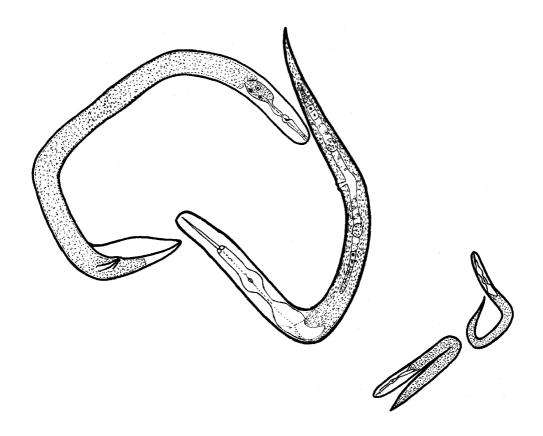
# AUSTRALASIAN NEMATOLOGY NEWSLETTER



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# From the Editor

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

# **January Issue**

The deadline for the January issue will be 30 December. I will notify you a month in advance so please have your material ready once again.

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# **Association News**

#### FROM THE PRESIDENT

I am just back from 6 weeks in Europe attending the European Society of Nematologists (ESN) meeting in Bulgaria, then visiting colleagues, a short holiday and then a Pine Wilt Nematode (PWN) meeting in Portugal, so a brief review of my impressions of what is going on in that part of the world and what it means for nematology in Australia seems appropriate.

The ESN meeting was attended by about 250 people from all of Europe, with a few from Asia, Australasia and North America. Out of a population base of perhaps 750 million including Russia, this seems like a very small number. But it is a large gathering when compared to about 30 people who attend the AAN biennial meeting. From a population of perhaps 25 million, it seems that Australasia is relatively well served with nematologists per capita. However, if one considers these figures on the basis of land area or agricultural production, then the situation is rather different. Australasia has a land area three quarters that of Europe, and agricultural production generally similar, although less intensive. Perhaps our nematological community is rather small after all.

This struck me particularly when considering the breadth of topics that a group of 250 people can cover. There were sessions on areas of study that are not well represented in Australia, such as virulence and pathogenicity, biodiversity, developmental agriculture, and interactions among nematodes. It was also pleasing to see marine, freshwater and vertebrate-parasitic nematodes represented at ESN. I know there are small research efforts going on for at least some of these topics within the geographic area encompassed by AAN, and I hope that AAN members will interact with researchers in other parts of nematology at least occasionally to draw on each others' experiences. It was gratifying that, for a small research community, Australian nematologists pull above their weight. The quality of the nematological science done in Australasia that I am familiar with is up to anything done in Europe, I am convinced. This is not suggesting that the work of anyone is unworthy, merely that everyone is working on demanding research with very tight budgets. Comparatively, I am convinced that the Australasian nematological community can say to our funders that we provide excellent value for money.

What were the principal nematological concerns at the conference? Genetics, plant-nematode interactions, physiology and biochemistry, virulence, management, developmental agriculture (heavily influenced by our very own Julie Nicol), quarantine, ecology, systematics, invertebrate associates, freshwater and marine nematodes, and associates of vertebrates. In terms of the species involved, there are concerns with the various Cyst Nematodes, Root Knot Nematodes, Root-Lesion Nematodes, Burrowing Nematodes, Stem Nematode, and with various fungal and bacterial biocontrol agents.

Overall, it was an excellent meeting, and the organizers are to be congratulated on their efforts.

The PWN meeting was smaller than ESN: it was attended by about 100 people. Delegates were mainly from regions where Pine Wilt is a major problem: Japan, China and Portugal. There were some delegates from North America (where Pine Wilt is native). There were 2 delegates from Australia, and 1 from New Zealand, reflecting the quarantine importance of this nematode. There was little overlap in the attendees of this meeting and ESN, which is rather sad in my opinion. My conclusion from this meeting, which was relatively specialized in terms of dealing with only one nematode species that occurs in very limited areas of the world, is that there can be a great diversity of approaches to similar problems. These different approaches can highlight different aspects, all of which must be integrated to build a picture of what is going on. For creatures with only about 1000 cells and 10 000 genes, the ways that these genes are arranged, expressed, and converted into a phenotype and its behaviour can be amazingly complex to tease out.

