

Rhabditida Mononchida Dorylaimida Areolaimida

Anticipated course cost is $1760 (including GST). This includes all materials and a printed course manual.

Details of course content can be varied to suit the interests of the participants. Please contact the co-ordinators to discuss any specific needs or topics desired for inclusion.

Dr Mike Hodda
CSIRO Ecosystem Science
GPO Box 1700
Canberra ACT 2601
Phone: 02 6246 4371
Fax: 02 6246 4000
Email: mike.hodda@csiro.au

or

Dr Kerrie Davies
School of Agriculture Food and Wine
The University of Adelaide
Waite Campus Glen Osmond SA 5064
Phone: 08 8303 7255

Email: kerrie.davies@adelaide.edu.au