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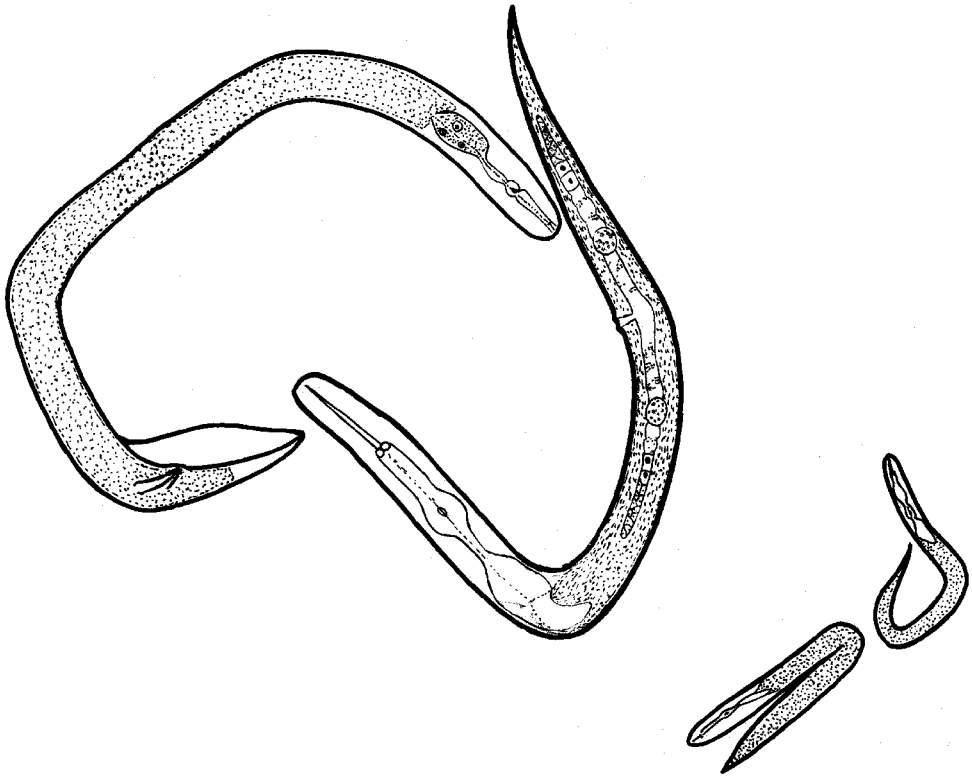
AUSTRALASIAN NEMATOLOGY NEWSLETTER



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From the Editor

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

July Issue
The deadline for the July issue is June 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 SECRETARY

GENERAL MEETING

7.25 pm, 25 SEPTEMBER 2001, CAIRNS

MINUTES

1. Minutes of the previous meeting in Canberra 1999 were presented by I. Riley.

2. M. Hodda presented a President's report, mostly discussing the request for financial support for IFNS meeting in Spain.

3. J. Lewis presented the Treasurer's report. The current bank account balance was $6199.62 and increase from $5,894.52 at the beginning of the period. Income was largely from membership dues. A payment of $800 was made to support the workshop in Cairns.

4. I. Riley presented a membership report. Membership stood at 73 with 43 financial members, 26 in arrears for less than one year and 4 for 1 to 2 years. Since, 1999 13 memberships were cancelled being more than 2 years in arrears, there were 19 new members, 1 death and 1 resignation. The system of payment by credit card though APPS had been used by 19 members and was appreciated. APPS has offered to continue the service next year.

5. Election of office bearers.

President – M. Hodda was elected unopposed.

Treasurer – J. Lewis was elected unopposed.

Secretary – I. Riley was re-elected unopposed.

Newsletter editor – J. Cobon was re-elected unopposed. However, Jenny has indicated that she won't continue beyond another term and a replacement Editor will be needed at the next meeting.

Committee member – No nomination for a NZ committee member to coordinate events associated with the conference was received.

6. Other business.

It was decided not to provide support for the IFNS congress as requested. M. Hodda will advise accordingly.

John Marshall provide a report for the IFNS meeting in Madrid. Matters included the request for funding for travel support for delegates from developing countries and affiliation with the International Federation of Plant Protection Societies.

The matter of declining support for nematology by Australia departments of agriculture and other research organisations was discussed. It was resolved that AAN should encourage APPS to join FASTS to support the lobby for science funding in Australia.

