AUSTRALASIAN NEMATOLOGY NEWSLETTER

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From the Editors

This year the end of January arrived just after Christmas, so a very special thankyou to all who took the time to send articles and news items for this newsletter. E-mail was not as magical as it first seemed, with several documents becoming scrambled. However, we think we have figured it out, so send those articles in for the next issue.

The dead line for items for the July issue is June 15. However you don't have to wait if you have any items, gossip or research findings that you would like to send in. We'd love to hear from you

We have included an update of the AAN members directory with this issue, as there are several new additions and other changes. If there are any errors or you know someone who wishes to subscribe to the Australasian Nematology Association please write to:

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Looking forward to see you at the Australasian Plant Pathology meetings in Christchurch in August, so start praying to your relevant funding body now!

Maria Scurrah, Sharyn Taylor, Terry Bertozzi

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Association News

ACCREDITATION OF NEMATOLOGY LABORATORIES

Graham Stirling, DPI, Brisbane

At the last general meeting of AAN which was held in Hobart last year, I offered to examine the issue of accreditation. I have therefore prepared the following paper in the hope of stimulating some discussion.

IS AN ACCREDITATION SCHEME NEEDED?

As the availability of nematicides declines, it is becoming increasingly difficult to control nematodes in intensively managed horticultural, vegetable and ornamental crops. Growers need help in making nematode management decisions and in the future, much of this help will come from pest management decisions and in the future, much of this help will come from pest management consultants and commercial diagnostic laboratories. That trend has already started, and in Queensland and northern NSW, for example there are at least six laboratories providing nematode monitoring and diagnostic services. The quality of the information they provide is variable. Some are excellent while others do not have the facilities or expertise required to provide a useful service. An accreditation scheme would provide growers with some protection by ensuring that laboratories met certain minimum standards.

WHO SHOULD OPERATE AN ACCREDITATION SCHEME?

In Australia, NATA (National Association of Testing Authorities) is recognised as the national laboratory accreditation authority and serves as the national focus for preparation of codes of good laboratory practice. A similar body is established in New Zealand.

Although there would be advantages in having a body such as NATA operate an accreditation scheme for nematology, laboratories process only a limited number of samples and the current fee schedule for NATA suggests that accreditation would cost a minimum of $3000 in the first year and more than $2000 per year thereafter.

I believe AAN is a more appropriate body to establish an accreditation scheme. It has the nematological expertise needed to establish codes of practice and to assess technical competence in nematology. Once the scheme is established and has been operating for a few years it may then be appropriate to hand it over to a national registration authority. However, AAN involvement in the formative years would minimise costs and enable nematologists to work with commercial laboratories and help them upgrade their service.

THE WAY FORWARD

Attached are some draft guidelines on how AAN might operate an accreditation scheme for nematology laboratories. Could you please consider them and comment by returning the questionnaire to me. Since I would like to make a recommendation to the next AAN general meeting (August 1995 in Christchurch) I hope to meet the following timetable:

January 1995	First draft of accreditation guidelines published in newsletter
March 1995	Deadline for comments on first draft
July 1995	Second draft of guidelines published in newsletter
August 1995	Discussion of accreditation scheme at AAN General Meeting and a decision on whether to proceed or not

PROPOSAL FOR AAN ACCREDITATION SCHEME FOR NEMATOLOGY LABORATORIES (FIRST DRAFT)

Objective: To prepare codes of good practice for laboratories providing services in plant and soil nematology and assess compliance with these criteria.

Operation:

1. AAN would elect an accreditation committee of three members (one from northern Australia, one from southern Australia, one from New Zealand) to develop and operate the scheme. The role of this committee would be to prepare codes of practice, to maintain a list of accredited laboratories and to ensure uniform standards were applied across Australian and New Zealand.

2. To enable most matters to be handles at a regional level, each of the three committee members would chair a regional subcommittee that would make decisions on laboratories within their jurisdiction.

3. Laboratories applying for accreditation would submit a document containing details of their organisation and management, quality control systems, staff qualifications, calibration processes, record keeping systems and extraction, counting and identification procedures. This application would be referred to the appropriate regional sub-committee, who would work with the applicant and help them achieve a satisfactory standard.

4. The accreditation committee would determine an appropriate fee schedule to cover various components of the scheme e.g. reviews of documentation, assessment visits, travel, submission of reference samples.

Guidelines for accreditation

1. There would be two levels of accreditation.

   i) **General.** Accredited as proficient in providing general nematological services on a national basis.

   ii) **Limited.** Accredited as proficient in providing nematological services on a limited scale (eg. in certain regions or on specific crops).

2. To obtain accreditation, a laboratory would have to satisfy specific criteria established by the accreditations committee.

The following general competencies would be required:

i) Management and staff have appropriate technical qualifications, knowledge and competence.

ii) The laboratory maintains a quality system appropriate for its size and has a commitment to good laboratory practice.

iii) Laboratory accommodation is suitable with regard to security, environmental conditions and space and equipment is in good working order.

iv) Methods for processing samples are appropriate, they are documented in a quality manual and they are regularly validated.

v) Appropriate procedures are in place to document, protect, retain and discard samples, to record results and to provide clear, unambiguous reports.

