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AUSTRALASIAN NEMATOLOGY NEWSLETTER

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From the Editor

The second issue for the year contains some interesting news and articles from members and abstracts from the nematology workshop held at Lincoln last year.

Those who have contributed with so little prompting, have made my task relatively easy. However, I am sure that there are many 'might-have-been' contributions out there that readers would be pleased to see materialise in the next issue.

January Issue

The deadline for the January issue is December 20. Please endeavour to prepare material well in advance, as December can provide many distractions. The January issue will contain a membership directory. If your contact details or interests have changed, please let Nora Galway know so that the list can be as accurate and useful as possible.

New publications list

The inclusion of a list of new publications of ANN members, although a worthy suggestion, has not captured the imagination of the membership. Only four members provided details of their papers, for which they are thanked. However, given the limited material, a list was not include in this issue.

An alternative approach may be to compile a list of Australasian contributions to nematology literature from the computerised version of Current Contents or the like. If there is a member willing to take on this task, I would be pleased to hear from you.

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Association News

FROM THE PRESIDENT

The president's page (well at least a few lines):

This is written as I prepare to go to the Third International Nematology Congress in Guadeloupe. While there, I will represent the association in discussions on the proposal that there should be an international federation of nematology societies. I have an open mind on this but would need to see the benefits for us before I would be persuaded of its merits. Appended to this newsletter are several pages concerning the proposed International Federation of Nematology Societies.

John Marshall, AAN President, Crop & Food, Lincoln

NEMATOLOGY WORKSHOP, PERTH 1997

A nematology workshop will be held in conjunction with the Australasian Plant Pathology Society Conference in Perth, 29 Sept - 3 Oct, 1997. The proposed topic is:

Nematode control - genes and microbes

The program could include biological control, transgenic and traditional breeding, ie ways of coping with no or fewer pesticides. Rob Potter of Murdoch University has offered to assist in the running of the workshop. A call for papers will be included in the next issue. In the interim, I would welcome any comments, offers of assistance, offers of papers, suggestions for keynote speakers, etc.

Workshop Convenor, Ian Riley, Agriculture Western Australia, South Perth

BOOKLET ON ADVISORY SERVICES FOR NEMATODE PESTS

In the January issue of the newsletter, I mentioned that AAN had been asked by RIRDC to prepare a final submission on our proposal to prepare a booklet on the operation of nematode diagnostic services.

We have been advised by RIRDC that our application was successful and Julie Nicol and myself will be preparing a draft publication in the next few months. Next year we will be asking members with experience in this area to review our draft manuscript.

Many thanks to RIRDC for their continued support of nematology in Australia

Graham Stirling, QDPI, Indooroopilly

Regional News

NEWS FROM CANBERRA

Nematode Collection

The development of a reference collection of plant-parasitic nematodes in Australia, housed at the Australian National Insect Collection (ANIC) within the CSIRO Division of Entomology in Canberra, is continuing with support from GRDC. From small beginnings, the collection is growing to cover all plant-parasitic nematode groups. To date, we have received material from all states except Tasmania and the Northern Territory, as well as from as far afield as Riverside, Wageningen, Rothamsted and the International Institute of Parasitology. As part of our commitment to maintaining the links between the collection and researchers all over Australia, we present this update of the status of the collection.

The collection currently consists of:

- over 1000 specimens of plant-parasitic nematodes on permanent slide mounts, covering different life stages, geographic and host variation of many important species;
- bulk specimens fixed and stored in formaldehyde;
- cryopreserved, living specimens of selected species;
- a computer database of all plant-parasitic nematodes in the collection, plus other species described from Australia;
- a library of relevant literature, including species descriptions, also completely computer catalogued.

We have a specialist curator (Frances FitzGibbon) and I have responsibility for identifications, management and overall development.

It is important to note that the collection is an actively used physical database of nematodes. It is being actively studied as well as rapidly expanded. So far I have concentrated my studies on *Pratylenchus*. Morphological variability in key characters between different species and geographically different populations of the same species is revealing interesting relationships within the genus. This work is allied with parallel studies of genetic relationships lead by John Curran, with Nora Galway and Felice Driver. Altogether, this work is aimed at understanding the complex relationships between plant hosts and their nematode parasites, including resistance, pathotypes and interactions between pathogens. This is essential for effective control. The collection is also being used as a resource for the development of molecular diagnostics for nematodes.

As the collection grows, it will become an increasingly valuable resource and repository for nematologists across Australia. The aim is to make information available and accessible, hence the emphasis on computer databasing. The more support and material received, the more informative and useful the collection will be. To this end, I seek further material and specimens. If possible, the material should be in culture (so that all material can be processed in identical fashion and inter-culture variation can be assessed). Original host and location records are also important to the value of the collection. Species identity is a bonus, but if unknown I can identify it for you. We can also handle material in other forms (for example bulk soil samples with high

counts of particular species of interest, or microscope slides). The species you are working on will almost certainly be of interest to someone, somewhere in the future, so taking a few minutes to deposit specimens and supporting information will save the next person a lot of time. And don't let those old slides or tubes sit in the corner until they are broken, dry out or disappear: send them to us, so that they will be cared for and curated until they are needed. Or take a minute to make one extra slide of the material you are currently studying and send it to us. Please feel free to contact myself or Frances to arrange shipment of material - we can be contacted on (06) 246 4371 (telephone) or (06) 246 4000 (fax), e-mail mikeh@ento.csiro.au or francesf@ento.csiro.au.