This brings me to what I think is another very important conclusion: that nematodes can behave very differently in different situations. Pine Wilt Nematode is a minor problem at best in its native range of North America on the trees endemic to that range. But it is a major problem outside that range - in Japan, Korea, China and Portugal - and on other species. For example, it can cause problems within its native range on exotics planted there. Added to this is evidence of a disease complex involving a bacterium as well as the nematode, and a strong effect of environment - particularly temperature - on pathogenicity, and it becomes obvious how much research there is still to do.

A corollary of these observations is that quarantine is particularly important for nematodes. It seems that it is not just exotic species we need to exclude, but a whole range of subspecific groups and even associated organisms.

Another corollary is that Australasia cannot rely on direct applicability of results from elsewhere: we have to do the research under local conditions with local populations. At both conferences there was widespread concern about succession, training and funding levels. There was a session on "Raising the profile of Nematology" at ESN, which was attended by about half the delegates. Alas, it seems to me that no-one has a definite solution to the problem of converting interest in nematode issues into concrete support at departmental, institutional, state or national levels. There are plenty of good ideas about getting nematology and nematode issues to students, farmers and the general public, but it seemed to me that most of the examples were local issues rather than general ones. I think we need to emphasise that there are many general problems in nematology that demand substantial research investments: things such as identification, virulence/resistance/tolerance, quarantine and population management. The alternative is many smaller, more specific problems that I fear will always be seen as small-scale problems appropriate for ad-hoc solutions involving interpretation of existing knowledge, rather than justifying a viable research community. I am hoping that an influx of people from overseas to 5ICN will encourage thinking about some of the larger issues of nematology and address this issue. It remains to be seen whether this will raise the profile of the discipline sufficiently to translate into funding.

In my talks with people about 5ICN, I have been noting the grants and schemes available for funding visits to Australia. Because it is a long way to come for many people, there is considerable interest in any form of assistance to get to Australia. Most of the grants and funding sources that I have found to date are for visits to enhance collaboration, rather than just to attend a conference. Most require visits by overseas researchers to laboratories in Australia for several weeks. So, I have been encouraging people to contact Australasian colleagues and start collaborations where appropriate with a view to visiting at the time of 5ICN.

Collaborations can, of course, be initiated from this end as well. If there are particular collaborations with people from overseas that local researchers think could benefit their research, then they should start trying to arrange visits and funding soon. 5ICN should be a good incentive for people to take time out from busy schedules to travel to Australia.

I was conscious of the distinctly different styles of the two conferences I attended. The ESN was tightly and completely organized. Organization of the PWN meeting was much looser. There were advantages of each that we should try to incorporate into the 5th International Congress. Each conference also had a noticeable local flavour. After much reflection, I think we should not try to copy anyone else, but have our own style: efficient, but flexible and creative. And, of course, friendly.

Mike Hodda

# **Regional News**

#### NEWS FROM VICTORIA

Motiul Quader joined the Department of Primary Industries, Knoxfield, Victoria on 13 June 2006 as a Nematologist. He will be working with Lila Nambiar in the Plant Health platform. Motiul has been in Australia for nearly twelve years. He has a diverse background in Nematology, molecular biology, plant pathology and breeding programs. He was working for DPI NSW before his current employment at Knoxfield. Motiul graduated from Bangladesh Agriculture University and did his MSc in Plant Pathology. He worked for National Research Institute in Bangladesh before coming to Australia in 1994. He obtained a post-graduate diploma in molecular genetics from the University of Queensland in 1997, then moved to Southern Queensland University for work and a part-time MSc in biology/breeding program. In 2000, Motiul moved to South Australia to take up a CRCV scholarship to do his Ph D in grapevine nematology at the University of Adelaide, with Ian Riley being his supervisor.

Initially Motiul will be working in nematode diagnostics and potato cyst nematode projects. He is also very keen to develop a research program, particularly in molecular tools for diagnostics. We hope to have a better collaboration in the future with other nematologists.

We welcome Motiul to our diagnostic team and wish him all best in the Nematology field.

