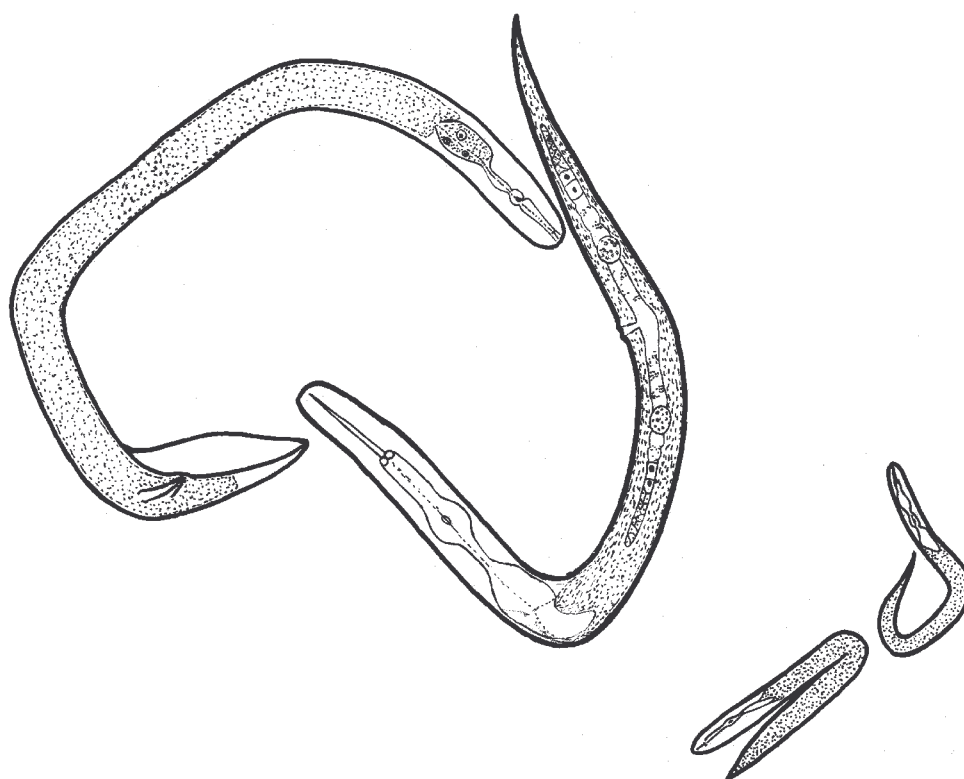


# AUSTRALASIAN NEMATODOLOGY NEWSLETTER



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# From the Editor

This is the first issue of the AAN Newsletter with me as editor. I want to pay special tribute to Jennifer Cobon, who for some years now has been 'editor *par excellence*' of our newsletter. Jenny, you have done a fantastic job, and produced great standards for your successors to adhere to. Thank you.

Thank you, too, to contributors to this newsletter.

## January Issue

The deadline for the January issue will be December 10th. 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

About the time this newsletter is printed, our bid for the 5<sup>th</sup> International Congress of Nematology will have been delivered for the initial consideration of the selection committee of the International Federation of Nematology Societies. The bid to host the Congress in Brisbane was largely put together by yours truly, with the assistance of the Brisbane Marketing Corporation, a government body charged with promoting Brisbane as an international destination. I would like to thank all the people who offered their assistance should the bid be accepted. Let us hope that the delights of Brisbane and Australia, nematological as well as touristic, will make people want to come here to share our knowledge.

Before the international congress will come our biennial meeting with APPS in September 2005 at Lorne, Victoria. Lila Nambiar and I are organising a nematological workshop in association with this meeting, which will focus on Nematological challenges for Australasia: exotic and native. The exact title is yet to be decided, as has the list of speakers, but we envisage discussing some of the recent quarantine issues that have arisen in Australia, some that have occurred recently overseas, together with recent findings about our local nematodes. It appears that we have a number of native species of plant-parasitic nematodes yet to be described, and we will be discussing identifying these from exotic species. We will hopefully have a full program by the next issue of the newsletter to whet your appetites. We are inviting several of the policymakers in the quarantine/biosecurity area to exchange views with the people on the ground. Such an exchange of views will hopefully improve the support and understanding of nematology in Australasia.

Nematology in Australia is facing a number of challenges: in training, in the stability of financial support, in the replacement of experienced people, and in the perception of nematodes as an issue. Far too frequently nematodes are either ignored because they are under the ground, or put in the too hard basket. I think that making people aware of what nematodes are and what they do, together with the past successes and current challenges is the antidote to the false perceptions about nematodes (which is then translated into the practical effects noted above). I hope that if people are aware of the real issues, the importance of nematological expertise and research will be self evident (at least in the longer term). I have published a number of general interest articles over the past few years pointing this out, in journals such as *Farming Ahead* and *Australian Cottongrower*. The current state of nematology in Australia may indicate that this sort of thing has not completely changed the position, but I hope that it has helped. If anyone has any other ideas about promoting our science, I would love to hear from you, and certainly assist if it will improve the status and support for nematology in Australasia. This is one of the main aims of our Association.