The AAN website remains at Queensland Museum but it was proposed to move it to CSIRO Canberra or the Waite Campus, Adelaide. It was decided that I. Riley should make arrangements for its move to Adelaide.

A vote of thanks was given to T. Pattison, G. Yeates and those that supported them in the running of a highly successful and appreciated workshop in Cairns.

Meeting closed at 7.55 pm.

Ian Riley

13TH APPS NEMATOLOGY WORKSHOP REPORT

A nematology workshop was held in conjunction with the 13th APPS conference in Cairns on September 24, 2001. The workshop was held at the Queensland Department of Primary Industries, Centre for Wet Tropics Agriculture at South Johnstone. The centre is located on the wet tropical coast in the middle of the banana and sugar cane production areas 80 km south of Cairns. The workshop was a slightly disrupted due to an airline collapse, but we were still able to get 17 participants all looking down microscopes at nematodes.

The Australasian Association of Nematologist's kindly contributed to the running of the workshop. Funds contributed by AAN were matched by Horticulture Australia which enabled a highly productive workshop increasing the skills of many of who attended by a renowned soil ecologist, Gregor Yeates.

Many thanks need to go out to Gregor Yeates and his wife Judy for their persistence with airline companies to make it to Cairns. Once they had arrived the return trip home to New Zealand was still not fully sorted out. Thanks to Gregor for the wonderful work he did in preparing for the workshop and explaining what the nematodes we were looking at were. The results of the nematode assemblages from the five different ecosystems investigated in the workshop is given in this edition. The investigation of soil ecology assemblages is gaining increasing importance as soil health becomes a more topical issue.

All participants at the workshop met again for the biennial meeting of AAN at a restaurant overlooking the Cairns inlet and were treated to a great seafood smorgasbord. Included in this edition are all the nematology papers submitted to the 13th APPS conference which I think highlights the diversity of nematology research in Australasia.

Thanks to everyone who attended the workshop and persisted with airline companies or found alternative ways of getting to Cairns for the nematology workshop.

Tony Pattison
13th APPS nematology workshop coordinator.

Workshop participants at QDPI, South Johnstone, September 2001.

FROM THE PRESIDENT

Another year over, another biennial meeting, another biennial nematodes "Identification & techniques" workshop (see incriminating photo following). Fortunately, it was not a case of another airline collapse -- there was only one (but it was an important one and great timing for the APPS conference organisers). The only thing that seems to go on forever are nematodes munching away at the roots of crops (and soil bacteria, and other nematodes, and the insides of insects -- but these are all other stories).

The biennial General Meeting was an opportunity to catch up with the people who made it (travel problems notwithstanding), but it is worth remembering that the major aim of the AAN is to encourage communication between people whose work involves nematodes. The general meeting is only one way that this occurs, the newsletter being

the other major means of communication between people. The articles need not be terribly formal, as any snippets that let people know of events or new findings will, I am sure, be of interest. The web site, when it is operational again, will hopefully also prove a useful means of communication, especially for those from outside the AAN. I hope people would agree that the AAN should be promoting communication about nematodes in all its forms. The AAN and its members can only benefit from interest in our subject.

With the major events for the society of the past 2 years reported at biennial GM, it is an opportunity now to look ahead. The membership of the AAN has remained more or less steady for quite a few years, which augurs well for interest in the subject on the ground. Interest in the various aspects of nematology at higher levels has, however, been variable. This is an issue for all nematology societies in the world, not just ours, so I am looking forward to discussions on this with the other nematology societies at the Fourth International Congress in 2002. Opportunities to swap ideas on promoting nematology should be one of the benefits of our affiliation with the international federation.

Finally, I also wish to again express the gratitude of the AAN to the office holders, particularly Jenny Cobon, John Lewis and Ian Riley, for their efforts for the past 2 years. May the next 2 years prove as smooth and productive.

Mike Hodda

PROPOSAL TO ESTABLISH THE AUSTRALASIAN NEMATOLOGY FOUNDATION

AAN recently received a request from the International Federation of Nematology Societies to sponsor a student to the next International Nematology Congress. The request was discussed at our general meeting in Cairns, but it was decided that we could not help because all AAN's funds are currently set aside for administrative purposes.

During the discussion, I indicated that we should consider establishing a fund that would enable us to respond positively to such requests in future. If AAN sought donations from retired and present members, funding bodies, chemical companies and other organizations involved in agriculture, and invested the money appropriately, the dividends could be used to further the science of nematology in Australasia. SON has already embarked on such a course of action in the U.S., with the N. A. Cobb Foundation raising more than $20,000 in its first year of operation.

