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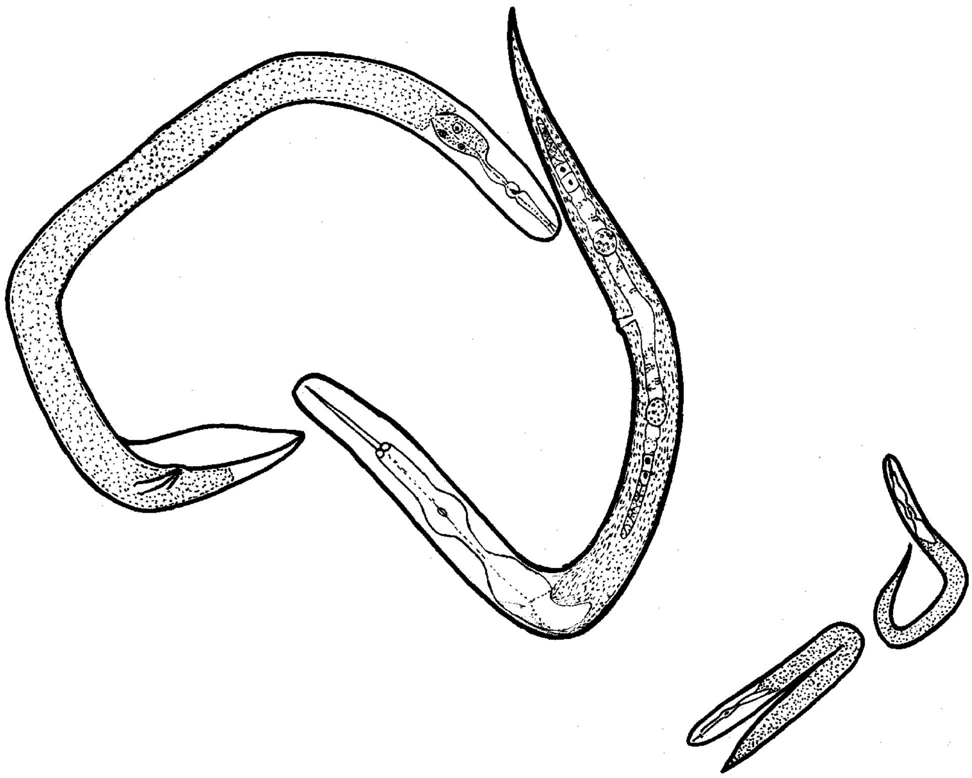
AUSTRALASIAN NEMATOLOGY NEWSLETTER



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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 will be June 15th. I will notify you a month in advance so please have your material ready once again.

Contacts

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

Dr Ian Riley
Secretary, Australasian Association of Nematologists
Department of Applied & Molecular Ecology
University of Adelaide — Tel: (08) 8303-7259
PMB 1 — Fax: (08) 8379-4095
GLEN OSMOND SA 5064 — Email: iriley@waite.adelaide.edu.au

Mr John Lewis
Treasurer, Australasian Association of Nematologists
SARDI, Plant Pathology Unit — Tel: (08) 8303 9394
GPO Box 397 — Fax: (08) 8303 9393
ADELAIDE SA 5100 — Email: lewis.john@saugov.sa.gov.au

Ms Jennifer Cobon
Editor, Australasian Association of Nematologists
Department of Primary Industries — Tel: (07) 3896 9892
80 Meiers Road — Fax: (07) 3896 9533
INDOOROOPILLY QLD 4068 — Email: Jennifer.Cobon@dpi.qld.gov.au

Association News

WORKSHOP NOTICE

Review of nematode resistance screening/breeding in Australasia

A Workshop of the Australasian Association of Nematologists

Friday, 6 February 2004

Plant Research Centre, Waite Campus, Urrbrae, South Australia

The program will commence with a keynote presentation by Dr Roger Cook – a global overview on development of nematode resistant crops – followed by a series of short presentations by individuals actively involved in screening/breeding for nematode resistance in Australasia. The focus will be the practicalities of screening including background, approach, successes and challenges. The culmination of the workshop will be a group discussion on ways to improve current practice, overcome constraints and set objectives.

Proposed program

8.45	Welcome	
9.00	Keynote presentation	Roger Cook, UK
10.00	Clover-*Heterodera*	Chris Mercer, NZ
10.30	Morning Tea	
11.00	Medics-*Pratylenchus*,	Rachel Hutton, SA
	Cereals-	Sharyn Taylor, SA
	Heterodera/*Pratylenchus*/*Ditylenchus*	John Lewis, SA
	Field crops-*Pratylenchus*	Grant Hollaway, Vic
12.30	Lunch and tour of campus and facilities	
2.00	Wheat-*Pratylenchus*	John Thompson, Qld
2.30	Tropical crops-*Meloidogyne*/*Radopholus*	Tony Pattison, Jenny Cobon, Qld
3.00	Potato-*Globodera*	John Marshall, NZ
3.30	Break	
4 00	Review and general discussion	
5.00	AAN meeting	
6.30	Dinner - Eagle on the Hill	

Registration

Register through Australasian Soilborne Diseases Symposium
(http://www.plevin.com.au/ASDS2004)

Fees: $95 ($65 concession- retired, students), GST inclusive

Register by 15 December 2003

Sponsors

Grains Research and Development Corporation
http://www.grdc.com.au/

South Australia Research and Development Institute, Field Crops Pathology
http://www.sardi.sa.gov.au/field_crops/pathology_quarantine/pathology_quarantine.htm

University of Adelaide, Plant and Pest Science
http://www.agwine.adelaide.edu.au/research/plant

Australasian Soilborne Diseases Symposium 8-11 February 2004
http:// www.plevin.com.au/ASDS2004

Convenors

Sharyn Taylor (08 8303-9381, taylor.sharyn@saugov.sa.gov.au); Ian Riley (08 8303 7259, ian.riley@adelaide.edu.au)

FROM THE PRESIDENT

Fresh from a stimulating week in Adelaide at the nematodes course, I thought I would be a little philosophical in this newsletter on the subject of nematode taxonomy. The reason for this is the special request we had at the course to discuss nematode taxonomy. (Apologies to those on the course who did not find it so interesting, and to those who are uninterested now.)