Lila Nambiar

## **NEWS FROM WESTERN AUSTRALIA**

News from the WA State Agricultural Biotechnology Center (SABC), Murdoch University--Mike Jones, Zhaohui Wang, Modika Perera, John Fosu-Nyarko, Kerry Ramsay, Angelina Ho, DAFWA collaboration with Vivian Vanstone

Professor Mike Jones was awarded the top Murdoch University Excellence in Research Medal for continuous high level contribution to research at the University, on 4 April 2006 at the Graduation Ceremony in Perth Concert Hall. A major part of his research has been in plant nematology.

Dr Zhaohui Wang continues to work on the ARC linkage project between Professor Mike Jones and Professor James Dale of Queensland University of Technology. He has generated a number of lines of transgenic tobacco. which contain different constructs for monitoring gene expression in root-knot nematode infected roots. T2 plants of these

transgenic tobaccos will be obtained later this year, and will be used to study plant responses to nematode infection.

In late February, Vivien Vanstone from DAFWA visited the SABC for a week. She is working with Modika Perera and Mike Jones on a jointly funded, ARC-Linkage project on protein based diagnostics for plant parasitic nematodes. During her visiting Vivien worked hard isolating nematodes (*Pratylenchus* species) collected at different sites in the WA cropping area. These extracts were used by Modika to generate protein profiles. In return Modika gave Vivien a taste of proteomics based in protein profiling of plant parasitic nematodes using MALI-TOF mass spectrometry. As a result of this collaboration Modika was able to establish protein profiles for *P. neglectus*, *P. pentrans* and *P. thornei* and to identify protein biomarkers that can be differentiate these species. Modika is planning to test these biomarkers further to confirm the results with blind samples.

Modika has also completed MALDI-TOF diagnostic protein profiles for stem nematodes (*Ditylenchus dipsaci*) of oat and lucerne races provided by Dr Sharyn Taylor from SARDI. She is writing up a paper on "Identification of oat and lucerne races of stem nematodes *Ditylenchus dipsaci*".

Research assistant Kerry Ramsay left our plant-nematology group in February after two years hard work. She has been appointed as a research assistant in Royal Perth Hospital working on cancer related research. We wish her a bright future with her scientific career.

Dr. John Fosu-Nyarko, a molecular virologist from Murdoch University, joined the plant-nematology group in January 2006 as a post-doctoral research associate to continue on the ARC Discovery Project started by Kerry. The project aims to construct giant cell specific cDNA libraries using Laser Microdissection and Catapulting (LMC) to study gene expression at the early stages of nematode infection. Total RNA, isolated from giant cells in tomato roots infected with root-knot nematode (M. javanica) at 4dpi and 10dpi using the LMC technique, has been used to generate first-and second-strand cDNA populations. Methods used include a modified BD SMART technology to obtain full-length cDNAs and a strategy for subtractive hybridization that involves hybridization to a solid-phase healthy root cDNA library on magnetic beads. Several protocols have been followed to clone and screen the cDNA. The current and most efficient of these involves tailing the heterogeneous population of cDNA with individual deoxynucleotides using a recombinant terminal deoxynucleotidal transferase before cloning into vectors tailed with complementary deoxynucleotides. In addition, a cDNA library is being created which makes use of the 22-nucleotide trans-splice leader sequence, common to about 70% of nematode mRNAs, as a 5' primer to amplify cDNA populations. This is expected to generate a library consisting of genes/gene homologues specifically encoded by nematode mRNA or directly involved in host-nematode interaction. The libraries are being screened and sequencing is ongoing to identify novel genes expressed at the early stages of infection.

Later this year, Juan Emilio Palomares Rius of the Department of Crop Protection, Institute of Sustainable Agriculture, C.S.I.C, Cordoba, Spain, will join the plant nematology group as a visiting academic for six months. Mr Ruis will be working with Dr Fosu-Nyarko to exploit the similarities in pathways involved in host plant interaction

with nematode and fungi. The collaborative project, involving Pablo Castillo of C.S.I.C, will involve the use of the LMC technology and a proteomics approach to exploit the genetic basis for the loss and maintenance of resistance of two cultivars of chickpea to *Fusarium oxysporium* F sp cicer and *Meloidogyne arteliellia*.

Angelina Ho finished her Honours project at the end of June, and submitted her thesis to the Research Office of Murdoch University. Using the GUS reporter gene, she investigated the promoter activities of two transcription factor genes in transgenic *Arabidopsis* and tobacco. Some of her transgenic lines of *Arabidopsis* showed root-specific expression of one promoter. Two of her transgenic lines were further studied by examining GUS expression in root-knot nematode infected roots.

