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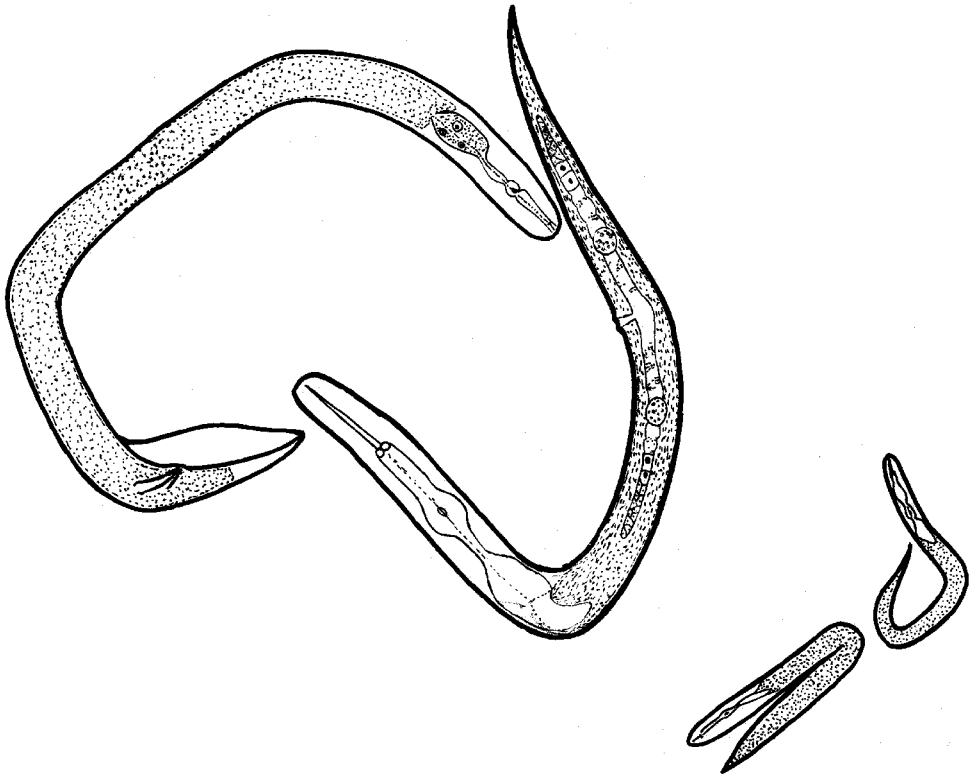
AUSTRALASIAN NEMATOLOGY NEWSLETTER



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From the Editor

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

January Issue

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

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ASSOCIATION NEWS

FROM THE PRESIDENT

I have just returned from the Fourth International Congress of Nematology at Tenerife, Canary Islands, and have several items of news which may be of interest.

In a conference of about 600 participants located about as far from Australia as it is possible to get on the face of the earth, it was good to see 5 Australians and a Kiwi there, even if one was the expatriate Julie Nicol. Sessions covered a wide range of topics within the broad field of nematology:

- Lesion Nematodes (Pratylenchidae)
- Cyst Nematodes (*Heterodera* spp)
- Root Knot Nematodes (*Meloidogyne* spp.)
- Pine Wilt Nematodes (*Bursaphelenchus* spp.)
- Marine & Freshwater nematodes
- Entomophilic Nematodes
- Resistance
- Chemical Control
- Biological Control
- Integrated Management
- Subsistence Agriculture
- Precision Agriculture and the use of GPS
- Phylogeny and Classification, Development
- Molecular Diagnostics
- Digital Images
- Quarantine
- Ecology, Food Webs, Biodiversity
- Parasitic Interactions between Nematodes & Plants

Volume 4 part 2 of *Nematology* has all the abstracts.

I found the congress very useful scientifically and in terms of discussing the challenges facing nematology. At the Congress I was invited to give a talk on "Nematode management in major crops in the Australasian Region", and I will present the paper from the talk in the next newsletter.

One of the main activities for the evenings was discussing and approving a final Draft Constitution for the International Federation of Nematology Societies (IFNS). In these discussions I represented the AAN in place of John Marshall, who normally sits on the IFNS Council for the AAN, but was unavailable. The Draft Constitution should be circulated to the nominated contact officers of the 14 Nematology Societies which make up the Federation (Afro-Asian, Australasian, Brazilian, Chinese, Egyptian, European, Italian, Japanese, Southern African, Indian, Tropical American, Pakistani, Russian and USA). It is intended that the member societies ratify (or otherwise) the Draft Constitution before the end of the year, so that the constitution will come into effect next year. As this timetable means that there will be no opportunity to present the exact text of the document to AAN via the newsletter, solicit comments, and amalgamate replies, I will outline the main points of the document below. If anyone has any comments, please contact myself, Ian Riley or John Lewis (the current executive) or John Marshall, the IFNS representative. Otherwise the AAN executive will take a decision on behalf of the AAN.

The constitution sets out the goal of the IFNS, which is to foster communication and collaboration between nematologists, to increase awareness of nematodes, and to advance the science of nematology worldwide. As this aim is very similar, though broader, than that of AAN, I would hope it is supported by AAN members.

The main purpose of the IFNS is to conduct an International Congress every 6 years. The congress just concluded was the 4th of these Congresses, with previous Congresses being in Guadeloupe (in the Caribbean), Veldhoven (Netherlands), and Guelph (Canada). The next Congress is due in 2008 (more on this below). Each of these Congresses has been hosted by one of the member Societies, who have assumed total financial and administrative liability for the meeting. The constitution formalises this role for FICN, while leaving the exact arrangements which will apply to future congresses open for negotiation. I think that all the AAN members at the congress would concur that these are very useful events for discussion and information exchange in ways which are not possible via email or telephone, and so this is a concept that AAN should support. However, I recognise that the sample of members present at the Congress is both small and biased towards those who think congresses are useful (and hence attend them). If there are strong views to the contrary from those who were not there, please let someone listed above know, and this will be taken into account.