Nematode taxonomy has been in considerable flux almost since day 1, when Linnaeus placed what we think were nematodes in the genus *Chaos*. One could argue that the name never should have changed. Recently, there have been moves to change many of the higher taxa (Classes, Orders, Families etc, even down to genus), based on molecular evidence. One of the aims of taxonomy is to maintain stability of names, so that the same thing is called the same name unless there is a very good reason for changing it. Imagine having to call me Mike one week, Bruce the next, and George the week after that! This is not merely an academic point. Part of the work that I do for the Australian Biological Resources Study, Plant Health Australia and AQIS is to record the species of nematodes found in Australia. How these are named, catalogued and retrieved depends on the classification of nematodes used. I have adopted a fairly conservative classification which will maintain many of the existing well-known (and loved!?) names. As these lists grow, I am endeavouring to include all names nematode species and higher taxa are known by, so that these can be searched electronically, and the names become more interchangeable. However, there are moves to insist on the new names, which people may find confusing.

There are also currently moves to shift to a different code of nomenclature, which will be uninomial with a single name for each taxon (as opposed to the current binomial code where there are both genus and species names quoted). This proposal has some merits for organisms such as nematodes where taxonomy is still uncertain in many areas, but is being strongly opposed on the grounds that changes such as those proposed will lead to instability of names. Do not expect anything to happen quickly, but I think it is worthwhile to keep people informed of what is going on, so that people with opinions or experiences can be heard. The AAN does have a voice (albeit a small one) at the foray considering changes such as these, through the International Federation of Nematological Societies, the Australasian Plant Pathology Society, and individual members (who have contacts with the International Commission on Zoological Nomenclature).

On a more social note, I hope to see as many members as possible at our rescheduled biennial meeting, which is on Friday 6th February in Adelaide. This is always a good chance to catch up on what is going on, swap ideas and the venue for the dinner, Eagle on the Hill, has been the haunt of nematologists from Adelaide for some time, so I hope we can expect a very pleasant evening. Items can be added to the agenda if there are issues which people would like discussed, or failing that, issues can be brought forward at the meeting under "any other business". There is also a day long workshop on breeding earlier on the Friday.

Mike Hodda.

42ND ANNUAL MEETING OF THE SOCIETY OF NEMATOLOGISTS

The 42nd Annual Meeting of the Society of Nematologists attracted 251 registrations, a satisfying 44% of the membership. The meeting was held in the extensive 290-ha Cornell University, Ithaca, NY, a campus of classic buildings, large trees and wide lawns. The grass areas had distinct single-genotype patches of white clover – mostly medium-leaf types with few leaf marks and … but enough of that for now.

The symposium and paper session on resistance were the draw card for me. Ben Mathews, USDA-ARS, Beltsville, MD, reviewed his microarray comparisons of non-infected or SCN-infected soybean roots at 0, 6, 12 hr and 1, 2, 4, 6 and 8 days after infection. The metabolic profiles at each time revealed a precision I hadn't seen before. His talk was a hit with the non-specialists as he explained how the microarray technique had been used, and illustrated the description by holding red and green torches. Reporting on the same nematode/host association, G. Lu, from Pioneer Hi-Bred Inc., described two promoters peaking in reaction at less than one week after infection. Even greater detail of the nematode secretion/plant response interaction came from Sadia Bekal et alia, University of Illinois, who have characterised the SCN oesophageal gland protein, chorismate mutase (CM). They presented an auxin suppression model indicating how CM may suppress host resistance. The gene encoding CM was probably acquired by horizontal transmission.

In the biocontrol session, Brian Kerry, BBSRC, Rothamsted, brought us up to date with the Cuban efforts to use *P. chlamydosporia* to control root-knot – he showed phyllograms of the relationship of Pc isolates and described the efforts to improve production and application methods. Harry Kaya, UC Davis, reviewed the whole field of biological control in nematology – timely as the opportunities for the use of pesticides in soil in the USA are diminishing. In the subsequent discussion session on the range of control options available, there was agreement that most soft nematicides don't work but that the negative results are never published. There was general concern on the lack of publication of zero findings leading to continual re-invention of the wheel! Industry, especially, e.g. turf managers, need to know what not to buy. Publication of a Journal of Irrereproducible Results was proposed by a delegate but someone replied that the Annual Supplement of the Annals of Applied Biology and the Fungicide and Nematicide Test publications already go some way to meeting that need.

The social life of the meeting was nurtured by a local bar with 52 beers on tap! The wine tour visited four wineries in the Finger Lakes region – at lunch I enjoyed hearing John Webster describe a 1959 Society of European Nematologists meeting addressed by such notables as Bingefors, Goffart, Oostenbrink, Seinhorst and Fredy Jones. But the top tale of the meeting was from the West Virginian who arrived at his shared room late in the evening and found his room mate asleep, so he went to bed quietly himself. In the morning, his room mate took herself out to the corridor immediately on finding a fully bearded male nematologist in the other bed! They conversed through the closed door and resolved that the late comer would sort out the administrative error and find another room.

Some new occurrences reported were *M. graminicola* in Florida, a new *Longidorus* sp. on pine seedlings, *Meloidogyne mayaguensis* in Florida, and *M. haplanaria* n.sp. on peanuts in Texas.

Several projects are preparing industries for the withdrawal of MeBr, e.g. a paper by Judy Thies on nematode control in pepper, but the final decision on MeBr is a political one yet to be made.

The 2004 SON Meeting will be held in Estes Park (near the Rocky Mountains National Park) the 2005 meeting will be held in Fort Lauderdale, FL, and hooray, the 2006 one will be held in Hawaii.

Chris Mercer, AgResearch Grasslands, Palmerston North, New Zealand.

PLANT-PARASITIC NEMATODE CD-ROM

As some of you may know, I have been putting together a CD-Rom on the plant parasitic nematodes of Australia. I can now announce that this is nearing completion and will be ready for purchase in the next two months. There will be a formal launch at the Soil Borne Disease Symposium in February 2004, but it is available before that.

It is set up as a Web Site and is written in HTML and so can be accessed by any internet program (ie Microsoft Internet Explorer). It is separated into three main sections: Techniques, Nematodes and Crops (Vegetables, Grains and Sugarcane). It give information about processing and identifying nematodes as well as which species have been recorded on what crop and some methods of control.

It is available for $20, which is at cost and is restricted to vegetables, grains and sugarcane as these are the industries that have supported the production of the CD-Rom.

If you would like to order a copy, get in touch with Dr. Jackie Nobbs at e-mail nobbs.jackie@saugov.sa.gov.au or phone (08) 8303 9626.

Jackie Nobbs.

NEMATODE SIEVES

I recently purchased two 20 micron and two 38 micron stainless steel sieves (200mm) direct from Glenammer Engineering Ltd, Scotland, at a total cost of about $1200 (at current exchange rates, air freight included). Quotes from Australian suppliers for a single 20 micron sieve were $1200 or more. If AAN members are looking to obtain sieves for collection of nematodes, I suggest they look at http://www.glenammer.com. My purchase was made on-line with credit card payment. Supply was initially delayed, as the firm needed to obtain additional 20 micron mesh, but once the sieves had been made, delivery was prompt. The quality of the product delivered looks excellent. I investigated US suppliers and although cheaper than local suppliers, none matched Glenammer at the time. Although it should go without saying, even though the products suited my needs, others must make such judgments for themselves and not act on my recommendation alone.