In addition to the plant-parasitic nematode collection, I am also continuing RIRDC-commissioned research on the free-living nematode fauna of agricultural soils in Australia, focusing on the impact of cropping history and cultural practice on the diversity and abundance of nematodes in soils from the southern wheatbelt. To date I have found over 70 species - Tylenchids, Rhabditids, Dorylaimids, Enoplids, and Monhysterids - and many are new to science. I have begun description of some of these species, including one from a genus previously found only on the Great Plains of the USA, so keep an eye out for the descriptions in the literature. These nematodes, too, will become part of a linked collection of free-living nematodes.

The basic infrastructure for a modern, efficient collection has been set up so the collection can be used as a repository for housing voucher material, for housing material orphaned or no longer studied, or for safe keeping of entire collections. An added bonus of donation is that we will computer catalogue collections making them more available to all bona fide users, including the donor. There are many different arrangements under which material can be deposited or donated (including some that offer taxation benefits). Please contact me for further information.

Mike Hodda, Australian National Insect Collection, CSIRO Division of Entomology, Canberra

NEWS FROM NEW ZEALAND

Nematology at Lincoln

We are making good progress in identifying populations of PCN that are virulent to a range of resistant potato genotypes. We have identified a single highly virulent population of Pa3 that after two generations was reproducing on the highest level of potato resistance at 10% of the same population growing on a fully susceptible host.

We have finally completed our work on the PCR primers that can detect and differentiate the two species in the same reaction. We have finally got it to the editor and hopefully will get it published.

Root knot nematode in carrots has become of interest to the carrot growers and we are in the near future putting together a small project to look a the biology, identification and control of the pest.

Nematodes in mushrooms still cause a bit of a problem but the resolution of this problem is one of cleanliness and internal hygiene. Associated with the mushroom industry is the nematodes from peat bogs. We looked quickly at a number of bogs – if you do it slowly you sink – to determine the level at which nematodes disappear. The idea was that the peat used by the mushroom industry should be taken from the nematode free layers. The trouble is that the pear producers stock pile the peat on bare ground and the nematodes migrate into the clean peat.

John Marshall, Crop & Food, Lincoln

NEWS FROM SOUTH AUSTRALIA

South Australian Research and Development Institute

A five year contract from GRDC awarded to SARDI, VIDA and the University of Adelaide will start on July 1st, 1996 and at present encompasses research into root lesion nematode and stem nematode. Research on cereal cyst nematode may be incorporated into the program in the future. (In South Australia, the CCN projects currently concentrate on efficient mass screening to accelerate the release of CCN resistant cereals. During 1996, 70,000 wheat, barley and oat plants are being tested).

In the new program, research on root lesion nematode will assess rotations, identify tolerance and resistance and provide management strategies for *P. thornei* (VIDA at Horsham) and *P. neglectus* (SARDI and University of Adelaide at the Waite Institute and PISA at Minnipa Research Centre). In addition, a new collaborative project between SARDI and the University of Adelaide will attempt to develop a "rapid" screening test for root lesion nematode. Initially the test will focus on wheat but eventually other crops are hoped to be included. A smaller project is also under way to determine the role of root lesion nematode in medic decline in Southern Australia.

Finally, (and sadly) only a reduced amount of funding for stem nematode research was obtained to continue at one third of the previous level to collaborate with oat, faba bean and pea breeding programs.

Other news: Maria Scurrah and Julie Nicol were awarded grants from GRDC to attend the Third International Nematology Congress at Guadeloupe in July this year. Julie Nicol also had the joy of finding a medium size frog in our mister - she leaped twice as high as the frog.

Maria Scurrah and Sharyn Taylor, SARDI, Adelaide

University of Adelaide, Department of Plant Science

The University of Adelaide, Waite Campus, has a number of students who have recently completed, or are continuing, research projects related to our root lesion nematode program. All have made significant contributions to the understanding of specific problems.

In early 1996, two students successfully completed PhD projects in the Department of Plant Science. Both were sponsored by the Iranian Ministry of Culture and Higher Education, and have returned home to take up university research and teaching positions.

Abdol Taheri's thesis, "Interaction between root lesion nematode, Pratylenchus neglectus, and root-rotting fungi of wheat", was supervised by Gil Hollamby and Vivien Vanstone (Department of Plant Science) and Stephen Neate (CSIRO Division of Soils, Adelaide). Kerrie Davies and Julie Nicol (Department of Crop Protection) assisted greatly in many aspects of Abdol's project.

Abdol examined the interaction between *P. neglectus* and a number of root-rotting fungi commonly associated with wheat in South Australia. In glasshouse, laboratory, field plot and microplot experiments, Abdol demonstrated a positive interaction between the nematode and the fungi *Bipolaris sorokiniana*, *Microdochium bolleyi*, *Pythium irregulare*, *Pyrenochaeta terrestris*, *Rhizoctonia solani*, *Fusarium acuminatum* and *F. oxysporum*. The nematode-fungus combination led to enhanced root lesioning and/or increased nematode multiplication in roots. Inoculation timing and growing temperature significantly affected the observed interactions.

Conversely, *Gaeumannomyces graminis* (the "take-all" fungus) showed a negative interaction with the nematode, especially when plants were inoculated with *P. neglectus* two weeks prior to inoculation with the fungus. In the field, *G. graminis* reduced yield of Machete wheat by 48%, *P. neglectus* reduced yield by 20% and *G. graminis* + *P. neglectus* reduced yield by only 14%.

