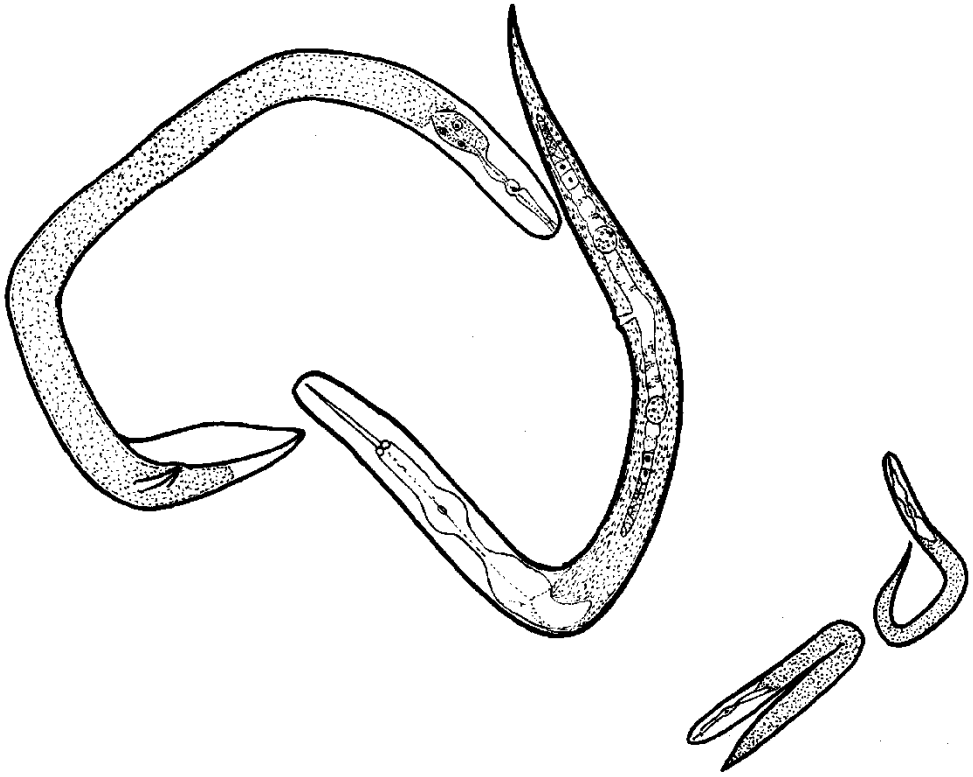
ISSN 1327-2101

# AUSTRALASIAN NEMATOLOGY NEWSLETTER



**Published by:** **Australasian Association of Nematologists**

**VOLUME 24 NO. 1 JANUARY 2013**

# From the Editor

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

**July Issue**

The deadline for the July issue will be mid June 2013. Kerrie Davies will notify you a month in advance so please have your material ready then.

*Katherine Linsell* on behalf of *Kerrie Davies*

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# Association News

## FROM THE PRESIDENT

Is there anybody out there? Does anyone read these words?

Last newsletter, I asked whether any members wanted to nominate topics or volunteer as session chairs for the next International Conference of Nematology. With the exception of my own students, there were no replies.

I also asked for suggestions and offers to organise a workshop for APPS in Auckland. Again no response.

I mentioned that the Australasian Nematology Support Fund (ANSF) had scholarships with minimal administrative loads for student members of AAN to attend conferences. That one managed a few applications. It also managed to start a debate among the committee managing the fund that maybe we should save up the fund this year so that we can offer larger scholarships for the sixth International Congress next year. So we were thinking of offering up to about $1500 for next year only. I hope this will get a reaction of encouraging people to attend.

I wonder whether the lack of reactions is related to the increasing demands placed on nematologists and other scientists. I know that I seem to be so busy most of the time that I cannot get everything I would like done (just ask my students or the newsletter editor) Is it lack of resources? One of the original reasons for creation of AAN was the perception of declining support for nematology in Australia. That was 24 years ago in 1989.

On a not entirely unrelated note, a former President of AAN recently announced his retirement. John Curran, although no longer active in nematology, retires in a few weeks time after 26 years at CSIRO, most recently as Director of Communications. John had a distinguished career in nematology, working on Mermithids, then entomophilics, then Root-Lesion Nematodes, before moving to genetics and finally into management. John was instrumental in developing the DNA test for soil nematodes now deployed by SARDI. I'm sure we all wish John well in his retirement. He said he plans to travel, learn Spanish and the take up the piano. On a very sad note, Gregor Yeates death in August 2012 was a huge shock to us all, as Gregor was the sort of person who seemed to go on forever with boundless energy. An obituary appears elsewhere in this issue. Those who worked on PCN will be saddened to hear of the death of Pat Haydock. Pat's passing will leave a huge hole in research on PCN.

I know I would have liked to have picked the brains of both these fine nematologists more than I managed over the years. Although both were prolific publishers, there was much that I learned from discussions with them both, and much more that I'm sure I could have learned, but now do not have the chance. I suppose that is a way of saying *carpe diem* and that there is value in seizing the opportunities to meet with and converse with fellow nematologists if the opportunity presents itself, and there are opportunities like those I described at the start of this missive. On that philosophical note, I will finish.

*Mike Hodda*

## FROM THE SECRETARY

## GENERAL MEETING
## 1.30 pm, 7 November 2012
## DRAFT MINUTES

### 1. ATTENDEES

Mike Hodda – President
Kerrie Davies – Newsletter editor
Katherine Linsell – Treasurer
Sarah Collins – Secretary

### 2. NEW MEMBERS

- KL happy to organise new memberships.
- SC will obtain a monthly report from Peter Williamson for any new members may be helpful to KL to reconcile new members with his APPS listings and payments.
- KL will send an updated list for membership to other committee members.
- KL to send out notices to members when necessary.

### 3. MINUTES

- Any AAN committee or group meeting to be recorded and published in newsletter.

### 4. CONFERENCE CALLING

- In past APPS has paid for the use of conference calling facilities where AAN required a committee meeting. APPS conference call arrangements have recently changed and cost of conference facilities have increased greatly.
- While APPS still supports use of conference facilities for Special Interest Groups it may be appropriate to utilise other methods for conference calls.
- SC, MH & KL all work in departments where conference call facilities are available so will utilise these in future on a rotational basis where possible to conduct meetings.

### 5. NEWSLETTER

- Costs $70-80 biannually for the international mailout.
- KD has adsorbed these costs until now.
- May need to begin to utilise internet options for all except those without necessary computer access.
- KL will assess costs after next mailout and change current setup if necessary.
- KD & KL to make increased attempts to get articles for newsletter from each state.

### 6. FINANCES

- Bank accounts still listed as Vivien Vanstone for treasurer so need to change account signatory to KL. Also need to change address to KL. SC to begin process and organise for signatories to attend ANZ while MH SC & KL in Canberra in November.
- Call for vote to officially make KL Treasurer for AAN as well as signatory and addressee for bank accounts held by AAN by SC.
- VOTE: MH moved the motion and KD seconded it. Therefore KL elected Treasurer and is to be instated as a signatory and addressee for bank accounts held by AAN.

**7. APPS WORKSHOP AUCKLAND 2013**

- SC is happy to help organise a workshop but committee feels that it would be best for the main organiser to be in NZ. Efforts have been made by MH and SC to locate and get interest for a local NZ Nematologist to be the organiser for a Nematology workshop. MH will send SC a list of potential contacts to be involved. SC has made several attempts to contact Nigel Bell with no success (impaired by time differences between NZ & WA) and will try again.
- If a NZ organiser cannot be determined efforts to organise workshop will cease.
- Idea for workshop: interesting Nematodes from different ecosystems e.g. - NZ, Asia, WA wheatbelt, tropical Australia.

**8. AAN STUDENT SCHOLARSHIP**

- Currently an open process where students can apply when necessary. Committee like this open system to continue rather than set dates for submission.
- Can students apply for AAN scholarship if they have already secured funding in the past from other avenues? Committee discussion determined that it is acceptable for students to apply for funding from several sources but these need to be stated and a budget assessed by the committee including ALL funding secured by the student for the relevant conference travel.
- If both APPS and AAN Conference funding scholarships have been requested by a student for the same conference, applications should not be granted where possible.
- MH suggested that funding could be saved for 2013 to be made available for 2014 International Congress in South Africa.
- MH to outline the scholarship for 2014 in Presidents report in next newsletter.

**9. NEMATOLOGY SESSIONS – CONFERENCES**

- Currently a split has developed in nematologists conference attendance between ASDS and APPS conferences.
- The last two APPS conferences (Newcastle and Darwin) have not held specific Nematology sessions. This has had the very unfortunate outcome of Nematology papers being held in clashing time allocations. Requests were made to the Darwin organising committee for Nematology sessions but this did not eventuate.
- SC is currently secretary of APPS management committee and will represent AAN special interest group request to hold Nematology sessions in NZ 2013 conference.
- The question was raised whether AAN make an effort to support one conference so that Nematologists get the best conference interaction in our field of expertise or begin organising a nematology bi annual meeting.

**10. NEXT MEETING**

- **April 2013 in Perth.**

*Sarah Collins*

## FROM THE TREASURER

Membership of the AAN currently stands at 84. At the time of publication, 26 of these members were un-financial. If you think this applies to you, please contact the treasurer at katherine.linsell@sa.gov.au.

Since 2011, 11 members have left AAN through career changes or retirement.