Ian Riley.

AUSTRALASIAN NEMATOLOGY FOUNDATION

This issue was first discussed at our general meeting in Cairns in 2001, after we received a request from the International Federation of Nematology Societies to sponsor a student to the International Nematology Congress. I then put forward a proposal (in the January 2002 edition of the newsletter) to establish a Foundation that 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.

The idea was to seek donations from retired and present members, funding bodies, chemical companies and other organizations involved in agriculture and invest the money appropriately. Income from dividends and capital gains would be used to further the science of nematology in Australasia. ANF was to be modelled on the Cobb Foundation in the USA. Anyone who has read the September 2003 issue of SON's Nematology Newsletter will be aware that this Foundation has started very successfully. Its initial fundraising target was $100,000 in five years, and with more than one year to go, it has already raised $80,000. The first Endowments from the fund were offered this year.

The enquiries I have made suggest that a Foundation of this nature could be set up in Australia as a Charitable Trust. ATO approval would be required to ensure contributions were tax deductible and earnings were tax free. The Trust would be administered by a Board of Trustees, and would need to be audited annually.

My current feeling is that considering the amount of money likely to be contributed to ANF in the short to medium turn, a Charitable Trust would be prohibitively expensive. Perhaps we could start in the following way.

1. Agree to establish the Australasian Nematology Foundation at the general meeting of AAN to be held in Adelaide in February. Decide on its objectives and elect three senior members to administer the Foundation. Consider transferring some of the funds in AAN's administrative account (e.g. $1000) to the Foundation to get it started.
2. Establish a separate bank account within AAN to hold funds contributed to ANF. AAN is currently registered with the ATO as a non-profit unincorporated entity, but this probably does not give us the right to offer tax-deductibility for donations.
3. Request that the committee elected to administer ANF make recommendations on the most appropriate structure for ANF prior to the next general meeting of AAN.

The most important issue to be addressed is whether there is general support for establishing a Foundation. I received almost no feedback from members when this proposal was first put forward in the newsletter.

If there is interest in proceeding, members must be found who have the commitment to make it happen. I am willing to be involved and Barry Thistlethwaite has shown an interest, but is anyone else willing to help?

Graham Stirling
graham.stirling@biolcrop.com.au

Regional News

NEWS FROM WESTERN AUSTRALIA

Vivien Vanstone, Sean Kelly & Helen Hunter

Department of Agriculture WA

vavanstone@agric.wa.gov.au

Vivien, Helen & Sean: Melbourne Cup 2003 (as if Nematology needs an excuse to wear silly hats!!).

The (mostly) serious stuff:

- **Trials**

  At sowing, bulk soil samples were taken from the Statewide CVT (Crop Variety Testing) trials, Cereal Breeding sites, large-scale field pea demonstrations, and pasture trial sites. These samples were provided by the various groups involved in sowing the trials (once we had educated them in the art of soil sampling for *Pratylenchus*). They must have realised how easy it really was, as some of them are already talking about "*when we do this next year*….", and one member of the cereal breeding team is determined to invent a soil sampling device to supersede the trowel.

  The *Golden Trowel Award* was instigated for the cereal breeding team taking the sample with the highest Prat count at sowing, and the *Prat of the Year* award for the Research Support Unit (RSU) collecting the CVT trial sample with the highest count. *Prat of the Year* for 2003 is Merredin RSU for 32 *P. neglectus*/g at Narembeen, and *The Golden Trowel Award* goes to the Blue cereal seeding team for 16/g dry soil *P. penetrans* at Narrogin. The prize consists of one carton per nematode, although this is still under negotiation.

- **Some Results**

WA 2003

***Pratylenchus*/g dry soil at sowing**	**Total number of sites**	**Number of sites with…….**				
		P. neglectus	***P. teres***	***P. penetrans***	**[A] Mixed species**	**[B] No i.d.**
>10	5 (3%)	2	-	1	2	-
3 – 10	14 (8%)	9	-	-	5	-
1 – 2	16 (9%)	12	3	-	1	-
<1	80 (44%)	48	12	-	4	16
0	67 (37%)	-	-	-	-	-
	182	71 (39%)	15 (8%)	1 (0.5%)	12 (7%)	16 (9%)

A "Mixed species" = *P. neglectus* &/or *P. teres* &/or *P. brachyurus*? &/or *P. coffeae*? &/or *P. penetrans* &/or *P. thornei*

B "No i.d." = no adults present for identification

Of the 5 sites with >10/g dry soil at sowing, one had 32/g *P. neglectus*, 2 had 12/g *P. neglectus*/*P. teres* mixed, and the site with *P. penetrans* had a population of 16/g dry soil. Wheat, barley and oat roots at the *P. penetrans* site were severely damaged, and preliminary investigation of the data indicates end of season *P. penetrans* levels are still in the range of 10 – 30 nematodes/g dry soil.

P. neglectus, followed by *P. teres,* was the species most frequently identified.
P. thornei was identified at only 3 of the sites, mixed with either *P. neglectus* or *P. teres*.

- **Sean's Stupendous Solo Sampling**
Intensive end of season plot sampling was conducted for a range of variety evaluation trials, based on the identity and populations of *Pratylenchus* determined for samples taken at sowing.

Sites with a range in densities for either *P. neglectus* (0.4 – 11.6/g dry soil at sowing) or *P. teres* (0.4 – 4.4/g dry soil at sowing) were chosen for each crop species. Samples for varieties and breeders' lines were taken to determine genotypic differences in nematode multiplication over the season (as an indicator of relative resistance/susceptibility), and to compare the two main species of *Pratylenchus* identified. Data are still being produced and analysed.

Sean sampled plots of individual genotypes for wheat, barley, oat, canola, lupin, chickpea and field pea. This sampling expedition involved a total travelling distance of 5,800km over 4 weeks, trowelling around 10,000 holes (although we did let him come back to Perth on weekends, but only so he could unload the car and put the samples in the cold room!).

The cereal breeding team has sampled various other trials (with either *P. neglectus* or *P. teres*), including the famous *P. penetrans* site at Narrogin. The pulse group has provided samples for field pea and faba bean trials (*P. neglectus*) to make sure that these crops really are resistant.

