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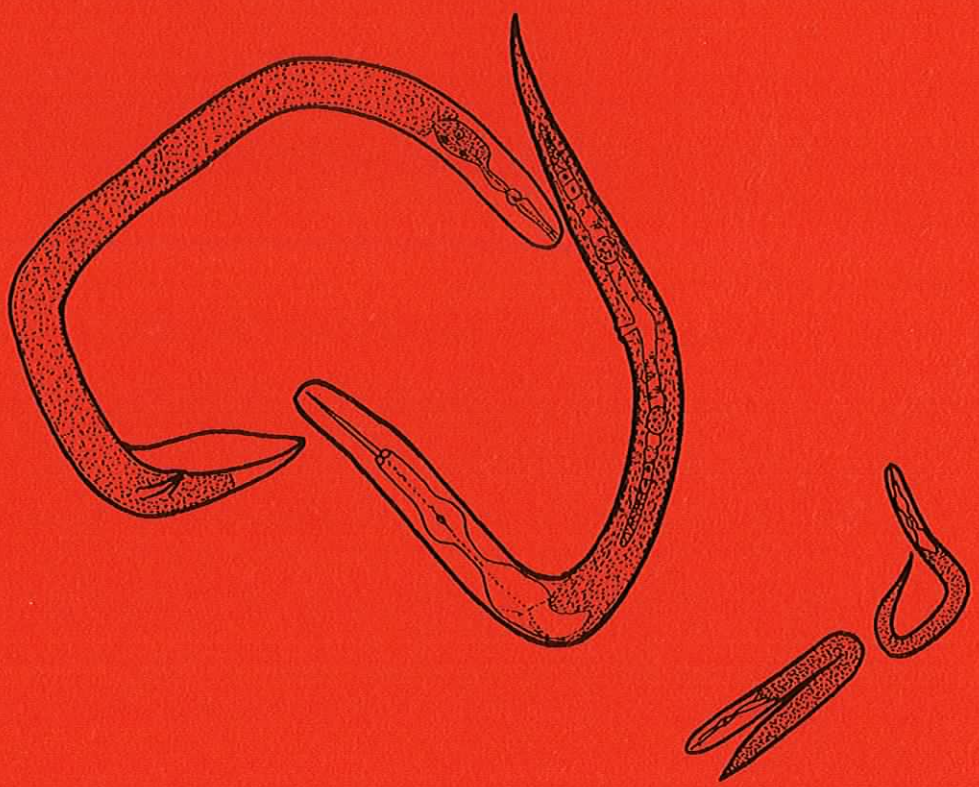
AUSTRALASIAN NEMATOLOGY NEWSLETTER



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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 20. 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
6.30 pm, 28 SEPTEMBER 1999, CANBERRA

MINUTES

1. Summary of previous meeting in Perth 1997 was presented by I. Riley.

2. J. Stanton presented a President's report (as attached).

3. In the absence of the Treasurer (R. Potter) a report was presented by I. Riley. The current bank account balance was $5,894.52 and there had not been expenditure since the last meeting. Deposits of membership dues by I. Riley had been $480 in 1999, $500 in 1998 and $180 in the last three months of 1997 i.e. following the meeting in Perth.

4. 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.

Committee member – T. Pattison was elected unopposed.

It was resolved that signatories for the Association's bank account be changed to M. Hodda, J. Lewis and I. Riley (any two to sign).

5. Membership report.

I Riley indicated the Association nominally had about 76 members of which 34 were financial, 18 were less than 1 year overdue and 24 that were more than 1 year in arrears. The matter of members in arrears was discussed and it was resolved to advise members more than 1 year in arrears that their membership be terminated.

6. Other business.

The association had been asked to provide suggestions for the scientific program for the Fourth International Congress of Nematology. Members were encouraged to make

suggestion to be provided to the committee by January 2000. AAN had been asked to provide funds to assist participants from developing countries to attend the Congress. It was felt that our income base was too small to be able to make a significant contribution and many were in effect contributing through SON. The association had been approached to consider hosting the congress in 2008. It was thought that Adelaide members could discuss this possibility.

Potential uses for current funds were discussed but no resolutions made.

The next nematology workshop will be held in conjunction with APPS in 2001 in Cairns. Tony Pattison offered to coordinate the workshop and suggested possible topics of marine nematodes or tropical plant parasitic nematodes.

Meeting closed at 7.20 pm.

AAN PRESIDENT'S REPORT – 1999

AAN has had another successful two years maintaining its financial membership at over 70, with about 80% from Australia and 10% each from New Zealand and other countries. We continue to produce two informative newsletters each year.

We now have a web site! It is hosted by the State Library of Queensland Community Web Publishing Project. It contains information about the society, newsletters, links and other interesting bits. The address is:

http://www.slq.qld.gov.au/cwpp/aan/

Thanks to Graham Stirling, Julie Nicol and Frances Reay, AAN has also completed and published its *Advisory Services for Nematode Pests: Operational Guidelines*. Free copies were given to each AAN member and additional copies are available from RIRDC.

For the fifth APPS conference in a row, AAN has put together a very useful workshop. Thanks very much to Mike Hodda, Felice Driver and John Curran for organising such a successful event.

AAN is now a member of the International Federation of Nematological Societies (IFNS). This puts us in a position to contribute to nematology internationally, but also imposes directions which we may not have envisaged previously. We are a small organisation with very few full-time nematologists so our collective effort will be much smaller than that of societies such as SON, ESN and ONTA. We should decide soon what our priorities are and how much we want to contribute to IFNS activities.

Thanks to all the committee members for their valuable contributions throughout the biennium.

Julie Stanton

FROM THE PRESIDENT

I would like to thank all those who came along to the recent General Meeting of the Association and nematology workshop in Canberra, held as usual in conjunction with the Australasian Plant Pathology Society Conference. I hope that everyone had a stimulating and productive time. The profile achieved by nematology, nematodes and nematologists within the larger APPS meeting was very gratifying and I hope bodes well for the future of our Association. The range of interests in nematodes, from plant pathology through taxonomy to ecology and free-living nematodes was also an indication of the strength of the discipline. Another indication of the interest in nematodes is that, by the time of publication, Kerrie Davies and myself will have presented courses on nematology to 25 students in Adelaide. We plan another course in about 2 years time, so please plan ahead and book early because we had to turn people away this time.