We have gained 15 new members since 2011:

- Gavin Ash, Agricultural & Wine Sciences, Charles Sturt University, NSW
- Natalie Banks, CSIRO Ecosystems Sciences, ACT
- Seona Casonato, Plant & Food Research, Te Puke, New Zealand
- Tim Clewitt, Leslie Research Centre, Department of Primary Industries, QLD
- Sjaan Davey, SARDI Soil Biology and Diagnostics, SA
- Dale Griffin, Crop Protection Research Pty. Ltd., VIC
- Shane Harvey, Westgate Research, NSW
- Christine Horlock, Biosecurity Queensland Control Centre, QLD
- Sadia Iqbal, Plant Biotechnology Research Group, Murdoch University, WA
- Lea Meagher, Ghent University, Belgium
- Sue Pederick, SARDI Horticulture Pathology, SA
- Matthew Rodda, Department of Primary Industries, VIC
- Alison Seyb, Department of Primary Industries, NSW
- Brady Smith, CSIRO Plant Industry, SA
- Jo-Anne Tan, Plant Biotechnology Research Group, Murdoch University, WA

*Katherine Linsell*

# Regional News

## NEWS FROM SOUTH AUSTRALIA

**The University of Adelaide**

For Kerrie Davies, 2012 was a busy year finalising various manuscripts for publication. *Fergusobia* was collected from *Leptospermum* in both Queensland and Victoria – a new host genus for the fly/nematode mutualism. The galls on the host species from Queensland have a typical fergusoninid gall form, but those from Victoria are so cryptic that Kerrie is now wondering just how many galls she has overlooked while collecting.

In August, Dorota Porazinska, who is working with Mike Hodda at CSIRO, visited Adelaide and described her 454 sequencing work in a well-received seminar on the Waite Campus.

In September and October, Kerrie was in the northern hemisphere. In Germany, she was able to catch up with Dr Suzanne Charwat, spending a happy week with Suzanne and her delightful family. In Florida, Kerrie spent three weeks in Robin Giblin-Davis's lab at the Fort Lauderdale Campus of the University of Florida. They have moved into a new building, and Robin now has access to a state-of-the-art molecular lab, and some nice new equipment including a microscope with automontage. Kerrie was working on descriptions of new species of *Schistonchus* from Central American *Ficus*, and planning new papers. One of these will deal with the generic status of '*Schistonchus*', which is polyphyletic. Her visit coincided with one from Meike Woehr, a Master's student from Pretoria, South Africa, who was there collecting *Parasitodiplogaster* from *Ficus*. After a near-miss with Hurricane Sandy (escaping the USA just before the airport at Newark was closed) on her return trip, Kerrie caught up with Matthew Tan in Singapore. He is working there in quarantine.

Just after Christmas, Kerrie will be heading back to the northern hemisphere, this time to meet her new twin grandchildren, and to lend her son and daughter-in-law a hand. She will be back in late February, to prepare for the short course on plant and soil nematodes that she and Mike Hodda will run in Perth in April.

*Kerrie Davies*

**SARDI**

***Cereal Nematology***

Katherine Linsell has continued work on the collaborative project between Biological Crop Protection, (Graham and Marcelle Stirling) and SARDI, which is developing DNA tests for the rapid assessment of free-living nematode communities in Australian cropping soils. Katherine presented some of this work in a poster at the ASDS in Fremantle in September. Katherine also presented her work on the genetic and biological resistance to *P. thornei* in wheat at Combio in Adelaide in September. Katherine attended the workshop 'Detection and Identification of Agricultural Pathogens using Next-Generation Sequencing Methods: Approaches, Advantages and Pitfalls' run by Mike Hodda and Dorota Porazinska at CSIRO Canberra.

Jackie Nobbs, Sue Pederick and Barbara Hall have been busy analysing samples through the SARDI Nematode Diagnostic service. The first known report of stubby root nematode on canola (*Brassica napus*) and vetch (*Vicia sativa*) were identified in samples from the SA Eyre Peninsula. Sequencing of the 18s rRNA region identified the species as *Paratrichodorus minor*.

Photo on right - Stunted or 'stubby' root systems of Canola caused by *Paratrichodorus minor*.

Jessika Aditya, graduate of Monash University, Malaysia campus, completed her Honours thesis, 'Characterisation of Barley Roots Responses to Infection by *Heterodera Avenae* Woll.' in November 2012. Her supervisors were Prof. Diane Mather, University of Adelaide, School of Agriculture, Food and Wine and Dr Matthew Tucker, University of Adelaide – ARC Centre of Excellence in Plant Cell Walls. The SARDI Nematology Group through John Lewis provided assistance and informal supervision with the nematological sections of this work.

Sjaan Davey and Alan McKay in collaboration with DPI Victoria are working on a GRDC-funded project screening cereal cultivars for resistance and tolerance to the root lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) and the cereal cyst nematode (*Heterodera avenae*) in the field. Data from the 2012 season is currently being analysed. Initial and final nematode populations are measured prior to seeding and post harvest respectively using the SARDI PreDicta B assay.

***Visiting Chinese Scientist***

The SARDI Nematology Group, through John Lewis, hosted Dr Hong-xia Yuan, a visiting scientist from the Department of Plant Pathology, College of Plant Protection, Henan Agricultural University, Zhengzhou, Henan, China. Cereal cyst nematode (CCN) is becoming more evident in China, with instances of severe crop damage. Dr Yuan spent seven weeks with SARDI to work with John Lewis and learn the range of techniques developed by SARDI to develop resistant and tolerant cereal varieties and manage this pest. Dr Yuan also had the opportunity to work with Dr Marg Evans to learn the techniques used for crown rot research in South Australia.

*Katherine Linsell and John Lewis*

## NEWS FROM ACT

**Nematode Biosystematics & Ecology - CSIRO Ecosystem Sciences**
The nematology group in Canberra has been a hive of activities with several nematode diagnostics workshops, two PhD students starting their field work, two PhD projects nearing completion, one project completed and applying for new projects. In addition, Mike, Natalie Banks and Sunil Singh completed a report on the nematode threats to the Northern Australia Quarantine Region.

Distinguished Visiting Scientist Dorota Porazinska from the University of Florida has completed the first phase of her project on using high-throughput sequencing methods to identify potential quarantine soil nematodes. There are some promising results on the sensitivity of the method in detecting species of root lesion and cyst nematodes directly from soil samples. The manuscript reporting the results is completed and hopefully will be published by the next newsletter in PLOS One. Considering the potential application and growing interest in the use of high-throughput sequencing methods, Dorota in collaboration with Mike and other researchers from CSIRO conducted a week-long workshop for diagnosticians working for the Plant Health Australia and the Subcommittee on Plant Health Diagnostic Standards. The workshop was attended by diagnosticians from all the states, plus NT.

Speaking of workshops, Mike is in high demand at the moment and is currently preparing for a workshop to follow up previous activities in Thailand in March. This time around, Natalie Banks will join Mike to conduct some preliminary surveys for her PhD project on movement of nematodes through trade networks. Afterwards the Thai collaborators will visit Mike's lab in Canberra as part of a Crawford Fund and Association of Southeast Asian Nations (ASEAN) Australia and New Zealand capacity building initiative. Then Mike and Kerrie Davies will present their biennial Nematodes in Cropping Systems workshop in Perth in April. A separate one-day workshop for the turf industry is also being planned for Perth just before the workshop.

When not planning fieldwork, Natalie Banks has been completing her literature review and plan of study. Kylie Crampton has started her field work and has established cultures of root lesion nematodes from grain growing regions in NSW. She will be testing the efficacy of various soil pathogens for control. She has identified field sites and strategies for her research.

Abdul Gafur's thesis is nearly complete and fills in gaps in the taxonomy of the subfamily Radopholinae, which includes burrowing nematodes (*Radopholus*) as well as several native genera like *Achlysiella*. As a result of findings from his project, he has had to venture into the taxonomy of the family Hoplolaimidae as well as Pratylenchidae where the burrowing nematodes have been classified. Nevertheless, he has some good morphological and molecular data that resolves some long standing issues with the taxonomy of the various groups. We probably don't need reminding that taxonomy of nematodes is slow and difficult work.

Sunil Singh came to realise that publishing papers can take a long time especially from thesis work after you have moved on to other projects. His paper on distribution and diversity of root-knot nematodes in agricultural areas of Fiji has lately been published (Singh SK, Khurma UR, Lockhart PJ. 2012. Distribution and diversity of root-knot nematodes in agricultural areas of Fiji. Nematropica 42:16-25.), almost three years after completing his MSc thesis. A lesson learnt, he now has three papers under various stages of review from his PhD work and is doing his thesis by publications.

*Sunil Singh and Mike Hodda*

## NEWS FROM QUEENSLAND

**Research Facility, Department of Agriculture, Fisheries and Forestry - Toowoomba**

The Leslie Research Facility (LRF) celebrated its fiftieth anniversary in September 2012. Nikki Seymour organised a very successful day for current and former staff and our collaborators to highlight the centre's achievements and current research. John Thompson prepared a plaque for grain grower, Alex Gwynne to thank him for his generous, long-term support of our nematode field work. Alex was instrumental in prompting researchers to discover that root-lesion nematodes were a cause of poor yields of wheat on the Darling Downs of Queensland.

Photo above right - Queensland Minister for Agriculture, Fisheries and Forestry, Hon. John McVeigh presented a plaque to thank grain grower, Alex Gwynne for the generous, long-term support of the Gwynne family for nematode research at the fiftieth anniversary celebration of the Leslie Research Facility (the former Queensland Wheat Research Institute) on 7 September 2012 (Photo: D. Herde)

Photo on right - A new foyer display (featuring Tim Clewett at the microscope) and the 37 wheat cultivars released by the Leslie Research Facility was prepared for our Fiftieth anniversary celebrations (Photo: D. Herde)

*Kirsty Owen*

In the northern grain region, a large range of crops are grown in summer and the host status of these to *Pratylenchus thornei* is being investigated as part of the GRDC-funded project - 'Northern Integrated Disease Management'. A summer crop rotation experiment was planted in December 2011 on our *Pratylenchus thornei* site (starting populations were between 2,000-3,000/kg soil to 90 cm deep). Several cultivar of soybean, mungbean, sunflower, maize and sorghum as well as a fallow treatment were included in the design. The season was very dry but the crops grew well on stored soil water. From preliminary results, one week after harvest, *P. thornei* populations were greatest at 0-15 cm but were found to 120 cm soil depth. Populations were highest after the soybeans (<10,500/kg soil at 0-15 cm) and least after sunflower and fallow (<3,800/kg soil at 0-15 cm). Maize and sorghum were poor hosts (4,600/kg soil). The experiment is still underway and in 2013 we will plant an intolerant and tolerant wheat cultivar on all plots and plan to hold a field day at the site in 2013. This project will finish in 30 June 2013 and negotiations for a new project will begin shortly.