- **Jackie & Wolfgang**

  We have enlisted the much needed assistance of Jackie Nobbs (SARDI Adelaide) and Wolfgang Wanjura (CSIRO Canberra) to investigate suspicious *Pratylenchus* specimens for us, and to confirm the ones we think we already know. There seems to be great morphological variation within species (or else we have even more species to contend with than we had originally feared!). Jackie is still ploughing through the samples (fixing, mounting and measuring) and Wolfgang is still producing sequence data so we can finally put it all together. Suspicious specimens are potentially *P. coffeae* or *P. brachyurus*, and perhaps others that even horrify the intrepid Jackie.

- **Nem Course – Adelaide**

  In December, Helen Hunter (DAWA Nematology), Lisa Blanch (AGWEST Plant Laboratories, Broadacre Crop Diagnostics) and Modika Perera (Plant Biotechnology Research Group, Murdoch University) attended the short course *Nematodes in cropping systems – identification and techniques*. Kerrie Davies (University of Adelaide) and Mike Hodda (CSIRO) expertly conducted the course at the glorious Waite Campus in Adelaide. Helen stayed on for an extra week to quiz SARDI and University colleagues on the vagaries of culturing, extracting and identifying *Pratylenchus* species, and had the chance to spend additional valuable time with Jackie Nobbs more closely investigating some of our WA *Pratylenchus* specimens.

- **Spreading the Wor~~d~~m**



With the assistance of our new mascot, *Wormy*, 2003 has been a big year for field days and grower group meetings, with the potential audience spread over at least 9 million hectares.

Lugging a microscope around to show people the nematodes has been a big hit. Farmer comments have included: "*Oh my goodness, they're alive!*" "*Are all those little things really in my wheat roots?*" and my all time favourite "*The only good thing about having your head in the sand is that at least you can see the Prats*".

Vivien also helped to demonstrate *Gross Science* for the Sci-Tech school holiday program. Although this was a potentially offensive use of innocent nematodes, the kids asked some amazing questions!

Seminars were invited by UWA and Curtin University. The seminar title *Prats from east to west*, however, gave rise to some unusual enquiries from the UWA history and anthropology departments.

- **Nematology Field Lab**

Funding was secured from DAWA for manufacture of a larger mister to cope with all the soil samples. Unfortunately (?!), this meant that a new location was needed for this equipment, as its placement in the current Plant Pathology Dirty Lab would have resulted in Nematology becoming rather unpopular. We managed to re-furbish an unused storeroom, and the Nematology Field Lab was born. The grand opening (with Helen's famous nematode cake) took place in July 2003.

- **Prat Breeding & Culturing**

We were lucky enough to secure the services of Caroline Versteeg (previously of QDPI South Johnstone, via SARDI, via University of Adelaide) for 3 months as a Visiting Specialist (affectionately known as *Visiting Specialist in Nematode Reproductive Therapy for the Captive Breeding Program*). Caroline (with the assistance of Helen, as shown above) was engaged to establish cultures of our pesky Prats. Due to the unknown identities of many of these nematodes, and their annoying habit of occurring in mixed species populations, it was necessary to initiate all the cultures from single nematodes.

About 1200 meticulously picked and washed single nematodes were placed on individual carrot pieces. These were extracted from the different trial site soils, with some donations from Diagnostics, and variously identified as *P. neglectus*, *P. teres*, *P. thornei*, *P. brachyurus*, *P. penetrans, P. coffeae* or *P. zeae*. Additional mystery cultures have only been named *Pratylenchus* sp. There has also been an attempt to breed the vagrant and rarely seen *Radopholus nativus* in captivity.

Unfortunately, contamination rates have been high, and as yet there is little sign of nematode activity. Hopefully, enough cultures will succeed for future use. It is anticipated that this could take at least 18 months of observation and sub-culturing. Once established, we hope that species identities can be confirmed through the magic of Jackie Nobbs and Wolfgang Wanjura.

- **Roger Cook**
  Following the February 2004 ASDS Symposium in Adelaide, Roger Cook of the Institute of Grassland and Environmental Research (Aberystwyth, Wales) will be spending about a week at DAWA (funded by the DAWA Visiting Scientist Program). We will have a chance to pick his brain about Cyst and Stem Nematodes and his work on nematode communities.

MORE NEWS FROM WESTERN AUSTRALIA

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

Mike Jones and Zhaohui Wang attended the 7th International Congress of Plant Molecular Biology held at Barcelona, Spain at the end of June. Both of them have had a great time over there, listening to congress lectures, talking to colleagues working on the same field, and looking around the charming city. Especially, exchanging latest progress with other plant-nematology groups around the world has given us the best opportunity to catch up with advances on the plant-nematode interaction research, and to modify our research directions. We presented our preliminary results of the microarray assay at the conference, which is the first microarray experiment on analysis of changes in gene expression in a full genome scale induced by root-knot nematode infection, using Affymetrix Arabidopsis ATH1 GeneChips (containing 24,000 genes). In this experiment, a total of 2448 genes have been identified with more than 2-fold changes in expression by comparing the transcript levels between dissected gall tissues and healthy root tissues, which are about 10% of the 24,000 genes arrayed on the microarray chips. Among the 2448 genes, 744 were up-regulated in nematode feeding site and 1704 were down-regulated. These genes have been classified into different functional groups according to Affymetrix annotation and other literature resources. Ten representative genes, five up-regulated and five down-regulated, have been selected from different functional groups. Sequence specific primers have been designed according to individual nucleotide sequences of the Affymetrix probes for these genes. Real time quantitative RT-PCR analysis has then been carried out to confirm the differential expression pattern generated by the microarray experiment.

Zhaohui and Mike finally got their giant cell specific mRNA differential display work published in September, after a long manuscript preparation and review process. The paper is titled 'Differential display analysis of gene expression in the cytoplasm of giant cells induced in tomato roots by *Meloidogyne javanica*', published in *Molecular Plant Pathology*, 4(5): 361-371. We are happy to provide reprints to anyone who is interested.

Modika Perera just returned from Adelaide attending the short course "Nematodes in Cropping Systems Identification and Techniques. She not only has benefited from the course, but also had an opportunity to meet with other nematologists, taxonomists and soil biologists from the University of Adelaide, UNE, SARDI, AQIS, DPI Victoria and CSIRO Entomology, Canberra. Overall she gained a wealth of knowledge in nematology and had a particular interest in identification and systematics of plant parasitic nematodes, which benefits her current research.

In addition Modika Perera with her colleagues Mike Jones and Vivien Vanstone (DAWA) is preparing a paper for publication on "***A novel approach to identify plant parasitic nematodes using MALDI-TOF mass spectrometry*".** This paper demonstrates proof-of concept that nematode species can be detected and differentiated by MALDI-TOF ms. Modika also has developed a simple and a rapid method for enzyme phenotyping using a single nematode. In the future she is planning to validate both methods developed using known nematode extracts, comparison with morphometric identification and by DNA- based methods.