On other business: of the items discussed at the recent General meeting, one which I would like to follow up is the suggestion to recognise the achievements of the nematologists who have contributed so much to what we now almost universally take for granted. The idea, as I understand, is to have links from the International Federation of Nematology Societies web site to pages for each of these people. I think it is important for it to be recognised that Australia has a very strong history of nematology and nematologists. I am tempted to claim N.A. Cobb for Australia because he started his career in nematology at the distinctively Australian named town of Wagga Wagga not far from Canberra where I write. Of course Canberra was just a sheep paddock in Cobb's time. Cobb spent over several years here before moving on to Sydney and thence back to the US after more than 10 years in Australia. In the US he is credited with founding the study of nematology, and even inventing the term.

Cobb aside, I would like to suggest 3 people who in their respective fields have made immense contributions to both nematology in Australia and the society or its forebears. All 3 are now retired but continue to make contributions to nematology.

Bob Colbran: nematode taxonomist and pathologist who described many species and nematode problems from Queensland.

Alan Bird: nematode anatomist, physiologist and microscopist, who has made many and varied contributions. Alan was editor of this newsletter for many years and author of many books on nematodes.

Warwick Nicholas: nematode taxonomist, physiologist, and culture specialist, who has made many contributions to the study of free-living nematodes. Warwick was also a former editor of this newsletter and author of 2 books on free-living nematodes.

I welcome suggestions and comments on this proposal, and intend actually creating the pages early in 2000 if there are no strong objections.

Mike Hodda

ALAN BIRD

We regret to inform you of the death in December of our long standing member Alan Bird. A full obituary will appear in the next issue of our newsletter.

THE CHINESE SOCIETY OF NEMATOLOGISTS JOINS THE IFNS

Dear Dr. Liao,

It is a pleasure to officially welcome you and the Chinese Society of Nematologists as the latest Society to become affiliated with the International Federation of Nematology Societies. We received positive votes from all IFNS Councillors responding on your request with most Councillors voting.

We shall look forward to working with you and your associates as we pursue the goal of advancing the awareness of nematodes and their study worldwide. Thanks again for the information on your Society as related to our new IFNS Web Page. Safia Siddiqi, who has developed this program, may contact you for additional materials (such as photographs) for the Web Page.

Please feel free to contact any of your fellow IFNS Councillors at any time as questions arise, or if we can be of assistance. Again, we are delighted to have the Chinese Society of Nematologists affiliated with our Federation. Within a few days, I will E-mail updated lists of the Presidents and IFNS Councillors (with their addresses) for each affiliated Nematology Society.

Sincerely yours,

Kenneth R. Barker

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WELCOME TO NEW AAN MEMBERS

Since our last newsletter we welcome 13 new members

Prof Diana Wall (Colorado State University, Fort Collins, USA)
Ruabete Takaniko (Koroniva Agricultural Research Station, Nausour, FIJI)
Sidney Suma (Ruma Sugar Ltd, Lae. FIJI)
Frank Hay (University of Tasmania, Burnie, TAS)
Caroline Veersteeg (QDPI, South Johnstone, QLD)
Rebecca Zwart (Leslie Research Centre, Toowoomba, QLD)
Fiona Cooper (Agriculture Victoria, VIC)
Sean Kelly (Agriculture Western Australia, WA)
Scott Patton (Cyanamid Agriculture, Scarborough, WA)
Emma Broos (University Of Western Sydney, Richmond, NSW)
Iqbal Zahid (University of Sydney, Orange, NSW)
Leigh Schmitzer (Taree, NSW)
Nigel Bell (Rurakura Research Centre, Hamilton, NZ)

NEW WEB SITE ADDRESS

There is a new biocontrol (natural enemies) of nematodes web site that may be of interest to some

http://sacs.cpes.peachnet.edu/nemabc/

POSTGRADUATE (PHD) SCHOLARSHIP IN BRISBANE

Project: "Flyash as a beneficial soil amendment and antagonist to plant-parasitic nematodes in horticulture"

Project Summary

Nematode damage to crop roots and subsequent economic loss is significant. This project will study environmental and ecosystem impacts of power station flyash addition to soils, as there are indications that flyash suppresses nematode activity, thereby increasing plant growth and crop quality. Positive research outcomes will have environmental and economic implications for power generation and farming industries. The former will gain from a beneficial use of an otherwise waste product and the latter from better crop yields, as well as from reduced use of expensive and highly toxic chemical nematicides.

Applying for the scholarship

Expressions of interest are sought from graduates in chemical engineering, environmental engineering, environmental science and agricultural science who have a good honours degree and a strong interest in applying their knowledge to solving practical environmental problems. Under the conditions of the granting organisation, these positions must be filled by candidates who are Australian citizens or candidates holding permanent resident status who have lived in Australia for at least twelve months.

The project is funded for three years with a stipend of at least $26,000 per year. It is anticipated that the project will commence in January 2000. If you are interested in applying for this position, please send a brief curriculum vitae, include the names of at least two academic referees and indicate your residential status.

For further information about the project, contact Professor Ashley Scott at j.a.scott@mailbox.gu.edu.au, phone (07) 3875 3661, fax (07) 3875 5288. For more information about the centre, see www.ens.gu.edu.au/CIEP/CIEP.htm

Regional News

NEWS FROM CANBERRA

Whilst in Canberra, many of you saw some of the *Pratylenchus* specimens in the nematode collection, and the sort of interactive key that can be built from such a collection. The support of GRDC is essential in building and maintaining the collection, and deserves the thanks of all the nematologists who will benefit in their research, as well as the grain growers who are the ultimate beneficiaries.

Latest additions to the collection include some several interesting specimens. We have been receiving quite a few stubby-root nematodes (*Paratrichodorus*), root-knot nematodes (*Meloidogyne*), a few root-lesion nematodes (*Pratylenchus*) and some more mermithids (*Hexamermis*). The stubby-root nematodes are quite widespread in sandier soils. Perhaps one to watch for the future.

The mermithids were taken from wingless grasshoppers and locusts and may have some role in regulation of the numbers of the insects, with high levels of parasitism in some areas. The root-lesion nematodes are a welcome addition to the collection of *Pratylenchus* which is continuing to add data on the species in Australia and their occurrence. The root-knot nematodes are also a welcome addition for a genus which is economically very important.

Many nematologists saw the sorts of interactive keys that can be built using data from an extensive collection. Conversely, the power of keys 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 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 you are 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 below.