*Kirsty Owen, Tim Clewett and John Thompson*

In the GRDC-funded project 'Genetic Options for Nematode Control' wheat germplasm has been released for use by Australian wheat breeding companies. Five cultivars (QT8343, QT8447, QT9048, QT9050, QT12242) with tolerance and resistance to *Pratylenchus thornei* were developed by staff at the Leslie Research Facility and were selected from crosses between GS50a (*P. thornei* tolerant and resistant) and Cunningham and Janz. The cultivars out-yield EGA Wylie, the most *P. thornei* tolerant commercial cultivar in the Australian northern grain region, on average by 2-8% (Table 1) and have retained levels of resistance similar to GS50a in both glasshouse and field testing (Table 1; Figure 1). Under the Australian Winter Cereals Pre-breeding Alliance guidelines, these cultivars are classified Tier 1 germplasm which means they are freely available without IP restrictions and will be distributed upon request under a material transfer agreement.

*Jason Sheedy and John Thompson*

A component of the 'Genetics Options for Nematode Control' project is to conduct surveys for root-lesion nematodes (RLN) in areas previously under-sampled in the northern grain region. In 2012, paddocks in Central Queensland and north-west NSW regions were sampled. Combining these samples with the samples received through 'Test your farm for nematodes' a total of 104 paddocks where tested in 2012. Of these 72% were found to have *Pratylenchus* (41 % *P. thornei*, 4% *P. neglectus*, 27% both *P. thornei* and *P. neglectus*). *Merlinius brevidens* was observed in 72% of these paddocks. Results are being used to construct RLN distribution maps for the northern region. The maps will operate using the Google map browser where RLN results can be searched on nearest town.

*Neil Robinson*

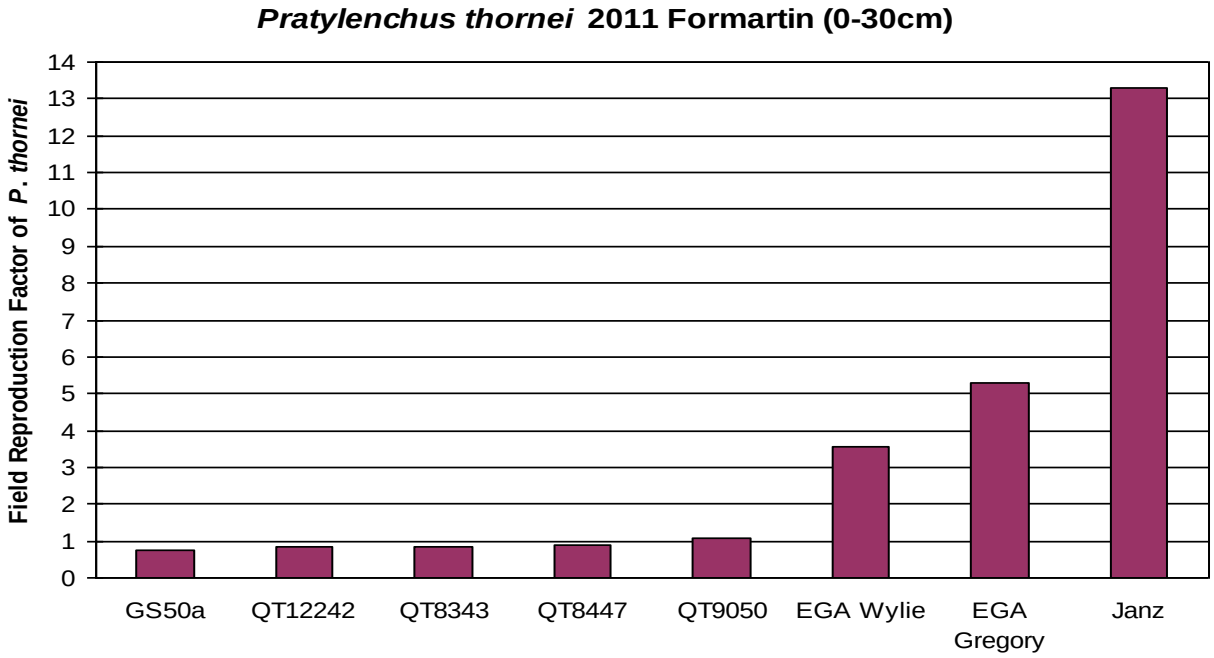


Figure 1. Root-lesion nematode (*Pratylenchus thornei*) resistant wheat cultivars reduce *P. thornei* populations compared to existing susceptible commercial cultivars (reproduction factor = final/initial population). Results are from a field experiment at Formartin, Queensland.

Table 1. Root-lesion nematode (*Pratylenchus thornei*) tolerant and resistant wheat cultivars which were released to Australian wheat breeding companies will out-yield current commercial cultivars in *P. thornei* infested soil.

| **Cultivar** | **Synonym** | **Pedigree** | ***Pratylenchus thornei*** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | **Tolerance (Field)** | | | | **Resistance (Glasshouse)** | | | |
| | | | **Yield (% of Wylie)** | **Classification[a]** | **No. Trials** | **No. Years** | **Reproduction Factor** | **Classification[b]** | **No. Trials** | **No. Years** |
| Cunningham | QT3826 | 3Ag3/4*Condor//Cook | 61 | I | 21 | 12 | 25.4 | VS | 14 | 11 |
| EGA Wylie | QT10198 | QT2327/Cook//QT2804 | 100 | T-MT | 11 | 7 | 14.4 | S | 7 | 7 |
| EGA Gregory | QT10776 | Pelsart/2*Batavia DH | 93 | MT | 14 | 8 | 11.4 | MS-S | 8 | 8 |
| GS50a | GS50a | Gatcher Selection 50a | 92 | MT | 21 | 12 | 3.6 | MR | 28 | 15 |
| Janz | QT3685 | 3Ag3/3*Condor//Cook | 61 | I-VI | 18 | 10 | 22.7 | VS | 23 | 14 |
| QT12242 | QT12242 | GS50a/3*Janz/Cunningham | 104 | T-MT | 5 | 3 | 3.1 | MR | 1 | 1 |
| QT8343 | QT8343 | GS50a/3*Cunningham/Janz | 104 | T-MT | 11 | 4 | 3.1 | MR | 17 | 12 |
| QT8447 | QT8447 | GS50a/3*Cunningham/Janz | 108 | T | 17 | 9 | 6.2 | MR-MS | 12 | 11 |
| QT9048 | QT9048 | GS50a/3*Cunningham/Janz | 103 | T-MT | 6 | 2 | 3.2 | MR | 15 | 10 |
| QT9050 | QT9050 | GS50a/3*Cunningham/Janz | 102 | T-MT | 17 | 9 | 5.4 | MR-MS | 11 | 10 |

[a]I, Intolerant, MT, Moderately Tolerant; T-MT, Tolerant-Moderately Tolerant; T, Tolerant.

[b]VS, Very susceptible; S, Susceptible; MS-S, Moderately Susceptible-Susceptible; MR-MS, Moderately Resistant-Moderately Susceptible; MR, Moderately Resistant.

## NEWS FROM VICTORIA

**DPI Victoria**

***Cereal Nematology***

DPI Victoria has been collaborating with SARDI on a GRDC-funded project to screen cereal crops in the field for resistance and tolerance to root lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) and cereal cyst nematodes. At multiple field sites, RLN numbers are manipulated using susceptible and resistant varieties. Test lines are evaluated for tolerance in the presence of elevated nematode populations, and varieties are also screened for resistance by assessing in-crop multiplication using soil sampling pre-sowing and post-harvest. The large number of soil samples generated are analysed using the DNA assay at SARDI. The data collected from these trials is assisting growers to minimise losses associated with these nematodes.

***Pulse Germplasm Enhancement***

Matthew Rodda, previously a postgraduate student at the University of Adelaide, started with DPI Victoria in October 2011, and has been working on the Pulse Germplasm Enhancement program. This project, funded by the GRDC and DPI Victoria, incorporates a component on RLN resistance in chickpea. Chickpea breeders in Australia are looking for a more cost effective method to screen lines for RLN resistance. Some resistance to RLN has been previously found in wild species related to cultivated chickpea, and backcrossed hybrids are in the breeding program, but this material has not been rescreened.

To date, the work on this project has been preliminary. Matthew has been investigating possible screening options including controlled environment and field screening. Matthew recently established a culture of *P. thornei* on carrot disk callus to use for experiments at Horsham. Danuta Pounsett, SARDI, provided starter cultures and methodology advice. Matthew has also gotten a misting chamber up and running at Horsham, donated by Ian Riley, University of Adelaide.

*Matthew Rodda and Grant Hollaway*

## NEWS FROM WESTERN AUSTRALIA

**Plant Biotechnology Research Group (PBRG) – Murdoch University**

The main focus of the work in the Plant Nematology Section of the Plant Biotechnology Research Group (PBRG) at Murdoch University is to understand the molecular basis of nematode-plant interactions, and then to use that information to develop new forms of synthetic resistance that is widely applicable and has the potential to confer broader resistance to plant parasitic nematodes.