Kerry Ramsay, a new member of the plant-nematology group at the SABC, has spent 5 months in the lab with Zhaohui as her supervisor, working on an ISC project in the last semester of her undergraduate study at Murdoch. She has carried out a quantitative RT-PCR assay to investigate the expression pattern of a novel MAP kinase gene in nematode infected tomato plants. She has also tried to use in situ RT-PCR technique during her project. Currently, she is applying for an Honours project supervised by Mike and Zhaohui, which will be commenced in February 2004.

Angela Hollams is gradually getting some results in her in situ RT-PCR studies for her Ph.D project. Using this method, she has confirmed the up-regulation of the actin gene and the MAP kinase gene in giant cells by localising the staining of the DIG-labelled antibodies in giant cells in fixed gall tissue sections. The difficulty of this method is to constantly generate high quality sections of nematode infected root tissues, which can not only reserve the cell structures but also keep the mRNA intact in the sections, and is easy to handle during the RT-PCR and staining process. Angela is working on the improvement of her sectioning, and more results will be delivered in the coming year.

NEWS FROM SOUTH AUSTRALIA

Terry Bertozzi and Aaron Mitchell completed their PhDs and graduated in December. Terry's thesis was entitled "Biology and control of the anguinid nematode associated with flood plains staggers" and Aaron's "Genetic and molecular biological studies of annual ryegrass resistance to *Anguina funesta*". Summaries are included in this issue of the newsletter.

Kerrie Davies and Mike Hodda ran a highly successful short course on nematode biology and identification at the Waite in December. The eleven participants came from NSW, SA, Vic. and WA.

Kerrie also travelled in Europe, visiting Adrian Evans (Silwood Park) and Rothamsted, UK, presenting a seminar on *Fergusobia* to Richard Sikora's group in Bonn, Germany and spent time visiting trial sites and talking nematodes with Julie Nicol while enjoying the sights in Turkey.

Ian Riley spent much of September and October in Maryland working with Norm Schaad (USDA) on bacteria associated with *Anguina*.

Sohbat Bahraminejad from Iran commenced his PhD studies with the group in June. He will be working on inducible nematicidal flavanoids in cereals. He will be examining genetic diversity in their inducible and constitutive concentrations, biological activity, inheritance and molecular biology. This work follows on from the findings of Imelda Soriano's project on mechanisms of plant defence against nematodes.

Mark Potter finished his postdoctoral studies on resistance of canola to *Pratylenchus* and has, for the time being, devoted his scientific skills to teaching the art of soft cheese making.

Zengqi Zhao has been busy sampling local conifers, plantation and native, and finding lots of new and interesting nematodes. Already he has collected a range of aphelenchid species, including some that look close to *Bursaphelenchus*. Work towards sequencing DNA from these will give us more clues on their identity.

Speakers at our campus-wide nematode discussion group for second semester were Robin Hardy, PIRSA, on nematodes in potatoes, Sharon Taylor, SARDI, on *Ditylenchus* and Ian Riley on *Anguina*/*Rathayibacter* associations.

Daivd Bird (North Carolina State University, USA) gave a special lecture in honour of his late farther, Alan Bird, to the Royal Society of South Australia in July. David's topic was "Cross-species gene flow and the evolution of parasitism in nematodes". His presentation was most appreciated by those from Waite who attended.

Ian Riley, University of Adelaide, Waite Campus.

NEWS FROM CANBERRA

I have recently completed Australian and world checklists to the nematode Order Aphelenchida. The Australian list will be available from the Australian Biological Resources Study web site, and the world list from my web site (http://ento.csiro.au/science/nematodes). Both lists have full bibliographic details of original descriptions, redescriptions and changed combinations of all species of

Aphelenchida. There are also distribution, lists of both plant and animal hosts, and ecological notes for many species, although these are not comprehensive. A total of 462 species are currently recognised, which have been called a total of 758 names. (If you want to find out how that works, have a look at the web site.) The aphelench with the most names was the ubiquitous *Aphelenchus avenae*, which has 19 different names. This is not surprising, as this "species" may yet turn out to be a species complex.

I hope that the host and distribution records may turn out to be useful as a record of what has definitely been found in Australia. Check it out, and if you have any feedback I would love to hear it.

The ANIC collection hall in which I now work is about to undergo a "seismic refit" to prevent damage to the collection in case of an earthquake: the staff, especially those in less protected offices are consequently expected to flood into the collection hall to complete some important taxonomic work on the collection if there is an earthquake. But seriously, it raises an issue which arose frequently during the compilation of the checklist mentioned in the last item: that is the importance of collections. Species and genera which were perfectly clear and well-defined when they were originally proposed, subsequently became unclear, incomplete, or imprecise due to advances in technology, changes in the concepts of taxonomic groups, and additional collections. This is how a species like *Aphelenchus avenae* finishes up with so many names. The worst part is that it is often impossible to check on what people have called *Aphelenchus avenae* because there are no specimens stored in an ongoing, curated collection.

At the time it may have seemed perfectly simple to record that *Aphelenchus avenae* occurred in the soil at a certain place. However, a few years later, someone else described a species called *Aphelenchoides solani*, which was rather similar (but not exactly the same) from the diseased leaves of solanaceous plants. It was noted that the two were rather similar: was the disease totally ubiquitous in the soil, as is *Aphelenchus avenae*? To cut a long story short, *Aphelenchoides solani* and *Aphelenchus avenae* appear to be variations of the same thing; synonyms for the one species. And the disease was caused by *Aphelenchoides fragariae*, which was only recognised as a different species after it was noticed that there were differences between what had previously all been called the same thing: *Aphelenchoides solani*. The point is that the confusion could only be sorted out by having collections of what people were calling *Aphelenchus avenae* (to see that they were the same as *Aphelenchoides solani*), *Aphelenchoides solani* (to see that some were the same as *Aphelenchus avenae* and some were different), and *Aphelenchoides fragariae* (to see that this was in fact the organism causing the leaf disease, and that it was not ubiquitous in the soil).

The ANIC Nematode Collection exists to record the diversity of nematodes, so that we can make progress in nematology. It is important to know and record what we are dealing with now, so that we can manage the present and future challenges which nematodes will present. For example, changes in populations such as the appearance of a new pathotype of *Heterodera avenae*, which will radically alter breeding and resistance strategies. Or deciding whether in fact we have 2 species of *Heterodera* and whether there is currently any difference in pathogenicity or whether one is likely to develop. Or deciding whether the *Pratylenchus* species of Western Australia are entirely different from those in the east. These are just 3 of the questions that are currently topical for which collections are vital. And it is impossible to tell what questions will arise in the future, but it is certain that they will. The seismic refit indicates how seriously this is taken by the ANIC. We are supported by GRDC and CSIRO to continue to build and maintain the collection, so please continue to make use of it by sending old material, voucher specimens and any interesting finds. They will be carefully curated and looked after for when they are needed. To make arrangements, contact me at the address inside the cover.