Mechanical root wounding did not enhance fungal activity, suggesting that the role of the nematode in the interaction is not merely in damaging roots, but brings about more complex biochemical and physiological changes in the host. *P. neglectus* did not appear to feed or multiply on fungi in agar plates, but nematodes were attracted to exudate from roots of plants that had been infected by some fungi.

In South Australian soils, where both *P. neglectus* and fungi are common, disease in wheat is caused by the combination of root lesion nematodes and fungi, with *P. neglectus* infection rendering roots more prone to fungal attack. It is not only the "major " fungal species that are involved in this interaction, but also many species of "minor" fungi not generally considered important in cereal root disease. Abdol's findings have implications in the interpretation of field results from both fungus and nematode assessments, especially resistance screening and breeding of cereals.

Mohammad Farsi's thesis, "Genetic variation for tolerance and resistance to *Pratylenchus neglectus*", was supervised by Tony Rathjen and Vivien Vanstone (Department of Plant Science) and John Fisher and Kerrie Davies (Department of Crop Protection).

In field and glasshouse experiments, Mohammad found that *P. neglectus* enhanced the severity of leaf yellowing for intolerant wheat genotypes, but controlling nematodes or adding nitrogen + phosphorus alleviated this symptom, compensating for reduced nutrient uptake by nematode infected plants. Leaf yellowing was strongly related to nitrogen deficiency and, to a lesser extent, to phosphorus deficiency. Genotypes with higher root growth rate were greener and had significantly greater shoot growth in nematode infested soil.

Using pots with a capacity of 650g of soil, Mohammad determined that adding 500 *P. neglectus* per pot and terminating tests at eight weeks after sowing was adequate to differentiate between resistant and susceptible plants, by ranking genotypes on the basis of number of nematodes per plant.

Durum wheat and triticale varieties tested showed lower *P. neglectus* multiplication than susceptible wheat varieties, and all lines containing rye chromosomal material supported low numbers of nematodes compared to wheat.

In Abacus triticale and two resistant, but non-adapted, wheats detected by the Waite Campus Wheat Breeding Program, time of nematode moulting and commencement of egg laying was delayed. There was also some indication that fewer nematodes initially penetrated the roots of resistant genotypes. Nematode invasion of resistant plants appeared to induce a hypersensitive reaction in root cortical cells. Considerable backcrossing of the non-adapted varieties to adapted recurrent parents will be necessary.

Sharyn Taylor, a part-time PhD student with the Department of Crop Protection and SARDI, is continuing her thesis on the biology of *Pratylenchus* spp. in South Australia. The project is funded by GRDC and supervised by Alan McKay (SARDI), Otto Schmidt and Kerrie Davies (Department of Crop Protection). Sharyn has investigated the depth distribution of *P. neglectus* in calcareous sands and clay vertisols, and also assessed the effects of different sampling techniques on the recovery of *P. neglectus* from soil samples. In the shallower sandy soil types, 73% of nematodes were found in the top 10 cm while in the deeper clays only 43% were found to

this depth. In dry soil of all types, the best sampling technique for maximum recovery of nematodes was an undisturbed core wetted to field capacity. Sharyn is also continuing work in determining losses caused by *P. neglectus*. Field trials in 1996 will investigate yield losses in varieties of wheat, barley, oats, chickpea, pea, lentil, canola, vetch, medic and faba bean.

Mark Potter is conducting a GRDC/APRA funded PhD project investigating *P. neglectus* on brassicas and related species. Mark is supervised by Tony Rathjen and Max Tate (Department of Plant Science), Kerrie Davies (Department of Crop Protection) and Phil Salisbury (Victorian Institute for Dryland Agriculture, Horsham).

When crop residues break down in the soil or plants are green manured, brassicas release chemicals with biocidal properties, which are effective against a number of plant pathogens, including nematodes. Mark has found considerable variation in ability of brassicas to host *P. neglectus*, and has developed a laboratory bioassay to assess activity of various brassica tissues against the nematode. Differences between species and varieties may be due to variation in the plant glucosinolate profiles. Chemical content of plant parts differs, and it is possible to breed plants with high concentrations in roots or leaves without negatively influencing seed quality.

Nematode reproduction on brassicas is a function of both the susceptibility of the crop and the nematicidal impact of the plant tissues. Interspecific hybridisation between species exhibiting significant differences in these traits is under way. By examining hybrid tissues, it is hoped that information relating to the inheritance of glucosinolate profile, susceptibility to nematodes and tissue biofumigation capacity can be gathered. The disease break potential of canola and other brassica crops within cereal rotations can thus be improved.

Mark has just spent several months with the brassica research group at the John Innes Institute, Norwich, England, to learn more about the chemistry and breeding of brassicas.

In 1995, Lesley Polomka completed an Honours project investigating "Resistance to *Pratylenchus neglectus* in wheat" under the supervision of Tony Rathjen and Vivien Vanstone (Department of Plant Science).

Lesley confirmed the resistance of an exotic wheat detected by the Waite Campus Wheat Breeding Program, and identified resistant F3 families for use in the breeding program. It was not possible to determine accurately the inheritance of resistance to *P. neglectus*, but there was some indication that resistance in the variety tested may be controlled by a single gene. As there was no clear segregation ratio amongst the F3 families, further analysis would be needed.

A non-destructive screening test for detecting resistant plants was examined, so that selected plants could be used directly as parents, rather than relying on progeny testing. Half the root system was removed and misted, then plants transferred to pots to continue growing. Due to the uneven distribution of nematodes within the root system, the chance of misleading results is increased, but this can be compensated for by screening a larger number of plants. The benefits would outweigh the disadvantage of increased chance of error.