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NEWS FROM QUEENSLAND

Leslie Research Centre, DPI, Toowoomba

It's a hive of activity in Toowoomba. The field experiments are harvested and the glasshouse experiments are now ready to collect. We've been lucky enough to find a *Pratylenchus neglectus* site to complement the usual *P. thornei* experiments with wheat and chickpea.

Jason Sheedy impressed everyone at the 9th Assembly of the Wheat Breeding Society of Australia with his talk - "Tolerance of Australian bread wheat (*Triticum aestivum*) varieties to the root-lesion nematode (*Pratylenchus thornei*) in a yellow spot (*Pyrenophora tritici-repentis*) year". He was awarded the prize for best oral presentation by a postgraduate student and was congratulated on his clear and logical presentation.

Nikki Seymour has disappeared for a short while on maternity leave. She had a girl on 5th December, which was a little earlier than expected. She had intended to put the finishing touches on her PhD thesis before the baby arrived. Michelle O'Reilly is back helping with some of Nikki's work.

Jason, Ros Reen and Rebecca Zwart are all currently at the Nematode Workshop in Adelaide. They were all looking forward to improving their nematode identification skills and re-visiting the Russian restaurant.

I've been awarded my PhD from Sydney University on "Resistance responses of grapevine to root-knot nematodes". (The abstract is included in this newsletter). I attended the Australasian Plant Pathology Conference and the Acquired Resistance workshop in Canberra in September (thankyou to RIRDC for the funds). At the workshop, Novartis, the suppliers of the systemic resistance activator, Bion, presented an optimistic future for the use of induced resistance for protecting plants. It was also a great opportunity to put a face to my fellow inducer of resistance against nematodes, Valerie Kempster. My new job here at the Leslie Research Centre is a GRDC project on "Cropping options for control of root-lesion nematodes". I've established high and low populations of *P. neglectus* and *P. thornei* at two field sites and will plant various summer crops to answer some questions that farmers have been asking for a while now about crop tolerance. Glasshouse experiments on resistance and 'antagonisms' to *P. thornei* in winter and summer crops are also well underway.

Kirsty Owen

NEWS FROM SOUTH AUSTRALIA

Andreas Hensel was successful in obtaining his Ph D from the University of Berlin. His research was conducted in Adelaide and resulted in his thesis entitled "Investigations of *Rhabditis necromena* (Rhabditidae, Nematoda), *Ommatoiulus moreleti* (Julidae, Diplopoda) and tripartite interactions with bacteria in South Australia". Also, Valerie

Kempster plans to have submitted her PhD thesis "Induced resistance to clover cyst nematode" by the end of the year.

Kerrie Davies and Mike Hodda ran two well-received nematode identification workshops at the Waite Campus during December. The first workshop had about 15 people including three international participants, one each from PNG, Fiji and Vanuatu supported by ACIAR. The second course was designed specifically for quarantine plant pathologists having 9 participants. In addition to 24 people receiving quality nematode training, AAN recruited 7 new members.

In October, Astrid Schmidt commenced six months research on leaf gall *Anguina* spp. towards her Diploma thesis at the University of Bonn. Also, Prof. Bill Bowers from the Chemical Ecology Laboratory, University of Arizona is spending sabbatical at the Waite investigating the effects of plant defence chemicals on plant-parasitic nematode feeding and reproduction.

Wim Wouts, Landcare NZ, Auckland, spent two months collecting and extracting nematodes mostly in SA and WA, funded by GRDC, SARDI, Agwest and University of Adelaide. The prime aim being to examine species diversity in *Pratylenchus* and *Radopholus* in agricultural soils and nearby native vegetation in southern Australia. In addition to collecting a lot of prats, Wim managed to find what appear to be two or more previously unrecorded species of cyst nematodes and many other nematological treasures.

Ian Riley

NEWS FROM WESTERN AUSTRALIA

News from WA State Agricultural Biotechnology Centre (SABC) Murdoch University, Western Australia – Mike Jones and colleagues

Major areas of research are in the molecular basis of host-parasite relations of endoparasitic nematodes (root-knot and cyst-nematodes). These include:

1. **Confocal Laser Scanning Microscopy of GFP expressing transgenic *Arabidopsis* with enhancer trap tagged cell lineages on infection with root-knot nematodes**

   In this work, transgenic *Arabidopsis thaliana* plants, in which specific cell lineages within the root have been tagged with the gene for Green Fluorescent Protein (GFP), have been used to study changes in gene expression in host root cells on infection with *Meloidogyne javanica*.

   A culture system has been designed to allow high resolution study of individual host-parasite relationships for up to 5 weeks from infection, using confocal scanning laser microscopy to look at GFP expression in giant cells and other surrounding tissues. Twelve lines of transgenic *Arabidopsis* out of 122

available have been studied in detail. Both up- and down- regulation of GFP expression has been observed.

For example, in one line in which only endodermal cells fluoresced green with GFP, infection with *M. javanica* resulted in switching off GFP expression around giant cells, but nowhere else in the root. One interpretation of these results is that there is no functional endodermis between giant cells and the surrounding gall tissues.

In another line in which the GFP expression was limited to phloem cells in control roots, there was very strong up-regulation of GFP within giant cells.

This approach promises to provide new information on changes in patterns of gene expression on infection of roots with nematodes. Because the transgenic plants were produced by enhancer trapping, it should also be possible to isolate the control sequences responsible for the changes of GFP expression on infection with nematodes. We are looking for support to continue this line of work.

John Blinco, Rob Potter and Mike Jones

2. Use of transgenic plants to study gene expression in roots infected with *M. javanica*

(i) *Adh-gus*

Transgenic *Arabidopsis* plants containing the *gus* reporter gene and *adh* (alcohol dehydrogenase) promoter have been studied after infection with *M. javanica*. The results indicate strong up-regulation of *gus* expression during egg laying. This gene is also up-regulated in non-infected roots by hypoxia, and this result supports the view that the partial pressure of oxygen in giant cells is reduced when there is maximum demand for nutrients by the associated nematode. These reduced oxygen levels leads to up-regulation of *gus* expression in *adh – gus* plants.