## Nematodes in Cropping Systems: Identification & Techniques Course 2005

The next course is scheduled for Canberra in November/December 2005, just before the Invertebrate Biodiversity & Conservation conference. If you are interested, or know of anyone who may be interested, please submit a non-binding expression of interest to Mike Hodda at CSIRO Entomology (address below). We need an indication of the level of demand for the course to decide whether it will proceed or not.

As in previous presentations of this course, Kerrie Davies and I, the co-convenors, will tailor the course to suit the needs of participants. However, we envisage the following.

The workshop will suit researchers and professionals working in agriculture, quarantine, green keeping, and soil biology, who need to understand the principles and practice of handling soil, plant and insect nematodes. It will provide hands-on experience in sampling, extraction, specimen preparation, culturing, diagnosis, and identification (including molecular techniques). There will be opportunity for interaction with experts in the field. Participants should have a degree which includes biology, agriculture, or soil science or have appropriate work experience to undertake the workshop. Less experienced participants can be supplied with recommended reading material prior to the workshop.

### Nematodes to be Considered

<i>Meloidogyne</i>	<i>Tylenchulus</i>
<i>Heterodera</i>	<i>Tylenchorhynchus</i>
<i>Pratylenchus</i>	<i>Morulaimus</i>
<i>Ditylenchus</i>	<i>Radopholus</i>
<i>Anguina</i>	<i>Bursaphelenchus</i>
<i>Scutellonema</i>	<i>Hemicycliophora</i>
<i>Paratrichodorus</i>	<i>Filenchus</i>
<i>Xiphinema</i>	<i>Tylodorus</i>
<i>Aphelenchoides</i>	<i>Heterorhabditis</i>
<i>Helicotylenchus</i>	<i>Steinernema</i>
Rhabditida	Mononchida
Dorylaimida	Areolaimida.

Anticipated course cost is \$1300+GST. This includes all materials and a printed course manual.

The course will only proceed if there is sufficient support. If you think you are interested please contact me at the address below. We will not require definite commitments until next year.

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# Regional News

## NEWS FROM SOUTH AUSTRALIA

### News from The University of Adelaide

The last few months have been anxious ones for nematologists on the Waite campus. The position of Lecturer in Nematology is unlikely to continue after December this year. This situation results from administrative problems together a perceived lack of funding for nematological research; and a new direction for the School of Agriculture and Wine, with greater emphasis on end-product quality and less on production (yield). If the position is lost, no Australian university will be teaching any sort of comprehensive course in nematology to undergraduates.

In May, Ian Riley taught in a seed health workshop on seedborne nematodes at the National Agricultural University China, Beijing, and visited Shanxi Agricultural University. He also attended ESN in Rome, where he presented a paper and a poster for Imelda, and caught up with many nematologists, including Johannes Hallman who visited the Waite some years ago.

Imelda Soriano has been busy finalising experiments and writing her thesis, which she will submit shortly. She has had two papers accepted, and will start a new job at the Waite – on rice improvement – in August.

Zengqi has now completed his sampling of pine forests in SE SA. He has found some interesting aphelench nematodes in the bark of both cultivated *Pinus* and native *Callitris*. He has begun to do morphological descriptions of these, and sequencing of them is underway. In addition, he is testing the possible pathogenicity to *P. radiata* of some of his isolates.

Sohbat Bahraminejad became the father of a beautiful daughter, and somehow continues his work despite sleep deprivation.

Elise Head has returned from maternity leave, and has now completed 2 years of data collection from her galled *E. camaldulensis* trees at Urrbrae Wetlands.

Kerrie Davies has been on the road again, this time with Prof. Robin Giblin-Davis (University of Florida) during July. Ian and Zhengqi joined them in Sydney for a weekend raiding sites, before they drove north to Cairns – sampling for ‘Fergs’ and *Schistonchus* along the way. It was a very successful collecting trip, with 4 new hosts of fergs recorded from *Melaleuca*, and the first Australian collections of adult *Parasitodiplogaster* (from *Ficus virens*) and of *Fergusonina* from *Syzygium*. In June, Kerrie presented the Alan Bird Memorial Lecture for the Royal Society of South Australia, on the *Fergusobia*/*Fergusonina* gall system – of course!

Ratina is a new Masters student from Lombok, who is beginning a project on the effect of actinomycete endophytes of roots on plant parasitic nematodes. Her first supervisor is Dr. Chris Franco, of Flinders University.

New Honours student, Matt Rodda, will start a project to examine biological and other determinants of local scale spatial variation in *Pratylenchus*. This will build on the precision agriculture work of the SARDI RDTS (Alan McKay and John Heap).

Nematology Discussion Groups were led by Simon Anistis (SARDI), who spoke on *Aphelenchus* and root disease interactions; and Chris Franco (Flinders), whose topic was endophytic actinomycetes and plant defence. We enjoyed the nematode workshop associated with the Soilborne Diseases Symposium.

