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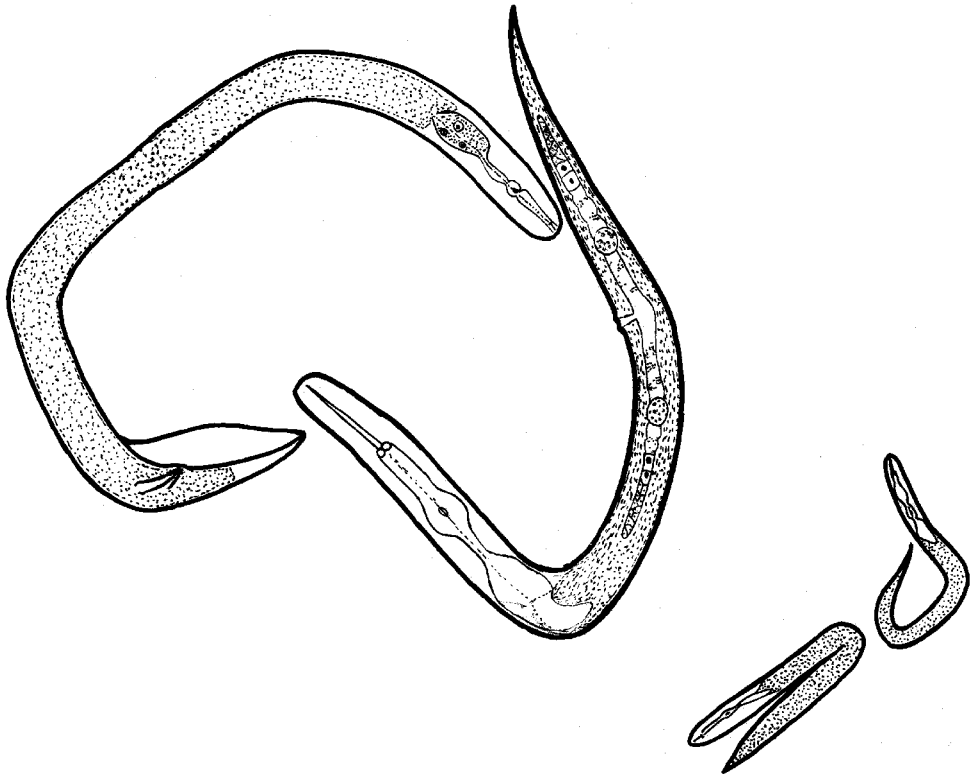
AUSTRALASIAN NEMATOLOGY NEWSLETTER



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From the Editor

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

January Issue

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

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Association News

FROM THE PRESIDENT

Thank you to all who participated in the last Biennial Meeting in Geelong, and thank you to the local organizers, Lila Nambiar and Grant Holloway. The workshop and meeting showed, I think, how a band of people small in number relative to the membership of APPS, can have a relatively big impact on Australian agriculture. AAN thanks all the speakers and participants in the nematology sessions, and we look forward to seeing everyone in Adelaide in 2007.

The completion of the biennial "Nematodes in cropping systems: identification & techniques course" recently has reminded me of the parlous state of nematology teaching in Australia. With Ian Riley's move to SARDI, and Kerrie Davies officially retired—I say "officially retired" because one would not know it from her current list of activities—there is no formal university nematology left east of Perth. This is a parlous state of affairs. It is inconceivable that there would be no undergraduate teaching in Entomology, and yet nematodes probably have a similar impact on agriculture to insects. Representations to Adelaide University about this in 2004 had little effect. Succession planning seems to be a hot management topic at the moment, so I suggest that nematologists mention to their clients in industry that we need more people coming through to maintain our intellectual capital, expertise base, human resources or whatever you want to call it. My personal experience is that it takes quite a few years to develop a real feel for nematology, so if the right buttons can be pushed now, we may see some results down the track.

The organization of 5ICN is continuing to advance, with a non-profit association separate to AAN being set up to administer the conference. This will be called 5th International Congress of Nematology Inc. (assuming that no-one has reserved that name already), and will be set up in the ACT.