*Current researchers at PBRG:*
Prof. Mike Jones
Dr John Fosu-Nyarko
Murdoch University Distinguished Collaborator: A/Prof. Derek Goto, Hokkaido University, Japan.

*PhD students:*
Jo-Anne Tan
Harshini Herath
Sadia Iqbal
Malathy Rathinasamy

*Recently completed students:*
Matthew Tan

***Activities***

Mike attended and presented at the MPMI meeting in Kyoto, Japan, and the Seventh Australian Soilborne Disease Symposium held at Fremantle. Mike, Jo-Anne and Sadia also attended the European Society of Nematologists (ESN) meeting at Adana in Turkey, and each were invited to give oral presentations.

Mike also attended Sadia's wedding celebrations in Faisalabad, Pakistan on 19-26 December 2012, and gave a seminar at the National Institute for Biotechnology and Genetic Engineering. This was a truly memorable experience, and Sadia's family looked after Mike really well. Here is a picture of Sadia on her wedding day to brighten up this report.

Mike and former student Derek Goto and now recognised by Murdoch University as a 'Distinguished Collaborator', currently at Hokkaido University, also organised the third and fourth Japan-Australia Symposia on Plant Sciences for Agriculture, at Hokkaido University and Murdoch University respectively, in January and December of 2012 (http://www.agr.hokudai.ac.jp/JAS-IV/). Both Symposia had strong plant nematology sessions, with Derek focussing on the processes involved in the formation of root-knot nematode giant cells.

*Mike Jones*

**Japan- Australia Symposium IV**

## S18: HIJACK OF A SYMBIOSIS SIGNALING PATHWAY BY PARASITIC NEMATODES FOR INFECTION OF PLANT ROOTS

Hikota Miyazawa[1], Shuhei Hayashi[1], Narumi Souda[2], Takuya Suzaki[3], Masayoshi Kawaguchi[3], Erika Asamizu[2] & Derek Goto[1]

*[1]Faculty of Agriculture, Hokkaido University, Sapporo, Japan; [2]University of Tsukuba, Tsukuba, Japan; [3]National Institute for Basic Biology, Okazaki, Japan.*

**Abstract:**
Root-knot nematodes (RKNs) are obligate parasites that establish specialised feeding sites in plants roots and remain protected at this single site for their whole life-cycle. RKNs do not show host specificity and are able to infect a broad range of important plant species, resulting in major losses to agricultural production worldwide. Understanding RKN infection site development at the molecular level is currently limited, particularly in the context of the broad host range. Most land plants form a beneficial interaction with symbiotic microorganisms, such as arbuscular mycorrhizal fungi (AMF). Studies using the model legume *Lotus japonicus* have revealed a plant genetic pathway regulating these interactions, which contains a common symbiosis pathway shared by both AMF and rhizobia. Using a new quantitative assay for *Lotus*, we show that loss-of-function of this pathway significantly represses the RKN infection cycle. This repression occurred after initiation of infection, suggesting a direct role in infection site development rather than an indirect effect related to root entry or target cell selection. Analysis of homologous genes in tomato confirmed that the requirement for this signalling pathway is representative of general RKN infection. These data reveal that RKNs achieve parasitic success in different host species by taking advantage of a common plant symbiosis pathway, deceiving the host into acceptance of infection site development (http://www.agr.hokudai.ac.jp/gotolab/)

JAS IV group photo, December 2012

# International News

## BELGIUM

Lea Meagher graduated from the University of Newcastle in 2009 with a Bachelor of Science. Lea then worked in the Pathology Department at BSES as a Research Assistant for 2 years. She is currently studying a Masters of Science in Nematology at Ghent University in Belgium after winning a Erasmus Mundas Scholarship. She has sent us an update after completing her first semester.

So my first semester of the Masters of Nematology course here in Ghent is drawing to a close, I'm in the middle of exams right now. The semester has been very intense but I have learnt a substantial amount from not only the main lecturers, such as Nic Smol, Wilfrida Decraemer and Wim Bert, but also guest lecturers who are specialists in their fields, including Eyualem Abebe, Sergei Subbotin and Roland Perry (who asked me to pass on his regards to those of you that know him as Rolo). We've had the opportunity to visit ILVO, the Institute for Agriculture and Plant Science Research, and to carry out some lab work there. It was intriguing to compare some of their culturing and inoculation methods to those currently used in Australia for PPN work. A visit to the laboratories for the Research of Aging, Physiology and Molecular Evolution for *Caenorhabditis elegans* was also extremely interesting as the technology being utilised there is world class.

I have decided to major in Agricultural Ecosystems, as opposed to Natural Ecosystems, and am looking forward to completing subjects such as behaviour and physiology of PPN, tropical plant nematology and systematics and life cycle of PPN along with virus vectors families, molecular and aging biology subjects in the coming months. I hear you are all enjoying a very hot, sweaty summer back in Australia, today we had more snow and the temperature was a chilly -11°C this morning. Best wishes to everyone for a happy, healthy and successful 2013.

*Lea Meagher*

# Research

## DISTRIBUTION OF SOUTHERN STING NEMATODE, *IBIPORA LOLII* (NEMATODA: BELONOLAIMIDAE), ON TURFGRASS IN AUSTRALIA AND ITS TAXONOMIC RELATIONSHIP TO OTHER BELONOLAIMIDS

Graham R. Stirling [1], A. Marcelle Stirling [1], Robin M. Giblin-Davis [2], Weimin Ye [3], Dorota L. Porazinska [2], Jackie M. Nobbs [4], Kenneth J. Johnston [5]
*Nematology 00 (2012) 1-15*

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Molecular evidence from sequences of three regions of ribosomal DNA (partial SSU, ITS-1, 5.8S and ITS-2, and D2/D3 expansion segments of LSU) is presented to show that the two belonolaimids described from turfgrass in Australia (*Ibipora lolii* and *Morulaimus gigas*) are identical. *Morulaimus gigas* is therefore considered a junior synonym of *I. lolii*. The decision to place the nematode in *Ibipora* rather than *Morulaimus* is supported by molecular studies which showed that *I. lolii* is not closely related to *Morulaimus* or *Carphodorus*, two belonolaimid genera that are only found in Australia. Survey data are presented to show that *I. lolii* is widespread on turfgrass around Newcastle in New South Wales and in Perth, Western Australia, where the infested area is increasing rapidly, largely because the nematode is being spread in planting material. *Ibipora lolii* damages all turfgrass species but is particularly damaging to kikuyu grass (*Pennisetum clandestinum*), the main grass used for sporting fields and recreational areas in warm regions of Australia. Data from an experiment in pots also show that the nematode multiplies to damaging levels on sugarcane. Symptoms on grasses are similar to those caused by the sting nematode, *Belonolaimus longicaudatus*, in south-eastern USA, but because the two nematodes are taxonomically different, *I. lolii* is referred to as the southern sting nematode. *Ibipora lolii* was not found in surveys of natural vegetation on the east and west coasts of Australia, suggesting that it is an introduced species, possibly originating in South America or the Caribbean, where other *Ibipora* species are found.

# GENE SILENCING IN ROOT LESION NEMATODES (PRATYLENCHUS SPP.) SIGNIFICANTLY REDUCES REPRODUCTION IN A PLANT HOST.

Jo-Anne C.H. Tan, Michael G.K. Jones, John Fosu-Nyarko.
*Experimental Parasitology 133 (2013) 166–178.*

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We have used Roche technology to sequence the transcriptome of mixed stages of the root lesion nematode (RLN) *Pratylenchus thornei*, assemble and annotate it (Nicol et al, I. J. Para 42, 225-237, 2012). In current work RNA interference in RLNs was assessed to study gene function in *P. thornei* and *P. zeae*. Conditions were optimised for 'soaking experiments', in which the RLNs were treated with double stranded RNA (dsRNA). Mixed stages of both *P. thornei* and *P. zeae* took up dsRNA in a basic soaking solution (M9 buffer, 0.05 % gelatine) containing 10-50 mM octopamine, 1-6 mM spermidine and 0.1-1 mg/mL FITC for 16 h without detrimental effects. Soaking in spermidine phosphate salt hexahydrate rather than spermidine or spermidine trihydrochloride improved uptake by nematodes, and also resulted in more effective gene silencing. Silencing of the genes *pat-10* and *unc-87* of both nematode species resulted in paralysis and slow and uncoordinated movements, although to a greater extent in *P. thornei*. QPCR analysis showed that there was also a greater reduction in transcripts of both genes in *P. thornei*.

Following dsRNA treatments, replicated axenic carrot 'mini' discs (10 X 10 mm) were inoculated with 50 nematodes each and cultured for 8 weeks: nematodes were extracted and counted from replicates each week. Untreated *P. thornei* and *P. zeae* increased in number 32-fold and 73-fold respectively over 8 weeks. However, for *P. thornei* treated with dsRNA of *pat-10* and *unc-87*, there was a substantial reduction in replication, with 81 % and 77 % reduction in numbers of nematodes respectively at 5 weeks. These results show that RLNs are amenable to gene silencing.

This work describes significant findings: it is the first study to demonstrate gene silencing in *P. thornei* and *P. zeae*, and the most detailed work on gene silencing in root lesion nematodes. It has recently been published in full:

# SILENCING THE EFFECTORS OF RNA SILENCING

Iqbal, Sadia; Fosu-Nyarko, John and Jones, Michael G.K.

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Root knot nematodes (RKNs, *Meloidogyne spp)* are crop pests. These sedentary endoparasites induce and feed from host cells (giant cells). Using RNA interference (RNAi) specific genes can be silenced by introduction of double stranded RNA (dsRNA) homologous to such genes into an organism. This technology has been used widely to study gene function in the free living nematode *C. elegans*, including identification of genes involved in the small interfering RNA (siRNA) and micro RNA (miRNA) pathways. In plant parasitic nematodes, RNAi has been used to study gene function either by *in vitro* feeding of juveniles with dsRNA or *via* a plant producing corresponding dsRNA/siRNA. However, there appear to be differences in the mechanisms of RNAi between free living and plant parasitic nematodes.