*Kerrie Davies.*

### **News from SARDI**

In February, the SARDI Group was busy with the 3<sup>rd</sup> Australasian Soilborne Diseases Symposium held in the Barossa Valley. As part of the Symposium, (and in collaboration with Ian) we hosted the visit of Dr Roger Cook (Institute of Grassland and Environmental Research, Aberystwyth, Wales) and ran the Nematology Workshop on resistance to plant parasitic nematodes. A fun and informative time was had by all, and we wish to thank all those who participated and attended.

GRDC funding for the SARDI Nematology has been restructured and, after July 1<sup>st</sup> 2004, screening programs for cereal cyst, root lesion and stem nematode will operate on a fee-for service basis, with funds provided by research projects and wheat, barley and oat breeding programs/companies.

Research projects (funded by South Australian Grain Industry Trust) are being undertaken in the development of tests for quantification of cereal cyst nematode from plant roots using DNA-based techniques and in assessment of pathogen/disease interactions using DNA-based tests coupled with Precision Agriculture zoning technologies.

John Lewis will take over the role of the SARDI Nematology Screening Program supervisor, so please contact John if you have any queries about the Screening services.

While Sharyn Taylor will remain as Leader of the Nematology group (with focus on nematodes in pastures and management of the SAGIT projects), she has also taken on the (sometimes challenging) job of Quarantine Manager at the Waite Campus.

Jackie Nobbs, our Nematode Taxonomist, will focus on sorting out the complicated assortment of Western Australian nematode species as well as nematodes from pastures. Jackie is still managing sales of the CD Rom on Plant Parasitic Nematodes (copies are still available!).

*Sharyn Taylor.*

## NEWS FROM WESTERN AUSTRALIA

### From the Western Australian Department of Agriculture

#### Highlights – 2003 Field Results

During the 2003 cropping season, samples were received at sowing from 182 field trials established across the State by DAWA Crop Variety Testing, Cereal and Pulse Breeding Programs.

*Pratylenchus neglectus* was the most frequent Root Lesion Nematode (RLN) species identified, occurring at 38% of trial sites. *P. teres* was the second most frequently identified species (at 8% of sites). This species of RLN is not known to occur elsewhere in cropping regions of Australia, and has been detected rarely worldwide. *P. thornei* was detected infrequently, and only at low levels in mixed populations with two or more other RLN species. Nematodes putatively identified as *P. brachyurus* or *P. coffeae* were also observed at low levels in mixed species populations. *P. penetrans* was identified at 2 trial sites.

The diverse range of RLN species present in WA cropping areas emphasises the need for growers to know not only the level, but also the identity, of RLN species present when devising rotational options to manage these nematodes. While this information is available for *P. neglectus* and *P. thornei*, it is yet to be developed for *P. teres* and *P. penetrans*.

Based on the identity and levels of RLN determined from soil samples taken at sowing, 36 trials were intensively sampled in September-October 2003 to enumerate RLN for genotypes of all field crop species, and for determination of nematode multiplication rates.

Field Pea and Faba Bean are recommended for use in rotations where medium-high levels of *P. neglectus* or *P. thornei* occur. Trials where *P. neglectus* was identified in 2003 showed that, for all cultivars tested, both Field Pea and Faba Bean were resistant. Although *P. neglectus* levels were initially high at sowing (6.0 – 10.0/g dry soil), nematode levels decreased over the growing season so that multiplication rates for all cultivars were < 1.0.

*P. penetrans* was identified in one Field Pea trial, where nematode multiplication rates over the growing season were 1.5 – 7.0, indicating susceptibility. Where *P. penetrans* occurs, use of Field Pea as a resistant break-crop for RLN is not an appropriate management option. The indication that Field Pea is resistant to *P. neglectus* but susceptible to *P. penetrans* further emphasises the need for identification of RLN species so that rotations can be tailored to the identity of the nematode species present.

*P. penetrans* was also identified in a cereal trial at Narrogin. This species of RLN is not usually associated with damage to cereals. Plants grew poorly, and severe symptoms typical of RLN infestation were observed on the roots of both Wheat and Oat. The Wheat cultivars assessed were susceptible to *P. penetrans* (multiplication rates 3.2 – 8.9). For the Oat trial, density at sowing was high (15.9/g dry soil), and this level was maintained or increased (up to 37.8/g dry soil) during the growing season. Since initial levels for Oat were high, multiplication rates appeared relatively low, so it was not possible to infer susceptibility from these data. However, since severe damage was observed to the Oat roots, this crop may be intolerant to *P. penetrans*. Although *P. penetrans* is infrequent in cereal cropping rotations, this nematode is capable of causing severe crop damage.

As expected, Canola cultivars were susceptible to *P. neglectus* (multiplication rates 3.2 – 19.5). Nematode levels by October were very high (13.6 – 61.5/g dry soil).



At sowing, both *P. neglectus* (97%) and *P. teres* (3%) occurred in a Barley trial at Katanning. When plots were sampled in October, *P. neglectus* accounted for 54% and *P. teres* for 46% of the RLN identified. Possibly, Barley is more susceptible to *P. teres* than it is to *P. neglectus*, leading to increased levels of *P. teres* during the season. At sowing, RLN density was 3.8/g dry soil, which had increased by October to 9.7 – 59.5/g dry soil.