Zhaohui Wang

# **News From Department of Agriculture and Food Western Australia (DAFWA)**

# Cereal Cyst Nematode

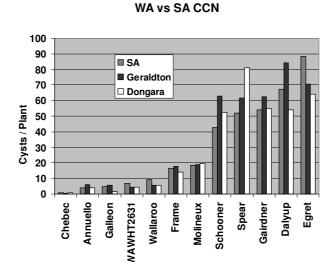
In 2005, two-year trials were established on heavily infested sites in The Northern Agricultural Region near Geraldton. Plots of wheat, barley, oat, pea and lupin were sown to manipulate soil nematode levels. Significant differences were measured in November from soil samples submitted to the SARDI Root Disease Testing Service.

Plots <u>were</u> to be split and over-sown with two susceptible wheat varieties (Wyalkatchem and Brookton) in 2006, to demonstrate to WA growers the benefits of crop rotation (particularly the use of resistant cereals and non-hosts) for the management of CCN.

However, due to "seasonal conditions" (i.e. no rain!), it was decided not to sow the trials this year, as yield data would be far from meaningful under these conditions. The trial areas will be kept weed-free for 12 months, soil sampled again at the end of this so-called "season", and the trials hopefully planted in 2007.

Crop	CCN	Eggs/g soil November 2005		
	Resistance/Tolerance	Wrigglesworth	Dongara	River
Carrolup Oat	Susc / Intol	88	15	65
Potoroo Oat	Res / Tol	11	3	18
Mundah Barley	Susc / Tol	33	25	64
Doolup Barley	Res / Tol	3	3	6
Janz Wheat	Susc / Intol	23	36	34
Yitpi Wheat	Res / Tol	4	3	7
Helena Field Pea	Non-Host	3	4	2
Tanjil Lupin	Non-Host	<1	3	3
Eggs/g soil March 2005		17	29	74

Milanka Matic and John Lewis (SARDI) have conducted host tests to compare WA (Geraldton and Dongara) and SA CCN to ensure that resistant varieties developed in SA and Victoria are relevant to WA. We are pleased to report that WA and SA CCN behave identically on resistant and moderately resistant cereals.



Milanka and John will be conducting further tests to investigate hatching requirements of WA compared to SA CCN. Soil has been washed and organic matter collected from infested paddocks in the Northern Agricultural Region (Geraldton and Dongara) and from the Central Wheat Belt (Northam). Milanka will establish hatcheries at varying temperatures. There has been some suggestion that CCN from the northern areas of WA have different temperature requirements and hatch earlier in the season than in SA and Victoria (Stanton and Eyres 1994 *Australasian Plant Pathology* 23:1-7). It was suggested that *Heterodera avenae* in WA may be a different pathotype, but from our tests, variety resistance/susceptibility seems identical.

## **Root Lesion Nematode**

Two-year trials were established at Katanning (4 *P. neglectus*/g dry soil) and Newdegate (6 *P. neglectus*/g dry soil) in 2005 to manipulate soil nematode densities during the season. Plots of Brookton wheat (susceptible), Wyalkatchem wheat (moderately susceptible), Sona chickpea (susceptible) and Kaspa field pea (resistant) were sown. Good plot-to-plot variation in nematode levels was established (<1 up to 120/g dry soil).

There was at least some rain in these areas, and plots in 2006 have been split and oversown with Brookton and Wyalkatchem to demonstrate to WA growers the benefits of crop rotation in terms of yield and nematode levels.

# **Pratylenchus penetrans**

*P. penetrans* is not usually associated with broadacre crops, but has been identified at high levels (up to 900,000/g dry root) causing significant damage to some wheat, oat and field pea crops.

In the glasshouse, *P. penetrans* multiplied on 8 field pea cultivars but *P. neglectus* did not. Barley, canola, triticale, oat, field pea, faba bean, durum, wheat, lupin and chickpea cultivars were all susceptible. Notably, this included crops that are resistant to *P. neglectus*: field pea, faba bean and lupin. Chickpea was the most susceptible, followed by lupin and wheat. Barley and canola were the least susceptible.