There was a strong correlation between number of nematodes per plant and nematodes per gram dry root. However, in some instances results were misleading and resistance rankings changed. F3 families had much higher root weights than the parental varieties, and nematodes per gram dry root was a more accurate indicator of resistance in this case.

James Neal is currently working on an Honours project in the Department of Agronomy and Farming Systems, Roseworthy Campus. James is supervised by Bill Bellotti (Department of

Agronomy and Farming Systems), Vivien Vanstone and Robin Graham (Department o f Plant Science).

James is investigating the interaction between *P. neglectus* and phosphorus nutrition in annual medic. The occurrence of both *P. neglectus* and phosphorus deficiency in the cropping soils of South Australia may be among the factors contributing to medic decline syndrome. Most medic varieties are relatively susceptible and intolerant to *P. neglectus*, and this has implications for the practice of wheat-medic rotations in South Australian farming systems.

Phosphorus deficiency compromises cell membrane integrity, causing root cell contents to leak into the soil. Nematodes may be attracted to these leaky roots. Supply of adequate phosphorus may therefore alleviate root damage and reduce nematode multiplication to some extent.

James' preliminary experiment has shown that *P. neglectus* significantly reduces root and shoot growth of Parabinga medic in pots, and nodulation of the plants is also affected adversely. With 5 *P. neglectus* per gram of soil, shoot and root dry weight we re reduced by up to 55%, and nodule fresh weight was reduced by 45% for eight week old plants.

Vivien Vanstone, Department of Plant Science, University of Adelaide, Waite Campus

University of Adelaide, Department of Crop Protection

In the Department of Crop Protection, Julie Nicol recently successfully completed her PhD project, funded by GRDC. Her thesis, "Distribution, pathogenicity and population dynamics of *Pratylenchus thornei* on wheat in South Australia", was supervised by John Fisher and Kerrie Davies (Department of Crop Protection) and Trevor Hancock (Biometry Unit, Department of Plant Science). Julie worked closely with Abdol Taheri and Vivien Vanstone (Department of Plant Science) and Sharyn Taylor (SARDI).

Julie surveyed plants and soils from the cereal growing regions of South Australia, and showed that for any soil type there was a 90% chance of finding either *P. thornei*, *P. neglectus* or both. While *P. neglectus* was found more commonly on sandy soils, and *P. thornei* in clay, soil type did not exclude either species.

An assay was developed for screening susceptibility of cereal and other hosts to *P. thornei* and *P. neglectus*, growing plants in sandy soil in small tubes for two months after inoculation with a non-damaging nematode density. In summary, most wheats were highly susceptible to *P. thornei*, while triticale, rye, oats and durum wheat ranged from moderately susceptible to resistant. Similar results were obtained with *P. neglectus*. The wheat accession AUS4930 was one of the least susceptible tested for *P. thornei*, but the most susceptible for *P. neglectus*. GS50A, a field selection obtained from Queensland, also seemed to have resistance to *P. thornei*. Preliminary studies suggested that the resistance mechanism acted post-penetration, but that further selection of parents was necessary to study genetics of inheritance of resistance.

Field studies on population dynamics and yield relations showed that *P. thornei* caused losses of up to 38%, but that initial density causing loss varied between seasons. The population dynamics of *P. thornei* followed the general pattern for nematodes, with high multiplication at low initial densities and reduced rates at higher initial densities.

Julie also did some collaborative work with Abdol Taheri, showing that *P. thornei* behaved similarly to *P. neglectus* in causing root disease of wheat when combined with the root-rotting fungi *Fusarium* spp. and *Microdochium bolleyi*.

Valerie Kempster has recently begun work on her PhD project, "Rhizosphere microbes as potential control agents for plant parasitic nematodes in pasture crops". She has a scholarship from the University of Adelaide.

Kerrie Davies, Department of Crop Protection, University of Adelaide, Waite Campus

NEWS FROM WESTERN AUSTRALIA

Agriculture Western Australia

Work on the fungus, *Dilophospora*, to control the bacterium and nematode associated with annual ryegrass toxicity (ARGT) in now focused on the methods for mass production and field scale inoculation. Recent problems with contamination of export hay with toxic ryegrass has highlight the pressing need for better control of the ARGT organisms and ryegrass. Along with other approaches, *Dilophospora* is likely to make a significant contribution to a long term solution to ARGT.

A quarantine compound has been constructed with funds from GRDC to permit the study of exotic, non-toxigenic *Clavibacter* spp. as potential antagonists of *Anguina funesta* and competitors for *Clavibacter toxicus* (the nematode and bacterium responsible for ARGT).

Some preliminary work is being undertaken in the Esperance mallee to assess numbers and effect of root lesion nematodes in wheat crops. Some high counts recorded in the district last season has prompted the study.

Ian Riley, Agriculture Western Australia, South Perth, WA

Research

POTENTIAL OF RHIZOSPHERE MICROBIALS FOR BIOCONTROL OF PLANT PARASITIC NEMATODES

Valerie Kempster, Department of Crop Protection, Waite Campus, University of Adelaide, SA

A survey of the permanent white clover stands in Australia was conducted in 1991 (Hinch et al., 1993; In: Mason (ed.), *White Clover, the Key to increasing Milk Yields*). Of the seventeen sites sampled, 94% were infested with *Pratylenchus neglectus*, 80% were infested with *Heterodera trifolii* and 70% with *Meloidogyne hapla*. All three of these nematodes are known to suppress nitrogen fixation and to act synergistically with other root pathogens, such as *Fusarium, Pythium* and *Rhizoctonia* (Nickle, 1991; In: *Manual of Agricultural Nematology*). It is estimated that these nematodes cost the Dairy Industry of Australia upwards of $20 million per annum.