Similarly, for Oat at the Katanning site, the proportion of *P. teres* increased during the season. At sowing, only *P. neglectus* was identified (7.1/g dry soil). By October, the RLN population consisted of 73% *P. neglectus* and 27% *P. teres*. As with Barley at this site, nematode densities for Oat in October were very high (14.5 – 52.6/g dry soil), and RLN had multiplied on all cultivars (multiplication rate 2.1 – 7.2).

In contrast, where only *P. neglectus* was identified, density remained low on Oat throughout the season, and multiplication rates were low (0.8 – 2.5).

### 2004 Trials

Samples provided at sowing from the State-wide DAWA Crop Variety Testing field trials are again being assessed for density and identity of RLN. Genotypes of all field crops from selected trials will be assessed in September-October to determine nematode multiplication rates.

Two of the 2003 Field Pea sites have been over-sown in 2004 with Wheat and Barley cultivars differing in levels of resistance/susceptibility to *P. neglectus*, to determine rates of nematode multiplication following the resistant Field Pea crop. It is anticipated that these trials will be over-sown again in 2005 to determine changes in *P. neglectus* levels in response to the cereal rotational sequences of:

susceptible followed by moderately resistant cereal

susceptible followed by susceptible cereal

moderately resistant followed by susceptible cereal

moderately resistant followed by moderately resistant cereal.

A Cereal Cyst Nematode (CCN) trial has been established on a moderately infested site at Moonyoonooka (15km East of Geraldton). Little information is currently available for most WA cereals. Wheat and Barley cultivars and advanced breeding lines will be assessed for CCN resistance/susceptibility relative to Eastern States cultivars with known levels of resistance/susceptibility.

WA CCN samples will be investigated by SARDI Nematode Taxonomist Jackie Nobbs to confirm (or deny) their identity as *Heterodera avenae*. John Lewis (SARDI Adelaide) also plans to perform comparative testing of WA and SA CCN populations by comparing reactions of a set of resistant and susceptible cereal cultivars. If WA cereals with resistance to CCN are to be developed, it is necessary to ensure that sources of resistance and markers developed in SA and Victoria are relevant.

A glasshouse trial has been established to investigate the ability of field crop species and cultivars to host *P. penetrans*. This involved collecting approx. 400kg of naturally infested field soil, mixing the soil, testing nematode levels, and planting 4 cultivars each of 10 crop species (Wheat, Barley, Oat, Triticale, Durum Wheat, Faba Bean, Field Pea, Lupin, Canola and Chickpea). More crop hosting studies using naturally infested field soil are planned for additional species of *Pratylenchus*, and for the Burrowing Nematode (*Radopholus*).

## Taxonomy

Jackie Nobbs (SARDI Adelaide) and Mike Hodda (CSIRO Canberra) continue to taxonomically investigate our many and varied WA RLN samples. Of particular interest is the nematode identified as “*P. teres*”. There are questions surrounding the validity of this identification, which Jackie and Mike hope to solve in the near future. I am opting for the new name *Pratylenchus quasi-pseudo-neo-terioides-up*, but doubt that this will be taxonomically acceptable! Mike is also attempting to define characters that can be used in routine microscopic identification of these nematodes from field samples.

Jackie is providing specimens to Diana Hartley (and previously to Wolfgang Wanjura, CSIRO Canberra) for sequencing. So far, Di has been able to group our specimens into either “Prat?” or *P. neglectus*. Identifications seem to be compounded by both intra- and inter-population differences, which may not indicate true species differences. Jackie is now performing taxonomy on individual nematodes, which she is then sending to Di for sequencing, so they know they are both looking at the same nematode.

In the meantime, Helen Hunter (DAWA Nematology Technical Officer) valiantly continues with routine microscopic identification and enumeration of RLN from field and Diagnostic samples.

Jackie is also investigating specimens of *Radopholus* recently collected from cereal crops. One acquisition with a high proportion of males is unlikely to be the previously identified *R. nativus*, as males are not reported for this species. Possibly, *R. crenatus* and/or *R. inaequalis* are present.

## Nematode Cultures

The taxonomic nightmare of WA RLN specimens will soon be aided by investigation of cultures established from single field-derived nematodes. From approx. 1300 cultures set-up on single carrot pieces in 70ml tubs by Caroline Versteeg and Helen Hunter 12 months ago, we now have at least 25 isolates (hopefully) flourishing on multiple carrot pieces in a total of 450 x 250ml tubs.

More isolates are likely to be developed from this process, as multiplication rates on the initial carrot pieces have been slow and variable.

Availability of these nematodes (once identified) as a source of inoculum for glasshouse pot trials will eventually enable hosting (and other) studies without the need to collect large amounts of naturally infested field soil.

One isolate of *Radopholus* seems to have successfully established in carrot culture, and attempts will be made to culture additional recently detected field specimens of *Radopholus*.