Another main purpose of IFNS is to maintain a web site. The aim of the web site is to provide links to the member society's own web pages, and information on books, events, people etc, which can be submitted by any of the member societies. Until now this service has been provided by a small grant from the European Society of Nematologists (ESN), with programming provided gratis by Safia Siddiqi of the Afro-Asian Society of Nematology (AASN) and friends. The draft constitution formalises this as a function of IFNS. There was a commitment from the Society of Nematologists

(SON) to sponsor this web site for the next 6 years. I hope this is not a controversial aim, and would be supported by AAN.

There was a great deal of discussion regarding finances for IFNS. Currently IFNS has no financial reserves and no budget, but following the success of the recent Congress, this may change. The constitution is deliberately non-prescriptive regarding finances, so that there is no obligatory financial commitment on any of the member societies, although societies may make voluntary contributions (But this does not apply to the hosting of an International Congress). It was hoped that IFNS would have a float that would act as seed money for future congresses following the success of the current congress. Hence future congresses should not necessarily be a potential financial burden on the host society.

The constitution also formalises the officers of IFNS – President, Vice-President and Secretary/Treasurer – and sets out their main roles. These are uncontroversial. The President convenes and chairs meetings of Council, the Vice-President does so if the President is unavailable, and the Secretary/Treasurer maintains membership lists and contact details. (The members of the Federation are the Nematology Societies, not individual people, so the IFNS has 14 members, and individuals are only listed as contact officers for the member societies.) The President is elected for 6 years, with an affirmation of support necessary at 3 years, and a mechanism for removal at any time if several societies propose a vote which is supported by a majority of Council. Changes to the Constitution require a majority of 75% of Council.

The way the IFNS will be run was decided to be detailed in a separate Procedures or Operations Manual, which can be more flexible than a Constitution, and offer suggestions only. There were some sensitivities about what an International Federation should prescribe for its member societies, and this was decided as the best way to both provide an ongoing record of how to do things (and how to improve where improvements are suggested), while not locking anyone in to any particular procedures. As a Federation, the aim is to accept the differences in the cultures and circumstances of the member societies, while ensuring that everyone is supporting the aims of the Federation.

In summary, I think AAN can gain a great deal and will lose nothing in accepting the constitution of IFNS. It will provide us with a seat at the table in international discussions about nematological issues, and valuable international publicity via the web site, and other publications that the IFNS may sponsor (such as conference proceedings). It will also give us a say in future International Congresses, and hopefully, better access to people or results from overseas.

While many AAN members are also members of other societies which are also affiliated with IFNS, this is true of many of the other members of IFNS, but because IFNS does not have a budget independent of the member societies, there is no disadvantage, financial or otherwise, in this situation.

One of the issues raised at the meeting was potential sites for the next congress. Although a decision is not necessary for several years, Australia was suggested as a possible site. A quick poll of the AAN members at the Congress revealed a great deal of

support for this idea. Of course, this poll included only those attending the Congress and who obviously think that Congresses such as this are worthwhile; otherwise they would not have travelled the rather difficult route to get there (over 40 hours flying and at airports in my case!). Those less supportive of such Congresses were not there to disagree. Hence I raise the issue here.

Personally, I think the advantages are considerable in having nematologists from all over the world come to a place which is much more accessible to most Australians than Tenerife. We can learn a considerable amount from them, as they can learn from us, and the benefits from research partnerships, collaborations, and other scientific relationships formed can be considerable. I find that direct discussions can be so much better than email or even telephones for bouncing ideas and getting people's impressions of what is going on, aspects which are not always included in formal presentations or papers. The disadvantage, of course is in the amount of organisation required, as well as the financial risk, although the latter will hopefully be borne partly by IFNS.

Of course, there is no guarantee that if Australia offers to host the next International Congress, that this offer will be accepted. There was some support from other societies expressed at Tenerife.

So, let discussion begin. We have some time to go before our next General Meeting, and if it is raised now people can have a chance to think about the idea and air their thoughts at the meeting or in the newsletter. I am sure that Jenny, our newsletter editor, would welcome any (non-libellous) contributions on the subject for the next newsletter.

For those of you avidly reading all the above hoping to find out what Nuccia Eyres and Lila Nambiar did after the incriminating photo published in the last edition of the newsletter**…**

Prepare for a great surprise…

Nuccia was actually serious and smiled politely for the camera. Yes, she could actually do it, and the most incriminating evidence of all is presented below.

Mike Hodda

Regional News

NEWS FROM NEW ZEALAND

A book by David Wharton (Department of Zoology, University of Otago) was published by Cambridge University Press as part of their popular science list on February 7th and released in Australia/New Zealand on May 6th. "Life at the Limits: Organisms in Extreme Environments" covers a variety of organisms but nematodes, of course, get a mention. Here's a description:

We are fascinated by the seemingly impossible places in which organisms can live. There are frogs that freeze solid, worms that dry out and bacteria that survive temperatures over 100°C. What seems extreme to us is, however, not extreme to these organisms. In this captivating account, the reader is taken on a tour of extreme environments, and shown the remarkable abilities of organisms to survive a range of extreme conditions, such as high and low temperatures and desiccation. This book considers how organisms survive major stresses, and what extreme organisms can tell us about the origin of life and the possibilities of extraterrestrial life. These organisms have an extreme biology, which involves many aspects of their physiology, ecology and evolution.

The book is available via Cambridge University Press's Melbourne office and at http://www.cambridge.edu.au/

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NEWS FROM SOUTH AUSTRALIA

Arrivals

For one week in March 2002, Ms Lee Davis from Ag Research, Ruakura Research Centre, Hamilton, New Zealand, visited University of Adelaide and SARDI nematologists. Lee was sponsored by the New Zealand-Australia Research Coordination Program, International Science and Technology Linkages Fund.

Lee works with Richard Watson and Nigel Bell in management, resistance, tolerance and biological control of nematodes in pasture. Pastoral land in New Zealand covers approx. 10 M ha, and pastures (*Trifolium* spp.) are affected by a number of nematodes: *Meloidogyne hapla, M. trifoliophila, Heterodera trifolii, Pratylenchus* spp., *Helicotylenchus pseudorobustus, H. labiatus, Paratylenchus nanus,* and *Paratrichodorus minor*.