The following nematological competencies would also be needed:

i) Facilities are available to adequately store samples prior to processing and to dispose of
nematodes and infested soil.

ii) The nematode extraction procedures are appropriate for the task and data on extraction efficiency and the variability of each procedure are available.

iii) Laboratory staff are adequately trained in nematological procedures and can identify genera of common plant-parasitic nematodes and all locally important species.

(Please fill in questionnaire at the last page of newsletter and send to Graham Stirling)

AAN Meeting
Lincoln August 1995

The AAN meeting and Nematology workshop are planned and as I told you in the July 1994 Newsletter the title of the workshop is:

Cyst and Root Knot nematodes

We are still trying to finalise the key note speaker from Germany but he is waiting to see what funding can be arranged. To date we have finalised two sessions; more will come.
There will be a session on the identification of the Heteroderidae. Wim Wouts has undertaken to run this session and he will be working from prepared material and a number of microscopes will be set up so that participants can get hands on.
There will be a session on molecular and biochemical identification of nematodes. This will be a presentation session. It has been suggested that discussion on the pro and cons of different methods would be interesting and informative.

Once again I repeat the call for suggestions of what you would like to have included in the workshop. To date I have had one suggestion.

Date
The workshops are planned to take place before the main conference, that is Friday 18 August. We can run on to Saturday if needed.
I have arranged for an informal AAN barbecue/wine/beer function in the afternoon. Venue to be decided based on numbers.

Cost
Cost of the workshop and social approx. $70.00.
Make sure that you book accommodation to include Friday, Saturday, and Sunday as well as the APPS meeting.
It has come to my notice that some members of AAN are not members of APPS and as such have not received any of the circulars. To offset this problem I have merged the AAN membership list with the APPS list. You should all receive the next 2 circulars.

APPS conference format
As far as possible, the nematology papers will be included in the Pathology sessions. However there will be specific concurrent nematology sessions if the papers warrant it.

Please get in touch with me if you need more information

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PLANT NEMATODES OF AUSTRALIA LISTED BY PLANT AND BY GENUS

R. McLeod, F. Reay and J. Smyth

The revision is close to completion. There have been some unexpected delays, however it is hoped to be ready for printing within the next few weeks.
The publication will comprise about 150 pages, with three sections. The first, listing nematodes by host or associated genus; the second providing a quick reference list of nematodes by genus and species; and the third section listing nematodes by genus and species with information about host range and geographical distribution.
Financial assistance has been provided by the Rural Industries Research and Development Corporation.
It is not intended that copies will be sent out automatically. There will be a nominal charge to cover postage and packing - a request form will be included in the next newsletter. Anyone interested in receiving a copy, please contact Rod McLeod or Frances Reay.

Rod McLeod, Biological and Chemical Research Institute, NSW Department of Agriculture, Plant Pathology Section, PMB 10, Rydalmere, NSW 2116. Tel: (02) 683 9777.

Frances Reay, South Australian Research and Development Institute, Field Crops Pathology,Waite Precinct, Hartley Grove, Urrbrae, S.A. 5064. Tel: (08) 3039391 or 3039361

Frances Reay

Regional News

News from the Southernmost Nematologists in the World (I think!)

Donald Ferns has recently started a project with me looking at recovery from anhydrobiosis in *Ditylenchus dipsaci*. The idea is to use micro electrodes to try and follow the recovery of nerve and muscle function following the reimmersion of anhydrobiotic nematodes in water.

Joan Lemmon in assisting me in a project to follow ultrastructural changes during desiccation in
D. dipsaci. We will be using freeze substitution in conjunction with scanning electron microscopy and transmission electron microscopy of thin sections and freeze fracture replicas. We hope that this will enable us to examine the ultrastructure at known water contents during desiccation.

Nhung To Bich is helping with a project looking at the interaction between osmotic stress and freezing tolerance in *Panagrolaimus davidi*, an antarctic nematode.

We have recently shown that *P. davidi* can survive intracellular freezing. It has always been thought that animals can only survive freezing if ice is confined to the extracellular spaces. This is the first demonstration of intracellular freezing in an intact animal. We are currently looking for cryoprotectant compounds in *P. davidi*.

David Wharton, Department of Zoology, University of Otaga, Dunedin, New Zealand.

CONFERENCE

RN Watson and CF Mercer will attend a Tripartite Australia/New Zealand/USA Workshop on Pasture Pathology in Mississippi during April 1995. This follows earlier tripartite workshops on 'Forage Legumes for Energy-Efficient Animal production' (Palmerston North, 1984) and 'Persistence of Forage Legumes' (Hawaii, 1988). This workshop will provide opportunities to link up and strengthen research in pasture pathology, including nematology, which seems to be characterised by small, somewhat disparate groupings (excepting Adelaide!) which are also probably still regrouping after the traumas applied to government research in the last decade. Unfortunately there is no Australian nematology delegate.