These cultured nematodes will also be characterised in future by Modika Perera (State Agricultural Biotechnology Centre, Murdoch University) using MALDI-TOF mass spectrometry to define species-specific protein profiles. We are hopeful that a recently submitted ARC Linkage Grant will be successful to allow Modika to continue this work. The project is also financially supported by DAWA.

## Diagnostic and Field Observations

An abundance of nematode problems has been diagnosed in samples submitted to Dominic Wright of AGWEST Plant Laboratories this season.

Of particular concern is the frequency and severity of CCN in cereal crops. No doubt this has occurred due to the intensity of cereal (particularly Wheat) cropping, the susceptibility of most WA cereal cultivars, and the cold wet conditions (favouring egg hatch) coinciding with seeding this year. Root symptoms are severe, and recent observations of affected crops indicate that CCN populations must have been developing to high levels for some time. For crops in the Geraldton area, development of “white females” on the roots was already obvious by late July. Extensive areas of individual crops are affected, including one case where the whole of a 500 acre Wheat crop has been devastated. Growers and agronomists are being educated in recognising the problem and implementing appropriate rotations and grass control. It is doubtful that many of these severely affected paddocks would be able to support growth of another susceptible cereal crop in the following season.

*Radopholus* has been identified from more crops than in previous seasons. This nematode was recognised causing economic damage to a WA Wheat crop by Ian Riley & Sean Kelly in 1998. Since then, identification of low levels of *Radopholus* in WA crops has been sporadic. However, severe damage and high to very high levels of *Radopholus* have been observed in several Wheat crops in different areas of the State this season. Root symptoms are severe (in some cases similar to RLN, but in most the roots are dark brown, thickened, lacking laterals and “noodle-like” in appearance), and crop growth patchy and retarded. In the worst cases, death of plants has occurred in patches throughout the crop, leading growers and agronomists to consider the cause as *Rhizoctonia*. On examination of the roots, the problem is obviously not *Rhizoctonia*. Furthermore, RLN are not concurrently identified from these most severely affected crops. Additional samples for investigation have been obtained from plants and soil inside and outside these patches. With the aid of Jackie Nobbs, we are investigating the species of *Radopholus* involved. We possibly have more than one species. At this stage, it is not possible to advise growers on appropriate rotations to manage these nematodes, and further investigations and host studies are planned.

As with all seasons, various *Pratylenchus* spp. have been identified from cereal and other crops. Notably, a very high level of *P. teres* was recently extracted from damaged roots of stunted and poorly growing Canola.

Needless to say, field trial sites for both CCN and *Radopholus* have already been selected for 2005!

### **Horticulture Funding for WA PCN Project**

HAL (with a Voluntary Contribution from the Potato Growers’ Association of WA) has funded a 3-year project:

“PCN Area Freedom for WA: Evaluation of the current status of Potato Cyst Nematode (*Globodera rostochiensis*) in WA”.

This project will be carried out by DAWA Nematology, Horticulture and Quarantine Plant Pathology, in collaboration with John Marshall (New Zealand Institute for Crop & Food Research, Christchurch).

PCN has not been detected in any WA potato crop since 1989. Despite extensive and ongoing State-wide testing and surveillance, combined with strict quarantine protocols, national and international market restrictions remain due to the past detection of PCN on 6 isolated properties (totalling approx. 15ha) in the Metropolitan area. We feel that WA is now in an excellent position to claim Area Freedom from this nematode. Additional testing and survey work will be

carried out over the next 3 years to strengthen this claim. PCN has not been a production constraint to the WA potato industry, but rather a market constraint.

A program of intensive and extensive sampling over the 3,000ha area of potato production will be implemented to demonstrate that:

- PCN does not occur in any WA potato field
- the nematode was successfully eradicated from the areas where it was originally detected
- and did not spread from these areas to other potato production regions.

Declaration of Area Freedom from PCN for WA will provide an “exit strategy” for growers in areas where PCN was historically detected, and aid the lifting of national and international market restrictions currently imposed State-wide due to past detection of PCN. Furthermore, Area Freedom will reduce the cost to growers, industry and the State of ongoing testing, surveillance and quarantine programs.

*Vivien Vanstone.*

## NEWS FROM QUEENSLAND

### **From Leslie Research Centre, QDPIF, Toowoomba**

Rebecca Zwart has completed her PhD on “Genetics of disease resistance in synthetic hexaploid wheat”. It was accepted with flying colours (see her abstract later in this newsletter) and she is now busy publishing her work. She continues to work on molecular markers for root-lesion nematode resistance in wheat and she is looking forward to starting her new job in Belgium in 2005.

Jason Sheedy is preparing the final draft of his Masters “Resistance to root-lesion nematode, (*Pratylenchus thornei*) in wild relatives of bread wheat and Iranian landrace wheat” and he expects to finish by the end of the year.

Kirsty Owen has recently returned to work after taking maternity leave for baby number 2. Work is already underway on the new GRDC-funded project “Cropping Options to limit root-lesion nematodes”. This project continues work from the previous project of the same name, but this time we’re paying particular attention to *P. neglectus* and the resistance/ susceptibility of wheat, and summer and winter rotation crops grown in the northern grains region.