(By the way, I am a former Novocastrian, I was in Newcastle when the earthquake hit there totally out of the blue many years ago, and it was an interesting experience, so you never know when it may happen!)

On a completely different topic, the fourth incarnation of the biennial “Nematodes in cropping systems: identification & techniques” course has come and gone again, in Adelaide this time. There were 11 participants this time, including Lila Nambiar, who has been to 2 of the previous courses. It was good to see PhD students and Post-Docs in nematology, indicating that there is interest in studying the group amongst younger people. Whether this interest translates into careers is another matter…

Mike Hodda.

Research

POSSIBLE TIMING OF INTRODUCTION OF PLANT PATHOGENIC NEMATODE SPECIES TO NEW ZEALAND

Gregor W. Yeates

Landcare Research, Private Bag 11052, Palmerston North, New Zealand

When compiling the 'Species 2000' list of nematodes for New Zealand only eight cyst nematode species were regarded as known alien species, with the origin of most of the 258 species of plant and soil nematodes being regarded as uncertain (Yeates, in press). The small size (~1 mm long), burrowing habit and lack of obvious effects mean that nematodes are overlooked unless a population eruption causes significant crop loss. Such a case occurred with potato cyst nematode (*Globodera rostochiensis*) at Pukekohe (Dale, 1972), leading to a range of management measures and the Potato Cyst Nematode Regulations 1974. On the basis of accounts of European settlement and plantings, trading of agricultural produce, and recorded damage, this note assigns periods for the introduction of 52 of the plant-pathogenic nematodes now known from New Zealand. Despite recent nematode surveys on both taxonomic (Yeates & Wouts, 1992; Loof et al., 1997; Sturhan et al,. 1997) and crop bases (Knight, 2001), the origins of the New Zealand nematode fauna remain poorly known. Many species of *Helicotylenchus* and *Criconema* recorded have affinities with Pacific and Pacific-rim countries, and, as the nematode faunae of most austral land masses remain scantily described, it is difficult to establish firm links; molecular techniques may help in the future.

The following commentary is included to indicate changes in land-use that could be related to introduction of plant and soil nematodes.

1. New Zealand's oldest standing European building is the Kerikeri Mission Station, built in 1821-22, and its garden was first dug in 1820 (New Zealand Historic Places Trust, 2003). The oldest standing European stone building, the Kerikeri Stone Store (1832), was meant to house mission supplies and large quantities of wheat from the mission farm (New Zealand Historic Places Trust, 2003). Clearly, by 1830 aspects of European agriculture were established. It is reasonable to assume that the various crops arrived with some of their contemporary pests and diseases; even if expressed, the diseases were scarcely even recognised in the settlers' homelands.

2. While little is known of pests and disease of the low intensity gardening by Maori, sealers and whalers, significant damage was wrought by native insects on crops planted by early settlers (e.g., Rutherford & Skinner, 1940).

3. Development of pastoral agriculture was associated with the import of many grass and clover species. Many plant species were brought deliberately, other ('weeds') were incidental imports – as were pests and diseases. The multiple imports of most pasture species not only increased the chance of particular pests and diseases arriving but also means that some pests and diseases may still have very local distribution patterns. There is a record of a 103 acre (42 ha) farm near Auckland that was in scrub and native grasses when bought in 1863 but was soon producing wheat and oats; the last crop was in 1930 when it changed to dairying, sheep and beef (Anon., 1990).

4. The origins of many horticultural crops are given in Wratt & Smith (1983). Seeds were the prime import for some (e.g., kiwifruit, *Actinidia deliciosa*, 1904; tamarillo, *Cyphomandra betacea*, ~1890) while in other cases plants were imported (feijoa, *Feijoa sellowiana*, 1908; avocado, *Persea americana*, 1919).

5. English, Australian and other exotic trees were planted from the settlement of communities and farms from about 1840. At Stoke, near Nelson, Thomas Marsden started planting trees in 1850 "… with trees he had brought from all parts of the world. Stories are told of ships' captains being commissioned to bring exotics from the lands they visited" (Horrocks, 1971). While acorns, pinecones and gumnuts could have been used, many of these trees were clearly brought growing in pots or tubs – with the associated nematodes, earthworms and other soil biota. How, and if, these trees, both ornamental and productive, were subsequently dispersed would affect the dispersal of any associated organisms.

Table 1. Stages of agricultural development in New Zealand and the number of alien nematode species considered to have been introduced during each stage. See also Table 2.

Period	Pattern of activity	Species introduced
pre-1840	sporadic settlement	2
1840-1860	organised settlements	11
1860-1880	extension of settlements throughout country	16
1880-1900	consolidation, improvement and diversification of farming	8
1900-1920	incremental improvement; diverse horticultural efforts	6
1920-1940	varying prices and fortunes on the land	2
1940-1960	pasture improvement; horticulture expanding	6
1960-1980	pasture improvement; explosion of horticulture	1

1980-2000	intensification, selection pressure on nematodes	

Undoubtedly, the various plants arrived with some of their contemporary pests and diseases. Even if expressed, the pests and diseases were scarcely even recognised in the early settlers' homelands. The multiple imports of many plant species not only increased the chance of particular pests and diseases arriving but also means that some pests and diseases may still have very local distribution patterns. Some nematodes (e.g., *Anguina tritici* of wheat; *Ditylenchus dipsaci* of oats) are closely associated with seeds and certainly impacted on 19th century agriculture in New Zealand (Kirk, 1899). Cyst nematodes (*Heterodera*, *Globodera*) are among those most likely to be introduced in debris, dust and soil associated with seeds, planting material and agricultural machinery. A wide range of nematodes, both plant-feeding and 'free-living', would have been associated with growing material in pots, in tubs or sacking-wrapped rootstocks – along with the associated earthworms and other soil biota. How, and if, these plants, both ornamental and productive, were subsequently dispersed would affect the dispersal of any associated nematodes.