The aim of this project is to study the differences that may exist in the RNAi pathways between plant parasitic nematodes and that of *C. elegans.* Genomic data mining and comparative bioinformatics has been used to identify similarities and differences in genes involved in the siRNA and miRNA pathways of these nematode groups. Protein domains and structures of identified RNAi effectors provide insights into their functions. *In vitro* feeding experiments to silence RNAs encoding protein domains of RNAi effectors followed by characterisation of expression of related genes and the fitness of nematodes can provide insights into the RNAi mechanism of RKNs. This study will identify candidate genes with potential for control of RKNs via *in planta* delivery of dsRNA/siRNA, and this can be extended to broader resistance to more than one nematode species.

# MOLECULAR APPROACHES TO DIAGNOSTICS FOR PLANT PARASITIC NEMATODES OF BIOSECURITY CONCERN

Matthew Tan

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The focus of this work was to assess existing methods and to develop new molecular approaches to detect and characterise plant parasitic nematodes of quarantine concern.

ITS-based PCR diagnostics were used to identify three *Pratlylenchus* (RLN) species, to compare different populations, and to study their relatedness. A similar approach was applied to cyst nematodes (*Heterodera* and *Globodera* species) of biosecurity interest.

In a protein-based approach nematode proteins were analysed using MALDI-ToF MS to generate distinct species-specific protein profiles for eight nematode species. Two dimensional protein gel electrophoresis was also undertaken to develop diagnostic biomarkers for nematode identification. Of 58 distinct protein spots for *Pratylenchus* spp. and 89 spots for *Heterodera* spp., 13 and 9 spots respectively were further analysed as potential diagnostic biomarkers. Individual proteins were excised and sequenced after trypsin digestion and the identities of 16 proteins were confirmed.

Two additional diagnostic methods were developed, termed anti-primer QPCR (aQPCR) and 'Multiplex anti-primer denaturation PCR' (MAD PCR). In aQPCR, an 'anti-primer' with a quencher of fluorescence reduces background fluorescence of unincorporated fluorescent label to give better signal to noise detection. An aQPCR multiplex assay system was developed to detect three *Pratylenchus* spp. at the same time. MAD PCR was developed to combine 'anti-primer' technology with 'auto-sticky' PCR, QPCR and fluorescent labels. The aim was to increase the level of multiplexing that could be achieved for nematode diagnostics. In the current work three RLN species were identified with such a multiplex system: with further development the potential exists to increase multiplexing substantially using this strategy.

A bottleneck in nematode diagnostics is often the time taken to extract nematodes from soil. A novel, rapid method was developed to extract nematode DNA from soil samples of 500 gm or more in 2 minutes using a modified blender and capture column, and was termed 'DNA Isolation Rapid Technique from soil'- 'DIRTs'. This extraction procedure was combined with molecular identification of nematodes by QPCR. The procedure for extraction and detection took < 4 hr. When the DIRTs extraction was combined with 'anti-primer' technology, three different RLN species were identified successfully in multiplex reactions. It is suggested that this extraction and detection system is sufficiently robust and rapid that, with further refinement, it could be used in field surveys for nematode detection and quantification.

Both protein and PCR-based procedures can provide robust approaches to detect and characterise plant parasitic nematodes.

# ROOT-LESION NEMATODE (*PRATYLENCHUS THORNEI*) REDUCES NUTRIENT RESPONSE, BIOMASS AND YIELD OF WHEAT IN SORGHUM–FALLOW–WHEAT CROPPING SYSTEMS IN A SUBTROPICAL ENVIRONMENT.

[1]Thompson JP, [2]Mackenzie J, [3]Sheedy GH
*Field Crops Research (2012) 137, 126-140.*

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Wheat in winter and grain sorghum in summer are the most important crops of the subtropical grain region of eastern Australia, where they are often grown in sequences of 1–4 years of each crop species separated by a long fallow period of 11–14 months. Growers have observed that the second wheat crop in sequence can appear nutrient deficient with poor growth and grain yield compared with the first or subsequent crops. To investigate this problem, wheat was grown in various field experiments as the first–fourth wheat in sequence after long fallow from sorghum sequences of various lengths (one–four years) to test for responses in biomass production and grain yield to fertilisers, biocidal fumigants and nematicides. Populations of root-lesion nematodes (*Pratylenchus thornei*) were greatest in the soil before the second wheat crop was sown than before the first or third wheat crops. Greatest responses in wheat growth were obtained to the nematicide aldicarb in various wheat sequence positions up to the fourth (up to 137% increased yield of second wheat). Aldicarb best protected the roots from *P. thornei* allowing increased nutrient response. The fumigants chloropicrin and dazomet caused substantial changes in soil microbial populations and available nutrients, but the systemic nematicides fenamiphos and aldicarb did not. Responses in grain yield were also obtained to N fertiliser and less frequently to P and Zn (41% increase of second wheat to NPZn fertiliser), with best overall responses to a combination of aldicarb and fertiliser. A range of tolerance to *P. thornei*, as judged by both grain yield from nil treatment and response to aldicarb and/or fertiliser, was identified among wheat cultivars. In comparison, one barley cultivar yielded maximally without treatment (up to 3.7 and 1.3 times the yield of the most intolerant and the most tolerant wheat cultivars, respectively). Integrated management using crop rotation with sorghum to reduce *P. thornei* populations combined with growing tolerant wheat and barley cultivars supplied with adequate fertiliser are practical measures to reduce the impact of *P. thornei*.

**INHERITANCE OF RESISTANCE TO ROOT-LESION NEMATODES (*PRATYLENCHUS THORNEI* AND *P. NEGLECTUS*) IN FIVE DOUBLED-HAPLOID POPULATIONS OF WHEAT.**

[1]Thompson JP, [2]Zwart ZS, Butler D
*Euphytica (2012) 188, 209-219.*

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Nematode species *Pratylenchus thornei* and *P. neglectus* are the two most important root-lesion-nematodes affecting wheat (*Triticum aestivum* L.) and other grain crops in Australia. For practical plant breeding, it will be valuable to know the mode of inheritance of resistance and whether the same set of genes confer resistance to both species. We evaluated reactions to *P. thornei* and *P. neglectus* of glasshouse-inoculated plants of five doubled-haploid populations derived from five resistant synthetic hexpaloid wheat lines, each crossed to the susceptible Australian wheat cultivar Janz. For each cross we determined genetic variance, heritability and minimum number of effective resistance genes for each nematode species. Distributions of nematode numbers for both species were continuous for all doubled-haploid populations. Heritabilities were high and the resistances were controlled by 4–7 genes. There was no genetic correlation between resistance to P. thornei and to *P. neglectus* in four of the populations and a significant but low correlation in one. Therefore, resistances to *P. thornei* and to *P. neglectus* are probably inherited quantitatively and independently in four of these synthetic hexaploid wheat populations, with the possibility of at least one genetic factor contributing to resistance to both species in one of the populations. Parents with the greatest level of resistance will be the best to use as donor parents to adapted cultivars, and selection of resistance to both species in early generations will be optimal to carry resistance through successive cycles of inbreeding to produce resistant cultivars for release.

**DIPLOID AND TETRAPLOID PROGENITORS OF WHEAT ARE VALUABLE SOURCES OF RESISTANCE TO THE ROOT LESION NEMATODE *PRATYLENCHUS THORNEI.***

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*Euphytica (2012) 186, 377–391.*

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The root lesion nematode *Pratylenchus thornei* is widely distributed in Australian wheat (*Triticum aestivum*) producing regions and can reduce yield by more than 50%, costing the industry AU$50 M/year. Genetic resistance is the most effective form of management but no commercial cultivars are resistant (R) and the best parental lines are only moderately R. The wild relatives of wheat have evolved in *P. thornei*-infested soil for millennia and may have superior levels of resistance that can be transferred to commercial wheats. To evaluate this hypothesis, a collection of 251 accessions of wheat and related species was tested for resistance to *P. thornei* under controlled conditions in glasshouse pot experiments over two consecutive years. Diploid accessions were more R than tetraploid accessions which proved more R than hexaploid accessions. Of the diploid accessions, 11 (52%) *Aegilops speltoides* (S-[B]-genome), 10 (43%) *Triticum monococcum* ($A^m$-genome) and 5 (24%) Triticum urartu ($A^u$-genome) accessions were R. One tetraploid accession *(Triticum dicoccoides*) was R. This establishes for the first time that *P. thornei* resistance is located on the A-genome and confirms resistance on the B-genome. Since previous research has shown that the moderate levels of *P. thornei* resistance in hexaploid wheat are dose-dependent, additive and located on the B and D-genomes, it would seem efficient to target A-genome resistance for introduction to hexaploid lines through direct crossing, using durum wheat as a bridging species and/or through the development of amphiploids. This would allow resistances from each genome to be combined to generate a higher level of resistance than is currently available in hexaploid wheat.

**Articles and Abstracts from the Proceedings of Seventh Australasian Soilborne Diseases Symposium', Ed WJ MacLeod. 17–20 September 2012, Fremantle, Western Australia**

# NEW APPROACHES FOR PLANT RESISTANCE TO NEMATODE PATHOGENS

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## ABSTRACT

Plant parasitic nematodes are an economically important group of soil pathogens of broadacre and horticultural crop plants. They reduce crop yields by ~7-15% directly by damaging roots and reducing the availability of water and nutrients, and indirectly by enabling other root pathogens to access roots. Having been a rather neglected group of pathogens, new resources provided by study of the free-living nematode, *Caenorhabditis elegans* have stimulated research to develop new forms of resistance to plant parasitic nematodes. The genome of *C. elegans* is the best annotated of any multicellular organism, and is highly amenable to gene silencing (RNAi), such that most of its 20,500 genes have been silenced, and many genes vital for its survival have been identified. This technology has now been mapped across to plant parasitic nematodes, and has enabled identification of a range of target genes. We have used 'Next Generation' sequencing to analyse the transcriptomes of two root lesion nematode species (*Pratylenchus thornei* and *P. zeae*) and one cyst nematode species (*Heterodera schachtii*), and then applied RNAi technology via transgenic plants to silence target genes in these species. The results provide proof-of concept that host resistance (up to 95% reduction in infection) to root lesion and cyst nematodes can be conferred respectively to wheat, sugarcane and Arabidopsis.