# Radopholus

Burrowing Nematode has now been identified from more than 30 locations throughout the WA cropping zone. The majority of these are *Radopholus nativus*, but another species is still being identified by Jackie Nobbs (SARDI Nematode Taxonomist),

Very high levels have been detected in wheat (176,000/g dry root) and barley (70,000/g dry root), moderate levels in lupin (12,000/g dry root), and low levels in canola (6,700/g dry root). Wheat and barley crops exhibit large areas of poor growth, patchiness, stunting & chlorosis.

*Radopholus* levels within patches of poor growth were very high (150,000/g dry root; 45/g dry soil), from the edge of patches moderate in the plants (16,000/g dry root) & high in the soil (33/g dry soil), & lower from apparently healthy areas of the crop (1,000/g dry root; 4/g dry soil).

We will be attempting to grow the two *Radopholus* species in pot culture, since other attempts at DAFWA and SARDI to culture these nematodes have not been successful.

## **Cultures**

Carrot cultures of *P. neglectus* have been developed from 11 WA cropping locations. These will be used as inoculum in comparative glasshouse studies between locations and between RLN species. Comparison will be made to SA *P. neglectus* (99C) which has been in culture now for about 15 years.

*P. thornei* has been cultured from one location, and *P. penetrans* from two. We have also obtained *P. thornei* cultures from SA and Victoria.

*P. teres* has proven difficult to establish in culture at both DAFWA and SARDI, and will now be produced in pot culture.

Helen Hunter maintains the DAFWA cultures. "Back-up copies" of WA cultures are maintained by Jackie Nobbs at SARDI.

#### **Pastures**

We are commencing screening pastures against Root Lesion Nematode (firstly *P. neglectus*). It was finally recognised by GRDC that part of the picture was missing in terms of rotations for nematode management in the WA cropping system. Ming Pei You's experience with pasture pathology and growing numerous species in the glasshouse will be utilised for this work, and an additional technical officer has been employed to assist with this. We also plan to screen weeds as part of this process.

# Potato Cyst Nematode

Sarah Collins is in the process of setting up bioassay experiments for PCN. Soil has been collected from the original sites in the Munster region where PCN was detected on

1986. The initial experimental plan included control plants inoculated with WA PCN sourced from New Zealand. However, due to the strict quarantine restrictions imposed on PCN in WA, plants will now be treated only with organic matter from the Munster sites, and no PCN positive control included. PCN susceptible potato will be grown in soil "baited" with organic matter.

For the area freedom survey, paddocks are being sampled on a 5 x 5 m grid, taking soil cores of approx. 50 g each to a depth of 15 cm. This equates to 400 sub-samples per hectare, creating bulk samples of approx. 20kg (all of which will be extracted by Fenwick can). "Big Bertha Mark II" has been constructed for this purpose.

To date, Sarah has sampled 29,600 50 g cores from 74 ha, resulting in 1.5 t of soil to be processed. This represents completion of just over one third of the planned sampling.

New Zealand PCN PCR protocols have been tested using our ready supply of CCN to ensure that primers, buffers and procedures are operational. CCN DNA has been extracted for use as a standard in PCR tests. Permits to acquire PCN DNA from New Zealand have been processed, and this will also be included as a standard to ensure detection protocols are functioning.

New Zealand PCR techniques for PCN will be perfected for WA soils. Organic matter extracts will be "seeded" with CCN, processed as for PCN, and tested with the PCR methods. Periodically, during PCN testing, soil from different potato growing areas will be tested using CCN cysts to ensure that the PCR testing is not giving "false negatives" for PCN due to inhibition of the reaction caused by soil factors.

# Nematode Short Course

Plans continue for Mike Hodda and Kerrie Davies to present their course on "Nematodes in Cropping Systems – Identification and Techniques" at Murdoch University in November this year.

Vivien Vanstone

## **NEWS FROM SOUTH AUSTRALIA**

Zhao Zeng Qi (University of Adelaide) had a busy 6 months writing papers and his thesis. He has had two papers published, and submitted his thesis at the end of June. We miss his cheerful face and helpful ways, and wish him well in his future career, wherever it takes him.

Ian Riley and Sharyn Taylor (SARDI) visited China for the Crawford Foundation CCN workshop in Baoding (see report in this newsletter).