(ii) Auxin responsive and chalcone synthase promoters

The expression of the auxin responsive promoter *(GH3)* fused to the *gus*A reporter gene in white clover, *Trifolium repens* cv. Haifa has been studied during the initiation of root galls by root-knot nematodes (*Meloidogyne javanica)* to investigate whether nematode infection affects auxin distribution in developing galls. In a search for a plant signal that would mediate changes in auxin location we studied the induction of the flavonoid pathway because flavonoids can act as auxin transport regulators. Three chalcone synthase (*CHS1, CHS2* and *CHS3*) promoter:*gusA* fusions were examined in transgenic plants and flavonoids were detected using fluorescence microscopy. Within 24 hr post inoculation, *CHS:gusA* expression occurred around the invading nematode. At 48 hr post inoculation, *CHS:gusA* expression and flavonoids

were detected throughout the infection site, followed by high *GH3:gusA* expression in the gall 48-72 hr post inoculation. Initially (48-72 hr post inoculation) high *GH3:gusA* expression in giant cell precursors was followed by low expression in the enlarging giant cells (96-120 hr post inoculation), suggesting that auxin is needed as a trigger for giant cell initiation but not for later enlargement. It is suggested that nematodes control auxin distribution in the root and that flavonoids could be responsible for controlling auxin accumulation.

Pokkwan Hutangua, Ulrike Mathesius, Mike Jones and Barry Rolfe (Murdoch/RSBS/ANU)

3. Use of fluorescent dyes to study changes in transport properties around giant cells.

The phloem mobile tracer, carboxymethyl fluorescein has been used to study changes in tracer accumulation on infection of intact *Arabidopsis* seedlings with *M. javanica* and *Heterodera schachtii*.

In intact seedlings, this fluorescent dye, introduced into cotyledons and leaves, moves as two fluorescent files down the phloem of the root. Without nematode infection, the fluorescence remains confined to sieve elements and is off loaded at the root tip, as determined by epifluorescence microscopy and confocal scanning laser microscopy. With nematode infection, the fluorescent tracer accumulates rapidly in the feeding cells, and can also enter the nematodes (this is usually the case for *M. javanica*, and occurred only rarely for *H. schachtii*).

The question posed here is whether there are sufficient plasmodesmata to allow symplastic off loading of the tracer at feeding sites, or whether the permeability of the cell membranes is altered at the infection sites such that the tracer can cross the membranes directly. The microscopic distribution of plasmodesmata suggests the latter explanation.

Pokkwan Hutangura and Mike Jones

4. Molecular studies on gene expression in giant cells.

(i) **DD-RT PCR**

Following Differential Display RT-PCR, comparing giant cell enriched and control root tissues, 12 up- or down- regulated transcripts have been identified. Their expression has been studied by Southern blotting and quantitative RT-PCR. One transcript found is of nematode origin, and this is being studied further. Up-regulated transcripts of plant origin have high homology with a cucumber basic blue protein and a gibberellic acid biosynthesis enzyme.

Audrey Ah Fong, Rob Potter and Mike Jones

(ii) **Gene expression in giant cell cytoplasm.**

A method has been developed to extract the cytoplasm directly from individual giant cells and to carry out DD-RT PCR from these cells to identify genes up- and down- regulated. A method has also been developed to carry out quantitative RT-PCR to look at relative levels of transcripts in giant cell cytoplasm compared with non-infected tissue. Actin mRNA is used to normalize mRNA levels for comparative results. This approach promises to provide new information on giant cell function because giant cell contents themselves are analyzed.

Zhao-Hui Wang, Rob Potter and Mike Jones

Research

SUSCEPTIBILITY OF WEEDS TO *PRATYLENCHUS NEGLECTUS* AND *P. THORNEI* (PART II)

Vivien Vanstone and Michelle Russ
Department of Plant Science, University of Adelaide, Waite Campus

Grass weeds (Poaceae) have been assessed for ability to host *P. neglectus* and *P. thornei* (Vanstone and Russ 1999). Complementing this study, a further 19 species from 9 families (18 dicotyledons and 1 monocotyledon), have now been tested. These weeds are common to crops, pastures and fallows in southern Australian broadacre dryland cropping systems (Table 1).

Methods

Seeds were germinated in Petri dishes on filter paper moistened with distilled water, or in seedling trays of moist UC soil mix. Petri dishes were placed at 5°C for 2 days, followed by 20°C for 2-3 days. Depending on plant type, seedling trays were kept at room temperature for 7-10 days; 5°C for 2 weeks followed by room temperature for 4 weeks; or room temperature 4-6 weeks.

Germinated seeds or transplanted seedlings were grown singly in 700ml plastic pots (17.5cm high, 9cm diam. at the top, tapering to 6cm base diam.) without drainage holes. Soil was a pasteurised (70°C for 45 mins.) sandy loam. Pots were placed in an ambient temperature glasshouse in controlled temperature waterbaths to maintain soil temperature at 20°C.

Plants were inoculated with 2000 (*Pi*) nematodes extracted from carrot cultures. Six replicates of each weed sample were grown, plus susceptible wheat (Machete) and moderately resistant triticale (Abacus) cereals for comparison.

Eight weeks after inoculation, soil was washed from the roots, and nematodes extracted by misting (*Pf*). Nematode multiplication rates (*Pf*/Pi) over the 8 week period were calculated.

TABLE 1. Weeds assessed for susceptibility to *Pratylenchus neglectus* or *P. thornei*.

(Bedstraw and onion weed have a fibrous root system, whereas all the other species tested have a taproot.)

Weed species	*Common name(s)*	*Family*	*Life span*
Arctotheca calendula	Capeweed, Cape dandelion	ASTERACEAE	Annual
Asphodelus fistulosus	Onion weed, Wild onion, Asphodel	LILIACEAE	Perennial
Bifora testiculata	Bifora, Carrot weed	APIACEAE	Annual
Brassica tournefortii	Long-fruited turnip, Wild turnip	BRASSICACEAE	Annual
Carrichtera annua	Ward's weed	BRASSICACEAE	Annual
Chondrilla juncea	Skeleton weed	ASTERACEAE	Perennial
Diplotaxis muralis	Sand rocket, Wall rocket, Goat weed	BRASSICACEAE	Perennial
Emex australis	Three-cornered Jack, Doublegee	POLYGONACEAE	Annual
Galium tricornutum	Bedstraw, Cleavers	RUBIACEAE	Annual
Hypochoeris radicata	Dandelion, Catsear, Flatweed	ASTERACEAE	Perennial
Lactuca serriola	Prickly lettuce, Milk thistle	ASTERACEAE	Annual / biennial
Malva parviflora	Marshmallow, Mallow	MALVACEAE	Annual / biennial
Oenothera stricta	Evening primrose	ONAGRACEAE	Perennial
Picris echioides	Ox tongue, Bugloss	ASTERACEAE	Annual
Raphanus raphanistrum	Wild radish, Wild mustard; Charlock	BRASSICACEAE	Annual
Rapistrum rugosum	Short-fruited turnip, Wild turnip	BRASSICACEAE	Annual / biennial
Rumex crispus	Curled dock, Sour dock	POLYGONACEAE	Perennial
Sisymbrium orientale	Indian hedge mustard, Wild mustard	BRASSICACEAE	Annual
Tribulus terrestris	Caltrop, Bindii, Puncture vine	ZYGOPHYLLACEA	Annual