The objectives and by-laws of the N. A. Cobb Foundation are presented in the 2000 Membership Directory of the Society of Nematologists. I believe we could do something similar but because AAN is relatively small, we may be able to operate with much simpler by-laws. I therefore propose that AAN set up a fund similar to the N. A. Cobb Foundation, and that it operates in the following manner.

Name of fund: Australasian Nematology Foundation (ANF).

Purpose: The Foundation would provide:

- grants to students for travel or to participate in nematology meetings
- support for special workshops, courses and programs that will improve communication amongst nematologists
- grants for worthy projects or publications
- support for any other activity that would strengthen the discipline of nematology in the Australasian region.

Organization: The Foundation shall function as a sub-committee of AAN and will be managed by a committee consisting of three AAN members. Members of this management committee must be senior members of the nematology profession, and will be elected at each general meeting of AAN. Their responsibilities shall be to seek contributions to the Foundation, invest its funds and expend interest moneys in a manner that fulfils the purpose of the Foundation.

Fund management: ANF funds shall be held in a separate account from the AAN administrative account and shall be audited at least once every two years. Funds shall be invested in a manner that ensures regular dividends are received and capital growth occurs. The management committee may expend interest moneys only. Expenditure of principal moneys shall require the approval of a majority of the AAN membership at a general meeting.

Before we can make a start, I need to know whether AAN members support the concept and whether the proposed management structure is appropriate. We will also need to find out whether regulatory authorities will allow AAN to incorporate the Foundation within its current organizational structure, recognize that it is a non-profit venture and agree that donations will be deductible for tax purposes.

Please take five minutes now to email me your thoughts. If you don't do it now, the issue will probably be forgotten. I need comments from a reasonable cross-section of the membership before we can proceed.

If I receive positive feedback from members and the organizational issues can be sorted out, I will put forward a formal proposal in the next newsletter. Rather than wait until the next general meeting, perhaps we can then arrange a postal vote to formally approve the proposal.

Graham Stirling
biolcrop@powerup.com.au

FOURTH INTERNATIONAL CONGRESS OF NEMATOLOGY

The details on the FICN Program, hotel availability and registration, guidelines on preparation of posters, and related materials are posted on the Federation/FICN Website

www.ifns.org/ifnscong.htm

Rather than using expensive mailings, the Federation and FICN will rely upon all nematologists to obtain FICN registration materials by visiting the above Website. Thus, your assistance with this endeavour will be much appreciated.

Ken Barker, President, International Federation of Nematology Societies (IFNS)

REPLACEMENT BOOK: ADVISORY SERVICES FOR NEMATODE PESTS

About 2 years ago AAN members received a copy of ' Advisory Services for Nematode Pests: Operational Guidelines'. The book was prepared on behalf of AAN by Graham Sterling, Julie Nicol and Frances Reay and was published by RIRDC.

Those who have used the book may have noticed that one section and several figures were missing. This was a mistake by RIRDC in the production and has now been rectified.

The amended version will soon be placed on the RIRDC website, where it can be downloaded for free. Alternatively, if you would like a replacement hard copy, please contact Carol Reeve at RIRDC. Her email address is

carolr@rirdc.gov.au

Graham Stirling

Regional News

NEWS FROM WESTERN AUSTRALIA

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

Zhaohui Wang has finished his Ph.D project on extraction of giant cell cytoplasm and analysis of the patterns of gene expression in giant cells by mRNA differential display RT-PCR, which resulted in his thesis entitled "Molecular Studies on Gene Expression in Host Plants Infected with the Root-knot Nematode *Meloidogyne javanica*". (The abstract follows this news item). He will submit his thesis before Christmas 2001. The first part of the results have been accepted for publication in the International Journal of Nematology (Vol.11: 219-225) "A novel approach to extract and analyse cytoplasmic contents from individual giant cells in tomato roots induced by *Meloidogyne javanica*".

Zhaohui Wang is now working with Mike Jones on an ARC funded project as a research assistant. *Arabidposis thaliana* has been chosen as the model system to be infected with *Meloidogyne javanica*, and the extraction of giant cell cytoplasmic contents from *Arabidopsis* is being undertaken. Global patterns of gene expression in giant cells will be examined using microarray technology with an Affymetrix GeneChip system.

A new member, Angela Hollams, joined the nematode group on May this year. Angela is a Ph.D student supported by a Murdoch University Postgraduate Scholarship. She is carrying out downstream analysis of a series of differential displayed cDNA bands isolated in the previous DD work. Specific expression of the identified genes in giant cells will be studied by promoter fusion analysis and *in situ* hybridisation.