To allow efficient cultivar evaluation and host range testing, nematode inocula are required. Cultures of some of the nematodes (*M. hapla, M. trifoliophila,* and *Heterodera trifolii*) have already been established at Ruakura in soil based media. The primary objective of Lee's visit to Adelaide was to learn the sacred art of culturing *Pratylenchus* aseptically on carrot pieces. Cultures will now be established at Ruakura.

Lee met with University of Adelaide and SARDI nematologists. Of particular interest was the potential relevance of Ian Riley's *Anguina* work to New Zealand grasses, and Rachel Hutton's pasture research. Lee spoke at the monthly meeting of the Waite Campus Nematology Discussion Group, and outlined current nematology research in New Zealand, in particular the pasture program at Ruakura. Funding willing, we hope to meet up with Lee again at the 2003 ICPP / APPS conference in Christchurch.

Siwi Indarti, Lecturer in Nematology and Pests of Stored Products, Entomology and Plant Pathology Department, Gadjah Mada University, Indonesia has been awarded a Crawford Fund traineeship to visit the nematology groups at the Waite Campus. Siwi works on *Pratylenchus*, *Radopholus* and *Meloidogyne* in banana with a focus on biocontrol. Siwi has not had the opportunity to travel outside Indonesia before, so I am sure she will find three months interacting with the Waite groups enjoyable and beneficial.

Elise Head has returned from a successful stint on maternity leave to continue her PhD (part-time) on the ecology of *Fergusobia*/*Fergusonina* in *Eucalyptus camaldulensis*. As a consequence of organisational restructuring within the University, Elise has established stronger links with the nematology group. So we welcome Elise back and we are glad to have her on the team.

Caroline Versteeg finally escaped the oppressing humidity of far north Queensland and is now attempting to acclimatise to Adelaide's chilly winter. Caroline will continue with her Masters research on brassicas for control of root knot nematodes in annual vegetable production comparing temperate and tropical systems.

Departures

Sharyn Taylor (SARDI) departed on June 5 for her 2002 'World of Nematology Discovery Tour'. Sharyn will attend the Fourth International Congress of Nematology, Tenerife, Canary Islands, Spain. A joint paper has been submitted with Julie Nicol: 'Global importance of cyst (*Heterodera* spp.) and root lesion (*Pratylenchus* spp.) nematodes'. Sharyn will also present three posters: 'Resistance to stem nematode (*Ditylenchus dipsaci*) in faba bean (*Vicia faba*) in Australia', 'Yield loss caused by root lesion nematodes (*Pratylenchus neglectus* and *P. thornei*) in Australia', and 'Comparison of PCR and misting chamber for the assessment of root lesion nematodes from wheat roots'.

Those of us not attending the Congress have loaded her up with posters, which will hopefully not exceed baggage limits. On behalf of Mark Potter, Sharyn is presenting 'The relationship between glucosinolates in canola (*Brassica napus*) and its ability to control the plant parasitic nematode *Pratylenchus neglectus*'. Sharyn is also lugging around four posters for Vivien Vanstone *et al.*: 'Use of the mistifier for extraction of root lesion nematodes (*Pratylenchus* spp.) from soil', 'Yield losses for barley, oat and wheat due to root lesion nematode (*Pratylenchus neglectus*) in South Australia', 'Weeds as hosts to root lesion nematodes (*Pratylenchus neglectus* and *P. thornei*), and 'Effect of applied phosphorus on density of root lesion nematode (*Pratylenchus neglectus*)'.

[Please note that *only* 87.5% of the SA posters at the conference involve *Pratylenchus*!!].

Following the Congress, Sharyn will travel to Rennes, France to meet with Roger Rivoal, INRA, and then to Turkey to spend three weeks with Julie Nicol, CIMMYT. Sharyn is also hoping to visit Wageningen, The Netherlands.

Contrary to previous premature reports, Vivien Vanstone (University of Adelaide) has not yet commenced as Plant Nematologist with the Western Australia Department of Agriculture, South Perth. Vivien will, however, be leaving the Waite Department of Plant Science (after 16.5 years) to begin the appointment in Perth on August 5, 2002.

Kerrie Davies, traveling *incognito* disguised as a grey nomad, spent much of May and June collecting *Fergusobia*/*Fergusonina* in Queensland and New South Wales.

Ian Riley was invited to talk on corynetoxin poisoning at the American Society of Microbiology meeting in Utah, USA in May. He managed to stretch the trip to include time with Norm Schaad, Bacteriologist with the USDA Foreign Disease Research Unit at Ft Detrick, Fredrick, Maryland and with Steve Alderman, Plant Pathologist with the USDA National Forage Seed Production Research Center, Oregon State University, Corvallis and John Griesbach, Nematologist with Oregon State Department of Agriculture, Salem. In both cases the interest was the *Anguina*-vectored bacterium in the genus *Rathayibacter*. Before returning home, Ian also visited Dr Nguyen Nogc Chau, Nematologist with the Institute of Ecology and Biological Resources. The emerging nematode issues of importance in Vietnam are *Radopholus* spp. in coffee and durian (this is notable because the populations in these hosts appear not be *R. similis*

and to differ from each other) and *Bursaphelenchus* in pines. It is hoped that nematological collaboration can be established between Australia and Vietnam. So if this is of interest to you, please get in touch with Ian.

Other News

The dissemination program for the fungus, *Dilophospora alopecuri*, an antagonist of *Anguina funesta* and *Rathayibacter toxicus* (the causal agents of ryegrass toxicity), has been actively extended from WA to SA this year. Ian Riley is acting as the local contact and we have seen some adoption of the fungus in areas of SA where ryegrass toxicity continues as a problem. It is hoped that the benefits will become more widely known and that the fungus will contribute to sustainable management of the problem in SA.

Speakers at our campus-wide nematode discussion group for first semester were Lee Davies on nematology work at Hamilton, NZ, Caroline Versteeg on brassicas for control of root knot nematode, Imelda Soriano on phytoecdysones and plant defence against nematodes and Elise Head on ecology of *Fergusobia*/*Fergusonina* in river red gums.