The use of nematicides is not appropriate in pasture situations. Thus the use of alternative, biological control against plant parasitic nematodes has been the research focus in the past 15-20 years (Stirling, 1991; In: *Biological Control of Plant Parasitic nematodes*). Chester (1933; *Quarterly Rev. Biol.* **6**:275-324) first wrote of acquired physiological immunity in plants and much recent research has been directed at the utilisation of this phenomenon in the control of plant pathogens, including nematodes (Cayrol, 1983; *Rev. Nematol.* **6**:265-273; Deverall, 1995; *Advances in Plant Pathology* **11**:211-228). Cross resistance is the induction of resistance by an organism from one taxonomic group to one from a different taxonomic group. There are many examples in the literature, e.g. the use of VAMs to induce resistance to *M. javanica* (Duponnois et al., 1995; *Nematologica* **41**:296-297); and endophytes inhibiting reproduction of root-knot nematodes (Elmi et al., 1990; *Ark. Farm Res.* **39**:3) More recently there have been reports of non-pathogenic *Bacillus* and *Pseudomonas* species and strains inducing systemic resistance to nematodes (Keuken & Sikora, 1995; *Nematologica* **41**:315; Hallmann & Sikora, 1994; *Int. J. Pest Management* **40**:321-325). Kuc (1995; In: *Innovative Approaches to Plant Disease Control*, 267-268) reasoned that resistance genes are not confined to phenotypically resistant cultivars, but are present, to be induced, in all plants.

This research and line of reasoning have led to the development of a project on the potential of soil microbes to induce a hypersensitive response, conferring non-specific resistance to nematodes. I began the project, in February, and will be supervised by Drs Kerrie Davies and Eileen Scott at the University of Adelaide, and Dr Jillian Hinch of the Victorian Department of Agriculture. Rhizosphere bacteria (specifically, *Bacillus* and *Pseudomonas spp)* are being extracted from soil taken from three of the sites from the nematode survey. These will be screened for both *in vitro* antagonism (cells and cell-free culture media) and their ability to induce resistance *in situ*, using both pots in greenhouse studies and microplots, with white clover as host plant. Biochemical and histochemical studies will be used to identify key signals or physiological pathways indicative of an induced resistance/hypersensitive response activity, to correlate with the observed biological phenomenon.

NEMATODES AS BIOCONTROL AGENTS OF HELICID SNAILS - AN UPDATE

Suzanne Charwat, Department of Crop Protection, Waite Institute, University of Adelaide, SA

Helicid snails are a serious pest of cereals in southern Australia. Following observations that an entomophilic *Rhabditis (Oeschius)* sp. would attack and kill the snails, a project funded by GRDC/RIRDC is under way to look at possible use of nematodes as biocontrol agents of white snails.

A survey of soils from snail infested areas of the Central Region, South East, Yorke Peninsular, and Eyre Peninsular in South Australia and the MIA in NSW was carried out. Isolates of bacteria likely to be pathogenic to snails were made from these and 77 isolates of fluorescent *Pseudomonas*, 70 of *Bacillus* and 3 *Bacillus thuringiensis* were found. In addition, 81 isolates were made from snail cadavers. All isolates are stored frozen for future work.

Using a technique of baiting soil with snails to trap nematodes, 18 nematode isolates have been obtained. Three nematode isolates have been made from snail cadavers collected in the field and two from local garden slugs.

Initial screening of nematodes for virulence to snails has been carried out using a soil-based bioassay. While most diplogasterid and panagrolaimid isolates caused little or no snail mortality, a cephalobid isolated from the Yorke Peninsular repeatedly killed 57 - 70% of snails. Of seven rhabditid isolates, an *Alloionema* sp. isolated from a garden slug caused the highest snail mortality.

Three inoculation densities (33,000, 66,000 and 100,000 dauers/100 g soil) of *Alloionema* were applied to containers with 7 snails and a total of 500 g soil. Each treatment was replicated three times and the mortality of snails was compared between treatments across sample dates (day 3, 6, 9 and 12) with ANOVA. Snail mortality was significantly higher in containers inoculated with 66,000 and 100,000 *Alloionema* sp. dauers/100 g soil compared to 33,000 dauers/100g soil. Significant differences in snail mortality were observed between day 3 and day 6. During this time snail mortality doubled and reached 60%.

For any use of nematodes as biocontrol agents mass-culture techniques are required to produce large numbers. We have tested a number of solid and liquid culture techniques and culture media for the cephalobid and *Alloionema*. A solid culture technique using 10% dog food agar proved most satisfactory. A Petri dish containing the dog food-nematode culture is placed in a container at 22°C and the base of the container is filled with water. Nematodes migrating from the medium are trapped in the water surrounding the Petri dish and can be easily collected from there. Containers can be harvested repeatedly over several weeks with numbers of dauers declining over time. Approximately one million larvae, nearly all dauers, were harvested from each Alloionema container after two weeks. This culture technique can be scaled up according to needs. Advantages of the method are that containers are set up quickly, yield high numbers of clean dauer larvae after a short incubation time and can be harvested over several weeks.