Once again, we are experiencing a very dry winter on the Darling Downs. We were lucky enough to get some wheat variety trials planted, but we’re now anxiously waiting for follow-up rain. Michelle O’Reilly has returned to work with our group for 3 months and we welcome Ian Dempsey to the “Cropping Options” project.

*Kirsty Owen.*

### NEMATODE COUNTING SLIDES

After many years of searching for replacements for our fragile, endangered Hawksley slides, Jason Sheedy found an American company that sells acrylic nematode counting chambers, that are very much like our well loved Hawksley slides. The company is “Chalex Corporation”, 5004-228<sup>th</sup> Ave SE, Issaquah, WA. USA 98029” The web address is: [www.vetslides.com](http://www.vetslides.com)

Follow the links to nematode slides. The on-line ordering system worked well, and the service was prompt. The slides are great to use and come with a secondary grid system, which is very useful on higher magnification. The cost was US\$45 per slide and there are discounts if you buy in bulk.

*Jason Sheedy.*

# Research

## GENETICS OF DISEASE RESISTANCE IN SYNTHETIC HEXAPLOID WHEAT

*Rebecca Zwart*

*Ph D Abstract, School of Land and Food Sciences, University of Queensland.*

Diseases causing substantial economic losses in the northern grains region of Australia include the destructive soil-borne pathogens, root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) and the stubble-borne fungal pathogen causing yellow spot (*Pyrenophora tritici-repentis*). Very effective sources of resistance to these diseases have been identified in synthetic hexaploid wheat lines. The genetics and nature of inheritance of disease resistance in these synthetic hexaploid wheat lines was investigated to give an insight into the most effective breeding strategy for durable resistance to multiple pathogens.

The inheritance of *P. thornei* resistance was investigated in five elite synthetic hexaploid wheat lines (Yallaroi/AUS24152, TAM870167/AUS18913, CPI133842, CPI133859, and CPI133872) using a half-diallel crossing design. The combining ability of resistance genes in the synthetic hexaploid wheat lines was compared with the performance of GS50a, the previous best source of *P. thornei* resistance used in Australian wheat breeding programs. Inheritance of *P. thornei* resistance was identified as polygenic and additive in gene action. General combining ability of the parents was more important in the inheritance of *P. thornei* resistance than specific combining ability. All synthetic hexaploid wheat lines investigated possessed better general combining ability for *P. thornei* resistance than GS50a. Thus, novel sources of resistance were identified in the synthetic hexaploid wheat lines that will provide alternative and more effective sources of resistance to be utilised in wheat breeding programs. CPI133872 was identified as the synthetic hexaploid with the best general combining ability for *P. thornei* resistance, indicating that use of this parent in breeding programs should prove to be an effective approach in breeding wheat with resistance to *P. thornei*. The presence of transgressive segregants in the resistant x resistant crosses between the synthetic hexaploid parents indicated that the synthetic hexaploid lines contain unique sources of *P. thornei* resistance, which have the potential to be exploited by pyramiding the resistance genes to obtain durable resistance to *P. thornei*.

The inheritance of disease resistance in the synthetic hexaploid wheat, CPI133872, was investigated in more detail through the molecular characterisation of the resistance genes. A CPI133872 x Janz doubled haploid population was phenotyped for resistance to *P. thornei*, *P. neglectus*, and yellow spot. A framework mapping strategy and quantitative trait loci (QTL) analysis was then used to determine the nature of the genetics of multiple disease resistance in the mapping population. All three of the diseases under investigation were quantitative traits that involved additive inheritance of resistance genes. QTL for *P. thornei* resistance were detected on chromosome arms 2DS, 6AS, 6AL, and 6DS. One QTL on chromosome 2DS was detected for *P. neglectus* resistance. Further mapping of chromosome 2D is required to determine if one gene is conferring dual resistance to both species of root-lesion nematode, or if there are two closely linked resistance genes that individually confer resistance to a single nematode species. QTL for yellow spot resistance were detected on chromosomes 3AL, 3DL, and 5BL. Resistance to root-lesion nematodes and yellow spot in CPI133872 was inherited from

## RESEARCH

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both the tetraploid durum (AABB) and diploid *Aegilops tauschii* (DD) wild progenitor of the synthetic hexaploid wheat lines.

The development of molecular markers that are closely associated with the respective resistance genes would accelerate the selection of desirable disease-resistance gene combinations. Incorporation of durable disease resistance into wheat cultivars of high agronomic quality will allow newly developed wheat varieties to withstand a range of pathogen threats.



**PROGRESS IN BREAD AND DURUM WHEAT BREEDING FOR  
RESISTANCE TO *PRATYLENCHUS NEGLECTUS***

R. K. Das and A. J. Rathjen

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Since 1990, the Waite wheat breeding unit has screened several thousands wheat cultivars from overseas and Australia. The most promising sources of *P. neglectus* resistance detected from this screening were *Triticum aestivum* cvv Virest (Aus 11984) and Persia20 (Aus5205), and *Triticum durum* (Aus10348) originating from Italy, Iran and Iraq, respectively. Virest and Aus10348 have winter habit and Persia20 has spring habit.