NEWS FROM QUEENSLAND

Experiences in international training

Following the success of a three-day nematology course in July 1992, the nematology group in Queensland recently embarked on a much more ambitious training project. It seemed like a great idea in the planning stages! The aim was to train potential collaborators in Asian countries. While a huge amount of interest was shown in the course very little money came forth so we decided to cancel it. Two people who eventually did pay still insisted on coming so we had them as training attachments for one month. We achieved quite a lot with these students from Fiji and Malaysia, particularly as they spoke very good English and had many years' experience in agriculture. However, it required a lot of effort on top of our normal work load and we breathed sighs of relief when it was over! If you are contemplating something similar, it is very worthwhile but be prepared for the extra work and disruption.

(Julie Stanton, QDPI, Meiers Road, Indooroopilly)

NEW PROJECT ON SUGARCANE

Despite the fact that economically important nematodes such as *Meloidogyne, Pratylenchus* and *Paratrichodorus* are widespread on sugarcane in Queensland and significant nematode damage is known to occur on light-textured soils, our understanding of nematode pest problems is negligible. Nematological work has been limited to a few nematicide trials done by entomologists at the Bureau of Sugar Experiment Stations (BSES) in the early 1980's and to Brendan Blair's current work on *Pratylenchus zeae* in north Queensland.

Fortunately, it has been belatedly recognised that nematodes may play a major role in a general yield decline problem that is now of concern throughout the sugarcane industry. Graham Stirling (DPI) and Peter Whittle (BSES) have recently received funds for a four year project which will establish the relative importance of the various nematode pests of sugarcane and determine their role in the yield decline syndrome. A position for a nematologist will be advertised early in 1995 and anyone interested in the job should contract

Graham Stirling on (07) 877 9392.

NEWS FROM ADELAIDE

The Field Crops Pathology Unit, SARDI has moved uphill to a brand new building called the Plant Research Centre. We share these facilities with other SARDI units including Administration, Horticulture, Seed Services, Adelaide University's viticulture and oenology section and PISA's plant pathology and Quarantine.

We are trying to adjust to a postmodern-architecture setting with no corridors, right angles, or sun light. Instead, we have an open plan office design, with laboratories, greenhouses, seed cleaning and storage in the same building. We are slowly realising that our work has not really changed; it just feels different.

The nematology group has many new facilities in this building including a shiny new root washing area with a mister for nematode extraction which can handle 432 samples (hopefully so can we).

Frances Reay has moved in with us and is currently working on Antarctic nematodes. At the moment she is investing in a pair of joggers to go between the old and the new building.

Franky Charman-Green has shown excellence in timing by giving birth to her daughter Alex, on December 6 (after CCN screening and GRDC submissions were drafted, but before the move). She is now taking time off for this new project.

We were visited by Adrian Evans from London University in September, and during December both Dr David Bird from Riverside California and Dr Robert Potter from Murdoch University in Western Australia were here. They both presented seminars on aspects of host-pathogen interaction using sophisticated molecular biology techniques.

Julie Nicol and Mohammed Farsi started writing up their PhD theses on *Pratylenchus* in wheat. Suzanne Charwat was granted her Ph.D degree on anhydrobiosis in July from Kiel University and in November began work on nematode-bacterial-snail interactions and nematodes as possible biocontrol agents of snails. Andreas Hensel continues his work on nematodes in millipedes. Kerrie Davies was appointed a temporary lecturer in nematology following John Fisher's retirement.

Maria Scurrah, Sharyn Taylor

EUROPEAN SOCIETY OF NEMATOLOGISTS - 22ND INTERNATIONAL SYMPOSIUM AUGUST 1994

Abdul Taheri (Dept. Plant Science, Waite Campus) and myself were fortunate enough to attend the 22nd International Symposium of ESN in Belgium during the 7-12th of August 1994. The conference was definitely a great experience and opportunity to meet over 200 nematode researchers in all fields. There were workshops on CCN, virus vectors, beet cyst nematodes and an area which seems to becoming more of interest and importance: nematodes in environmental studies.

Other aspects of nematology were covered in the conference with over 100 poster presentations. Abdul and myself presented a poster on the first Australian morphometrics and descriptions of the two species of Pratylenchus(*P. thornei* and *P. neglectus*) in South Australia. Abdul presented a poster on fungal interactions with *P. neglectus* on cereals. I also presented a paper on a bioassay for *P. thornei* resistance in cereals and non-leguminous hosts.

Emphasis was placed upon the reduction of the use of chemicals to control nematodes. In Europe there is a similar concern as here, of the dwindling presence of traditional nematode taxonomists. In addition Dr Ken Evans (Rothamsted) gave a very entertaining seminar on the distinctions (good or otherwise) between the traditional and molecular nematologists.