In order to find methods for long term dry storage of our nematodes two approaches were tried: embedding of nematodes in alginate gel beads with different fillers and/or coatings and incorporation into clay pastes. Alginate, a water soluble polysaccharide gum has been used to encapsulate a range of biological control agents. However, evaporation of water from alginate gel beads was too rapid for the survival of nematodes, and fillers were incorporated into or used as coating material of the beads at various combinations and concentrations. Rate of water loss from beads stored at 8°, 16° and 22°C was monitored during 6 days and varied significantly

between some treatments. To assess nematode survival, half of the beads from each treatment were placed in water and emerging nematodes counted over 2 - 3 days. The other half was desiccated at 0% relative humidity for 24 hours before rehydration to test if nematodes had reached anhydrobiosis.

Nematodes could be recovered from non desiccated beads dehydrated at 8° and 16°C but not at 22°C and no nematodes survived the desiccation treatment. Generally, liberation of nematodes from beads was slow, even when beads were placed in a citrate buffer solution to solubilise the alginate matrix.

Clays are known for their good water holding capacity and two clay types were tested. Concentrated nematode suspension (5 ml of either nematode) was pipetted over the surface of the clays in Petri dishes and incubated at 22°C or 16°C for 1 week. Treatments were then split. One half was rehydrated and the other half desiccated for 24 h at 0% RH before slow rehydration. Numbers of emerging nematodes were recorded over 6 days.

One week of dehydration at either 16° or 22°C in either clay type provided the right water loss conditions for the cephalobid to enter anhydrobiosis and to survive 24 h at 0% RH. We are currently monitoring survival of this nematode with prolonged storage in clay. Dehydration during storage at 16°C and 22°C was too fast for *Alloionema*. In order to optimise desiccation survival of *Alloionema*, dehydration in clay at lower temperatures will be tested. However, given the moist habitat and cryptic behaviour of slugs, it is possible that *Alloionema* does not naturally encounter higher levels of dehydration.

BIOLOGICAL CONTROL OF PLANT PARASITIC NEMATODES WITH *PAECILOMYCES LILACINUS*

R.. Holland and K. Williams, Macquarie University, Sydney, NSW

During the past few years the work of the Australian Technological Innovation Corporation Pty Ltd has been focussed towards the development of a strain of *Paecilomyces lilacinus*, originally isolated in the Philippines, for use against plant parasitic nematodes. Research is based at Macquarie University, Sydney.

The fungus occurs worldwide and normally lives in the soil but is a facultative parasite of nematodes and some insects. Within the species there are many different isolates. These differ in their growth temperature profiles and in nematophagous ability. A few of these isolates, including the one we are developing, when assayed for nematophagous ability against root knot nematodes infected 100% of available egg masses.

We have successfully developed an *in vitro* assay which is used for the screening of new strains and for the quality control testing of the bio-nematicide.

The ultrastructure of spores has been examined with TEM, and a rodlet layer identified from shadowed replicas on the outside of spores. Spores grown in liquid culture lack this rodlet layer and the spore wall layers differ in thickness.

Both electron and light microscopy has revealed that eggs are killed when the fungal hyphae penetrate the egg. Hyphae grow quickly within the egg until all the egg nutrients are used and the eff is full of hyphae. The fungus then makes special hyphae called conidiophores which penetrate the egg shell again and then make numerous conidia or spores on the outsides of the eggs of

different ages. The hyphae have been seen to grow over eggs, and seem to need time to recognise the egg surface. Sometimes a dens network of hyphae form on an egg, with appressoria at the terminal ends of the hyphae. The possibility of toxins killing eggs prior to infection has been ruled out as often infected eggs surround an uninfected egg which develops normally. A diffusible toxin would most likely affect adjacent, uninfected eggs.

P. lilacinus is well known to target root knot nematodes but is also effective against *Radopholus similis*, *Rotylenchulus reniformis* and cereal cyst nematode, *Heterodera avenae*, especially white females, for which we have established an *in vitro* assay for infection.

Workshop Abstracts

The following are abstracts of papers presented at the AAN workshop held at Lincoln University, August 1995 in conjunction with the 10th Biennial Australasian Plant Pathology Society Conference.

MODERN APPROACHES TO IMPROVING OUR UNDERSTANDING OF HOST PLANT AND NEMATODE PARASITE INTERACTIONS.

F. M. W. Grundler, Institut für Phytopathologie, Universität Kiel, Germany

Sedentary nematodes, e.g. *Heterodera schachtii*, are obligate biotrophic parasites which induce specific modifications in root cells. The cells form a characteristic syncytium with functions that are essential for nematode development. The functions are reflected by ultrastructural, physiological, biochemical and molecular alterations. The host *Arabidopsis thaliana* is optimally suited to study these alterations for a number of specific properties.

The infective juveniles select specific cells of the vascular cylinder for syncytium induction. Via the stylet, secretions are released. The affected cell responds with a number of ultrastructural changes. The walls to adjacent cells are partially dissolved. The differentiating syncytium becomes structurally isolated by a strongly thickened cell wall. The surrounding root tissue is triggered to adopt features of secondary growth.

As micro-injection experiments showed, the syncytium is symplastically isolated but a specific yet unknown mechanism ensures the unloading of assimilates in the area of the syncytium thus providing nutrient supply. Marker substances were transported in the phloem and subsequently enriched in the syncytium and the feeding nematode.