GENERAL MEETING 2005

Draft Minutes

Date: 27 September 2005
Time: Commencing 6.00 pm
Venue: Cat Restaurant Bar Lounge, 90 Little Malop Street, Geelong

1. Apologies were received from K. Davies, J. Lewis, J. Marshall, M. Quader, N. Seymour and R Watson.

2. Minutes of the previous meeting in Adelaide 2004 presented and accepted by the meeting. There was no business arising.

3. M. Hodda presented the President's report. The report largely covered planning for the International Congress on Nematology for 2008 (5ICN) to be run by members of AAN. Several letters were written on behalf of AAN to Adelaide University expressing concern over nematology teaching. Also, a vote of thanks was given to L. Nambiar and G. Hollaway for organising the workshop in Geelong.

4. I. Riley presented the Treasurer's report prepared by J. Lewis. The current bank account balance was $9645, an increase from $7629 at the beginning of the period. Income mainly came from membership dues and the workshop held in association with ASDS in Adelaide (about $1000 each).

5. Membership stands at 67 down by 9 since the last general meeting. This consists of 44 financial members, 22 in arrears for less than one year and 2 for more than one year. Since January 2004 there has been 14 new members (3 rejoined), 1 resignation and 19 cancellations. Credit card payment through APPS was used by 22 people (including 3 new members and 2 mistaken payments to be deducted from the 2006 payment from APPS) in 2005 and 22 people (including 1 new member) 2004.

AAN has registered a domain name – nematologists.org.au – with the website now hosted by openmonkey.com on a Linode virtual server in Nashville TN, USA. The secretary has user access to the server and can maintain the web site directly. Previously the University of Adelaide was the host for the website, but this meant a difficult URL and no direct access to maintain the site. Consequently the content was not well maintained. The current arrangement is provided gratis, as was the arrangement with the University. However, a small annual payment to the administrator of openmonkey.com would be appropriate.

In August 2005, a MoinMoin wiki, called NemWiki, was established on our website, allowing all members (and at this stage anyone with an interest) to directly contribute to the development of AAN web content provided it is consistent with the aims of the Association. The secretary is currently the administrator of NemWiki. Registration of users has been slow and apart from minor corrections and directory additions, no users have contributed additional content. It is recommended that (1) a full directory of members be added (consistent with privacy restrictions) to NemWiki to increase its usefulness and (2) the existence of NemWiki is promoted to the global nematology community through SON and Nema-L list servers.

6. Election of office bearers.

President – M. Hodda was re-elected unopposed
Treasurer – J. Lewis was re-elected unopposed
Secretary – I. Riley was re-elected unopposed
Newsletter editor – K. Davies was elected unopposed

7. Other business.

Following the Treasurer's report it was decided that, at J. Lewis's suggestion, that the funds should be transferred to an account with higher income earning potential. J. Lewis has offered to investigate the options.

It was also decided that membership dues should be increased from $10 to $15 per year from 2006.

I. Riley proposed that a full membership list be added to NemWiki to make it more useful. This would include institutional contact details only. The meeting agreed to this suggestion. G. Walker called for a vote of thanks for the establishment of a strong web presence and for NemWiki. Although the newsletter is now provided electronically, it was decided to continue to also publish it in print form.

An committee consisting of 10 people from Australia and New Zealand meet the previous evening to begin planning for 5ICN in 2008. It was decided to incorporate 5ICN Inc. as a separate entity from AAN. However, it was requested that AAN provide a loan to allow "5ICN Inc." to commence operation. It is planned to produce a surplus from 5ICN, the constitution of 5ICN Inc. will specify that excess funds be disbursed to APPS, AAN or a new organisation such as an Australasian Nematology Foundation. There are some restrictions under Australian law that limits the disbursement of funds from not-for-profit incorporated bodies. A loan of $5000 was sought and approved by the meeting.

G. Stirling raised the matter of workshops and other activities in association with 5ICN that could be held in other cities. This was considered a worthwhile suggestion and members encouraged to pursue suitable ideas.

Also, the meeting was advised that D. Bird, an adjunct professor of Murdoch University might hold a workshop in WA on molecular nematology within the next year.

Unless otherwise arranged, the next meeting will be held in Adelaide in August 2007.

Meeting closed at 7.00 pm.