Dwiratna Anugrahwati will be awarded her Masters degree for her work on endophytic actinomycetes against *Meloidogyne javanica*. Ratna did research for her project at Flinders University with Chris Franco and nematology help from the Waite Campus group. She has now returned to her job at Fakultas Pertanian Universitas Mataram, Lombok, Indonesia. Her thesis abstract is included in the newsletter.

Kerrie Davies (University of Adelaide) continues to slog on with descriptions and revision of the gall-forming genus *Fergusobia*. Her American colleagues, Robin Giblin-Davis (University of Florida) and Weimin Ye (North Carolina) are finalising work on a phylogenetic tree of the *Fergusobia*, which will enable Kerrie to finish her 'mini-monograph' on the genus. As part of the undergraduate subject 'Plant Pathology', she presented two lectures and a prac. class on plant and soil nematodes; so that group of students may actually remember that nematodes exist. 'Fred' Bartholomaeus provided 6 months technical support for work on nematodes in sycones (fruits) of Australian *Ficus*, and finished early in July. She measured and prepared preliminary descriptions of 11 new species of *Schistonchus*.

Ian Riley

## **NEWS FROM NEW ZEALAND**

The 45th Annual Meeting of the Society of Nematologists was held in a resort hotel on the windward side of Kauai, an island west of Honolulu. The beach setting was spectacular as the trade winds blew warm air day and night, a wonderful antidote for the two kiwis (Chris Mercer and Nigel Bell) coming from a cold winter.

The opening social, Ho'olaulea, was pool-side and we were greeted by Brent Sipes and his committee who hung leis around our necks and put glasses of mai tai in our hands.

Some highlights of the three days of sessions for us were:

- The introductory talks by Dick Mack and Mindy Wilkinson on biological invasions and a biological history of Hawaii since settlement. There are many parallels with NZ as Hawaii also has a high degree of endemism, vacant niches now filled by invaders, huge weed problems in native ecosystems, and a constant debate on how much money to spend on biosecurity. It was fascinating to learn that Hawaii had no native earthworms, amphibians or trees with showy flowers.
- Ernie Bernard's talk on nematode invasions which mentioned the recent finding of *Globodera pallida* in the USA for the first time. He described his studies of succession in habitats newly created by retreating glaciers or recent volcanism.
- Valerie Williamson's coverage of her "omics" (genomics, proteomics, metabolomics) approach to studying resistance, particularly how do invading *M. hapla* suppress plant defences. Her review paper is now available on line in Trends in Genetics 22(7) July 2006.
- Melissa Yoder presented a poster describing a new fixative (DESS) for nematodes
  which enabled both morphological and molecular examination of each specimen,
  see http://nematol.unh.edu/protocols.php for the recipe.
- The intricate work of Wim Bert on Tylenchid reproductive organs was very impressive and matching those morphological results with molecular data gave added robustness to existing Tylench phylogenies.

Chris Mercer

# **Training**

# CCN WORKSHOP, BAODING, CHINA

The First International Cereal Cyst Nematode (CCN) Training Workshop was held in Baoding, China, from 8–12 May 2006. It was organised under the auspices of the Crawford Fund, an activity of the Australian Academy of Technological Sciences and Engineering. The workshop was initiated by Dr Albert Rovira and Professors Tang Wenhua and Ma Ping, who also assisted with its organisation.

The Workshop was held at the Plant Protection Institute, Heibei Academy of Agricultural and Forestry Sciences, Baoding, Heibei.

Drs Sharyn Taylor and Ian Riley (SARDI), Julie Nicol (CIMMYT) and Professors Peng Deliang, Li Honglian, and Dr Chen Shulong from China gave presentations. The program was flexible and altered to suit the needs of the participants. The following topics were covered:

- Introduction to the life cycle of CCN, survival and dispersal strategies, an overview of CCN research in Australia and basic English terminology used in the workshop.
- Basic morphology of nematodes and identification of CCN.
- Techniques used for extraction and assessment of adult and juvenile CCN stages from plants and soil.
- Techniques and tools used for sampling CCN adult and juveniles from soil.
- Definitions of plant resistance and tolerance to nematodes.
- Nematode population dynamics and relationship of population dynamics to resistance and tolerance.
- Assessment of plant resistance to CCN using field trials, glasshouse and laboratory tests.
- Assessment of plant tolerance and yield loss using field trials.
- Design and analysis of data from nematode field trials.
- Biocontrol and disease suppression.