Forest and Wood Products Research and Development Corporation has indicated their support for a PhD student to undertake a study of aboveground nematodes of conifers in Australia, in particular *Bursaphelenchus* and closely related taxa. The project was initiated by Ian Smith, Department of Environment and Natural Resources in Victoria and will cover conifer populations in Vic, SA and NSW but is most likely to be based at the Waite.

Vivien Vanstone, University of Adelaide
Ian Riley, University of Adelaide

NEWS FROM NEW SOUTH WALES

NSW Biodiversity Research Network

What is the NSW Biodiversity Research Network?

The NSW Biodiversity Research Network has been established to facilitate communication and cooperation among stakeholders in research on biodiversity in NSW. These stakeholders include government, private and community organisations, and individuals - both those who do the research, and those who use the research. Currently biodiversity research is coordinated to some extent within government agencies, but not across all research organisations. There is a need for more consultation among organisations before research programs are established, and for greater coordination of existing programs.

One of our goals is to identify gaps and priorities for biodiversity research in NSW, and to outline these in an upcoming website and NSW Biodiversity Research Strategy document. Our website will also summarise and link the research of relevant organisations, and thus serve as a central point of reference for biodiversity researchers and students in NSW. We also aim to form enduring links among people, by providing

opportunities for communication, such as an electronic mailing list, newsletters, and meetings or mini-symposia. The outcomes will be increased awareness of biodiversity research priorities among stakeholders, better coordination of biodiversity research and funding bids across agencies, and an improved basis for biodiversity management and conservation in NSW.

The initiative for this Network arose out of the NSW Biodiversity Strategy (hard copy published by the National Parks and Wildlife Service, 1999; available online at http://www.npws.nsw.gov.au/services/index.html). The Network Steering Committee now holds regular meetings of interested agency and university partners. Current and past members of the Steering Committee include representatives from National Parks and Wildlife Service, the Australian Museum, the Royal Botanic Gardens Sydney, the University of Sydney, the University of Wollongong, Macquarie University, NSW Fisheries, the Department of Land and Water Conservation, CSIRO, the Zoological Parks Board, NSW Agriculture, and NSW State Forests.

If you would like to get involved, or receive further information, please contact:

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NEWS FROM WESTERN AUSTRALIA

News from WA State Agricultural Biotechnology Center (SABC), Murdoch University--Mike Jones, Zhaohui Wang, Angela Hollams and Simon Humphris

The nematode-plant interaction group in SABC has got fresh blood, Simon Humphris. Simon is gaining work experience on molecular research with Zhaohui Wang following up on the downstream analysis of the previous differential display experiments. Simon has got an offer from Murdoch University for a mid-year honours project starting in July, 2002. He is going to work on enhancer trapping in host plants infected by root-knot nematodes, using transgenic *Arabidopsis thaliana* plants as a model system generated with different upstream activation sequence (UAS)-GAL4-GFP vectors. These *Arabidopsis* lines have cell lineages tagged with GFP, such as endodermis, phloem, vascular parenchyma and cortex. Cell specific gene expression induced by or related to nematode infection can be monitored by confocal laser scanning microscopy.

Zhaohui Wang has ordered the Affymetrix *Arabidopsis* full-genome genechips for his microarray experiment. These chips have recently been released by Affymetrix, which contain 22,000 *Arabidopsis* gene sequences and ESTs. The entire pattern of gene

expression in host roots related to nematode infection will be examined in the future using these chips by microarray.

Another set of microarray instruments were installed and tested in the SABC a few days ago. They are a GeneTAC ™ G3 work station and a GeneTAC UC-4 slides scanner produced by PerkinElmer. The GeneTAC ™ G3 is a multifunctional robot work station which can be used to manage cDNA library (for example colonies picking, replicating and griding) and print bio-chips for cDNA microarray. Zhaohui has been fully trained on the operation of these equipment to secure our access to both the Affymetrix and GeneTAC microarray instruments. Any interesting genes identified by microarray using Affymetirx could be potentially useful to make our own chips using the G3 work station for further studies.

Angela Hollams is still working on her mRNA differential display project. She has re-amplified a number of cDNA fragments generated from previous differential display work. Some of them have been cloned into pGEMT vector for screening and sequencing. Dot blot combined with quantitative RT-PCR is being developed to identify the true positive cDNA fragments whose expressions are up- or down-regulated in giant cells in host root induced by the nematode pathogen. She has been physically unwell in the past half year, which has kept her away from her Ph.D study for a while. Fortunately, she is recovering and back to her normal lab work.

Mike Jones is very busy as usual on his duty as the director of SABC. Although his application on nematode studies for an ARC linkage grant was successful, the joint chief investigator for this project, Dr Shashi Sharma at the Department of Agriculture WA, has been transferred to quarantine section. Mike is waiting for another senior nematologist to be appointed in AgWA to start this project.

Research

PRATYLENCHUS NEGLECTUS: COMPARISON OF CROP RESISTANCE AND CEREAL YIELD LOSSES IN SOUTH AUSTRALIA

Vivien A. Vanstone and Michelle H. Russ

Department of Plant Science, University of Adelaide, Waite Campus, Glen Osmond, SA 5064

Wheat is the principal host of *Pratylenchus neglectus* in southern Australia. By comparison, barley, oat, canola and vetch are moderate hosts. Lupin, lathyrus, pea, faba bean, lentil and narbon bean are resistant. From the least to most susceptible, crops were ranked lathyrus < pea < faba bean < lentil < narbon bean < lupin < vetch < barley < oat < canola < durum wheat < wheat.

Mean yield loss recorded in South Australia for intolerant wheat (1995 - 1998) is 12 - 20% (a loss representing approx. $40 - $60/ha). Trials in 1999 and in 2000 were assessed to compare losses for barley, oat and wheat. By exploiting the natural variation in nematode density between and within trial sites, plot yields could be compared over a range of nematode densities. Negative correlations between yield and final nematode density (*Pf*) were significant in 1999 for barley (r = 0.795), oat (r = 0.827) and wheat (r = 0.659). The negative relationship between yield and *Pf* was also significant in 2000: r = 0.789 - 0.875 for barley, r = 0.654 - 0.892 for oat, and r = 0.524 - 0.828 for wheat.