Regional News

NEWS FROM CANBERRA

The fourth biennial "Nematodes in cropping systems: identification & techniques course" was run by Mike Hodda and Kerrie Davies at ANU in November/December. This year there were 12 participants from WA, Victoria, NSW, Qld and NZ. The finale on Friday afternoon included a wild storm that flattened trees, drenched anyone in the open, and closed the airport for an hour.

Figure 1. Participants in the Nematology Short Course, Canberra, 2005.

Mike Hodda has started on an ABRS grant to describe local Aphelenchida and add them to his interactive electronic key. The project will run for 3 years. He has also started on a diagnostic protocol for *Bursaphelenchus* spp. for AFFA, which is due to be delivered at the end of April 2006.

Mike Hodda has also had a student funded by CSIRO for 10 weeks over the summer. She is Ema Falez, an ecology student from Deakin University who is starting Honours in 2006. While in Canberra, she has been studying the Aphelenchida associated with native trees and insects around Canberra, with the subsidiary aim of supplying

specimens for Mike's ABRS project on the systematics of Australian Aphelenchida. Her project has been wildly successful in finding large numbers of nematodes from the bark and wood of standing dead trees. To the time of writing, Ema has yet to find how the nematodes get from tree to tree, but the insect trapping part of her study has only just begun. Ema will write a report on her studies that will be in the next newsletter.

John Curran has moved from Entomology to CSIRO headquarters to lead the internal communications team. A farewell was held on December 13th, with many in CSIRO Entomology, not just nematologists, wishing him well in his new role. John is a former President of AAN and we hope that he will not be lost to nematology forever.

NEWS FROM WESTERN AUSTRALIA

From the WA State Agricultural Biotechnology Center (SABC), Murdoch University--Mike Jones, Zhaohui Wang, Modika Perera, Kerry Ramsay, Angelina Ho, DAWA collaboration with Vivian Vanstone

Professor Mike Jones attended the American Society of Nematologists Annual Meeting in July at Port Lauderdale in Florida, avoiding hurricanes, and presented a talk on Laser Microdissection and identification of nematodes using MALDI-TOF mass spectrometry.

Dr Zhaohui Wang is working on an ARC linkage project with Professor Mike Jones and Professor James Dale (Queensland University of Technology). He has prepared several transgenic constructs which will be tested out next year for potential resistance to root-knot nematodes. Zhaohui attended the 2nd Asian Plant Pathology Conference in Singapore at the end of June. He presented a talk on progress of the plant-nematology research at the SABC, especially the application of Laser Microdissection to studying plant-nematode interactions. Also in September, Professor Mike Jones, Zhaohui and Dr Modika Perera attended the AAN Annual General Meeting in Geelong, associated with the APPS Meeting.

Research assistant Kerry Ramsay continued work on constructing a giant cell specific cDNA library using Laser Microdissection and Catapulting (LMC). This involved isolating the contents of giant cells from tomato roots infected with root-knot nematode (*M. javanica*) at 4 dpi and 10 dpi, using the LMC technique, and extracting the total RNA from these samples. Following reverse transcription, various methods were attempted to amplify the cDNA including *in vitro* transcription (IVT) and PCR amplification. Following successful amplification, a strategy for subtractive hybridization was developed involving hybridization to a solid-phase healthy root cDNA library on magnetic beads. The subtracted cDNA library is currently being screened with the aim of identifying novel genes expressed at the early stages of infection.

In September, Kerry spent a week working at ANU in the Biochemistry and Molecular Biology Institute with Dr Ulrike Mathesius, on a collaborative project on the involvement of the flavonoid pathway in nematode infection. The work involved infection of *Medicago trunctula* in which flavonoid deficiency had been induced using RNAi against chalcone synthase (CHS), the enzyme catalysing the first committed step of the flavonoid pathway, with root-knot nematode (*M. javanica*). It has been proposed

that the interactions between the nematode and the host plant in initiation of giant cells are similar to those occurring between rhizobium bacteria (*Sinorhizobium meliloti*) and legume plants in nodule development. Plants deficient in the flavonoid pathway cannot nodulate. However, early results suggest that inhibiting the flavonoid pathway does not stop root-knot nematode infection and giant cell formation.