Short presentations by workshop participants provided a summary of:

- Environmental conditions in their province.
- Major crops grown.
- Types of plant pathogens/diseases most commonly found.
- Background to their area of research and expertise.

Practical sessions were given in the Nematology Laboratories at Hebei Academy, and included:

- A demonstration of basic extraction techniques for CCN from soil and opportunity for participants to use these techniques.
- Use of microscopes to assess CCN juveniles, cysts and eggs.
- Assessment of nematodes extracted from soil and plant samples from each province represented.
- Practical demonstrations of soil sampling equipment.
- Visit to a CCN field trial (established by Dr Chen Shulong) demonstrating the use of replication in field sites, comparisons between nematicide treated and untreated plots, comparisons between tolerant and intolerant varieties.
- Practical demonstrations on the use of GPS units and soil sampling equipment and strategies at the CCN field trial.

Group sessions were also held, in which participants were divided into teams to evaluate areas of research required to establish or confirm levels of CCN, and to determine yield loss caused by CCN and/or potential control strategies. Chinese and international scientists were included in each group and presentations were given by participants at the end of the workshop to summarise future directions for CCN research in each province. These presentations highlighted the information obtained from the workshop and provided a basis for development of a strong network in CCN research in China.

Participants came from 5 provinces in China, (Qinghai, Inner Mongolia, Gansu, Henan and Hebei), representing all major irrigated and rain-fed wheat growing areas of China. Most participants had moderate English skills, however 3-4 had poor understanding of spoken English. Interpreters, all with significant experience and expertise within plant pathology, were provided for discussion and practical sessions. This greatly increased the understanding of topics and assisted with feedback between participants and trainers.

A major outcome of this workshop was the establishment of a network between researchers and experts in China for assessing yield loss and management strategies for CCN. This network is strengthened by the inclusion of scientists from Australia and Turkey to assist with design of field trials, implementation of sampling strategies and data analyses to fast track progress in defining the impact of CCN in Chinese agriculture.

The workshop provided the basis for participants to commence or extend surveys to further determine the distribution of CCN, as well as design and conduct field trials to assess levels of yield in wheat cultivars in different environments in China. As a result of information provided within this workshop and also from the Crawford Fund Master Class held in May 2005, funding will be sought by Chinese and international scientists to continue and expand research on CCN in China, as this pathogen is seen as a major limitation for wheat production.





Practical sessions on extraction techniques for nematodes



Participants visiting field trials for assessment of yield loss caused by CCN



Participants in the Workshop.

# Research

## LONGEVITY OF ANGUINA TRITICI

Ian T. Riley & Zahraalsadat Mirmoeini Crop Pathology, SARDI, Waite Campus, Urrbrae SA 5064, Australia

Second-stage juveniles (J2s) of *Anguina tritici* are renowned (Womersley *et al.* 1998) for their ability to survive for decades in a dry, dormant state (anhydrobiosis). Limber (1973) showed that J2s from 32-year-old galls could be revived and were still able to invade wheat seedlings. The revived J2s remained active for over one year stored in water alone. Limber (1980) also showed that a small percentage of revived J2s from similarly aged galls were able to move vertically (up to 190 mm) through a moist soil column. However, it appears that the ability of J2s from old galls to initiate new galls and complete their life cycle has not been examined.

Wheat cv. Spear was grown in 200 mm pots of peat-sand mix in a controlled environment room (20/16°C, 12 h light/dark cycle) for inoculation with *Anguina tritici*. Seeds were germinated in a misting cabinet and transplanted (10/pot, 4 pots) on 17 February 2006 and inoculated with ten pre-soaked (overnight at 5°C) galls placed on the surface of the growth medium. The galls were collected in December 1991 from Carnamah, WA and mostly stored in a cold room (5°C) since. For two of the pots, J2s revived from several pre-soaked galls were suspended in water, checked for viability (movement) and pipetted onto the plants at weekly intervals for three weeks commencing 28 February. The plants were watered liberally with a soft spray nozzle every 1 to 2 days. Heads (mean of 60/pot) were harvested on 27 June, after withholding water for two weeks, then threshed and sieved.

A mean of 12 and 89 galls per pot were produced in pots inoculated once and four times, respectively. The galls contained large numbers of viable juveniles only. The lack of eggs indicates that all progeny had reached the survival stage.