Yield losses for each cereal genotype were estimated by regression of plot values for grain yield against *Pf.* In 1999, mean yield loss was 10.3% for barley, 9.5% for oat, and 3.8% for wheat. Mean yield loss in 2000 for barley was 5.4%, for oat 8.4%, and for wheat 6.7%.

Although barley and oat are more resistant than wheat to *P. neglectus*, they suffer comparable yield loss and are therefore as intolerant as wheat.

Introduction

Pratylenchus neglectus is widespread in dryland cropping areas of South Australia. *P. thornei* also occurs, but *P. neglectus* is the most common species of root lesion nematode in this region. Although *P. neglectus* has a wide host range, including cereal, pulse, oilseed and pasture species, crop species and cultivars vary in levels of resistance and tolerance to this nematode.

Resistant crop species and cultivars can be used in crop rotations to reduce *P. neglectus* density. Susceptible wheat is the principal host, with successive crops of most cultivars likely to increase nematode density, hence increasing the potential for yield loss to

subsequent intolerant crops. Mean yield loss of 12 - 20% due to *P. neglectus* has been recorded for intolerant wheat cultivars in South Australia (Vanstone *et al*. 1995, 1998; Taylor *et al*. 1997, 1999).

Compared to wheat, barley and oat are moderate hosts of *P. neglectus* (Taylor *et al*. 2000; Vanstone *et al*. 2000), but little is known of the relative tolerance of barley and oat and the magnitude of potential yield loss. However, Taylor *et al*. (1999) indicated that oat cultivar Echidna was intolerant to *P. neglectus*, since yield was 22% greater after a resistant compared to a susceptible cereal.

Comparison of yield with *Pf* provides the best estimate of loss caused by root lesion nematodes (Smolik and Evenson 1987; Plowright *et al*. 1990; Prot and Savary 1993; Todd and Oakley 1996). Yield loss for wheat in South Australia has been determined previously by relationship of yield with *Pf* for *P. neglectus* and *P. thornei* (Vanstone *et al*. 1998; Taylor *et al*. 1999).

Although *Pf* offers no predictive value in estimating yield loss in the current season, assessment of the relation between *Pf* and yield should reveal tolerance or intolerance of crop cultivars which can then be exploited or avoided in future rotational sequences in areas where *Pratylenchus* potentially limit crop production.

Methods

Trials were sown in May - June at sites naturally infested with *P. neglectus*, and plots mechanically harvested in November - December.

Initial (*Pi*) *P. neglectus* density was determined three weeks after sowing, and final density (*Pf*) in Spring (October - November), co-inciding with grain development for the cereals.

Twelve to fifteen random samples (25mm diameter to 100mm depth using a hand trowel) of soil plus root material from each plot were bulked. Nematodes from a 200g sub-sample of soil (plus roots) were extracted by misting (10 sec. mist duration at 10 min. intervals) at 25 C over 4 days (Vanstone *et al*. 2001). Nematodes (adults plus juveniles) were counted microscopically (40 - 50 x magnification) from a 1ml sub-sample of the mister extract for each sample. Fifty gram sub-samples of soil were oven-dried (40 C for 24 h) to determine soil moisture, and nematode density calculated on a soil dry weight basis.

Nematode multiplication rates (*Pf/Pi*) were calculated by dividing final (*Pf*) by initial (*Pi*) densities.

Low levels of ectoparasitic nematodes (*Paratylenchus, Tylenchorhynchus, Merlinius* and/or *Filenchus*) were detected at all sites, but *P. thornei* and *H. avenae* were absent.

Relationship between plot yield and *Pf* was determined for each genotype of the cereal species by simple, linear regression. Yield loss was estimated from the difference between the actual mean genotype yield and the maximum yield predicted by the regression (i.e. the *y*-intercept).

Results

Relative to wheat, barley and oat were more resistant to *P. neglectus* (Table 1). Mean *Pf* in 1999 for barley was 6.0, for oat 11.6, and for wheat 32.8 *P. neglectus*/g dry soil (Table 3). Within each crop there was variation in level of resistance. The most resistant wheats (Excalibur, Worrakatta and Krichauff) resulted in 72% fewer nematodes than the most susceptible (Machete). The difference in *Pf* between the most (Doolup) and least (WI3102) resistant barley was 79%. Euro was the most resistant oat cultivar, and resulted in 40% fewer nematodes than the most susceptible oat (Echidna). Nematode density for barley and oat, respectively, was 82% and 64% lower than for wheat.

For the cereals (particularly wheat) *P. neglectus* densities in 2000 (Table 4) were lower than in 1999 (Table 1). Mean *Pf* values in 2000 for barley, oat and wheat were, respectively, 4.9, 6.3 and 9.9 *P. neglectus* per g dry soil. Mean *Pf/Pi* for barley was 1.0, for oat 1.6, and for wheat 2.5.

Table 1: Final nematode density (*P. neglectus*/g dry soil) and estimated yield loss for barley, oat and wheat sampled from South Australian field trials in 1999. Average *Pi* = 8.2 *P. neglectus*/g dry soil (3 weeks after sowing). Percent yield loss estimated from the difference between actual yield and the maximum yield (*y*-intercept) predicted from simple, linear regression of yield with *Pf*. n = 4, n = 6 and n = 16 for each barley, oat and wheat genotype, respectively.