## INTRODUCTION

Plant parasitic nematodes cause an estimated $125 billion pa in crop losses worldwide. Because they are soil-borne, these crop pathogens have been relatively intractable to work with, but with the rapid advances in genomics and molecular biology, genomic resources developed for *C. elegans* can now be applied to develop new approaches to host resistance to these soil pathogens. We have applied these resources to develop resistance to root lesion nematodes (*Pratylenchus* spp.) that attack wheat and sugarcane, and the beet cyst-nematode, *Heterodera schachtii*. Using new sequencing technologies (Roche 454 FLX, Illumina), we have undertaken transcriptome analyses of *P. thornei* and *P. zeae*, and *Heterodera schachtii.*

Following annotation and comparative genomic analyses, we identified a series of potential target genes which if silenced by RNA interference (RNAi) would confer host resistance. Two approaches to test the effects of silencing target genes were undertaken – 'soaking' J2 nematodes in dsRNA, and delivery of dsRNA to nematodes via transgenic plants. Methods were established to generate transgenic plants of wheat, sugarcane and Arabidopsis, and lines of different transgenic events were challenged in soil or sand with J2s of the different nematode species.

The results provide clear proof-of-concept that RNAi can be used to confer host resistance to nematode pathogens both in dicotyledonous and monocotyledonous crop plants.

## MATERIALS AND METHODS

**Nematodes.** *P. thornei* cultures were derived from single nematodes infecting wheat plants in WA; *P zeae* was isolated from sugarcane in Queensland and maintained on sorghum plants. Both were kept on carrot pieces *in vitro.* *H. schachtii* cysts were collected from infested Brassica plants near Perth. **Next generation sequencing.** RNA isolated from nematodes was sequenced by Roche 454FLX or Illumina technologies at Murdoch University, and the reads assembled and annotated using various software packages (1). **Plant transformation.** Transgenic plants of wheat and sugarcane containing constructs that generated dsRNA *in planta* were generated after particle bombardment and selection *in vitro.* Arabidopsis plants were transformed by the floral dip method using Agrobacterium as vector. **Nematode challenge.** Mixed stages of *Pratylenchus* species bulked up on carrot discs, or J2s of *H. schachtii* hatched from eggs in cysts were added to replicated plants grown in sand (wheat, sugarcane) or a sand soil mixture (Arabidopsis), and following up to two month's culture, nematodes present were extracted by a mister, or cysts numbers were counted, and dry root weights recorded.

## RESULTS

A reduction in nematode replication was found when five target genes in *H. schachtii* were silenced using RNAi. A typical result is shown for target gene *Lev-11* in Figure 1, in which replicates of different transgenic events reduced the percentage infection of roots by between 10 and 90%. Similar results were obtained both for wheat infected with *P. thornei* and sugarcane plants infected with *P. zeae*. In addition, in 'soaking' experiments, cross-protection was conferred by RNAi using a sequence from *P. thornei* against *P. zeae* and vice-versa, indicating that careful choice of target sequences may provide broader spectrum resistance.

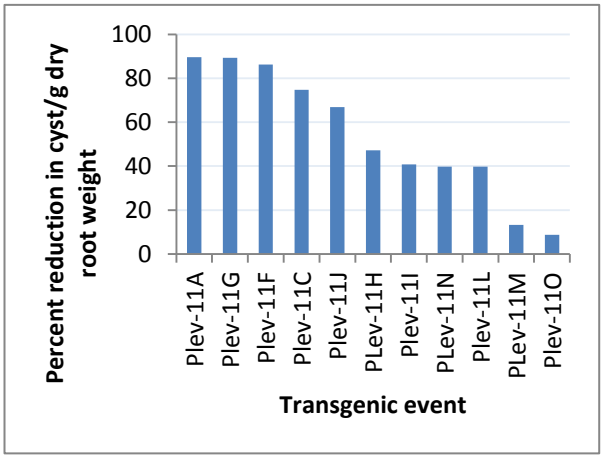


***Figure 1.*** *Different events of transgenic Arabidopsis plants expressing dsRNA to lev-11, challenged with H. schachtii in soil (10-15 replicate lines per event). The percentage reduction in cysts is expressed per gram dry root weight to allow for differences in root growth of different plants.*

## DISCUSSION

This work demonstrates that silencing of plant parasitic nematode target genes using RNAi, by delivery of dsRNA via a host plant, can be used to confer host resistance to these nematodes. Significantly, this can be achieved in both dicotyledonous and monocotyledonous crop plants, to two economically important species of root lesion nematodes and to one economically important cyst nematode, the beet cyst nematode. This work also provides the first evidence that one target sequence can silence gene expression in two different species of plant parasitic nematodes.

## REFERENCES

(1) Nicol, P. et al (2012). De novo analysis and functional classification of the transcriptome of the root lesion nematode, *Pratylenchus thornei,* after 454 GS FLX sequencing. Int. Journal or Parasitology **42**, 225-237.

## FREE LIVING NEMATODES AS INDICATORS OF THE BIOLOGICAL STATUS OF AUSTRALIA'S CEREAL-GROWING SOILS

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The impact of management practices on the biological status of cereal-growing soils was analysed through nematode community analysis in selected treatments from nine field trials across Australia (3 SA, 1 VIC, 1 QLD and 4 WA). In general, soils with pasture rotations had more structured and diverse communities than continuously cropped soils, indicating that pasture inclusion in farming systems results in a more diverse soil food web with many trophic links, multitrophic interactions and higher predatory activity. Tilled (CT) soils had higher total free-living nematodes (FLN) numbers, particularly bacterivores, than reduced (RT) or no-till (NT) soils. The addition of lime to an acid soil (pH 4.7) increased the proportion of fungivores and omnivores in the nematode community, indicating that the soil biological status had improved. At many sites, the number and diversity of FLN was relatively low, suggesting that management practices which deplete soil C (e.g. excessive tillage) were a contributing factor. Since manual nematode community analysis is laborious and requires specialised taxonomic skills, molecular technologies are being developed to allow routine FLN identification and quantification in soil. DNA probes have been designed around the 18s rRNA region to specifically identify common FLN groups, including the predatory Mononchida, omnivorous Dorylaimida and fungivorous Aphelenchoidea.

## WHAT IS THE IMPACT OF WINTER GRAIN CROPS ON *PRATYLENCHUS THORNEI* GROWN IN ROTATION WITH TOLERANT AND INTOLERANT WHEAT?

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Management of the root-lesion nematode, *Pratylenchus thornei* (*Pt*), is central to wheat production in the northern grain region of Australia and relies on rotation with resistant crops and growing tolerant wheat cultivars. We determined the residual populations of *Pt* to 90 cm soil depth after winter grain crops and their impact on the growth of subsequently planted tolerant and intolerant wheat cultivars. A weed-free fallow and 5–6 cultivars each of faba bean, chickpea, barley and wheat were treatments on two areas of land, 1) 4,500 *Pt*/kg soil at 0–45 cm following 7 months fallow after wheat and 2) 2,100 *Pt*/kg soil at 0–45 cm following 14 months fallow after sorghum. Six months after harvest there were 10,000–22,700 *Pt*/kg soil 0–45 cm after most barley, faba bean and commercial wheat cultivars; 2,600–6,000 *Pt*/kg soil 0–45 cm after wheat cvv. QT9050, GS50a, chickpea cv. PBA Hattrick and fallow. There was a negative, linear relationship between *Pt* (to 90 cm) and biomass of the intolerant wheat cv. Strzelecki (max. $R^2 = 0.70$ $P<0.001$ at 0–45 cm). There was no impact of residual *Pt* on the tolerant wheat cv. EGA Wylie. The tolerance of cv. EGA Wylie was robust but breeding resistant barley, faba bean and wheat will improve management options.

**SIMULTANEOUS SELECTION OF WHEAT PLANTS WITH RESISTANCE TO ROOT-LESION NEMATODES, CROWN ROT AND YELLOW SPOT.**

Sheedy, JG, Thompson JP, Reen RA

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Root-lesion nematodes (*Pratylenchus thornei* [Pt] & *P. neglectus* [Pn]), crown rot (CR; *Fusarium pseudograminearum*) and yellow spot (YS; *Pyrenophora tritici-repentis*) are four economically important diseases of wheat that commonly occur in combination and cost northern growers $137 M annually. Therefore, we need to develop germplasm with combinations of disease resistance to minimise yield loss in these situations. To achieve this, the established screening methods for each disease were combined and where necessary modified, to allow testing of up to 4 diseases simultaneously. Initial experiments tested every disease combination on up to 42 fixed cultivars covering resistant to susceptible disease reactions to develop a procedure that could be used to select individuals with resistance to Pt, Pn, CR and YS. This procedure was used to select resistant individuals in an experiment of 21 check cultivars and 132 segregating $F_3$'s exposed to all 4 diseases. The check cultivars in the $F_3$ experiment were significantly correlated with long-term rankings for YS (r = 0.96***), CR (r = -0.61**) Pt (r = 0.84**) and Pn (r = 0.52*) and we were able to select nine F3's (7%) with resistance to all four diseases. This procedure will likely hasten the development of multiple-disease resistant parents, suitable for introduction into commercial breeding programs.