If anyone has any questions or would like to see the abstracts, please do not hesitate in contacting me.

(Julie Nicol, University of Adelaide, Department of Crop Protection, Waite Campus)

Research

PASTURE NEMATOLOGY an awakening?

Nematode impacts in pasture species has been recognised by nematologists in New Zealand and Australia since the 1960's but recognition beyond the immediate discipline in terms of vigorous funding commitment has been very tardy to say the least. More specialist topics where the problem is easily identified such as stem nematode in lucerne and annual grass nematode mitigated toxicity have attracted some attention but this has not been successful in spawning a wider involvement in pastoral nematode ecology. Reasons for the lack of research commitment can only be attributed to the lack of sufficiently compelling and irrefutable evidence of the impact of nematodes as a limitation on pasture performance over other more visually appealing or politically competitive disciplines, including entomology, soil fertility and pasture management to name a few.

In the 1970's Yeates et al. demonstrated the effects of root knot and clover cyst nematodes on white clover vigour and Colman and Brown showed widespread presence of these nematodes on clover roots in NSW and Victoria respectively, but the implications were obviously not appreciated, or were ignored by research priority setters. After all clover seemed to persist as a perennial, if fickle, junior component in pasture which was known to be sensitive to a wide range of biotic and abiotic factors.

In the 1980's KW Steele, a soil scientist, noted a very large response in N-fixation by clover after application of high rates of fensulfothion to control insect pests on his trials. It was in an effort to verify the extent and magnitude of such responses in pasture that nematode impacts were demonstrated in a way that could, in the Australasian pastoral context, no longer be overlooked - viz a 50% response in N-fixation averaged of 16 pasture sites. While some misgiving concerning the heavy rates of pesticide use on non-target organisms are valid, it seems that clover nematodes markedly reduce the clover vigour in NZ pastures. Interestingly, similar treatments applied on pasture at Kyabram, Victoria, in the absence of these two clover nematodes, failed to produce a response and furthermore clover yields under irrigation of 20000 kg DM/ha were recorded.

Interest in pasture nematology is finally increasing to a more respectable level with new initiatives towards problem definition in NSW (Bill Faulkerson et al., Wollongbar) and Victoria (Linda McLiesh/Gordon Berg). Recent studies in NZ in a summer-dry environment show not only the degree to which summer drought can compromise clover plant survival, but the extent to which this is accentuated by nematodes.

As we are now finding, the greater challenge lies in moving from problem definition into the area of problem management. This is being tackled using three main approaches:

- reducing plant nematode burdens through resistant/tolerant white clover selection
- reducing nematode burdens in soil through enhanced bioregulation
- reducing nematode impacts through pasture management to improve clover persistence.

In the first area some progress has been achieved in terms of reducing the root carrying capacity for individual nematode species (programme of Chris Mercer et al.). Seedlines from this programme are being evaluated in grass swards at three sites and further selection cycles are continuing for resistance and tolerance.

Evaluation of biological controls of clover nematodes is in the early stages of determining pathogen species from pasture soils which invade nematode stages and their relative pathogenicity (fungi - Frank Hay; bacteria - Upali Sarathchandra et al.).

Research work is also looking at soil amendments which enhance soil microbial activity. Management options to improve legumes persistence and performance include an evaluation of the practice of deferred summer grazing and use of alternative perennial legumes to white clover, especially Caucasian clover (Richard Watson et al.). This study also involves the monitoring of nematode population changes in relation to the establishment of Caucasian and white clover after long term non-host rotation for clover nematodes, in this case maize.