Seed-gall nematodes (*Anguina*) are host-specific and would have been intimately associated with seed introductions. Indeed, *A. tritici* was an economic problem in the late 19th century (Kirk, 1899). Now, due to seed-cleaning, *A. tritici* and *A. agrostis* are difficult to find in New Zealand. Cyst-forming nematodes of introduced plants such as white clover (*Trifolium repens*) and potato (*Solanum tuberosum*) are unequivocally introduced. The stem and bulb nematode, *Ditylenchus dipsaci*, would have been associated with imported oats and other plants (e.g. onion, lucerne); it would have multiplied on such crops, becoming widespread and probably supplanting any endemic populations in agricultural areas. The 'garlic race' of *D. dipsaci*, which caused problems in the 1980's, may have been a later introduction. The foliar nematodes *Aphelenchoides fragariae* and *A. ritzemabosi* occur widely and, while the latter has been collected from a fern in native forest (Knight et al., 2002), the dominant populations are assumed to be introduced. Several nematode vectors of plant viruses (e.g. *Longidorus elongatus, Xiphinema diversicaudatum* and *Trichodorus primitivus*) are apparently introduced from Europe (Brown & Topham, 1985; Boag et al., 1997; Sturhan et al., 1997).

There are problems associated with dating the introduction of 'recently described' species that include New Zealand in their ranges (e.g., *Meloidogyne fallax* Karssen, 1996; *M. trifoliophila* Bernard & Eisenback, 1997). Some of these species probably reflect discrimination using molecular techniques or recent selection through plant breeding. Further, many plant-associated (e.g. *Paratylenchus, Tylenchus*) are too poorly studied in New Zealand to attempt any analysis.

Table 2. List of 52 plant-feeding nematodes regarded as alien and introduced to New Zealand. For some species, indications of host, habitat and distribution (W, widespread; L, localised) are given in addition to surmised period of introduction.

Nematode	Host / habitat		Period
Longidoridae			
Longidorus elongatus (de Man, 1876) Micoletzky, 1922	Pastures etc	W	1840-1860
Xiphinema americanum Cobb, 1913	Avocado, feijoa	L	1900-1920
X. diversicauadatum (Micoletzky, 1927) Thorne, 1939	Fruit trees, bowling greens	L	1860-1880
X. radicicola T. Goodey, 1936	Nelson record	L	1860-1880
Trichodoridae			
Paratrichodorus pachydermus (Seinhorst, 1954) Siddiqi 1974		L	1900-1920
Trichodorus primitivus (de Man, 1880) Micoletzky, 1922	Cultivated areas.	L	1860-1880
Aphelenchoidoidea			
Aphelenchoides fragariae Ritzema Bos, 1890	Foliar nematode	W	1840-1860
A. ritzemabosi (Schwartz, 1911) Steiner, 1932	Foliar nematode	W	1840-1860
Criconematidae			
Criconema annuliferum (de Man, 1921) Micoletzky, 1925	Kiwifruit	L	1860-1880
Crossonema civellae (Steiner, 1949) Mehta & Raski, 1971	Passionfruit	L	1880-1900
Mesocriconema rusticum (Micoletzky, 1915) Loof & de Grisse, 1989	? damp pastures	L	1860-1880
M. xenoplax (Raski, 1952) Loof, 1989	Fruit trees	L	1880-1900
Ogma palmatum (Siddiqi & Southey, 1962) Siddiqi, 1986	Fig, Nelson	L	1920-1940
Anguinidae			
Anguina agrostis (Steinbuch, 1799) Filipjev, 1936	Browntop seed gall nematode	W	1840-1860
A. tritici (Steinbuch, 1799) Chitwood,	Wheat seed gall nematode	W	Pre

1935			1840
Ditylenchus destructor Thorne, 1945	Potato tubers etc	?	1840-1860
D. dipsaci (Kuhn, 1857) Filipjev, 1936	Stem & bulb nematode	W	Pre 1840
Subanguina radicicola (Greeff, 1872) Paramonov, 1967	Grass root gall nematode; Southland	L	1860-1880
Tylenchulus semipenetrans Cobb, 1913	Avocado, persimon, passionfruit	L	1860-1880
Belonolaimidae			
Geocenamus nanus (Allen, 1955) Brzeski, 1991	Pastures in Southland & Otago	L	1860-1880
Quinisculcius capitatus (Allen, 1955) Siddiqi, 1971	Tobacco in Motueka	L	1900-1920
Tylenchorhynchus dubius (Bütschli, 1873) Filipjev, 1936	Stunt nematode; ?drought susceptible soils	L	1860-1880
T. maximus Allen, 1955	? drought susceptible soils	L	1860-1880
Heteroderidae			
Globodera pallida (Stone, 1973) Behrens, 1975	Potato cyst nematode	L	1940-1960
G. rostochiensis (Wollenweber, 1923) Behrens, 1975	Potato cyst nematode	L	1940-1960
H. avenae Wollenweber, 1924	Cereal cyst nematode	L	1860-1880
H. cacti Filipjev & Schuurmans Stekhoven, 1941	Cactus cyst nematode	L	1920-1940
H. fici Kir=yanova, 1954	Fig cyst nematode	L	1840-1860
H. humuli Filipjev, 1934	Hop cyst nematode	L	1860-1880
H. schachtii Schmidt, 1871	Beet cyst nematode	L	1860-1880
H. trifolii Goffart, 1932	Clover cyst nematode	W	1840-1860
M. fallax Karssen, 1996	Root knot nematode – potato	L	1960-1980?
M. naasi Franklin, 1965	Root knot nematode – cereals	L	1940-1960
M. trifoliophila Bernard & Eisenbach, 1997	Root knot nematode – clover	W	1860-1880?
Hoplolaimidae			
Helicotylenchus canadensis Waseem, 1961	? Southland introduction	L	1860-1880

H. exallus Sher, 1966	Saltmarsh at Nelson	L	1840-1860
H. leiocephalus Sher, 1966	? grasslands	L	1940-1960
H. paxilli Yuen, 1964	? tree imports	?	1840-1860
H. paraplatyurus Siddiqi, 1972	Tamarillo, kiwifruit	L	1880-1900
H. serenus Siddiqi, 1963	? horticultural	L	1900-1920
H. varicaudatus Yuen, 1964	Also known from Europe & India	L	1880-1900
H. vulgaris Yuen, 1964	Orchard, Kurow	L	1880-1900
Rotylenchus buxophilus Golden, 1956	Trees	L	1880-1900
R. robustus (de Man, 1876) Filipjev, 1936	Pathogenic, trees	L	1880-1900
R. uniformis (Thorne, 1949) Loof & Oostenbrink, 1958	Pathogenic, feijoa	L	1880-1900
Scutellonema brachyurus (Steiner, 1949) Andrassy, 1958	Avocado, kiwifruit	L	1900-1920
Pratylenchidae			
Pratylenchus coffeae (Zimmermann, 1898) T. Goodey, 1951	Root lesion nematode	?	1940-1960
P. crenatus Loof, 1960	Globally in light soils, on Graminae	?	1860-1880
P. pratensis (de Man, 1880) Filipjev, 1936	? from European clay & peat soils	?	1840-1860
P. thornei Sher & Allen, 1953	Globally widespread, especially in dry zones	?	1840-1860
P. vulnus Allen & Jensen, 1951	Globally widespread in warmer areas – woody hosts	?	1900-1920
Zygotylenchus guevarai (Tobar Jiménez, 1963) Braun & Loof, 1966	Passion fruit	L	1940-1960

In Table 2 estimated times of first introduction of 52 species of plant-pathogenic nematodes have been allocated to the periods given in Table 1. The immaturity of the science is highlighted by many dates of introduction being before dates of description. These are estimated new, first introductions, many species will have been, and continue to be, introduced repeatedly. In the absence of formal records and voucher specimens the allocated periods are somewhat arbitrary, if not circular. While further information will hopefully emerge to clarify some actual dates of introduction this note provides a starting point.