Several approaches were taken to analyse the biochemical and molecular background of the syncytial differentiation. One aimed at the purification and sequencing of proteins expressed specifically as a response to the pathogen attack at the feeding site. In this way a myrosinase could be identified which is expressed specifically in the peridermal tissue covering the expanding syncytium. Its role in the pathogenesis is, however, not yet clarified. In a second approach transgenic *Arabidopsis* lines were produced (by MOGEN, NL) which harboured a promoterless gus construct. A screening was performed in order to identify lines with specific gus expression in syncytia. Lines with expression in syncytia and other plant tissue were found. Two were taken to isolate and analyse regulatory sequences tagged by the gus gene.

PROGRESS IN BREEDING WHITE CLOVER FOR RESISTANCE TO THE CLOVER CYST NEMATODE

C. F. Mercer, J. J. Grant, and J. van den Bosch, AgResearch, Palmerston North, NZ

The clover cyst nematode (*Heterodera trifolii*) is one of two important root-infecting nematodes which debilitate white clover in New Zealand pasture. Recurrent phenotypic selection over four cycle resulted in widely divergent counts of cysts between seed lines selected for resistance from seed lines selected for susceptibility. Many very resistant lines and some apparently immune genotypes have been identified. Counts of juveniles in stained roots of partially resistant and susceptible germplasm indicate that resistance works primarily at the post-infection stages but some evidence exists for minor pre-infectional resistance. Cysts on a resistant plant were smaller and contained fewer eggs than those on a susceptible plant further reducing the eggs per plant count.

SCREENING FOR RESISTANCE AND TOLERANCE TO CEREAL CYST NEMATODE IN A WHEAT BREEDING PROGRAM

Gil Hollamby, Roseworthy Campus, The University of Adelaide, SA, Alan McKay and John Lewis, SARDI, Adelaide, SA

Resistance to cereal cyst nematode is a high priority in cereal breeding programs in southern Australia. In wheat there are several genes for resistance but introgressing them simultaneously with other desirable genes into new varieties has been slow. Mostly because these genes have been in unadapted parents and because accurate screening of large numbers of genotypes has been difficult.

Controlled environment tests have been used for screening potential parents, for checking homogeneity of advanced lines suitable for routine screening of large numbers in the early generations of a conventional breeding program.

Special field nurseries have been planted at CCN infested sites for digging to rate resistance on white cyst numbers. Large uniform field infestations are difficult to find so many susceptible plants escape infection, some soils are hard to dig at the white cyst stage and in some seasons cysts do not develop, so selection is not always effective.

In 1994 a new pot test screening procedure was developed and automated such that 30,000 plants were screened by six people in three weeks. After screening desirable resistant plants can be carried through to maturity for seed. The system and its use in the Roseworthy breeding program will be detailed.

This system of plant propagation and its integration with specially written computer software to manage planting and data processing lends itself to many other uses e.g. for foliar disease screening or DNA analysis.

ENGINEERING RESISTANCE TO ROOT-KNOT NEMATODES IN PLANTS

R. H. Potter, J. Sowden, B. Carson, A. Ah-Fong, S. Herbert, S. J. Washer and M. G. K. Jones, State Agricultural Biotechnology Centre, Murdoch University, Perth, WA

Root-knot nematodes (*Meloidogyne* spp.) are economically important pests of crops worldwide. Chemical control by toxic nematicides has now been banned in some countries/regions, and natural sources of resistance are not available for all crops. Alternative control mechanisms are therefore required. The adult nematode feeds from specialised giant-cells which the nematode itself induces in the stele of the host plant root. Interference with the development of giant-cells will prevent the nematode from completing its life-cycle, therefore we are concentrating on molecular approaches to understand the host plant response. A novel PCR-based cDNA cloning strategy is being followed, using magnetic beads, to clone genes specifically expressed in the nematode-induced giant-cells. The expression of these genes may be suppressed in transgenic plants to interfere with giant-cell development. Cloning the promoters of genes expressed specifically in the giant cells will, further, provide an opportunity to express anti-nematode genes in precisely the cells required. Possible anti-nematode genes which could be used include proteinase-inhibitors, BT genes and collagenase genes. The late approaches are also being studied.

MOLECULAR IDENTIFICATION OF *MELOIDOGYNE* SPP.

Julie Stanton, QDPI, Indooroopilly, QLD, Andrew Hugall and Craig Mortiz, Department of Zoology, University of Queensland, St Lucia, QLD

We have developed a polymerase chain reaction-based method of identifying single Meloidogyne eggs, juveniles and females. The test can also detect components of mixed populations. It is based on mitochondrial DNA and uses four primers to amplify two DNA products in a single reaction. Digestion of amplification products with one or two restriction enzymes differentiates seven molecular types. Further developments of the test are proceeding to allow routine and rapid detection of nematodes in soil and roots. Identification of molecular type will allow prediction of host reaction within a narrow range based on cropping system and production area.

PROGRESS TOWARDS THE DEVELOPMENT OF BIOCONTROL PRODUCTS FOR USE AGAINST ROOT-KNOT NEMATODES

Graham R. Stirling, QDPI, Indooroopilly QLD

There have been numerous attempts over many years to utilise the nematophagous fungi for biological control purposes. In many of these attempts, root-knot nematode has been the target pest. However, there are not yet any examples of the successful commercial use of these fungi for biological control. Stirling (1991) analysed the reasons for this lack of progress and noted that the fungi were introduced into soil as a fungus/substrate mixture. Since such substrates provide a food source for resident soil micro-organisms, the introduced fungus does not necessarily establish in this competitive environment. Also, possibilities for commercialisation of mass production systems involving such solid substrate fermentation systems are limited.