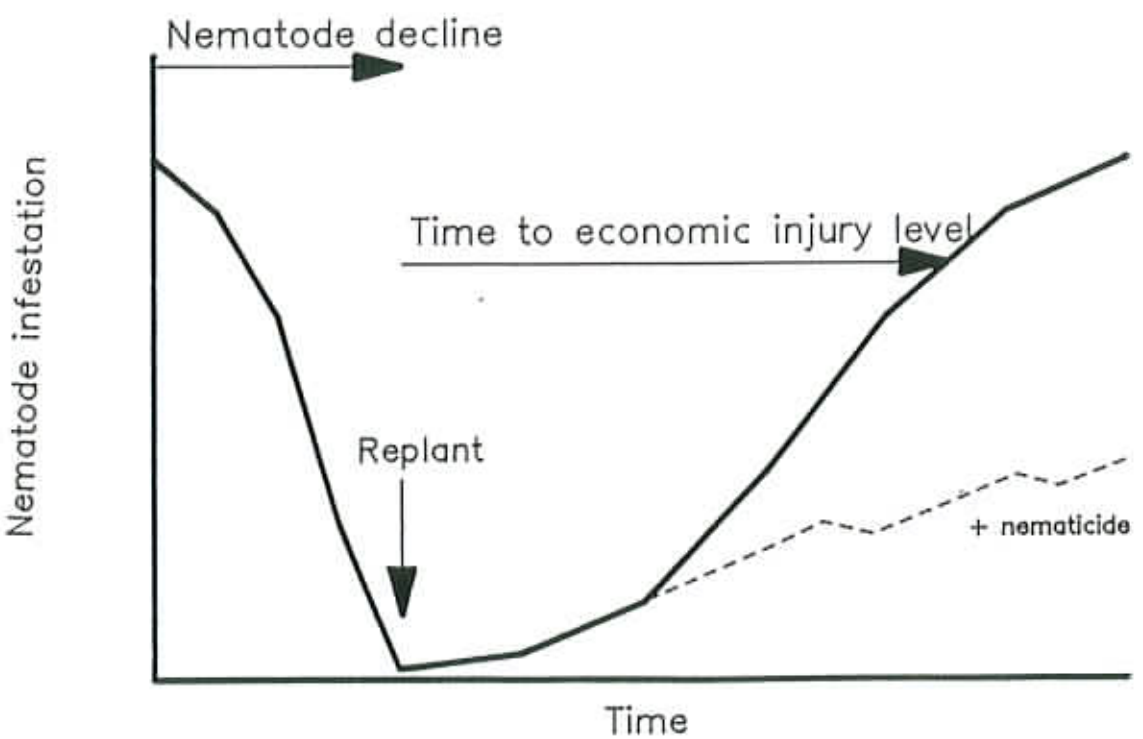
The above mentioned groups welcome communication in pasture nematology and the prospect of collaborative research. Contact with above named individuals may be established through the AAN listings or Richard Watson (listed).

CONTROLLING *RADOPHOLUS* ON BANANAS IN AUSTRALIA AND AFRICA

For the past year, QDPI (Tony Pattison in particular) has been tackling the problem of minimising chemical use to control burrowing nematodes on bananas. Nematicide application costs about $1500/ha/year and every year, several workers are hospitalised in north Queensland because of Nemacur poisoning.

The aim is to be able to sample a crop, advise growers whether damage in the following year is likely to be economic and make recommendations on management options. Such options might be 1) to apply nematicide, 2) to manage the crop to maximise its tolerance to the nematode or 3) to pull out the crop, manage the land so that nematode decline is maximised and replant.

To achieve this, we have a several pronged approach. Essentially, we want to put values on the population dynamics graph which shows nematode infestation (root damage) from the time a damaged crop[is pulled out, replanted and nematode population builds up again. Firstly, a trial has been established to assess the potential of various rotation and organic amendment options to reduce nematode populations at the end of the cropping cycle. Secondly, Tony is monitoring 13 existing crops for nematode infestation and plant growth. It seems too early at this stage to draw many conclusions except that there is a huge amount of variation! In another trial, Tony is assessing the use of mulching around the plant base to improve root growth and therefore tolerance to the nematode.



Although not major components of our work yet, resistance and biological control may be the only options in countries with third world agriculture. I recently had the opportunity to visit the International Institute of Tropical Agriculture (IITA) in Uganda, Nigeria and Benin and to look at their nematology program which is run by one postdoctoral fellow, Paul Speijer.

Bananas are usually grown in small family gardens with a large number of different cultivars. There is no cropping cycle as such; if a plant dies, it is simply replaced by another. The potential for strategies such as rotation seems minimal although it may be possible to rotate small amounts of land within the garden. Chemicals are rarely used because of their cost.

Resistance and biological control may be useful where only one nematode is of major importance. However, in many regions in these countries, *Radopholus, Pratylenchus coffeae, P. goodeyi* and *Helicotylenchus multicinctus* are all abundant. In such situations, approaches which are not species-specific are required.

Nematode control in developing countries certainly appears more difficult from that in Australia. Many options are not available. Although labour is cheap, other costs are too high for the non-commercial farmer and land is scarce. In addition, nematological expertise is in short supply. I guess they will always be behind the eight ball.

(Julie Stanton)

NOTES ON IMPORTANT POINTS ABOUT EXTRACTION AND PENETRATION OF *Pratylenchus thornei* AND *Pratylenchus neglectus* IN WHEAT.

Julie Nicol and Abdul Taheri

Much work on *Pratylenchus thornei* and *Pratylenchus neglectus* is being carried out in Australia, particularly on cereals. Both species have been shown to cause yield reductions on wheat, and hence are of economic importance to the cereal industry.

Current laboratory procedures rely heavily upon mister extraction of the nematodes, especially in South Australia,to measure the numbers in the root. However, this poses some very important questions; one of which is the effect of soil type and consequently penetration of nematodes into the host, and secondly how effective the mister extraction procedure is for the recovery of nematodes.

The wheat variety Machete was used to investigate the penetration efficiency and extraction of *P.thornei* and *P.neglectus* using four different soil types.