# Thesis Summary

## CHARACTERISATION OF BARLEY ROOTS RESPONSES TO INFECTION BY *HETERODERA AVENAE* WOLL

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*Heterodera avenae* Woll. (cereal cyst nematode; CCN) infects the roots of barley (*Hordeum vulgare* L.) by forming feeding sites (syncytia) near vascular tissues. Syncytia form in both resistant and susceptible barley cultivars, but resistance leads to fewer nematodes developing into mature cysts. The mechanisms of syncytia formation and collapse in barley are not well understood.

Responses to infection by cereal cyst nematode were investigated in the roots of susceptible and resistant barley cultivars through nematode infection assays, histological analysis of developing syncytia, cell wall compositional analysis and cell wall-related gene transcriptional analysis.

Syncytium development differed between susceptible and resistant cultivars, as well as among resistant cultivars. In susceptible plants, syncytia continued to develop until at least 25 days after inoculation (dai). In resistant plants, syncytia developed more rapidly than in susceptible plants, but by 11 dai they showed signs of degeneration, including accumulation of large vacuoles and reduced cytoplasmic activity. No clear differences in monosaccharide composition or (1,3;1,4)-β-D-glucan levels were identified between treatments. However, immunolocalisation and transmission electron microscopy (TEM) showed an accumulation of arabinoxylan and (1,3;1,4)-β-D-glucan in syncitial cell walls at 18 dai. Transcripts of the cellulose-like synthase genes *HvCslF3*, *HvCslF6* and *HvGS10* were elevated at dai 14 in syncytia of infected roots, with *HvGS10* expression elevated only in resistant cultivars. This coincided with a key time point in *H. avenae* GAPDH expression, where nematode viability apparently diverged between resistant and susceptible cultivars.

Different resistance mechanisms in CCN-resistant barley cultivars that relate to the speed of syncytium formation. Moreover, cell wall biosynthetic enzymes are induced during the CCN-infection process and possibly act as part of the plant pathogen response.

# Obituary

## GREGOR WILLIAM YEATES (1944-2012)

Gregor Yeates died on 6 August 2012 in Palmerston North, New Zealand, after a short illness.

Gregor was born in Palmerston North, New Zealand on 19 May 1944. He was educated at Palmerston North Boy's High School and completed a B. Sc. (Hons) at Canterbury University. Gregor completed a year of his PhD studies at Massey University before returning to Canterbury to finish his degree in 1968 under the tutorage of Prof. Wally Clark. Gregor married Judith (née Killick) on 31 August 1968 – Judy had been working with J. B. Goodey at Rothamsted Experimental Station (now Rothamsted Research), UK. They returned to Rothamsted that year for Gregor's post-doc appointment, then, with Judy as an assistant, Gregor worked with Malcolm Luxton at Molslab, University of Aarhus, Aarhus, Denmark, in the International Biological Programme studying the nematode populations in beech forests. This was the beginning of his extensive network with overseas soil biologists.

Gregor returned to the Soil Bureau, Department of Scientific and Industrial Research (DSIR), at Taita in 1970 to join the Soil Biology section led by Dr John Stout. Here, he demonstrated the effects and economic impact of clover root nematodes (*Heterodera* and *Meloidogyne*) on white clover growth and symbiotic nitrogen fixation in New Zealand pastures. This 1970s work underpinned the DSIR, MAF Technology and, subsequently, the AgResearch plant breeding programmes. In the 1992 restructuring of science in New Zealand, Gregor moved with other staff to the Landcare Research Crown Research Institute located on theMassey University Turitea campus. Canterbury University awarded a DSc to Gregor in 1986. He retired from Landcare Research in 2009.

During a career that spanned more than 40 years, Gregor made a significant contribution to the science of nematology, primarily through his work in nematode ecology. His strength as an ecologist was his broad understanding of nematodes, which developed from a strong foundation in taxonomy that he established early in his career. Gregor was unusual amongst nematode taxonomists in that he described plant-parasitic and free-living nematodes in groups as diverse as the Tylenchida, Araeolaimida, Oncholaimidae, Ironidae, Alaimidae, Mononchidae, Plectidae, Diphtherophoroidea, Acrobelinae, Dorylaimida, Monohysterida, Chromadorida and Rhabditida. This breadth of knowledge of the nematode community gave him an exceptional insight into the ecological roles of a wide variety of nematodes.

In the 1970s, he was one of the first nematologists to encourage his fellow scientists (who were primarily interested in plant nematodes) to expand their horizons and encompass the whole nematode community. His first paper on feeding groups in plant and soil nematodes was published in 1971, while he reviewed the early work on nematodes in terrestrial ecosystems in the *Journal of Nematology* in 1979. The decade of the 1970s saw an enormous expansion in our knowledge of many aspects of soil biology and ecology, and Gregor was one of a limited number of nematologists who ensured that the role of nematodes was recognised and investigated. A conference held in Arizona, USA, in 1980 stimulated interest in the structure and function of soil nematode communities, with a paper by Yeates and Coleman being particularly important because it highlighted the key role of

nematodes in nutrient cycling and the beneficial impact of some groups of nematodes on plant growth. A paper by Yeates *et al.* in 1993 entitled 'Feeding habits in soil nematode families and genera – an outline for soil ecologists' has become the most commonly cited source of information on the ecological classification of nematodes – cited 903 times (Google Scholar).

Gregor established the positive correlation between nematode abundance and pasture herbage production, in contrast to previous ideas of nematodes always being detrimental; the related nematode: microbial interaction superseded the energy flow approach used in the IBP study in the Danish beech forest. This underpinned much work, both individually and internationally, on the relations of nematodes to their environment, the contribution of soil nematodes to ecosystem processes, biodiversity studies and in assessing the potential impact of both land use management and environmental changes on ecosystem function.

Gregor conducted environmental impact assessments, in several countries, on the use of the nematode-trapping fungus *Duddingtonia flagrans* (or *Arthrobotrys flagrans*) for biological control of gastro-intestinal nematodes of grazing animals. On-farm economic returns using feed additives were demonstrated in Sweden. He was instrumental in discovering and recording the occurrence of *D. flagrans* in New Zealand. Gregor provided impetus for the development, in New Zealand, of a novel delivery mechanism for *D. flagrans* suitable for extensive grazing systems.

iven the complexity of the biological environment in which nematodes live, it is impossible to make major contributions on the ecology of nematodes without some understanding of the many other organisms that share the same habitat. Gregor recognised the biological complexity of soil and, wherever possible, made observations on components of the soil food web other than nematodes. This was evidenced by his studies on the population dynamics and community structure of arthropods, earthworms, flatworms, enchytraeids, tardigrades, planarians and protozoa, and his co-authorship of papers on the response of the soil microbial biomass to plant litter decomposition.

Gregor received many awards, distinctions and invitations to collaborate and to speak. Perhaps the most prestigious of these were: Nuffield Foundation Commonwealth Travelling Fellowship (1977-1978), Nematology Department, Rothamsted Experimental Station; Fellow of New Zealand Society of Soil Science (1995) '*In recognition of distinction in the application and / or advancement of Soil Science*'; Fellow of the Royal Society of New Zealand (1998), '*In recognition of distinction in research and the advancement of science*'; Honorary Member of the Royal Society of New Zealand Manawatu Branch (2004), '*In recognition of his services to the branch over many years*'; Norman Taylor Memorial Award of the New Zealand Society of Soil Science (2006) '*In recognition of outstanding contributions to Soil Science in New Zealand*'; Fellow of the Society of Nematologists (2007) '*For outstanding contributions to nematology*'.

Gregor had a work rate and a publication record which made the rest of us reflect on our own. He had over 290 scientific papers, chapters, invited reviews, *etc*. (plus one in press and another eight in preparation), 310 'communications' being abstracts, conference papers, reports, *etc*., including 40 items to newsletters as New Zealand correspondent. The top cited paper has been mentioned above and there appear to be 17 papers with more than 100 citations each (Google Scholar). Gregor was proud of the book he edited and contributed to with Vince Neall, *Plains' science: inventions, innovations and discoveries in the Manawatu* (2011). The book celebrates the science discoveries made in the Manawatu.

Gregor contributed much to his local community. He was a City Councillor on the Upper Hutt City Council in the 1970s and concurrently a member of the Hutt Valley Drainage Board. The Palmerston North Science Centre benefitted from his work on committees which extended to mentoring school pupils, especially those doing Science Fair and 'Crest' activities. Recently, he was that 'cool scientist' taking science activities at Bunnythorpe School.

Gregor also served the New Zealand Rhododendron Association in several roles.

Gregor was appreciated by nematologists around the world for his thorough reviewing of their manuscripts and was on the editorial boards of numerous scientific journals (recently eight). On retiring, his refereeing rate fell from about 50 manuscripts per year to about 40. He had a reputation for being meticulous in every endeavour he did, particularly appreciated by his post-graduate students – he brought out the best in them.

Gregor was a courteous gentleman with a caring nature. His legacy is one of an extraordinary work ethic and high standards in work and service.

The New Zealand Maori have a saying, 'Kua hinga he totara i te wao nui a Tane' meaning the totara (tree) has fallen in the sacred forest of Tāne, repeated whenever a great person dies. This is apt for Gregor considering his love for the New Zealand forest and his studies of its ecology.

Gregor is survived by his wife Judy, their two sons Peter and Stuart, and a granddaughter.

*Chris Mercer and Graham Stirling*

**References**

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(This obituary was first published in *Nematology* (2012) 14, 1019-1021; it is published here by permission of Brill, Leiden, The Netherlands.)

Gregor was a member of the AAN for several years and his contributions will be missed.