These resistant materials were used to incorporate resistance into the existing agronomically suitable cultivars. From repeated testing in both the glasshouse and field, the following lines

Pn6 -(Yk\*JzVr)\*Wk)\*Yr10Mx)/B19,

Pn10-(Ps20\*WA)/22,

Pn12-(Yk\*JzVr)\*Wk)\*Yr10Mx)/B113 \*Wkb)/A12

Pn13-(Yk\*JzVr)Wk)\*Yr10Mx)B113\*Wkb)/A33,

Pn22-(Bk\*WAVr58B\*WkPmHT\*MwSch)/B21 and

Pn24-( Bk\*WAVr58B\*WkPwmHT\*MwSch)/B24

were selected as resistant parents (Das, *et al.*, 2004).

Currently, Pn6, Pn10, Pn12 and Pn13 are used as resistant parents, since Pn22 and Pn24 are of late maturity. In 2003,1086 progeny (F<sub>4</sub>) were planted in two locations. On the basis of glasshouse screening and field performance, 411 progeny were selected and planted in six locations in 2004. A second group of 404 progeny were planted in two locations in 2004 for further selection.

The tetraploid wheat Aus10348 was used to incorporate resistance into Tamaroi and Kalka. A number of progeny have been selected from (Aus10348\*Tamaroi)\*Tamaroi and (Aus10348\*Kalka)\*Kalka after repeated testing in the glasshouse. A number of these progeny will be available for field testing next year.

**Reference:** Das, R. K., V. A. Vanstone, M. H. Russ and A. J. Rathjen. 2004. Development of bread and durum wheats for resistance to *Pratylenchus neglectus*. Third Australasian Soil Borne Diseases Symposium Proceedings, Poster Presentation. Barossa Valley, South Australia 207 -208.



**TAXONOMY, BIOLOGY AND PATHOGENICITY OF NEMATODES  
ASSOCIATED WITH PINE TREES AND OTHER CONIFERS IN AUSTRALIA**

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**Aims and objectives**

- To survey the above ground nematode fauna of *Pinus* and related conifers in south-eastern Australia.
- To describe and characterise *Bursaphelenchus* spp. and any morphologically similar taxa detected.
- To examine the biology and pathology of the *Bursaphelenchus* spp. detected.

**1 Preliminary Surveys in Plantation *Pinus* and Native *Callitris***

**1.1 Survey method**

Stands of *Pinus* were surveyed at Kuitpo and Mount Gambier, South Australia. To give a representative sample, five compartments were selected with establishment dates of 1948, 1962, 1985, 1986 and 2000 in Kuitpo, and ten compartments with different locality were selected in Mount Gambier. A “W” transect method was used in Kuitpo, Two parallel rows of trees were selected in Mount Gambier and 50 trees were sampled in each compartment. A total of 700 *Pinus radiata* and 50 *Pinus pinaster* trees were sampled.

For comparative purposes, native *Callitris* woodland in SA was also sampled, including roadside stands north of Burdett and the Tailem Bend Forest Reserve. Forty *Callitris preissii* trees at six sites were sampled.

Both bark and wood samples were collected from 1.5 m above the ground on the south-eastern side of the tree. A borer (5 mm diameter) was used for wood sampling and an axe for bark sampling. Trunk circumference (at 1.5 m) and condition was recorded for each tree. Sampled trees were marked by yellow waterproof paint in Kuitpo Forest to facilitate re-sampling, if needed.

**1.2 Extraction, fixation and mounting**

After the samples were collected, nematodes were extracted in a misting cabinet. Nematodes recovered were examined and classified to Order under a compound microscope. Established methods were used to heat kill and fix the nematodes for mounting. Eighty seven samples of aphelenchids and tylenchids were fixed and 130 specimens of 10 morphospecies have been mounted ready for identification.

### 1.3 Preliminary results

- The nematodes found in Kuitpo Forest, Tailem Bend Forest and Mount Gambier Forest belonged to five orders, Aphelenchida (fungal and plant feeding), Rhabditida (bacterial feeding), Mononchida (predatory), Tylenchida (plant feeding) and dorylaimida. The percentage of nematodes found were:

Kuitpo and Tailem Bend Forest:	64	aphelenchids
	30	mononchids
	3	rhabditids
	3	tylenchids
	0.3	dorylaimids

Mount Gambier Forest:	75	aphelenchids
	10	mononchids
	12	rhabditids
	2	tylenchids
	0.5	dorylaimids

- No nematodes were found in the wood samples or young shoots of *Pinus radiata*. Nematodes were only found in bark samples.
- In Kuitpo Forest, for *Pinus radiata*, 82 to 98% of trees in the 1961, 1985 and 1986 compartments contained nematodes. No nematodes were found from the young pines in the 2000 compartment. For *Pinus pinaster*, 90% of trees in the 1948 plantation contained nematodes.