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Review

BIOLOGY AND CONTROL OF THE ANGUINID NEMATODE ASSOCIATED WITH FLOOD PLAIN STAGGERS

Terry Bertozzi

PhD Thesis Summary, University of Adelaide

Flood plain staggers is a poisoning of livestock similar to annual ryegrass toxicity (ARGT). In 199 1, it resulted in the deaths of 1722 cattle, 2466 sheep and 11 horses in northern New South Wales (NSW) and over the past 30 years, has caused livestock losses in south-eastern South Australia (SA). The toxins involved are produced by the bacterium *Rathayibacter toxicus*, which is carried into the grasses *Polypogon monspeliensis* and *Agrostis avenacea* by nematodes belonging to the genus *Anguina*. Both hosts dominate in flood prone pastures, which may remain inundated for up to five months of the year. This study was initiated to examine the distribution, biology and ecology of the nematode and bacterium associated with flood plain staggers and to use this information to examine potential pasture and livestock management practices that could be employed to reduce the impact of flood plain staggers to the livestock industry.

Surveys conducted in SA and NSW showed that the incidence of the nematode and bacterium is greater than indicated by reported outbreaks of flood plain staggers. The organisms appear to be restricted to flood prone areas and pastures adjacent to watercourses. While both *P. monspeliensis* and *A. avenacea* were found to be infested in NSW, infested *A. avenacea* has not been found in SA.

Host range studies demonstrated that the nematodes from both provenances can reproduce in either host. No galls were induced by the nematodes in closely related grasses that are known hosts of other species of *Anguina*, indicating that a new species may be involved. An examination of nematode populations from both provenances and hosts by allozyme electrophoresis showed that while the nematodes within each provenance were genetically uniform, there was some genetic variation between provenances. Morphological and cytological evidence indicated that they are likely to be the same species but different from described species of *Anguina*. This was corroborated by sequencing the internal transcribed spacer regions of the nematodes from both provenances.

Monitoring of nematode populations from SA over two seasons revealed that the nematode can initiate galls in the vegetative shoot apical meristem in addition to ovary initials. Shoot galls can be initiated at any time of the year, which may play an important role in nematode survival during flooding. Although the nematodes are unable to invade host plants in flooded conditions, experiments also indicate that nematodes can survive for at least three months in flooded soil and are able to induce galls in both hosts once conditions become favourable. The nematodes only complete one generation within a gall but can have two or more generations per year by repeated gall formation.

Agronomic methods that have been effective in controlling other *Anguina* species, particularly *Anguina funesta*, were examined in the field. While significant reductions in seed galls were achieved by heavily grazing pastures in spring, it appears that shoot galls are not consumed and thus there will be a limited effect on nematode populations. Excellent control of the host grasses was achieved with herbicides. However, where pastures remained inundated for long periods of time, no pasture regenerated on the treated areas, reducing the productivity and increasing the risk of erosion and degradation.

The plant pathogenic fungus *Dilophospora alopecuri* was found to be associated with nematode populations in *P. monspeliensis* and assessed as a potential biocontrol agent, as it is already providing useful control of *Anguina funesta* in Western Australia. This strain was tested against other available Australian strains of the fungus for its effectiveness in reducing nematode populations. The strains examined were neither host nor vector specific. The strain isolated from *P. monspeliensis* was the most aggressive train tested and reduced nematode numbers significantly. An investigation of the strains by allozyme electrophoresis showed that *D. alopecuri* is either highly variable or consists of several species.

GENETIC AND MOLECULAR BIOLOGICAL STUDIES OF ANNUAL RYEGRASS RESISTANCE TO *ANGUINA FUNESTA*

Aaron Mitchell

PhD Thesis Summary, University of Adelaide

Annual ryegrass toxicity (ARGT) is an often fatal poisoning occurring in grazing animals following the ingestion of seedheads of the annual ryegrass, *Lolium rigidum*, infested with the corynetoxin-producing bacteria, *Rathayibacter toxicus*. Breaking the disease cycle, through the use of lines of *L. rigidum* resistant to the nematode, *Anguina funesta*, can be used to reduce the risk of ARGT outbreaks. In *L. rigidum*, resistance to *A. funesta* appears to be under the control of two unknown, but complementary genes. This study explored alternate approaches towards the allocation of genotype for lines of *L. rigidum* with respect to resistance to *A. funesta*.

A genetic approach involving the analysis of numbers of progeny, resistant and susceptible to *A. funesta,* from factorial crosses to infer parental genotypes was employed. Allocations of the resistance genotypes were possible for a number of *L. rigidum* lines.

Two alternate molecular approaches were taken, in an attempt to isolate molecular markers linked to the regions of the *L. rigidum* genome responsible for resistance to *A.*

funesta. A total of 62 arbitrary 10-mer oligonucleotide primers were used to screen a pair of putative near isogenic lines (NILs), differing in resistance to *A. funesta* (R799 and S1150), for RAPD markers linked to the genes conferring resistance. RAPD reactions with the primers OPAM-1 and OPAM-08 yielded products with genomic DNA from R799 but not S1150 as template. However, the association of these products with resistance was not maintained across an extended range of *L. rigidum* lines. A more targeted molecular approach used degenerate oligonucleotide primers, designed on highly conserved motifs of the nucleotide binding site (NBS) region from the proteins encoded by many recently cloned plant resistance genes, to amplify resistance gene analogues (RGAs) in *L. rigidum*. This is the first record of the presence to RGA sequences in *L. rigidum*. A total of 91 cloned, amplified products were analysed, from which 22 were sequenced and assigned to one of four classes, each exhibiting high levels of similarity to previously cloned RGA sequences in other plant species. Each class was detected in low or moderate copy number in the *L. rigidum* genome. *L. rigidum* genomic sequences hybridised by class 2 and class 3 RGA sequences are presented as potential markers of resistance.