Angelina Ho, an Honours student with Mike and Zhaohui, joined the plant-nematology group in July. Her project is to characterize promoters of two Transcription Factor (TF) genes in Arabidopsis. These two TF genes have been identified with root-specific expression by RT-PCR. Angelina cloned the promoter region of the two TF genes, and attached the promoters to reporter genes (GUS and GFP) in a binary vector. The constructs were then transformed into Agrobacteria, and Arabidopsis plant transformation was carried out using flower dipping and vacuum infiltration. Analysis of the expression of the TF promoters will be conducted upon harvesting of the seeds from transgenic plants.

Dr Modika Perera attended a recombinant antibody training workshop for diagnostics held in November at the Department of Primary Industries, Indooroopilly, Queensland. It was an intensive workshop facilitated by Wouter van Wyngaardt, Onderstepoort Veterinary Institute, Onderstepoort, Republic of South Africa. The participants were given hands on practical training on the entire panning process for screening antibody libraries, using a large semi-synthetic single-chain Fv phage display library based on chicken immunoglobulin genes (Nkuku recombinant antibody library). During the workshop, antigens from different plant pathogens were selected for hi-affinity antibodies using this library. The overall result of the workshop was very promising, and this library will be a valuable tool for immunodiagnostics of pathogens including nematode diagnostics. This workshop was organised by the CRC for National Plant Biosecurity and was funded by Plant Health Australia and the Australian Government through the "enhancing diagnostic expertise network" project. Modika is looking forward to using the tools and techniques learned from this workshop in the next step of her current project to develop specific antibodies to different nematode species.

For the past two months Modika has been very busy analysing protein profiles of *Pratylenchus neglectus* isolates in collaboration with Dr Vivien Vanstone, and developing MALDI-TOF based diagnostic protein profiles for stem nematodes (*Ditylenchus dipsaci*) of the oat and lucerne races (provided by Dr Sharyn Taylor from SARDI). The results obtained so far are promising, and work is still in progress.

Modika has also published a couple of papers recently. These are as follows:

M.R. Perera, K. L. Bayliss & M.G.K. Jones (2005) New Australian record for infection of trees in Paulownia plantations by root-knot nematodes. ***Australasian Plant Pathology* 34**: 419-420**.**

Modika R Perera, Ruben D. Flores Vargas & Michael G.K. Jones (2005) Identification of aphid species using protein profiling and matrix-assisted laser desorption/ionization time–of–flight mass spectrometry. ***Entomologia Experimentalis et Applicata* 117**: 243-247. [Although this paper is on identification of aphids, the method is relevant to nematodes as well]

NEWS FROM SOUTH AUSTRALIA

Zhao Zeng Qi (University of Adelaide) presented a paper at the Nematology workshop in Geelong. In October, he visited nematology labs in China, Japan and Florida and gave seminars on his work on aphelenchid nematodes in conifers. Zengqi also learnt much about pinewood nematode and appreciated greatly the generous welcome of his hosts. He has now finished his experimental work and is busy writing papers and his thesis.

Ian Riley (SARDI) also visited China and gave a seminar at Anhui Agricultural University on the MLA-funded project to develop DNA assays for organisms in pasture soil, including species of *Heterodera*, *Meloidogyne* and *Pratylenchus*.

Dwi Ratna Anugrahwati finished her project on the activity of endophytic actinomycetes against *Meloidogyne javanica*. Ratna did research for her Masters at Flinders University with Chris Franco and nematology help from the Waite Campus group. She has now returned to her job at Fakultas Pertanian Universitas Mataram, Lombok, Indonesia.

An Tae-Jin, a plant pathologist with the Korean National Institute of Crop Science, Ginseng and Medicinal Crops Division, arrived in Adelaide in late December as a visiting scientist for 6 months. He will work on control of soilborne pathogens using microorganisms and compost, primarily with Dr Steve Barnett with support from Drs Ian Riley, Trevor Wicks, Greg Walker and Ross Ballard. On return to Korea, Tae-Jin faces the challenge of research on biocontrol of root-knot nematode in ginseng.