Results

P. neglectus *(Table 2)*

Twelve of the weeds did not host *P. neglectus* (*Pf/Pi*=0.1-0.9). Six weeds (Ward's weed, marshmallow, curled dock, three-cornered Jack, long-fruited turnip, caltrop) were poor hosts (*Pf/Pi*=1.3-1.9). Only wild radish (*Raphanus raphanistrum*) was classified as a good host (*Pf/Pi*=2.3).

P. thornei *(Table 3)*

None of the tested weeds hosted *P. thornei* (*Pf/Pi*=0.03-1.0).

TABLE 2. Multiplication rates (*Pf/Pi*) of *Pratylenchus neglectus* in weeds, and difference in weed *Pf/Pi* from cereal controls.

Weed species	*Pratylenchus neglectus* *Pf/Pi* [a]	SE [b]	Difference from cereal	
			Machete	Abacus
Skeleton weed	0.1 a	0.05	-2.8 ***	-0.8 ns
Dandelion	0.1 ab	0.03	-2.8 ***	-0.8 ns
Onion weed	0.2 abc	0.03	-2.7 ***	-0.7 ns
Evening primrose	0.3 abc	0.07	-2.6 ***	-0.6 ns
Short-fruited turnip	0.3 abc	0.06	-2.6 ***	-0.6 ns
Ox tongue	0.3 abc	0.13	-2.6 ***	-0.6 ns
Bifora	0.5 abcd	0.07	-2.4 ***	-0.4 ns
Short-fruited turnip	0.5 abcd	0.15	-2.4 ***	-0.4 ns
Sand rocket	0.5 abcd	0.09	-2.4 ***	-0.4 ns
Bifora	0.6 abcde	0.07	-2.3 ***	-0.3 ns
Bedstraw	0.6 abcde	0.11	-2.3 ***	-0.3 ns
Indian hedge mustard	0.7 abcde	0.13	-2.2 ***	-0.2 ns
Prickly lettuce	0.7 abcde	0.04	-2.2 ***	-0.2 ns
Capeweed	0.7 abcde	0.11	-2.2 ***	-0.2 ns
Prickly lettuce	0.8 abcde	0.17	-2.1 ***	-0.1 ns
Indian hedge mustard	0.9 abcdef	0.24	-2.0 ***	0 ns
Ward's weed	1.3 abcdefg	0.40	-1.6 **	0.4 ns
Marshmallow	1.5 bcdefg	0.35	-1.4 **	0.6 ns
Curled dock	1.5 cdefg	0.26	-1.4 **	0.6 ns
Three-cornered Jack	1.5 cdefg	0.13	-1.4 **	0.6 ns
Long-Fruited Turnip	1.7 defg	0.39	-1.2 *	0.8 ns
Caltrop	1.7 defg	0.32	-1.2 *	0.8 ns
Long-fruited turnip	1.9 efg	0.32	-1.0 *	1.0 ns
Wild radish	2.3 fgh	0.72	-0.6 ns	1.4 **
Wild radish	2.3 gh	0.56	-0.6 ns	1.4 **
Abacus	0.9 abcdef	0.14	-2.0 ***	-
Machete	2.9 h	0.46	-	2.0 ***

ns not significant; * significant at $P<0.05$; ** significant at $P<0.01$; *** significant at $P<0.001$

[a] Values followed by the same letter do not differ significantly at $P<0.05$; species were classified as a non-host (resistant, $Pf/Pi<1.0$), poor host (moderately resistant, $1.0<Pf/Pi<2.0$) or good host (moderately susceptible/susceptible, $Pf/Pi>2.0$).

[b] Standard error of mean.

TABLE 3. **Multiplication rates (*Pf/Pi*) of *Pratylenchus thornei* in weeds, and difference in weed *Pf/Pi* from cereal controls.**

Weed species	*Pratylenchus* *Pf/Pi* [a]	SE [b]	Difference from cereal control	
			Machete	Abacus
Evening primrose	0.03 a	0.01	-5.8 ***	-2.3 ***
Skeleton weed	0.1 a	0.02	-5.7 ***	-2.2 ***
Dandelion	0.1 a	0.04	-5.7 ***	-2.2 ***
Three-cornered Jack	0.2 a	0.04	-5.6 ***	-2.1 **
Ox tongue	0.2 a	0.04	-5.6 ***	-2.1 **
Short-fruited turnip	0.2 a	0.04	-5.6 ***	-2.1 **
Short-fruited turnip	0.2 a	0.07	-5.6 ***	-2.1 **
Sand rocket	0.2 a	0.05	-5.6 ***	-2.1 **
Bedstraw	0.3 a	0.08	-5.5 ***	-2.0 **
Capeweed	0.3 a	0.04	-5.5 ***	-2.0 **
Prickly lettuce	0.4 a	0.06	-5.4 ***	-1.9 **
Onion weed	0.4 a	0.09	-5.4 ***	-1.9 **
Ward's weed	0.5 a	0.09	-5.3 ***	-1.8 **
Long-fruited turnip	0.5 a	0.06	-5.3 ***	-1.8 **
Long-fruited turnip	0.5 a	0.08	-5.3 ***	-1.8 **
Indian hedge mustard	0.5 a	0.11	-5.3 ***	-1.8 **
Wild radish	0.5 a	0.07	-5.3 ***	-1.8 **
Prickly lettuce	0.6 a	0.09	-5.2 ***	-1.7 **
Curled dock	0.6 a	0.08	-5.2 ***	-1.7 **
Caltrop	0.7 a	0.13	-5.1 ***	-1.6 *
Indian hedge mustard	0.7 a	0.07	-5.1 ***	-1.6 *
Bifora	0.7 a	0.20	-5.1 ***	-1.6 *
Bifora	0.7 a	0.10	-5.1 ***	-1.6 *
Wild radish	0.8 a	0.12	-5.0 ***	-1.5 *
Marshmallow	1.0 a	0.34	-4.8 ***	-1.3 *
Abacus	2.3 b	0.24	-3.5 ***	-
Machete	5.8 c	0.62	-	3.5 ***

ns not significant; * significant at $P<0.05$; ** significant at $P<0.01$; *** significant at $P<0.001$ [a] Values followed by the same letter do not differ significantly at $P<0.05$; species were classified as a non-host (resistant, $Pf/Pi<1.0$), poor host (moderately resistant, $1.0<Pf/Pi<2.0$) or good host (moderately susceptible/susceptible, $Pf/Pi>2.0$).
[b] Standard error of mean.