Mike Jones was also successful in his application of an ARC linkage grant, submitted jointly with the senior nematologist, Dr Shashi Sharma, at the Department of Agriculture Western Australia. This project focuses on a novel approach to identification of nematode species using mass spectrometry.

The SABC has also been invited to be an international associate of the Centre for the Biology of Nematode Parasitism (North Carolina State University, USA), with collaboration with David Bird and colleagues.

NEWS FROM SOUTH AUSTRALIA

SARDI, Nematology Group

Michelle Russ, Margaret Wyszynski and Julie Lindner joined the Nematology Group at SARDI in June 2001. Michelle will be processing millions of soil samples in the next 3 years as we evaluate resistance and tolerance to *P. neglectus* in field trials across South Australia. Margaret assists with the laboratory screening of zillions of cereal lines for resistance to *P. neglectus* and *P. thornei* and has quickly mastered all of intricacies of DNA extraction. Julie assists with the production of our *Pratylenchus* and stem nematode inoculum and I'm sure has come to love carrots and all they mean to Nematology!

Big congratulations go to Danuta Szot who finished her Diploma in Biomedical Science. Its been a tough 4 years and we give a big cheer to Danuta for this achievement (and heartfelt thanks for the excellent job she's done while studying).

Sharyn Taylor and Vivien Vanstone attended the Soil Biology Workshop in Canberra. The workshop was remarkable for its paucity of nematode discussion and made us realise we need to educate the rest of the soil biologists about the wonderful world of nematodes (just as they succeeded in educating us on the finer points of soil physics, chemistry and furry roots).

Screening for resistance to cereal cyst nematode is almost complete for 2001, with harvest and assessment wrapping up in early December. This year, John Lewis, Milanka Matic and Tony Debicki (ably assisted by a casual staff of 10 at seeding and harvest) screened a total of 130,000 plants for the cereal breeding programs in South Australia and Victoria. Results are sent out to plant breeders in December/January and we are currently writing project proposals to continue this screening service as well as research into definition of resistance in field sites.

Sharyn Taylor, SARDI

Adelaide Univesity, Plant Science

Vivien Vanstone completed her 5 year period of Root Lesion Nematode GRDC funding at the end of December, accompanied by the obligatory Final Report prepared with Sharyn Taylor and Grant Hollaway. With the assistance of Sharyn's group at SARDI (particularly Michelle Russ and Brett Malic), trial work was wound up, and Michelle managed to mist and count all the samples by Christmas. A paper on yield loss in barley and oat due to *P. neglectus* was submitted to AJEA, and several more are gestating on the lounge room floor. Vivien was "out and about" during 2001, attending and presenting at the Cairns Conference, attempting to raise the profile of nematology (or at least mention the word "nematode") at the Canberra GRDC Root and Soil Biology Workshop, and attending the Mike Hodda/Kerrie Davies nematology short course in Canberra.

From February 2002, Vivien will be taking up the position of Senior Nematologist at WA Department of Agriculture. Projects will involve *Pratylenchus* (of course!)

management and resistance, plus investigation of the importance to WA cropping of stem, cereal cyst and burrowing (*Radopholus nativus*) nematodes. "Collaborative" visits to WA will be essential for many of you, I'm sure, and the spare room will always be ready!

Vivien Vanstone, Adelaide University

Adelaide Univesity, Applied and Molecular Ecology

Max Dewdney has joined the group to undertake research towards a PhD on *Pratylenchus* species interactions. The project is funded by GRDC. Max comes from from NZ with a background in ecology of fresh water invertebrates.

Working with Mark Potter, Tara Sallows has completed her honours thesis on glucosinolates, *Pratylenchus* and canola quality mustards (*Brassica juncea*).

Kerrie Davies has continued her peripatetic nematology with a field trip to Victoria in October collecting *Fergusobia*. Kerrie and Mike Hodda ran a short course on nematode identification in Canberra in early December. As with the previous course, there was strong interest from participants. This time there was an increased emphasis of nematodes of forest trees.

Imelda Soriano and Ian Riley won recognition for the most dedicated participants in the APPS conference in Cairns, having driven from Adelaide. Nematologists to the fore again, one way or the other. Ian also travelled to Taiwan to teach the nematology section of an international workshop on seed health testing.