With Mike Hodda, Kerrie Davies (University of Adelaide) presented another short course on "Nematodes in Cropping Systems" in Canberra in early December. Information flows both ways at these workshops, and this one was no exception. Kerrie was successful in obtaining an ABRS grant for 2005/06, for technical support for work on nematodes in sycones (fruits) of Australian *Ficus*. 'Fred' Bartholomaeus has joined her lab. She has previously worked with rabbit fleas and snails, and is being indoctrinated into the mysteries of plant nematodes. (Fred has been overheard muttering things like 'wow! what a beautiful nematode' while working at the microscope, so the indoctrination seems to be successful).

Dr Roger Dennis, from Geissen University, Germany, visited Adelaide in August, and presented a seminar on his work on angiogenesis induced by *Schistosoma.* Roger did his Ph.D. at Adelaide in the 1970's, and was supervised by John Fisher and Prof. 'Buddy' Rogers.

NEWS FROM NEW ZEALAND

From Nigel Bell, AgResearch, Hamilton

Nigel Bell has been hosting Dr Dieter Sturhan, a visiting nematologist from Germany, in his team's lab at Ruakura, Hamilton. Dr Sturhan's visit was funded from an AgResearch fellowship and aimed to add his expertise and experience to a multi-agency publication on the distribution of *Meloidogyne* nematodes in New Zealand. As part of this initiative, a meeting was held on 14 November in Palmerston North with nematologists from AgResearch (Nigel, Chris Mercer, Richard Watson and Lee Aalders), Crop and Food Research (Farhat Shah) (John Marshall was absent due to a missed flight!), Landcare Research (Gregor Yeates), Biosecurity NZ (Karen Knight) and AgriQuality (Graeme Page) present. This is the first such meeting of New Zealand nematologists to occur, and prompted some interesting discussion and strengthening of contacts within the field. Dieter presented some of his findings from previous New Zealand visits, including occurrences of *Globodera* spp. and entomopathogenic (EPN) nematodes. A database of *Meloidogyne* distribution in New Zealand has been started and disseminated to all participants. It will be the basis for the *Meloidogyne* distribution manuscript, and for addition of future finds.

After the meeting Gregor took Dieter, Nigel, Richard and Lee to his PhD sampling site of some 40 years ago. We had a dig in the Himitangi Beach dunes and watched the whitebaiters catch their tiny prey.

Back at Ruakura, Dieter worked with us on identifying *Meloidogyne* and *Heterodera* species from J2 specimens, and *Steinernema* from their infective stages. Dieter sampled many non-agricultural sites and we now know what such interesting creatures as *Neodolichodorus*, *Paralongidorus* and *Pakira*, amongst others, look like "in the flesh". Dieter also travelled to Christchurch to work with Karen Knight and Trevor Jackson (AgResearch). While with Karen, Dieter conducted a number of trips into the field to collect material from local pockets of native vegetation. During these survey exercises he was able to locate additional specimens of *Paralongidorus* and *Globodera* spp. In the laboratory he was able to view recent finds of virus vector nematode species and the archive collection of *Meloidogyne*. Observations of the latter will be incorporated into the *Meloidogyne* distribution paper. Despite the short duration of Dieter's stay in Lincoln a fair amount was achieved.

Trevor and Dieter worked on EPNs that Trevor has in culture.

We very much enjoyed Dieter's visit and the expertise he has imparted. This, along with our meeting of other nematologists, will significantly progress both the *Meloidogyne* project and other areas of research for some time to come.

Figure 1. Nematologists at AgResearch Grasslands on 14 November 2005. Dieter Sturhan (Germany), Richard Watson (AgResearch) (back), Graeme Page (AgriQuality) (back), Chris Mercer (AgResearch), Lee Aalders (AgResearch) (back), Gregor Yeates (Landcare Research), Nigel Bell (AgResearch), Karen Knight (Ministry of Agriculture and Forestry), Farhat Shah (Crop and Food Research).

Research

BIOCONTROL ORGANISMS AND HUMAN HEALTH

Greg Walker

SARDI Plant Research Centre, GPO Box 397, Adelaide 5001

Nematologists who have worked with biocontrol organisms are probably familiar with the potential threat to human health of some strains of such common organisms as the fungus *Paecilomyces lilacinus* which is commonly found associated with *Meloidogyne* egg masses. Some bacteria may pose an even greater threat in this regard and have the ability to become transmissible to humans.