Conclusions

- The nematodes were unable to survive in most of the weeds. Initial inoculum density was not maintained. *P. thornei* did not multiply, and *P. neglectus* multiplied in only 7 of the 19 species tested.

- Wild radish (*R. raphanistrum*) was the only weed classified as a good host to *P. neglectus*. This weed may be particularly important as a nematode host, since plants can continue to grow after crop maturity and harvest (Cheam and Code 1998).

- Many significant weeds are members of the Brassicaceae. Some of these were poor or good hosts of *P. neglectus*, but none hosted *P. thornei*. Similarly, cultivated varieties of *Brassica napus* (canola) are susceptible to *P. neglectus*, but moderately resistant to *P. thornei* (Vanstone *et al.* 1998).

- Susceptibility to root lesion nematode was not related to plant family, root type, root texture, or life span of the plant.

- Most grasses that have been tested were also poor or non-hosts of *P. neglectus* and *P. thornei* (Vanstone and Russ 1999). Only barley grass (*P. thornei*) and wild oat (*P. neglectus*) were hosts. Although wild turnip is a good host to *P. neglectus* (*Pf/Pi*=2.3), wild oat was far more susceptible (*Pf/Pi*=3.1-10.9) to this nematode.

- If susceptible weeds are not controlled, management practices implemented by growers to protect crops from root lesion nematodes will be undermined.

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DEVELOPMENT OF *PAECILOMYCES LILACINUS* AS A BIOCONTROL AGENT - SPECIFICITY TO NON TARGET ORGANISMS.

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The nematode egg parasitic fungus, *Paecilomyces lilacinus*, Bioact strain 251, is currently undergoing development as a biocontrol agent against plant parasitic nematodes. The main thrust of research during the past year has been to address some environmental concerns.

A biocontrol agent needs to be specific to its target pest. Research has concentrated on specificity testing of plants and invertebrates. Vertebrate toxicology studies for this fungus have been completed elsewhere with every indication that *Paecilomyces lilacinus* is safe for mammals.

Plant roots

A previous histological study of the interactions of the fungus *Paecilomyces lilacinus* with the nematode *Meloidogyne incognita* on tomato roots, suggested that *P.lilacinus* colonised root tissue (Cabanillas *et al*, 1988). To confirm this report of invasion of plant tissue by *P. lilacinus*, a selection of plant species have been challenged with *P. lilacinus* in a series of replicated experiments. A detailed study on one tomato variety was undertaken, where tomato roots were exposed to *P.lilacinus* in pots, on agar plates and in root-tissue culture, and a more general study on a range of other plants (6 additional tomato varieties, cotton, capsicum, wheat, barley, potato, banana and pineapple). An additional study was undertaken with banana, where *Radopholus similis* was also added to some pots to test whether the fungus would enter roots which had been damaged by this nematode. Plants were tested with the fungus at approximately ten times the normal field dose. No fungal hyphae have been detected within the roots of any of the plants tested. Roots infected with *Rhizoctonia solani* did have internal hyphae, which demonstrated that detection methods were adequate.

Invertebrates

A selection of invertebrates has also been tested for specificity. These were entomopathogenic nematodes (2 species), earthworms, slaters, brine shrimp, collembola, termites, cockroaches, beetle larvae, fly pupae, paper-nest wasps, and ants. Two doses of *P. lilacinus* were tested; a low dose comparable to expected exposure in the field, and also a high dose at 10 or 100 times the low dose. Only termites (*Heterotermes ferox)* and ants (*Camponotus intrepidus)* at the 100 times exposure showed significant mortality after 2 weeks. Termites had no significant mortality after one week but 66% mortality at the high dose after 2 weeks. At the low dose there was no significant difference to control treatments which had no *P. lilacinus* added. Ants had no deaths after one week but at 2 weeks had 22% mortality at the 100x dose. All other invertebrates tested had either no deaths or a non-significant result.

When Paecil is applied to soil for treatment of nematodes, very moderate doses are used. For example, on tomato, 1g containing 2 to 5 x 10^9 viable spores is applied to each plant

and would be distributed around the plant. Some spores would move with water down into the top layer of soil. It is unlikely a termite or ant would be exposed to many spores simultaneously after application to soil. As invertebrates could only be exposed to low spore numbers, it can be concluded that *P. lilacinus* is not a threat to the invertebrates tested at normal field doses.

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PROGRESS IN THE APPLICATION OF NEMATOPHAGOUS FUNGI – *PER OS*

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Background

Although the use of nematophagous fungi as 'a new approach to the eelworm problem' was advocated in C.L. Duddington's (1957) *The Friendly Fungi*, their application to plant pathogenic nematodes has not proved easy. In 1991 Graham Stirling (1991 p9) wrote 'there are still no widely accepted examples of the contrived use of an antagonist to control a plant parasitic nematode', and neither Evans *et al.* (1993) nor Perry & Wright (1998) described practical examples. While fungal pathogens of cyst-forming nematodes have provided more fertile ground (e.g. Kerry, 1995; Bourne & Kerry, 1999; Pyrowolakis *et al.*, 1999) that area, like the use of nematode-trapping fungi for migratory stages, has not seen any generalised, practical application. In population terms, the problem is one of dispersing, and maintaining, adequate numbers of infective propagules of the nematophagous agent throughout the potential range of the target species.

Practical use of entomopathogenic nematodes has been more successful, but according to Kondo & Kaya (1998: p77) "... although nematode infection may successfully occur, the insect cadavers do not always produce nematode progeny, and the nematodes fail to establish and recycle in the soil environment." "Reapplication is often needed," and "... their use will be restricted to high value crops or to areas where chemical pesticides cannot be used due to environmental or human health concerns."