Speakers at our campus-wide nematode discussion group for second semester were Greg Walker on associations between nematodes and carrot defects, Mark Potter with reports of his international travels, Max Dewdney on stream invertebrates and pastoral management in NZ and Motiul Quader on molecular identification of *Meloidogyne*. The year ended with a meal at the Eagle on the Hill, a favoured venue overlooking the Adelaide Plain.

We have also had some interesting nematology lab group meetings twice per month for most of the year. These provide a place for practice runs of seminars, discussion experimental plans and finding. A recent lively discussion was generated by Val Kempster on post-genomic nematology based on Grant and Viney (2001, IJP).

Ian Riley, Adelaide University

More news from South Australia

The rhizosphere microflora is to a plant, as people at rush hour is to New York City. We know that the rhizosphere, the zone of soil in close association with plant roots is rich in substances extruded from the roots, and this supports the growth of soil-dwelling bacteria and fungi. Where there are prey, there will be predators, and the bacteria and fungi that live within this very rich region compete for carbon or nutrients, and they provide food for flagellates, ciliates, amoebae and nematodes. Some nematodes, of

course, feed directly on the plant roots themselves, and so a very complex, multi-variate food web exists in this region. Sometimes nematodes in association with other microbiota can be disastrous, for example when infestation of cotton crops occurs with root-attacking nematodes and together with *Fusarium* wilt fungus, almost total crop failure can result.

With the advance genetic modification in plants there is potential to insert genes to express other compounds such as that expressed by root-knot-nematode-resistant cotton which inhibits the juveniles from developing into adults, and therefore arrests the spread of plant-pathogenic nematodes. There are already patents which describe *Bacillus thuringiensis* protein susceptibility in nematodes.

The project that I am currently involved with is examining the environmental effect of genetically modified crop plants on the microbiology of the rhizosphere. This includes nematodes, but in the context of their environment, and the interactions with all the other soil microbiota within the rhizosphere of crop plants.

So far I have learnt that to the untrained eye these blighters are remarkably similar, and won't stay still to have their photos taken. I think I have much to learn about them, but thankfully I have been able to call on Kerry Davies' nematode team here, and 'bug' Ian Riley occasionally for help with identification. Thanks to the members of this group for allowing me to sit in on their excellent lunchtime lectures.

Diana Walter, CSIRO.

NEWS FROM QUEENSLAND

Leslie Research Centre, DPI, Toowoomba

Rebecca Zwart and Jason Sheedy have impressed many with their presentation skills. Rebecca was awarded the prize for "Best student oral presentation" at the 10th Wheat Breeding Assembly held in Mildura, Victoria. Her talk, "Inheritance of root-lesion nematode (*Pratylenchus thornei*) resistance in synthetic hexaploid wheat" had a perfect mix of humour, good science and clear explanations. Jason, a previous winner of the same Wheat Breeding Assembly award, continued his successful oratorical ways and was awarded second place in the APPS Postgraduate Seminar Awards day for his talk "Wild relatives of wheat as a source of resistance to the root-lesion nematode *Pratylenchus thornei*."

Rebecca is off to Christchurch, New Zealand, to attend a statistical genetics course. She plans to return with impressive knowledge of analysis of quantitative trait loci. She has been awarded a tutorial scholarship by the organisers of the course, North Carolina State University and a GRDC-training award.

We welcome two "embryo" nematologists to our group. Jan Wood is working with Nikki Seymour to phenotype double haploid populations of bread wheat for molecular

markers of resistance to root lesion nematodes. Christine Donkin is working with Rebecca on molecular markers for disease resistance for the northern region.

Nikki and I thoroughly enjoyed the nematology workshop at the APPS conference in Cairns. No more excuses for not looking long and hard at those free-living nematodes.

We've had some good rain for summer crops after a very sad winter season on the Darling Downs. I have planted a summer crop experiment using several varieties of sorghum, maize, sunflower, millet, panicum, and soybean to look for crops that may actively decrease *P. thornei* populations.

Kirsty Owen

NEWS FROM CANBERRA

This photo illustrates the strength of the great diversity of viewpoints of nematologists from Australia & New Zealand. (As President of the AAN, I am naturally concerned with publicity for nematology, and so am the only one looking at the camera!)

Those of you wanting to see what Nuccia Eyres (3rd from right, middle row), and Lila Nambiar (2nd from right, middle row), did next will have to read the next newsletter.