The experimental set up involved :

4 soil types

- **Ulns:** Urrbrae loam non sieved (clay loam)
- **Uls:** Urrbrae loam sieved (clay loam)
- **Ps:** Palmer sand (sand from the Palmer region)
- **Rs:** Roseworthy sand (sand from the Roseworthy region)

2 nematodes

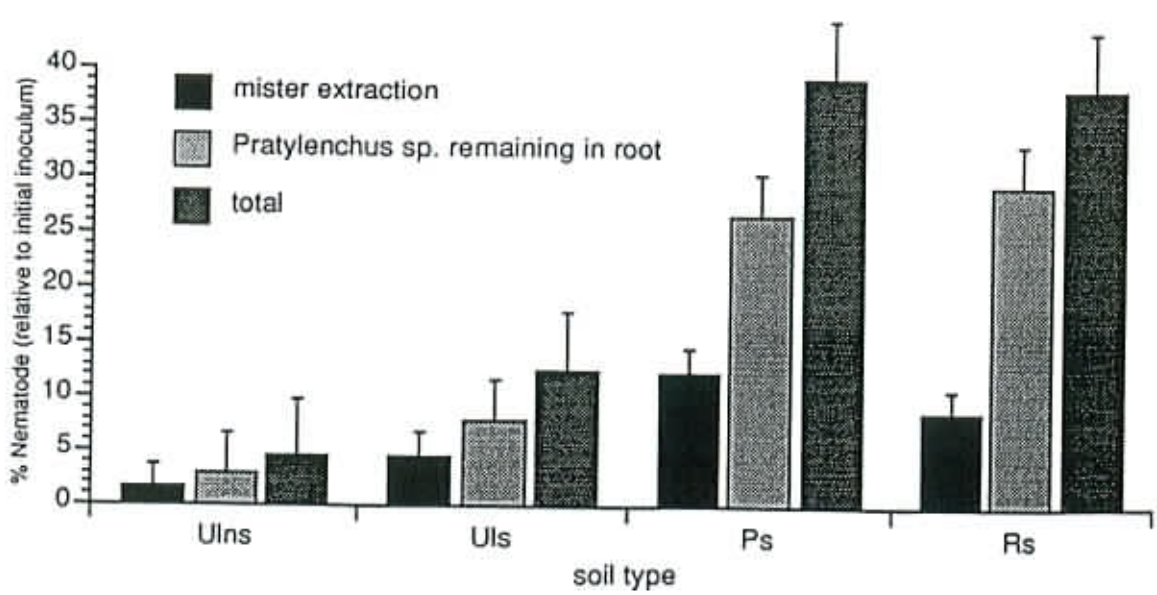
P. thornei and *P. neglectus*

1 inoculation density: 2000 *P. thornei* or *P. neglectus*/plant

1 harvest time: 1 week after inoculation

Pre-germinated Machete plants were inoculated with either *P.thornei* or *P.neglectus* and harvested 1 week after inoculation and placed on the mister for four days. Nematodes collected were counted. The remaining roots on the mister were stained with acid fuschin lactoglycerol and the nematodes counted in order to determine the total of nematodes per root system. The data was analysed using a completely randomised design of six replicates.

Fig 1 The effect of soil type and recovery by mister extraction of *Pratylenchus* sp. from Machete wheat roots.



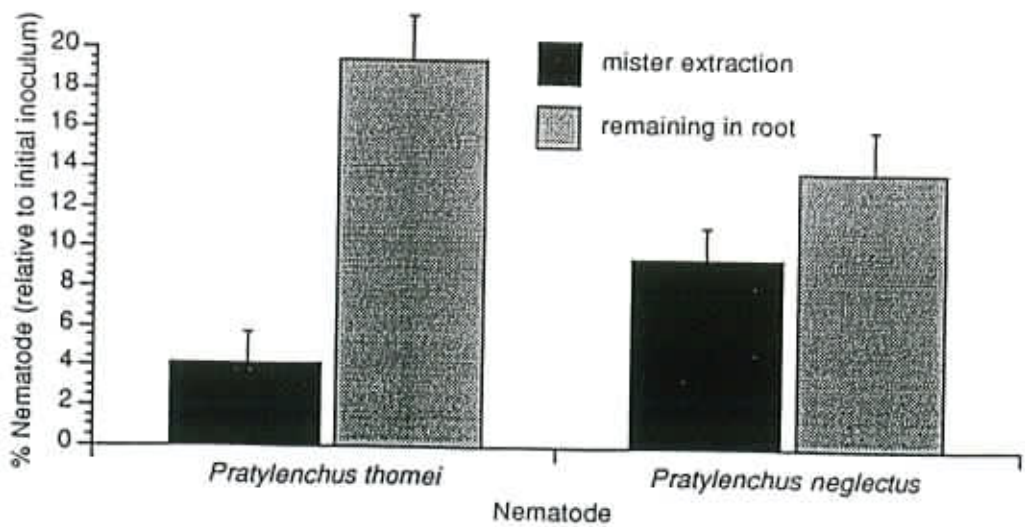
The nematodes were found to act similarly regardless of soil type. Overall the actual effectiveness of the original inoculum is quite low; from Figure 1 it can be seen the sandy soil was by far the best medium ($P<0.05$) to allow maximum penetration (up to 40%) which supports the theory of maximum nematode movement. However, the clay loam (Urrbrae loam) was very inefficient, particularly if not sieved, with significantly fewer nematodes (only 5%) penetrating the roots of Machete.