In both these groups of potential biological control agents, small populations are present in the environment but successive, inundative releases appear to be needed for successful biological control. That is, to over-ride the barriers which have evolved.

The *Duddingtonia flagrans* story to date

Duddingtonia flagrans is a predacious fungus for which isolations have been reported from the environment in Australia (NSW, Queensland, WA, SA), Canada (Ontario, Nova Scotia), England, Denmark, France, India, Malaysia, Mexico, Russia and the USA (California). Some systematists place *D. flagrans* in the genus *Arthrobotrys*, a widely studied genus of the nematophagous hyphomycetes. They trap nematodes by means of specialised hyphal structures. *D. flagrans* forms adhesive three-dimensional hyphal nets in which nematodes are trapped, and produces large numbers of thick walled resting spores (chlamydospores).

D. flagrans can be cultured on appropriate substrates, the chlamydospores extracted and fed to sheep, cattle, horses and other grazing animals by incorporation with various feed supplements. The chlamydospores pass through the intestinal tract, apparently unscathed, and germinate once the faecal material is deposited. The resulting mycelium with its 'traps' has proved effective in reducing the number of 'infective larvae' of gastro-intestinal nematodes which survive to migrate to herbage and thence to re-infect grazing animals. An introductory account of such applications of nematophagous fungi is given by Larsen & Faedo (1998).

In a grazing trial in New South Wales, delivery of *D. flagrans* to sheep via salt-licks provided control of gastro-intestinal nematodes comparable with that from a conventional drenching regime (Dick, 1996). There are several publications describing successful control of nematode parasites of calves, cattle, horses and sheep. (e.g. Wolstrup *et al.*, 1994; Dick, 1996; Fernández *et al.*, 1997, 1999). In August and September 1999, I visited trials using *D. flagrans* for control of gastro-intestinal nematodes of sheep and cattle presently being conducted in Denmark and Sweden.

Systems for delivering chlamydospores to grazing animals have to be developed for a range of farming systems and the efficacy of parasite control confirmed under diverse climates, stock management and parasite burdens. However, use of *D. flagrans* to overcome both environmental concerns about anthelmintic use and the rapidly emergence of nematode resistance to the three currently used families of anthelmintics certainly looks promising.

Is this the silver bullet livestock farmers want?

- If the trapping is essentially a physical, density-dependent process, as long as chlamydospores continue to be administered, it seems that significant reduction of 'infective larvae' on herbage and thus of parasite burden will continue to be achieved.
- Inclusion in a livestock management regime should permit relaxation of the parasite control component of grazing patterns that were part of farm management before the advent of drenches.

What are the potential problems?

- *Duddingtonia* could affect populations of other nematodes and organisms. A study of the New South Wales trial mentioned above showed no detectable changes in the taxonomic or functional composition of the soil nematode fauna during the first year

of that trial (Yeates *et al.*, 1997). Such studies need to be repeated under different soil and grazing regimes. As such trials will in effect normally be on sites with a history of drench use, it will be necessary to distinguish between those effects due to application of *Duddingtonia* and those due to the withdrawal of drenches.

- That *D. flagrans* has already been reported from 10 countries suggests that it is already widespread in the environment and that its use as a biocontrol agent is unlikely to have any additional impact on non-target organisms.
- As long as repeated inundative release is required there seems little potential problem.
- If farm management practices are modified to achieve 'natural on-farm cycling' of *D. flagrans*, those conditions are likely to be so specific that they do not present an environmental risk. Any 'escape' would presumably just merge into the low-level population already present.

What are the messages for plant nematologists?

- Successful biological control can be achieved when resistant propagules (chlamydospores) are delivered (*per os*), thoroughly mixed with the media (in the gastro-intestinal tract) and germinate in the medium (dung) before competing saprophytic fungi arrive.
- Perhaps, the 'thermal treatment' in the gut puts *D. flagrans* at a temporary competitive advantage in organic-rich substrates. Manipulation of the microflora in seedbeds and rooting media may be worth investigating. Although it has not yet been reported from mineral soils, incorporation of *D. flagrans* chlamydospores into pasteurised propagating mix could be a starting point.

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Review

"RESISTANCE RESPONSES OF GRAPEVINES TO ROOT-KNOT NEMATODES"

University of Sydney, 1999.

Abstract from Kirsty Owen's PhD thesis

The resistance of glasshouse-grown grapevines to a mixed population of *Meloidogyne javanica* and *M. incognita* was investigated.

The first part of the thesis described experiments testing the stability of resistance of the grapevine rootstock, Ramsey (*Vitis champini*), against root-knot nematodes at high temperatures. There was no reproduction by the nematodes in Ramsey grapevines grown at 30 and 32°C or 32/ 27°C day/ night temperature and it was thus concluded that its resistance was stable at the tested temperatures.

Part 2 describes the effect of application of Bion WG50, a well characterised activator of systemic induced resistance, to Cabernet Sauvignon grapevines (*Vitis vinifera*) inoculated with root-knot nematodes. In all experiments Bion WG50 was applied to the foliage of the grapevines at the rate of 50 μgmL^{-1} active ingredient (a.i.).

Before thorough assessment of the activity of Bion WG50 in the slow growing grapevines, a preliminary set of experiments using tomato seedlings was designed. Bion WG50 did not cause any change in nematode penetration, development or egg production in tomatoes.

A single treatment of Bion WG50 applied one week before or at the same time as inoculation of 4-week-old or 10-week-old grapevines grown at 19–24°C or 16–21°C, respectively, caused a substantial decrease in nematode egg production 10 weeks after inoculation. Further investigations demonstrated that while nematode penetration of the treated grapevines was not affected 72 hours after inoculation, the total number of nematodes found in the roots of treated plants 3 or 4 weeks after inoculation was significantly less and there were also fewer mature nematodes. Bion WG50 did not cause an effect on nematode egg production when 8-week old plants, grown at 19–24°C, were treated.

The following chapter in Part 2 described experiments in which Bion WG50 was applied directly to nematode eggs or juveniles (at the rate of 1–50 μgmL^{-1} a.i.) to test for toxicity. The effect on egg hatching and mortality of the juveniles was assessed, then the treated nematodes were inoculated onto tomato seedlings, and penetration, egg production and the number of males that developed was assessed. There was no direct

effect by the active ingredient in Bion WG50 on the nematodes at any of the stages tested.