News from the collection. Latest additions to the collection include some Stubby-Root nematodes (*Paratrichodorus*), and Stunt Nematodes (*Tylenchorhynchus*), as well as our old friends Root-Knot Nematode (*Meloidogyne*), and Root-Lesion Nematode (*Pratylenchus*). There have also been quite a few Aphelenchs from various genera. The Aphelenchs have been mainly focussed on associates of insects and conifers, but specimens associated with other habitats would be most welcome, because there are

some plant parasites which should be of concern as well as more innocuous species and I am proposing some work on identification guides to the group.

The power of identification guides depends on the range of material available for study. Which brings me back to the collection, and the plea to keep sending us material. It is only through building a the collection by including as much geographic, host crop and seasonal variation as possible, that the systematics and identification of nematodes can advance. Likewise it is the only way that we can recognise previously undiagnosed problems, and new threats identified. So when doing a study of any particular nematode problem, send us some specimens, so that we can add them to the collection where they will add to the data that will be the basis of future nematode systematics, identification, host and geographic records. As a specialist collection, we have the best possible curation equipment, expertise and a special purpose building for biological collections. We also have a separate unit creating specialist collection management software to ensure that the specimens are as accessible as possible. If you want to donate material, in whatever form (fixed or unfixed, mounted on slides or not, in pure or mixed culture), please contact me at the address at the front of the newsletter. The support of GRDC is gratefully acknowledged.

The photo below could be used for a funniest caption competion.

My suggestions: "This new *Bursaphelenchus* species really is dangerous! It's just eaten a hole in the bench";
or "Kerrie Davies illustrates the correct way to crack a joke without losing any of the specimens being handled at the recent nematodes short course".

Does anyone have any better suggestions?

Mike Hodda

Research

DIVERSITY OF QUEENSLAND NEMATODE ASSEMBLAGES B THE WORKSHOP SAMPLES

Gregor W Yeates and A (Tony) B Pattison
Landcare Research, Palmerston North, NZ and
Centre for Wet Tropics Agriculture, QDPI, South Johnstone, Qld.

Introduction

At the workshop on nematode diversity which the Association held at South Johnstone on 24 September 2001 participants looked at nematodes from five samples. This note summarises the analyses that we made in preparation for the workshop. We know of no similar previous analyses from Queensland.

Materials and methods

The samples were collected in the Innisfail area on 22 September. Each was made up of 12 cores 45 mm in diameter from 0-10 cm soil + litter depth, except for the bowling green from which there were 9 cores from 0-8 cm depth. The total counts presented are the mean of duplicate 250 g (fresh weight) samples extracted on trays with 0.25 litres of water for ~24 hours and then concentrated using a 25 micron sieve. After counting the samples were fixed with boiling 8% formaldehyde, bulked and then random specimens identified to nominal genera.

The *rainforest* sample was from ~60 year old regrowth on steep cut-over land at South Johnstone. The *intensive banana* crop (South Johnstone) regularly had trash removed from around the base of plants and received some 185 kg N per year (as urea and potassium nitrate). In the *sustainable banana* crop ("Pacific Coast Eco Bananas") trash is left around the plants and N inputs were about130 kg per year (60.6 kg since 1 Jan 2001 applied in various forms other than urea). In both cases cores were taken within rows of banana plants about 5 years old. The couch *bowling green* at the South Johnstone Bowling Club was known to be infected with Longidoridae. *Stabilised sand* was taken from under large, widely spaced trees at Etty Bay. The first three samples came from sites with similar, silty loam soil texture.

Results and discussion

- Nematodes were most abundant at the two most intensively managed sites, intensive banana and bowling green (Table 1). These sites also had the most plant-parasitic nematodes, with burrowing nematode (*Radopholus similis*) comprising 94% of the fauna under intensive banana.

- Relatively low numbers of bacterial-feeding nematodes were recovered; while the unusually dry conditions may have suppressed their populations not even juveniles were common - perhaps they passed through the single sieve. (Centrifugal flotation and sieving yielded and average of 1250 nematodes /250 g compared with 4110 /250 g obtained by the tray method.)

- Predacious Mononchidae were recovered from three sites, with the most being found in the bowling green which also had the most Cephalobidae (Table 1). Similar correlations between Cephalobidae and Mononchidae have been reported by Yeates & Wardle (1996) - whether the correlations are causal or reflect similar requirements in the two groups is unknown.

- Leptonchidae (a family of Dorylaimida which is regarded as fungal-feeding) were not recovered from bananas under either management regime (Table 1).

- A similar number of taxa was identified from all sites except the intensive banana (6 *vs* 13 -16) (Table 2). This difference is more marked when species richness (SR) is considered as this includes an adjustment for identification effort; many more specimens were identified from under intensive banana.