This simple experiment has demonstrated that anhydrobiotic *Anguina tritici* J2s, in addition to surviving for 15 years, retain the ability to invade the host plant, initiate galls and complete their lifecycle. The numbers of galls produced was small compared to the number of juveniles applied but still represented a multiplication rate up to 8 times. The low relative humidity and lack of dew or rain splash in the growth room are unlikely to be ideal for *Anguina* invasion, so it is postulated that greater invasion and multiplication would have occurred in a field situation.

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# **Thesis Abstract**

# ACTIVITY OF ENDOPHYTIC ACTINOMYCETES AGAINST MELOIDOGYNE JAVANICA

#### DWI RATNA ANUGRAHWATI

Summary of thesis submitted in partial fulfilment of the requirements for the degree of Master of Biotechnology Studies, Department of Biotechnology, Flinders University of South Australia 2005

Plant-parasitic nematodes have been recognised as major pests of various economically important crops. Following public concerns on the potential effects of pesticides on human health and the environment, and the awareness of more sustainable control strategies, there has been renewed interest in searching for alternative methods which are safe and efficient.

The role of endophytic actinomycetes as biocontrol agents has been studied, especially in inhibiting fungal plant pathogens. However, little is known on their role in suppressing nematode infestation. Therefore, the main aim of this study was to observe the potential of endophytic actinomycetes in suppressing plant-parasitic nematodes.

Methanol extracts of 42 strains of endophytic actinomycetes were screened *in vitro* to determine their nematicidal effects on the root-knot nematode *Meloidogyne javanica*. Metabolites from 84% of the strains tested significantly reduced the motility of nematodes, while 21% caused juvenile mortality. Metabolites of *Streptomyces somaliensis* PM 143 and *Streptomyces peruviensis* EN 26 showed the highest nematostatic effect *in vitro*, with a significant reduction in motility of *Meloidogyne javanica* by 54% and 44%, respectively.

Further evaluation was carried out *in planta* to test the nematicidal activity of eight strains representing various level of nematicidal activity *in vitro*. Cucumber (*Cucumis sativus* L.) seeds treated with spores of these endophytic actinomycetes were germinated in sandy soil infested with 375 juveniles of *M. javanica*, and the level of nematode invasion detected 2 weeks after inoculation. Even though the number of nematodes invading the root system was not affected, *Streptomyces somaliensis* PM 143 and *Streptomyces peruviensis* EN 26, as well as *Streptomyces somaliensis* PM 349, were able to inhibit nematode growth and reduce the number of root galls in cucumber plants.

Growth promotion activity was also assessed using cucumber plants treated with endophytic actinomycetes, grown in the absence of pathogens. None of the treatments showed significant differences on shoot and root length, nor on root dry weight. The limited growth promotion activity in this experiment was probably due to the host specificity of these endophytic actinomycetes, since they were originally isolated from cereal plants. Nevertheless, in nematode-infested condition, most of the actinomycete-

treated plants showed less reduction of root growth compared to untreated control plants.

An evaluation of the bactericidal and fungicidal activities of the selected endophytic actinomycetes was carried out *in vitro*. The endophytic actinomycetes with high nematicidal activity showed no detectable bactericidal activity, but possessed moderate activity against *Pythium irregulare* and *Gaeumannomyces graminis* var *tritici*. Bioassays indicated that isolation of the nematicidal compounds needs to be carried out in order to characterise their chemical structure.

This preliminary study has revealed that some endophytic actinomycetes are effective in the control of plant parasitic nematode infections. These microorganisms are a potential source for novel biocontrol agents that can be used in an environmentally sustainable manner and that further screening is warranted to obtain more effective strains.

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There are 68 species of criconematids known to be present in New Zealand, and 47 of these are endemic. This fauna covers all 68 species, 16 of which are new to science, and includes detailed descriptions for the female of each species, including morphometrics and illustrations, supplemented by SEM micrographs of morphological details. Details of males and juveniles are given if available.

A diagnosis is presented to distinguish each species from other New Zealand species. Locations and plant species associations of each species are indicated and the significance of each species as a taxonomic unit is commented upon. Lists of nematode species present at various localities and associated with individual plant species are given in appendices, and keys to all taxa covered are provided.

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