Burkholderia (formerly *Pseudomonas*) *cepacia* is one such bacterium. It has been widely promoted as a biocontrol agent (Deny®, Blue Circle®, Intercept®) for important root pathogenic fungi such as *Fusarium*, *Phytophthora*, *Pythium* and *Rhizoctonia*, and for some foliar diseases too (such as *Alternaria*). Some reports indicate activity against some nematodes although a recent report failed to demonstrate control of *M. incognita* (Roberts *et al*., 2005). It was first described in 1949, as the cause of Slippery Skin of onions. An increasing number of genomically distinct species ('genomovars') have been described within the *B. cepacia* species complex but only some have been phenotypically differentiated. The factors associated with the ability of different genomovars to infect humans and with virulence are poorly understood. Genomovar III is most commonly associated with cystic fibrosis (CF)-patients in Canada (Speert *et al*., 2002), but other genomovars are also found. Genomovar III is commonly associated with plants (Balandreau *et al*., 2001) and different strains can coexist in complex communities in the plant rhizosphere (Ramette *et al*., 2005). People with CF or the immuno-compromised are particularly susceptible to infection by *B. cepacia*, and can suffer rapid lung collapse followed by death, and virulent strains have spread between CF-sufferers. It is not limited to CF-sufferers, and can opportunistically invade hospital patients, wounds or troops operating in moist environments ('foot rot'), although this is rare.

B. cepacia is commonly found in soil and in moist environments, is resistant to a broad range of antibiotics, is metabolically versatile, has a large genome and is able to mutate and adapt rapidly to new environments. It has been suggested that highly transmissible strains evolve randomly and because of this a moratorium on the widespread agricultural use of this organism was proposed (Holmes *et al*., 1998). The effects of different strains on plants may also require further study, as genomovar III has been associated with finger-tip rot of banana (Lee *et al*., 2004).

Even with strains of organisms deemed to be safe to humans, there remains the possibility of the development of transmissibility to humans or of opportunistic infection of particular individuals. Our knowledge is never complete enough to be

entirely sure of the risks. Plant pathologists working with biocontrol organisms need to take special precautions to safeguard their health and of those who may subsequently come into contact these organisms in the field. Generation of aerosols is a particular concern and viable bacteria, introduced into irrigation water and spread by aerosols, have been detected 200 m or more away from outlets. Before widespread application to the environment is considered, the biology of these organisms needs to be thoroughly studied to allow a realistic assessment of risks.

Thesis Abstract

FACTORS WHICH DRIVE SPATIAL VARIATION WITHIN PADDOCKS OF *PRATYLENCHUS NEGLECTUS* AND *P. THORNEI*: CASE STUDIES IN SOUTH AUSTRALIA

Matthew Rodda

Honours Thesis, Plant Science, The School of Agriculture, Food and Wine, The University of Adelaide

It is desirable to know the nature and cause of spatial variation of *Pratylenchus* (root lesion nematodes, RLN) population density within-fields, because the factors responsible may be manageable. This study investigated the spatial patterns of *P. neglectus* and *P. thornei* in two case study paddocks in South Australia: examining characteristics of and reasons for spatial variation in population density. Three main hypotheses were tested: that spatial variation is due to variability in a) abiotic soil properties, b) the presence of weeds as alternative hosts, and/or c) the presence of antagonistic soil microorganisms (soil suppression). Soil sampling at sites around the paddocks was conducted to determine the population density of RLN, as well as study these other characteristics. Considerable variability in RLN density existed within each of the sampled paddocks. The link between the 'management zones' delineated for precision agriculture in each paddock and *Pratylenchus* density was not strong.

The first two hypotheses were tested through observational studies and the last was tested through experimental manipulation of the biological component of the soil. Two experiments assessed the possibility of soil suppression at sampling sites: the survival of RLN in gamma-irradiated and untreated field soil incubated for three weeks, and the invasion and multiplication of RLN in a wheat host (cv. Machete) grown in selectively treated field soil. No significant correlation was found between the presence of weeds or abiotic soil characteristics and RLN density. Evidence of soil suppression was seen in some sites, including the majority of those from the paddock at Crystal Brook, but this did not correlate with or explain the variability of RLN density observed within the two paddocks. Given the methods used, the variability of *Pratylenchus* observed was not driven by factors analysed.