In Mount Gambier *Pinus radiata* forest, 24%, 64% and 72% of trees in the 1950, 1952 and 1969 compartments contained nematodes; 78%, 96% and 98% of trees in the 1989, 1977 and 1987, 1985 and 1965 compartments contained nematodes respectively, and 100% of trees in the 1970 and 1981 compartments contained nematodes.

Nematodes were extracted from all *Callitris preissii* sampled along the roadside, and from 95% of those in the Tailem Bend Forest Reserve.

- From morphological observation, there appear to be one or two species of *Bursaphelenchus* and two species of *Laimaphelenchus*. These are potential new records for Australia.

### 2 Sequence Analysis

Sequence analysis of PCR amplified ribosomal ITS region is a simple and convenient method for nematode molecular taxonomy. Nematode DNA was extracted from specimens from Knoxfield, Heidelberg Park and Tailem Bend. Primers flanking the ITS region between the 18S and 28S ribosomal genes were used in PCR reaction. The amplified PCR products were cloned into T-Easy vector and sequenced.

The ITS sequences of the nematodes from Knoxfield, Heidelberg Park and Tailem Bend were obtained and the Clustal method was used to compare ITS1, 5.8S and ITS2 of *Bursaphelenchus* species.

For nematodes from *Callitris preissii*, there is 35.4% similarity with *B. abruptus* in ITS1, 90.2% similarity with *B. conicaudatus* in 5.8S and 25.9 % similarity with *B. xylophilus* in ITS2 region.

For nematodes from *Pinus radiata* at Heidelberg Park, there is 33.1% similarity with *B. abruptus* in ITS1, 86.3% similarity with 4 *Bursaphelenchus* species in 5.8 S, and 28.4 % similarity with *B. xylophilus* in ITS2 region.

For nematodes from *Pinus radiata* at Knoxfield, there is 34% similarity with *B. abruptus* in ITS1, 78.6% similarity with *B. conicaudatus* in 5.8S and 30.4% similarity with *B. abruptus* in ITS2 region.

### **3 Pathogenicity test**

Nematodes from infested trees in Melbourne have been maintained in cultures of the fungus *Botrytis cinerea*. To test their pathogenicity, three species were inoculated on to 3-year-old *Pinus radiata* trees grown in a shade house. Two presumptive *Bursaphelenchus* species from Knoxfield and Heidelberg Park and a laboratory culture of *Aphelenchus avenae* (as a negative control) were inoculated to 15 replicate trees each. These are being maintained and watched. It is now 3 months since the trees were inoculated and no definite symptoms are visible. Attempts will be made to recover nematodes from all inoculated trees.

# Book Review

## REVIEW OF *IN THE BEGINNING WAS THE WORM*

By Andrew Brown, Published by Pocket books, 244pp, \$19.95

Reviewed by **Rod McLeod**

So, you think that extracting, counting and mounting nematodes can be tedious. I know I sometimes did during my many years in Nematology. Then spare a thought for Eileen Southgate, one of the heroes of *In the beginning was the worm*, by freelance journalist Andrew Brown. Brown's book tells the story of the epic project that for the first time sequenced the genome of an animal. Three themes inter-weave through the text. The first is why such a boring and inconspicuous animal as *Caenorhabditis elegans* was chosen for the project. The second theme is that scientific progress often depends on long-term, monotonous, hard work, such as that of the researchers in the *C. elegans* genome project. The third is the human story behind the scientific achievements.

In one just one aspect of the enterprise, technician Eileen Southgate, working with John White and Nichol Thomson, traced the nematode's entire nervous system down to the last synapse. This involved cutting consecutive sections one twentieth of a micron, thick along the entire length of the animal. Three thousand sections were regularly cut from single specimens. Eileen Southgate and John White prepared the sections for electron microscopy, photographed them and integrated the results into an overall picture. This exhaustive mapping of the nervous system took eighteen years of Eileen's life. Three of the project's leaders, Sydney Brenner, Bob Horvitz and Sir John Sulston, shared the Nobel Prize for their work. Sulston and Bob Waterston went on to run major parts of the public human genome project.

Besides telling an engaging story, Brown does well in explaining complex concepts and problems: for example, the explanation of the interaction between the genome and protein configuration in determination of development. There are many appealing analogies: mutations are described as broken genes, cutting sections is likened to sawing logs in a sawmill, sections are "salami" one twentieth of a micron thick, an organism's DNA is not a Rosetta stone program governing its development.

Many technical terms are, of necessity, included. These are used with commendable accuracy, and are handled in ways that should not repel or antagonise the uninitiated reader. Although nematodes are called worms in the title, the author acknowledges that they are not worms in the sense that worm usually refers to earthworms. Who would object and spoil such a good title?

This book could be read with profit by most nematologists. It is, however, written for a wider readership; my impression is that it is written well enough to hold the interest of readers with a general interest in science. If you have come across another book about soil or plant nematodes that you could recommend as a good read to a non-nematologist friend, I would like to know about it.