Genotype	Crop	*P. neglectus*/g dry soil (*Pf*)	Estimated Yield Loss (%)
Doolup	Barley	2.1	2.8
VB9524	Barley	3.5	6.3
Mundah	Barley	4.1	6.8
Barque	Barley	4.1	6.0
Lofty Nijo	Barley	4.2	9.2
Keel	Barley	5.2	0
WI3107	Barley	5.5	22.9
Arapiles	Barley	5.9	21.4
Franklin	Barley	6.2	27.1
WA2052	Oat	7.1	0
WB232	Barley	7.2	8.8
Gairdner	Barley	8.3	7.5
SV91024-7	Oat	8.6	0.3
Chebec	Barley	8.9	9.9
Sloop	Barley	9.1	3.6
Euro	Oat	9.3	0
SV91139-27	Oat	9.6	0
WI3102	Barley	10.0	11.4
SV91108-3	Oat	10.4	0
SV073-10-14	Oat	10.4	2.1
Hotham	Oat	10.5	32.9
Mortlock	Oat	10.9	19.7
Numbat	Oat	11.2	18.4
Quoll	Oat	14.0	8.8
Krichauff	Wheat	14.2	0
SV92040-54	Oat	14.7	0
Worrakatta	Wheat	15.0	0
Potoroo	Oat	15.2	13.6
SV92070-86	Oat	15.2	0
Echidna	Oat	15.5	37.2
Excalibur	Wheat	20.4	3.6
RAC873	Wheat	22.5	1.1
Tamaroi	Durum Wheat	22.6	12.9
Yitpi	Wheat	25.6	3.1
RAC897	Wheat	26.5	4.6
Kukri	Wheat	28.3	4.2
RAC875	Wheat	28.5	0
Camm	Wheat	28.6	3.0
Diamondbird	Wheat	31.0	8.1
Sunco	Wheat	31.1	11.7
WI98053	Wheat	32.5	3.3
Westonia	Wheat	33.0	0
WI98046	Wheat	34.2	1.2
RAC903	Wheat	34.6	1.5
Spear	Wheat	34.8	9.1
Carnamah	Wheat	35.4	2.6
H45	Wheat	36.7	0
RAC893	Wheat	37.1	3.8
Silverstar	Wheat	37.1	3.9
Giles	Wheat	37.5	1.8
Goldmark	Wheat	38.0	10.1
WI98056	Wheat	38.7	13.3
WI98049	Wheat	42.0	0.4
Chara	Wheat	49.7	0.7
Machete	Wheat	58.4	8.0

Canola and vetch were moderate/susceptible hosts, while lupin, lathyrus, pea, faba bean, lentil and narbon bean were resistant (Table 2). Canola was more susceptible than barley, similar to oat, and less susceptible than wheat (Table 3).

From least to most susceptible, crops were ranked lathyrus < pea < faba bean < lentil < narbon bean < lupin < vetch < barley < oat < canola < durum wheat < wheat (Table 3). Although few genotypes of lathyrus, pea, faba bean, narbon bean or durum wheat were included in these trials, results were consistent with data collected in previous seasons.

Table 2: Final nematode density (*P. neglectus*/g dry soil) for pulse and canola sampled from South Australian field trials in 1999.

Genotype	Crop	*P. neglectus*/g dry soil (*Pf*)
Kalya	Lupin	0.3
WA2005	Lupin	0.4
WL612	Lupin	0.4
Chalus	Lathyrus	0.7
BC Lathyrus	Lathyrus	0.7
Gungurru	Lupin	0.7
Tanjil	Lupin	0.7
Tallerack	Lupin	0.8
Digger	Lentil	0.9
Belara	Lupin	0.9
Wonga	Lupin	1.0
Parafield	Pea	1.0
Fiesta	Faba Bean	1.1
ILL7180	Lentil	1.1
Moonah	Lupin	1.3
Cassab	Lentil	1.4
Ansak	Lentil	1.5
Northfield	Lentil	1.5
Cumra	Lentil	1.8
SA3354	Vetch	2.4
Cazar	Bitter Vetch	2.5
N9035*002	Narbon Bean	2.9
SA33555	Vetch	3.2
Languedoc	Vetch	4..8
SA33585	Vetch	4.2
SA33600	Vetch	4.7
Cummins	Vetch	4.9
Morava	Vetch	5.1
Blanchfleur	Vetch	6.1
SA33224	Vetch	6.4
Monty	Canola	7.1
Surpass 600	Canola	8.4
Dunkeld	Canola	8.6
Charlton	Canola	9.5
46CO1	Canola	9.9
47CO2	Canola	10.8
BLN1990	Canola	11.5
BLN1400	Canola	12.7
BLN1216	Canola	13.5
Rainbow	Canola	13.8
Mystic	Canola	14.5
Grouse	Canola	16.1
RL8	Canola	17.1
Oscar	Canola	18.0

Table 3: Initial (*Pi*) and final (*Pf*) densities of *P. neglectus* (nematodes/g dry soil) for crops sampled from South Australian field trials in 1999. Range in *Pf* and *Pf/Pi* (nematode multiplication rate) is also indicated for each crop. Data for individual genotypes are presented in Tables 1 and 2.

Crop	Average *Pi*	Average *Pf*	*Pf* Range	*Pf/Pi* Range	No. of Genotypes Sampled	Replicates Sampled per Genotype
Lupin	1.1	0.7	0.3 - 1.3	0.3 - 1.2	9	4
Lathyrus	5.2	0.7	0.7 - 0.7	0.1 - 0.1	2	8
Pea	5.2	1.0	-	0.2	1	8
Faba Bean	5.2	1.1	-	0.2	1	8
Lentil	3.4	1.4	0.9 - 1.8	0.3 - 0.5	6	4
Bitter Vetch	5.2	2.5	-	0.5	1	8
Narbon Bean	5.2	2.9	-	0.6	1	8
Vetch	6.2	4.6	2.4 - 6.4	0.4 - 1.0	9	4
Barley	8.2	6.0	2.1 - 10.0	0.3 - 1.2	14	4
Oat	8.2	11.6	7.1 - 15.5	0.9 - 1.9	14	6
Canola	8.2	12.3	7.1 - 18.0	0.9 - 2.2	14	6
Durum Wheat	8.2	22.6	-	2.8	1	16
Wheat	8.2	32.8	14.2 - 58.4	1.7 - 7.1	26	16

Table 4: Final *Pratylenchus neglectus* density (*Pf*), multiplication rate (*Pf/Pi*), and mean plot yield for barley, oat and wheat genotypes assessed from South Australian field trials in 2000. Percent yield loss estimated from the difference between actual yield and the maximum yield predicted from simple, linear regression of yield with *Pf*. n plots of each genotype were assessed.