Further analysis revealed that there was a significant nematode species effect , with both the mister extraction and the numbers of stained nematodes remaining in the root system (Fig 2). From the mister extraction 42% of the *P.neglectus* exited the root in four days, while for *P.thornei* the number leaving the root is significantly less (only 16%), almost 3 times fewer. However, when the nematodes were counted inside the root there were significantly greater number of *P.thornei* left (84%) with much less *P.neglectus* (58%). With the total numbers of nematodes (mister extraction plus the remaining nematodes in the root) , there was found to be no difference between numbers. Why *P.neglectus* exits the roots faster than *P.thornei* is unknown!

The results from this relatively simple experiment have very important inferences for the interpretation of data. Research dealing with nematode resistance is very much reliant upon numbers, with many researchers making reference to a multiplication rate (final density/initial density (usually inoculum density) on a particular host. It is important to remember, depending on the soil medium, that this will influence the initial number of nematodes penetrating the root and hence the final number obtaining from that host. Furthermore it is not strictly valid to make comparisons between the two nematode species with relation to mister extraction, as from this experiment three times as many *P.neglectus* had exited the root than *P.thornei* over a four day period. Of course if different time periods and initial inoculum densities were considered the results may be different again!.

It is important to have an understanding of the nematode penetration in different soil types and extraction efficacy of the method you are using. If comparative data is required a standardised method should be used. Caution should be taken if comparison with actual numbers are made with different experimental techniques and methods.

Fig 2 The effect of mister recovery of *Pratylenchus* sp. from Machete wheat roots.



Department Crop Protection, Department of Plant Science, Adelaide University

A RAPID CEREAL CYST NEMATODE SCREENING TEST

A new procedure to screen cereal plants for resistance to cereal cyst nematode (CCN) has been developed by the SARDI's Field Crops Pathology Unit to cope with the increasing numbers of lines that the breeders want assessed. This technique enabled the CCN program to increase the number of plants screened from 3,000 to 60,000 plants per annum in 1994. The long term aim is to screen 100,000 plants per annum.

When handling this number of plants everything is on a large scale. Eliminating as many tasks as possible, no matter how small, was a high priority. To eliminate all of the paperwork a software program was developed to manage all of the information relating to each plant tested, including line identification and results. The breeders provide a list of all of their lines to be assessed on disk, and the software producers a seeding layout.

To save on cost of glasshouse space, the plants are grown outside in 150 cc seedling tubes containing infested soil and slow release fertiliser. The tubes are stored in groups of 50 in wire baskets. These baskets have been called "McKay crates" by some bemused colleagues. Each basket has a unique number, which combined with the crate grid reference, 5 rows X 10 columns, enables the location of each plant to be stored in the computer, eliminating the need for tags. Scarce space at the Waite was solved by a terracing a steep paddock.

The plants also grow much better outside, particularly as the tubes are open at the base enabling the roots to tap moisture and nutrients in the soil below the baskets.

To establish the experiment required 12 tonnes of inoculum. This was produced by blending concentrated CCN infested field soil with sand and slow release fertiliser. CCN concentrations in the final inoculum was 16 eggs per gram. To mix this material we had to hire a cement truck for several hours.

To assess the plants we had planned to mechanically wash the soil from the roots and quickly classify each plant as resistant or susceptible based on a visual assessment of the number of CCN females that had developed. But the soil was too difficult to wash due to the amount of clay in the field soil containing the CCN cysts. Fortunately there were enough females developing on the outside of the root ball to enable the plants to be rated quickly, one person could assess up to 1,000 plants per day. Four work-stations were set up to allow 4 people to simultaneously enter results of plants being assessed.

Last year was the first large scale attempt to use this technique to screen breeders lines. In general the test worked well, however we did not anticipate problems with birds. Once a few wily birds discovered what was in the crates, they were amazingly cunning in wiping out several experiments involving about 30,000 plants. Suffice to say we are looking at a number of options to keep the birds off the crates until the seedlings are established.

Encouraging results have also been obtained using this system to screen oats and faba beans for resistance to stem nematode, wheat for resistance to common root rot, cereals for resistance to smuts, peas for resistance to black spot, ryegrass for resistance to *Anguina funesta*.

John Lewis, Franky Charman-Green, Alan McKay, Milanka Matic.

NEW ROOT-KNOT NEMATODE IN NEW ZEALAND

Chris Mercer
AgResearch Grasslands, Palmerston North

New Zealand pasture nematologists are coming to terms with the fact that an important root-knot nematode species has been misidentified. Nematode parasites of our pasture clover species have been studied since the 1970s and data on distribution, pest status, development rates, pest relationships and resistance have been published.