# Other News

John Sagun, a teacher from Marrara Christian College in Darwin Northern Territory was awarded a $3000 environment grant from the NT government (EnviNT Grant) this year. The grant was used to purchase materials and equipment for nematode research to be carried out by 5 classes from years 7-9 (Total of 125 students). This enabled John's students to learn the importance of using biological control as an alternative to products with harmful chemicals by performing a research project about the mortality rate of beetle larvae to *Heterorhabditis* nematodes. As a result, John's students were awarded first and second prize from the Young Scientist Competition in the Northern Territory for the said projects. A report detailing the research written by some of the Marrara students' is detailed below.

## COMPARING THE MORTALITY RATE OF BEETLE LARVAE TO *HETERORHABDITIS* ENTOMOPATHOGENIC NEMATODES USING FILTER PAPER AND SAND BIOASSAY

Ysabela Dinglasan and Jared Curtis
*Marrara Christian College, Year 7B*

**ABSTRACT**

*Heterorhabditis* was first described from an infected caterpillar collected in Brecon Australia. These nematodes are widely distributed in North and South America, Australia and Europe. A number of biological control agents have considered using parasites for prevention of pests. The purpose of this experiment was to test *Heterorhabditis* nematodes for biological control of beetle larvae. One hundred and fifty *Heterorhabditis* nematodes were placed in 16 Petri dishes with 8 live beetle larvae in each. Three quarters of the Petri dish was then sealed with laboratory film to let the flow of oxygen into it. It was labelled with the infection date and the Petri dish code. Each Petri dish was then placed in an incubator for 48 hours. Based on the result, the infection of all the beetle larvae was more successful using filter paper which yielded an average mortality rate of 7.4 per 8 larvae or 92%. Five of 11 Petri dishes had a mortality rate of 100%. This result is very high and should be taken into consideration to use these nematodes for biological control. The main contribution of this experiment is the effectiveness of *Heterorhabditis* nematodes for biological control of other potential pests in the Northern Territory.

## INTRODUCTION

Biological control is the control of a pest by the introduction of a natural enemy or predator. Biological control in the NT initially concerned plants, natural insects or predators but humans are trying to use other things as well, such as parasites and plant diseases. A parasite that can be used for biological control is *Heterorhabditis* entomopathogenic nematodes.

Nematodes are simple round unsegmented worms. Most lives in damp spots under the soil near plants to hide from UV radiation. If these nematodes are exposed to UV radiation, they die. *Heterorhabditis* nematodes are extraordinarily lethal to most insect pests but safe to plants and animals. In the NT, insect pests are commonly killed with pesticides. When pesticides are used, they go into the soil and out to the ocean, which can harm some marine animals and even humans since we are on top of the food chain. These nematodes can actually be a replacement for pesticides. Instead of using pesticides that can harm the environment, we can use these *Heterorhabditis* nematodes to conserve the environment.

## AIMS AND HYPOTHESIS

To test the mortality rate of beetle larvae to *Heterorhabditis* Entomopathogenic nematodes after 48 h and comparing the result using filter paper and sand bioassay.

Using 150 nematodes per 8 beetle larvae, the mortality rate will be 50% in both filter paper and sand bioassay.

## METHODS

1. Put 2 x 9 cm filter paper in each Petri dish. There will be a total of 8 Petri dish for each filter paper and sand experiment trials.
2. Use a grid slide to count 150 juvenile *Heterorhabditis* nematodes under the microscope from the nematode culture. This nematode culture was collected and cultured at 25°C in an incubator and stored at 15°C in a refrigerator.
3. Transfer 150 nematodes onto Petri dish by spraying the grid slide with water. Place 8 live beetle larvae into each of the Petri dish. Spray the top of the filter paper and sand in the Petri dish with water to avoid the drying up of samples.
4. Seal only ¾ of the Petri dish with laboratory film to conserve moisture while letting the flow of oxygen into it.
5. Write and label each Petri dish including the infection date. Place the Petri dishes in an incubator for 48 hours.
6. Watch the change of colour in beetle larvae every 24 h. Infected larvae will change from yellow to brick red.
7. Count the mortality rate starting from 48 h from the date of infection by counting the number of infected beetle larvae and by dividing it by the total of original larvae used.
8. To prove the infection, dissect one beetle larvae and collect the adult *Heterorhabditis* female. Take photos using a microscope camera. To prove the infection further, put the beetle larvae into White traps and collect the infective juveniles in water. Record and analyse the result.

## RESULTS

**Table 1.** Mortality rate of Beetle larvae in filter paper

| Sample no. | 48 h after infection |
|---|---|
| 1001 | 8 |
| 1002 | 8 |
| 1006 | 8 |
| 1007 | 6 |
| 1009 | 7 |
| 1011 | 8 |
| 1012 | 6 |
| 1015 | 8 |
| Average | 7.38 |
| Percent | **92.19%** |

**Table 2.** Mortality rate of Beetle larvae in sand

| Sample no. | 48 h after infection |
|---|---|
| 2001 | 3 |
| 2002 | 2 |
| 2003 | 4 |
| 2004 | 3 |
| 2005 | 4 |
| 2006 | 4 |
| 2007 | 3 |
| 2008 | 2 |
| Average | 3.13 |
| Percent | **39.06%** |

## DISCUSSION

The mortality rate of beetle larvae was tested by *Heterorhabditis* entomopathogenic nematodes and was compared using sand and filter paper at room temperature. One hundred and fifty infective juveniles were counted under the microscope and were applied to each Petri dish. After 48 h, the beetle larvae in the petri dish were observed for a brick red coloration which indicates infection. The mortality rate was established by counting the number of infected beetle larvae over the original number tested.

Our experiment found that 92% of all the beetle larvae were infected by *Heterorhabditis* entomopathogenic nematodes in 48 h on filter paper compared to 39% using sand. Comparatively, according to the study of Mahar *et al*., (2007), the mortality rate of Mustard beetle larvae treated with *Heterorhabditis indica* was 97.5% at 30°C. Moreover, a study conducted by Mannion *et al*., (2000) stated that there was a 100% mortality rate after 21 days when *Heterorhabditis maretatus was used against* Japanese beetle larvae. A research by Olgaly Ramos-Rodriguez *et al*., found that *S. riobrave* caused a mortality rate of 80% on beetle larvae. Our results are similar to the scientists because we achieved a similar mortality result. Some of the experimental errors are due to not having exactly 150 nematodes in the Petri dish, which probably affected the infection of the beetle larvae which caused some of the beetle larvae to survive. We can improve this experiment by counting or having exactly 150 nematodes by using more sophisticated equipment such as high-definition microscopes. Our high mortality rate result using filter paper indicates that *Heterorhabditis* entomopathogenic nematodes could be efficient for biological control of invasive insects in the NT.

## CONCLUSION

This experiment had a result of 7.4 average mortality rate per 8 larvae which is equivalent to 92% using filter paper which indicates the high probability of utilising *Heterorhabditis* as a biological control agent against insect pests in the NT. The sand bioassay, on the other hand, yielded an average mortality rate of 3.1 per 8 larvae or 39%. Both results showed evidence that *Heterorhabditis* are pathogenic against beetle larvae and should be taken into consideration to use these entomopathogenic nematodes for controlling insect pests for agricultural purposes instead of depending on pesticides which could harm the environment.


## ACKNOWLEDGEMENTS

The students would like to thank the Northern Territory Government for granting us $2000 to be able to do our experiment. We would like to thank Clare Stanley who provided the materials. We also want to thank Mr Sagun for helping us achieve this successful and challenging experiment.


## REFERENCES


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# Upcoming Events

Society of Nematologists

**52ND ANNUAL MEETING OF THE SOCIETY OF NEMATOLOGISTS (SON)**
**Venue:** Cleveland, Ohio, USA
**Date:** 14-17 July 2013
For more information go tohttp://www.nematologists.org/

**FOURTH WORKSHOP OF THE INTERNATIONAL CEREAL CYST NEMATODE INITIATIVE (ICCNI)**
**Venue:** Friendship Hotel Grand, Beijing, China
**Date:** 22-24 August 2013
For more information go to http://www.icppbj2013.org/file/workshop/2thWorkshop.asp

**Workshop Topics:**
1. Global status of the distribution of CCN
2. The economic importance and population dynamics of CCN on wheat
3. Control strategies of CCN in wheat using host resistance
4. Control strategies of CCN other than host resistance
5. Use of molecular tools for research with CCN
6. CCN genome and parasitism genes
7. Biological control and IPM

**Registration and Abstract Submission:**
Registration and abstract submission of this workshop are bundled with the 10th ICPP registration system. **Early bird registration before** 28 February 2013

For more information and intention to participate, please contact (Dr Deliang Peng, dlpeng@caas.net.cn; Dr Amer A Dababat, a.dababat@cgiar.org)

APPS 2013

**NINETEENTH AUSTRALASIAN PLANT PATHOLOGY CONFERENCE**
Protecting our crops and native flora

**Venue:** Auckland, New Zealand - University of Auckland
**Date:** 25-28 November 2013

**Abstract submissions** January-June 2013. **Early bird registration:** May-August 2013

**The program themes covered at the Symposium are:**

| | |
|---|---|
| Plant-pathogen Interactions | New and Emerging Diseases |
| Application of New Technologies | Population Genetics |
| Biological Interactions and Plant Disease | Epidemiology |
| Disease Management | Biosecurity |


**SIXTH INTERNATIONAL CONGRESS OF NEMATOLOGY (ICN)**
Ensuring the future of nematology by encouraging student participation, relying on experience and empowering developing nations to ensure global food security

**Venue:** Cape Town, South Africa
**Date:** May 2014

The congress will be presented by the Nematology Society of Southern Africa (NSSA).

More details and information on the Congress will be soon uploaded on the website (http://www.6thicn.com/) and at (http://www.ifns.org/). Also check out the website (Nematology Society of Southern Africa) at www.sanematodes.com for updates or contact Mieke Daneel (mieke@arc.agric.za), Driekie Fourie (driekie.fourie@nwu.ac.za) or (susie@goingafricaconferencing.com).