Genotype	*Pf*/g Dry Soil	*Pf/Pi*	Actual Mean Yield (kg/plot)	Predicted Max. Yield (kg/plot)	Estimated Yield Loss (%)
Barley (n = 8)					
Franklin	2.8 a	0.6 a	1.1	1.2	8.3
Keel	3.7 a	0.7 a	1.3	1.3	0
Schooner	4.9 a	0.9 a	1.2	1.3	7.7
Gairdner	5.3 a	1.2 a	1.0	1.1	9.1
Barque	6.3 a	1.2 a	1.3	1.4	7.1
Sloop	6.5 a	1.5 a	1.2	1.2	0
Oat (n = 16)					
Marloo	4.8 a	1.5 a	1.1	1.2	8.3
Euro	6.4 a	1.3 a	1.7	1.8	5.6
Quoll	6.8 a	1.4 a	2.1	2.2	4.5
Echidna	7.2 a	2.3 a	1.7	2.0	15.0
Wheat (n = 16)					
WI99072	4.3 a	0.9 a	6.0	6.0	0
Stylet	6.6 ab	1.6 a	6.4	6.5	1.5
RAC891	6.6 ab	1.4 a	6.6	7.6	13.2
Camm	7.2 ab	1.8 a	6.3	6.9	8.7
Yitpi	7.6 abc	1.8 ab	5.3	5.6	5.4
Kukri	7.9 abc	1.7 ab	5.6	5.9	5.1
H45	8.1 abc	1.8 ab	6.1	6.9	11.6
Chara	12.3 bcd	3.3 bc	6.0	6.6	9.1
WI99069	14.4 bcd	3.6 c	5.8	6.0	3.3
Machete	16.3 cd	3.5 c	5.0	5.7	12.3
Mitre	17.9 d	5.6 c	6.1	6.3	3.2

In 1999, yield loss estimated for barley was 10.3%, for oat 9.5%, and for wheat 3.8% (Table 1).

Although nematode densities were lower, and fewer genotypes were tested, similar losses were recorded in 2000: 5.4% for barley, 8.4% for oat, and 6.7% for wheat (Table 4).

Discussion

Relative levels of resistance/susceptibility for cereal, pulse and canola genotypes are useful when planning rotations to manage *P. neglectus* populations in cropping soils. *P. neglectus* did not multiply under the pulses assessed in 1999, while multiplication rate for even the most susceptible vetch, barley, oat and canola genotypes was only 1.0 - 2.2. Wheat was the most susceptible crop, with multiplication up to 7.0.

Barley and oat have moderate resistance compared to most wheat cultivars (Taylor *et al*. 2000; Vanstone *et al*. 2000), but they are as intolerant as wheat, thus suffering comparable yield losses. This has implications for crop rotation sequences employed by growers. Since barley and oat are less susceptible than wheat, growing these crops in rotations is expected to reduce nematode density and therefore decrease potential for yield loss to subsequent crops. However, growth of susceptible wheat will result in high nematode density. As barley and oat cultivars can be intolerant to *P. neglectus*, these crops then risk significant yield loss if grown after wheat. Although barley and oat crops will reduce nematode numbers, this benefit needs to be balanced against the potential yield loss to these crops.

Yield losses estimated in the present study are comparable to those reported from South Australia in previous cropping seasons. In 1997 (Taylor *et al.* 1999) and 1998 (Vanstone and Russ 1999), yield loss was estimated by regression of yield against *Pf.* Mean loss for intolerant wheat tested in 1997 was 13%. In 1998, mean loss for intolerant wheat was 12% and for intolerant barley 13%. In 1995 (Vanstone and Taylor 1996) and 1996 (Taylor *et al*. 1997), response to nematicide (aldicarb, 2.5kg/ha a.i.) was used to determine yield loss, and values were similar to those estimated by regression analysis of yield against *Pf* (Taylor *et al*. 1999).

Although yield losses and nematode numbers will vary with site and seasonal conditions, these results indicate potential losses for cereals and will be useful when planning crop rotations and selecting cultivars. With *P. neglectus* in 1999 and in 2000 reducing cereal yields by 7%, this nematode is clearly a constraint to crop production in southern Australia. These yield penalties represent significant financial loss to the grower (approx. $17/ha for barley, $9/ha for oat, and $21/ha for wheat).

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DNA METHODS TO DISTINGUISH *MELOIDOGYNE ARENARIA, M. INCOGNITA* AND *M. JAVANICA* FROM VINEYARDS

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Summary:

The North Carolina (NC) host test and mtDNA were used to distinguish a collection of root-knot nematodes from South Australian vineyards. The PCR of D3 expansion region of 28S rRNA gene and intergenic sequences of ribosomal DNA (IGS-rDNA) were also made to distinguish *Meloidogyne arenaria, M. incognita* and *M. javanica*. The NC test differentiated *M. incognita* but not *M. arenaria* race 2 from *M. javanica*. The combination of the NC test and mtDNA analysis differentiated between *M. arenaria, M. incognita* and *M. javanica*. The differentiation of these species with D3 expansion region of 28S rRNA gene was not possible. The PCR of IGS-rDNA from single female of each species produced distinct banding patterns that differentiated the species from each other. These species-specific banding patterns were reproducible across a range of individual nematodes of each species collected from different geographical locations of Australia. This method also produced DNA fingerprint variability within some individuals of each species.

Introduction

Root-knot nematodes (RKN, *Meloidogyne* spp.) may causes up to 60% yield losses in grapevines. Three RKN species *M. arenaria, M. incognita* and *M. javanica* are the most common in Australian vineyards. Hence, determination of species identity in vineyards is benificial for the development of an efficient management strategy for RKN.

Objectives

To evaluate the potential of NC host test and DNA methods for the identification of *M. arenaria, M. incognita* and *M. javanica* from vineyards.

Methods

The NC host test (Hartman and Sasser, 1985) and mtDNA method (Powers and Harris 1993) were used to determine the species identity of *M. arenaria*, *M. javanica*, *M. incognita*. These identified species were used in the evaluations of D3 of 28S rDNA and IGS-rDNA. The amplified D3 regions of these three species were sequenced and analysed.