The next chapter in Part 2 described the effect of application of Bion WG50 (50 µgmL^{-1} ai) to 4- or 8-week-old Cabernet Sauvignon grapevines on ß-1,3-glucanase activity in the leaves and roots. There was a small increase in activity in the roots of 4-week-old treated grape vines 5 days after treatment, and in the leaves 7 days after treatment. The greatest increase in ß-1,3-glucanase activity was found in the leaves 28 days after application. No change in ß-1,3-glucanase activity was detected in 8-week-old grapevines 7 days after application of Bion WG50.

The final chapter of the thesis discussed the results from Part 2 with reference to other studies of systemic induced resistance. It was concluded that systemic induced resistance was most likely activated by Bion WG50 application to grapevines and that the activated resistance may affect the physiology of giant cells and thereby nematode feeding and development in the roots. This is the first report of decreased infection by a nematode after treatment of grapevines with a chemical inducer of resistance.

INVESTIGATIONS OF *RHABDITIS NECROMENA* (RHABDITIDAE, NEMATODA), *OMMATOIULUS MORELETI* (JULIDAE, DIPLOPODA) AND TRIPARTITE INTERACTIONS WITH BACTERIA IN SOUTH AUSTRALIA

Abstract from Andres Hensel's PhD thesis

The diplopod *0. moreleti* is a species accidentally introduced in Australia, where it has developed large populations and can be a severe nuisance, mainly by invading houses in large numbers in the south-eastern part of Australia. A rhabditid nematode, *R. necromena,* indigenous to the Adelaide region (South Australia), can be ingested by *0. moreleti* and enter its haemocoel. Previous work suggested that the nematode can kill the diplopod by bacterial induction of septicaemia. As a follow-up of previous work on the potential of this nematode for biological control of *0. moreleti* and its large-scale application in the Adelaide region in 1989, this study investigated the distribution of *R. necromena* in this region, its temporal development in populations of *0. moreleti,* its putative effect on the host and the role of bacteria in such an interaction. The following results were obtained:

A survey of 46 sites in the Adelaide region and on the Fleurieu Peninsula revealed that nematodes occurred in the haemocoel of all populations of *0. moreleti* during winter and that the *R. necromena* is widely distributed. Analysis of the temporal development of the nematode-infection in *0. moreleti* up to 18 months at 20 sites in the Adelaide region showed that the infection undergoes seasonal changes. The prevalence and the intensity of nematode-infection in the populations of *0. moreleti* increases during winter and decreases in spring and summer. In autumn, when *0. moreleti* has its main activity, only a few nematodes were found in some populations.

Subsequent investigations of the reasons for these seasonal changes revealed that *O. moreleti* can eliminate *R. necromena* juveniles from the haemocoel during the moults of this diplopod. Adult *O. moreleti* have two annual moulting periods in the Adelaide region. In this study, *R. necromena* was isolated from the soil during summer, suggesting that infections of *O. moreleti* in winter may come from a few aestivating dauer juveniles in the soil. *R. necromena* has potential for rapid population growth which could lead to rapid colonisation of the soil and subsequent infection of the host populations through ingestion by detritivorous diplopods like *O. moreleti*. A facultative association between R. *necromena* and *O. moreleti* may be possible.

In order to confirm an adverse effect of *R. necromena* on *O. moreleti*, laboratory and field tests were conducted and population densities of *O. moreleti* compared with the level of nematode infection in the Adelaide region. Laboratory experiments did not show a dose-response curve even with high nematode numbers found in the haemocoel. More than 1000 nematode juveniles were frequently recovered from the haemocoel of a single, living *O. moreleti*. Field trials over three months at two sites in the Adelaide region also showed a lack of effectiveness of *R. necromena* in causing mortality to *O. moreleti*, even at high densities of nematodes and diplopods. Comparison of population densities of *O. moreleti* with the level of nematode infection at 10 sites in the Adelaide region gave no correlation between population densities of the diplopod and either prevalence or intensity of nematode-infection.

In order to investigate the reasons for lack of adverse effects, the significance of bacteria in this interaction was further investigated. Surprisingly, only one bacterial type was found to be carried by *R. necromena* into the haemocoel of *O. moreleti*. Cluster analysis of gas chromatographic profiles of fatty acid methyl esters (GC-FAME) showed that probably the same bacterial species were carried by *R. necromena* into the haemocoel at widely separated sites in the Adelaide region. At five out of six sites, bacterial isolates clustered within the same level of criteria used, indicating the same subspecies. These bacteria belong to the Enterobacteriaceae.

Both injection of these bacteria into the haemocoel and topic application with the nematode showed that bacterial isolates differed significantly in pathogenicity to *O. moreleti*. One isolate was genetically labelled with green fluorescent protein (GFP) and was used to show that infective dauer juveniles of *R. necromena* do not retain the bacterium in the intestine and that the bacteria did not specifically attach to the cuticle. This suggests that there is no specific association between *R. necromena* and bacteria found together with the nematode in the haemocoel of the diplopod.

In order to further investigate the possible origin of these bacteria from *R. necromena* from the diplopod's haemocoel, bacteria from the intestine of *O. moreleti* were further studied. Adult *O. moreleti* can retain bacteria in the midgut during the moult. Cluster analysis of GC-FAME profiles of these bacteria showed that *O. moreleti* carries similar bacteria in its alimentary tract to those isolated from the nematode juveniles. Preliminary genetic characterisation of one of the predominant isolates, using sequence analysis of the 16S rDNA, indicates that a new species of the Enterobacteriaceae may have been isolated which occurs in the gut of the diplopod as well as in facultative association with the nematode.

These results suggest that a predominant bacterial component in the alimentary tract of *0. moreleti* is carried into the haemocoel by the nematode *R. necromena* in the Adelaide region. Interestingly, the bacteria appear to differ in pathogenicity to the diplopod depending on the site from where the diplopod was recovered. Such differences in pathogenicity are discussed together with the results obtained by previous authors suggesting that *R. necromena* had potential to control *0. moreleti.* Further investigations are required to explore possible adaptive changes in the gut bacteria of *0. moreleti* to become less pathogenic in the haemocoel, where diplopods enter an environment with nematodes penetrating the morphological and physiological barrier between intestine and haemocoel.