The root-knot nematode causing conspicuous galling on white clover in New Zealand has been referred to as *Meloidogyne hapla*. However, during my stay at Texas A & M University a year ago, Prof Jim Starr compared my nematode culture with his own cultures of *M. hapla* and found differences in size and gall character.

Since then I have raised single egg mass cultures of the new species and of *M. hapla* collected off kiwifruit and vegetable hosts. The North Carolina host differential test has shown distinct differences in host range, eg, both species parasitise white clover but only *M. hapla* parasitises tobacco and tomato. There is a clear distinction in the type of root gall formed on white clover. *M. hapla* galls are smaller, bear more side roots, mostly contain one female, and have a visible egg mass which protrudes from the root surface early in its development. The undescribed *Meloidogyne sp* causes much larger swellings of the root which frequently contain several females; egg masses do not break through the epidermis until the gall degrades, and galls frequently contain hundreds of eggs of juveniles within an intact epidermis.

We are embarking on a programme to identify and characterise the new species. The morphology of the new species is being described by a specialist in the *Meloidogyne* genus, Prof J E Eisenback.

Our involvement is to compare the DNA banding patterns of the new species with known root-knot species using PCR and primers which have already been used by others to distinguish species of *Meloidogyne*. We will also compare the isozyme phenotypes of known species with the new one.

We have no information on how widely *M. hapla* is distributed in New Zealand pasture. The undescribed species, with its large galls has been observed on white clover throughout the North Island and in parts of the South Island. We now need data on the distribution of population densities of *M. hapla* in order to compare its importance to the undescribed species.

Our resistance screening programme has identified germplasm in white clover and other clovers resistant to the new root-knot species. We must now determine if this resistance is also effective against *M. hapla*. If there is no cross-resistance and if *M. hapla* is widespread and abundant, we will have to include it in our resistance breeding programme for white clover.

NEMATODES AS BIOCONTROL AGENTS OF HELICID SNAILS

A new research project is underway at the Waite Campus, University of Adelaide, in which we will examine the possible use of nematodes as biocontrol agents of the introduced snail species *Cernuella virgata, Theba pisana* and *Cochlicella* sp., also known as white snails.
The project is jointly funded by GRDC and RIRDC, reflecting the increasing concern about snails in crops and pastures in the agricultural and horticultural districts throughout SA, the Wimmera and Mallee in Victoria, southern NSW and across parts of Western Australia (Blesing, 1993).

It has been shown that bacterial feeding Rhabditid nematodes will attack slugs or snails (Poinar, 1989; Jaworska, 1993; and Wilson *et al.* 1993). Wilson *et al.* (1994) tested 16 different bacterial isolates associated with the slug killing nematode *Phasmarhabditis hermaphrodita,* or from dead slugs. They found that two isolates gave good yields of nematodes when cultured with them and these nematodes were consistently pathogenic to slugs. One bacterial isolate was identified as *Moraxella phenylpyruvica* and is now used for rearing the nematode as a commercial biocontrol agent. Preliminary work in this laboratory has shown that some Australian nematodes will attack Helicid snails, and up to 100% mortality was achieved in petri dishes. Nematodes used in this experiment carried a mixed bacterial flora.

While entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae always form a mutualistic relationship with a gram negative bacterium, a less specific relationship between nematodes and bacteria is found in nematodes of the families Rhabditidae, Diplogasteridae, Panagrolamidae and Cephalobidae. We have found that two or more different bacterial colonies are commonly present when surface sterilised nematodes are placed on Nigon's agar.

Soil samples from snail infested areas on the Yorke Peninsula, the west coast of South Australia, Callington and the Murrumbidgee Irrigation area in NSW are currently being screened for fluorescent *Pseudomonas* species and *Bacillus* spp. Additionally, the soil is being used for snail traps (set up in the same manner as *Galleria* traps) for the isolation of snail-infesting nematodes. So far 57 fluorescent *Pseudomonas* isolates, 42 *Bacillus* isolates, two *Bacillus thuringiensis* isolates and 12 nematode isolates have been obtained. In a first step, nematodes will be grown on the different bacterial isolates to select strains of bacteria that provide good yields of nematodes. Selected nematode/bacterium combinations will then be tested for pathogenicity to snails. Two bioassays have been developed. The first screening of nematode/bacterium combinations against snails will take place in petri dishes and any successful combinations will be screened again using a soil-based bioassay.

(Suzanne Charwat, Kerrie Davies and Heather Fraser, Department of Crop Protection, Waite Campus, University of Adelaide, SA)

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Breeding for cereal cyst nematode resistance and tolerance in wheat
Root lesion nematode tolerance breeding
Pratylenchus sp.

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Resistance in clover hybrids
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Pratylenchus thornei in wheat
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Molecular diagnosis of *Meloidogyne*. Biological control of *Meloidogyne* and *Radopholus similis*.

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Pratylenchus thornei and *Merlinius breviden*.
Identification of nematodes
Control methods, especially through resistance breeding

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Biological control
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