- The number of taxa identified is reflected in both the diversity indices (H', λ) and evenness (J').

- The ratio of bacterial-feeding nematodes to bacterial-feeding + fungal-feeding nematodes [B/(B+F)] is sometimes used to indicate whether the nutrient cycling at the site sampled is rapid (bacterial dominated; higher values for the ratio) or slower (fungal dominated; lower values). While the present samples had relatively low proportions of both bacterial and fungal-feeding nematodes and results must be treated with care, the two "least disturbed" (= lowest artificial input) systems (rainforest, stabilised sand) had distinctly lower values (Table 2).

- The overall Maturity Index (ΣMI) (which does not discriminate between "free-living" and "plant-parasitic" nematodes) had its greatest values at the rainforest and bowling green sites; the latter reflecting the abundance of Longidoridae.

- During the course of the workshop specimens of the genera *Falcihasta* Clark, 1964 (Dorylaimida: Belondiridae) and *Pakira* Yeates, 1967 (Araeolaimida: Leptolaimidae) were identified among the samples. These are both new records for Australia and will be duly recorded by Mike Hodda - both genera were first described from New Zealand.

Table 1. Abundance of total nematodes and various nematode groups per 250 g moist field soil (- represents 'not detected')

Vegetation	Nematodes	Plant parasites	Bacterial feeders	Rhabditidae	Cephalobidae	Mononchidae	Leptonchidae
Rainforest	2602	329	55	-	27	-	466
Intensive banana	9252	8666	176	176	-	-	-
Sustainable banana	1402	781	366	32	127	32	-
Bowling green	5112	2624	1040	-	995	497	271
Stabilised sand	2191	538	423	192	77	77	192

Table 2. Indices of the nematode assemblages. Equations for the indices are given by Yeates & Bird (1994) and by Yeates & Bongers (1999)

Vegetation	Specimens identified	Taxa identified	SR	H'	λ	J'	B/(B+F)	ΣMI
Rainforest	95	13	2.64	2.13	0.16	0.83	0.11	4.34
Intensive banana	158	6	0.99	0.35	0.87	0.20	1.00	3.04
Sustainable banana	88	16	3.35	1.95	0.24	0.70	0.96	3.06
Bowling green	113	13	2.54	1.97	0.20	0.77	0.79	4.05
Stabilised sand	57	16	3.71	2.56	0.09	0.92	0.58	3.05

Conclusions

Results from this limited sampling show differences in the nematode assemblages among sites similar to those found elsewhere. The difference between high and low input banana sites was marked. Differences in input strongly affect the nematode assemblage; diversity was markedly lower at the high input site, and there were many more plant-pathogenic nematodes at this intensively managed banana site.

Acknowledgements

We are grateful to DPI for use of their facilities for the workshop, to the sponsors of the 13th Australasian Plant Pathology Conference for support, and to Frank Sciacca for access to his banana fields.

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At the suggestion of Tony Pattison (QDPI, South Johnstone) the remainder of the research segment of this edition of our newsletter includes all the papers written by members of our association who attended the 13th Biennial Conference of the Australasian Plant Pathology Society in Cairns, September 2001.

Review

MOLECULAR STUDIES ON GENE EXPRESSION IN HOST PLANTS INFECTED WITH THE ROOT-KNOT NEMATODE *MELOIDOGYNE JAVANICA*

Murdoch University, 2001

Abstract from Zhaohui Wang's PhD thesis

Root-knot nematodes are economically important phytopathogenic endoparasites that invade more than 2,000 species of horticultural and crop plants in sub-tropical and tropical regions of the world, including Australia. Infection by root-knot nematodes induces the redifferentiation of provascular cells of the host roots into multinucleate feeding cells called 'giant cells', which are surrounded by a gall. Giant cells form by repeated mitosis without cytokinesis, and develop wall ingrowths typical of transfer cells. Giant cells act as sources of nutrients for the development of the nematode parasite, and break down after the nematode has completed its life cycle.