Results

The isolate identity determined by NC, mtDNA methods have presented in the Table 1. A 300 bp band was amplified from the D3 expansion of rRNA genes of three *Meloidogyne* spp. (Fig. 1A). The sequences of D3 region are highly conserved among the species studied. The IGS-rDNA-PCR produced three different banding patterns for each *Meloidogyne* species (Fig. 1B). The mtDNA-PCR produced 1.1 kb bands for *M. arenaria* and 1.7kb and 556bp bands for *M. incognita*/*M. javanica* (Fig. 1C) but no restriction cut was found for 1.7 kb or 556 bp bands with enzyme *Hinf*I (result not shown). The IGS-rDNA-PCR also produced variability within the individuals of *Meloidogyne* spp. (Fig 2), even though the individuals fell within the same group by cluster analysis (Fig. 3).

Table 1. The identity of *Meloidogyne* spp. from vineyards by NC host test and mtDNA analysis.

Vineyard locations	NC differential host test type	mtDNA type (Powers and Harris 1993)	Species identity
Winkie (34^{O}18'S,140^{O}31'E)	*M. arenaria* race 2 /*M. javanica*	*M. arenaria*	*M. arenaria*
New Residence (34^{O}22'S,140^{O} 24'E)	*M. arenaria* race 2 /*M. javanica*	*M. arenaria*	*M. arenaria*
,,	*M. arenaria* race 2 /*M. javanica*	*M. incognita* /*M. javanica*	*M. javanica*
McLaren Vale (35^{O} 13'S,138^{O} 32'E)	*M. arenaria* race 2 /*M. javanica*	*M. arenaria*	*M. arenaria*
Padthaway (36^{O} 36'S,140^{O} 29'E)	*M. arenaria* race 2 /*M. javanica*	*M. incognita* /*M. javanica*	*M. javanica*
Adelaide	*M. incognita*	*M. incognita* /*M. javanica*	*M. incognita*
,,	*M. arenaria* race 2 /*M. javanica*	*M. incognita* /*M. javanica*	*M. javanica*

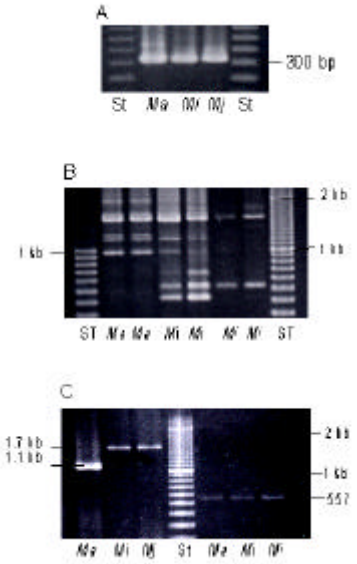

Fig. 1. PCR products of (A) D3 expansion region of 28S rRNA gene, (B) IGS-rDNA and (C) mtDNA (lanes 1 to 3 and 5 to 7 are PCR products for primers described by Powers and Harris, 1993 and Stanton *et al.*, 1997 respectively) of *Meloidogyne arenaria* (*Ma*), *M. incognita* (*Mi*) and *M. javanica* (*Mj*). St are 100 bp DNA ladder

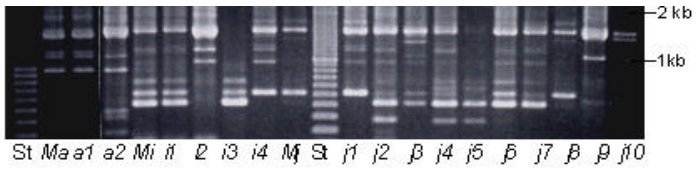

Fig. 2. Lanes *a*1, *a*2, *i*1 to *i*4 and *j*1 to *j*10 are IGS-rDNA-PCR variants of *M. arenaria* (*Ma*), *M. incognita* (*Mi*) and *M. javanica* (*Mj*) respectively. St 100 bp DNA ladder.

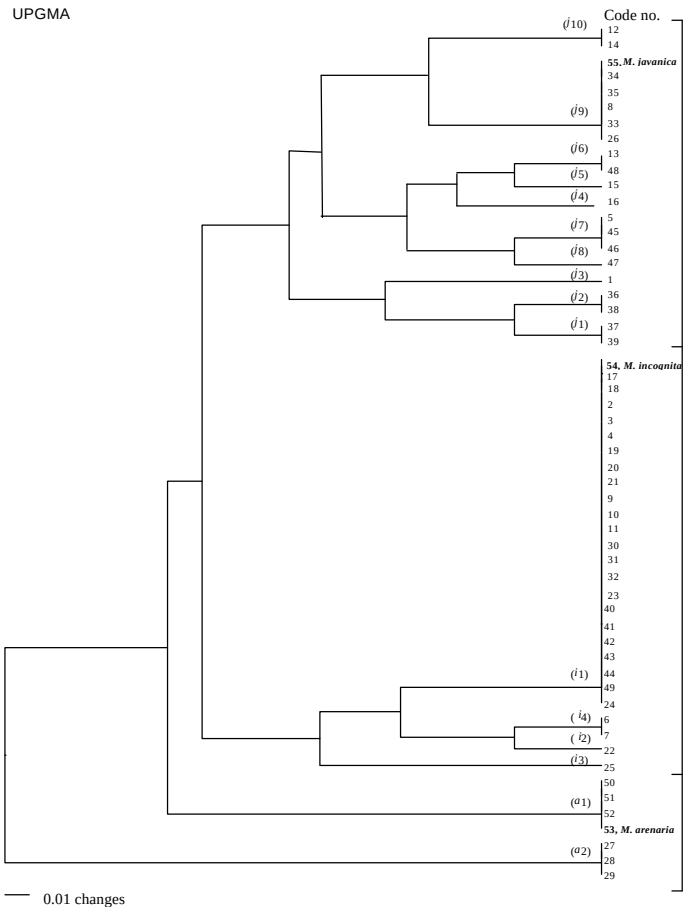

Fig. 3. Dendrogram illustrating IGS-rDNA based general relationships of individual root-knot nematodes with *M. arenaria*, *M. incognita* and *M. javanica*. Number within parenthesis correspond to genetic types in each species.

Conclusions

It is essential to use at least two methods to identify the species *M. arenaria, M. incognita* and *M. javanica* from grapevines. Sequences in D3 are highly conserved. IGS-rDNA-PCR method could be applied to the examination of intraspeific variation and potentially development of race specific diagnostic marker(s).

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