The pronounced morphological and physiological changes associated with infection by the nematode, especially in giant cells, are the result of altered gene expression in host root cells. Since giant cells are the only root tissue from which the nematode can feed, these giant cells are the subject of much molecular investigation on plant-nematode interactions. The study of gene expression in giant cells will provide new information on the host-parasite relationship, and lead to novel strategies for engineering host plant resistance.

mRNA differential display reverse transcription polymerase chain reaction (DDRT-PCR) was used to study changes in gene expression in giant cells during the compatible interaction between tomato (*Lycopersicon esculentum*) and the root-knot nematode *M. javanica* at 25 days post-inoculation. Methods of direct extraction of cytoplasmic contents from individual giant cells were developed using a modified pressure probe system. The giant cell origin of the extracted cytoplasm was confirmed by the presence of multiple nuclei in extracts after staining with different fluorescent dyes. mRNA was isolated from the extracted giant cell cytoplasm using magnetic Dynabeads, and its use to study gene expression in giant cells was evaluated by RT-PCR analysis. A series of experiments were undertaken to improve the sensitivity and reliability of DDRT-PCR in the gene expression studies. With a total of 44 primer combinations, 81 differentially displayed bands were isolated from differential display (DD) gels.

Although DDRT-PCR offers several advantages over other methods for the isolation of differentially expressed genes, it can also lead to artefacts. An efficient method was developed to identify true up- or down-regulated genes from a relatively large number

of DD bands being analysed. This involved single pass direct sequencing of the re-amplified DD bands, with the same anchor or arbitrary primers used in differential display reactions to generate these DD bands. Of the 81 DD bands, 27 produced readable sequences, of which 16 were selected for further analysis. Sequence specific primers for these 16 DD bands were designed and used to carry out real-time quantitative RT-PCR to re-confirm the differential expression of these genes and relatively quantify it in giant cells. The differential expression of the 15 genes, 14 up-regulated and 1 down-regulated in giant cells, were successfully re-generated by the quantitative RT-PCR assay. The expression of one gene, ZW30050025, was only detected in giant cell cytoplasmic contents but not in non-infected control, and so could be giant cell specific. Further study of this gene needs to be undertaken, since it could be used in engineering plant resistance.

Of the 15 differentially expressed genes, transcriptional regulation of ZW2703003 showed about 56-fold increase in giant cells compared to healthy root tissue at 25 days post-inoculation, and a 10-fold decrease for ZW1307002. However, in analysis of the time course of expression of these two genes, a dilution effect on the regulation of gene expression was observed when giant cell enriched tissue was used as starting material. These results indicate that the use of giant cell cytoplasmic contents will provide more accurate information on differential gene expression in giant cells, and so help understanding of the plant-nematode interaction.

The functions of the differentially expressed genes in maintenance and development of giant cells were predicated by database similarity searches. At the amino acid level, the deduced products of several genes, ZW0805001, ZW0903001, ZW0903002 and ZW30050020, shared strong identities with different types of ribosomal proteins. The up-regulation of these ribosomal protein genes, which in turn reflects the high translation activities, agrees with the predicted high metabolic activity in giant cells. Similarly, other genes encoding proteins with homology to those involved in high metabolic activity were also found to be up-regulated in giant cells. These genes included a Histone H3 gene (ZW0103005), a S-adenosylmethionine decarboxylase (SAMDC) gene (ZW0103003), and a cysteine synthase gene (ZW0103001). By using 5’ RACE, more sequence information of the coding region of genes ZW2703003, ZW1008005 and ZW1307002 was obtained. The deduced amino acid sequence of ZW1008005 showed 40% identity over 146 amino acid residues with the unique C-terminal region of TDY (Thr-Asp-Tyr) type mitogen-activated protein kinase, indicating a complex single transduction pathway involved in the function of giant cells. The down-regulated gene identified in this study, ZW1307002, shared very weak identity with metallocarboxypeptidase inhibitor (MCPI). The real role of ZW1307002, as well as the high up-regulated gene ZW2703003, remains to be determined by further investigation. Other genes identified in this study to be up-regulated included two genes probably involved in the pathogen responses, a cytochrome *c* reductase gene (ZW3107005) and a glyceraldehyde-3-phosophate dehydrogenase (GAPDH) gene (ZW0103004). Although further analysis is necessary to understand the significance of the altered regulation of these genes in relation to giant cell function, the results obtained in this study are new, and add to the known pattern of genes with altered expression in nematode feeding cells.

The results presented indicate that extracted cytoplasmic contents can be used as an appropriate starting material in DDRT-PCR analysis to identify differentially expressed genes in giant cells. The development of direct sequencing combined with real-time quantitative RT-PCR assay provides an efficient approach to verify changes of the transcripts level in giant cells, particularly when a large number of DD bands are being analysed. With more genes isolated and identified, a better understanding of the molecular events in giant cells in the plant-nematode interactions will be achieved, which should contribute to the development of new strategies to provide plant resistance against these pathogens.

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