For the last five years, QDPI has been collaborating with Crop Care Australia Ltd on the development of a biological nematicide. Our aim is to produce a granular product that has

enough biological activity to control root-knot nematode on an annual crop such as tomato. Considerable progress has been made towards this goal:

- Australian isolates of nematophagous fungi have been assessed for biocontrol potential. *Verticillium chlamydosporium* isolate LS53 (an egg parasite) and *Arthrobotrys dactyloides* isolate A4 (a nematode-trapper) were selected on the basis of performance in a number of screening tests.
- Both isolates have been mass produced by liquid fermentation and adequate biomass yields have been obtained in commercially acceptable media. Optimum fermentation parameters have been defined.
- Fermented biomass has been mixed with kaolin, vermiculite and gum arabic and this mixture has been granulated and dried to produce formulations with good handling characteristics and commercially acceptable crush strengths. Many of these formulations have retained viability following storage for more than 12 months.
- Formulated products containing the two fungi have been tested for activity in numerous glasshouse tests and significant nematode mortality has been observed. Formulations containing *V. chlamydosporium* generally parasitise 50-80% of first generation egg masses while formulations of *A. dactyloides* reduce galling by 60-90%.
- The best formulations are now being tested against root-knot nematode on tomato in the field.

Stirling, G. R. (1991). 'Biological Control of Plant Parasitic Nematodes' (CAB International: Wallingford UK).

SEEDLING INVASION OF NINE COMMERCIAL WHITE CLOVER CULTIVARS FROM A FIELD SOIL CONTAINING HETERODERA, MELOIDOGYNE AND PRATYLENCHUS NEMATODES

R. M. Watson, G. Dickie, F. J. Neville and N. L. Bell, AgResearch, Ruakura Agricultural Research Centre, Hamilton, New Zealand

Seed of three white clover cultivars commercially available in New Zealand, from within each of three leaf-size classes (Small: Grasslands Prop, G. Tahora, G. Prestige; Medium: G. Huia, G. Pitau, G. Demand; large, G. Kopu, G. Sustain, Aran), were sown into seed-trays containing a field soil naturally infested with nematodes. Some trays were frozen before planting to exclude plant-parasitic nematodes before planting. Soil freezing was only partially effective in eliminating clover cyst nematode (*Heterodera trifolii*) but was effective against root knot (*Meloidogyne* sp.) and lesion (*Pratylenchus* spp.) nematodes. There was a considerable growth advantage to seedlings growing in soil which had been frozen. There was no difference in nematode infection in roots of clover seedlings in the large and medium leaf size clovers but in the small leafed clovers cv. Prop contained only about half the number of root knot nematodes as the other seed lines. This provided a growth advantage to Prop seedlings growing in the presence of nematodes on non-frozen soil.

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June 20, 1996

Dr. John W. Marshall, President
Australasian Association of Nematologists
Crop & Food Research Ltd.
P. O. Box 4704
Christchurch, NEW ZEALAND

Dear Dr. Marshall:

The enclosed sheet is a draft of a handout for summarizing the "IFNS" proposal that we plan to use for the brief plenary session at the upcoming Nematology Congress in Guadeloupe. This handout on the proposed International Federation of Nematology Societies includes the following:

1. IFNS Agenda for Guadeloupe.
2. Recommended issues to consider.
3. Related issues to "ponder".
4. Tentative operational Guidelines.

Under the Agenda, please note that we have added the option for follow-up Mail ballots in the event that this could be required in the by-laws of some Nematology Societies.

Should you have additional suggestions on any of the issues or wording of the "Handout", please forward them to me by e-mail (KRBPP@Unity.NCSU.edu) or FAX (919-515-7716) by July 1, 1996. Your inputs on the proposed "Finances" and "name" of subsequent Nematology Congresses (World or Internatiional) will be most helpful.

Thanks much. Hope to see you in Guadeloupe.

Sincerely yours,

Ken Barker

Kenneth R. Barker
Chair-IFNS Study Group

enclosure(s)

cc: Members of "IFNS" Study Group
Dr. Rodrigo Rodríguez Kábana

PROPOSED INTERNATIONAL FEDERATION OF NEMATOLOGY SOCIETIES

For consideration at "THINC" (Guadeloupe) -- July, 1996

Recommended procedures for considering proposal at Guadeloupe:

IFNS AGENDA FOR GUADELOUPE MEETING:

1) JULY 8th at 7:00 am --- Meeting of IFNS Study Group (Room_____).
2) JULY 8th at 9 am --- Presentation of IFNS Proposal at THINC Plenary Session (Room____).
3) JULY 9th at 4:30 pm --- Discussion/action on IFNS proposal by respective NEMATOLOGY SOCIETIES.
4) JULY 10th at 8:00 pm --- Discussion and establishment of IFNS (Room_____).
5) JULY 11th at 7:30 am --- First meeting and organization of IFNS REPRESENTATIVES/ COUNCILORS (Room_____).
6) JULY 10th thru DECEMBER 10, 1996 --- where necessary, given interested Nematology Societies would do mail ballot on their affiliation with IFNS and advise Council of results.

THE IFNS STUDY GROUP RECOMMENDS THAT THE FOLLOWING ISSUES BE CONSIDERED AT GUADELOUPE:

ISSUE #1 - Ratification of concept and formation of an IFNS:

- Request the president or other officers of each Nematology Society canvas their respective Executive "Board" and/or members to determine whether their Society will become a member of the proposed Federation. If this cannot be done before Guadeloupe, these decisions would be made during the first 3 days at that meeting. Each NEMATOLOGY SOCIETY would appoint representative(s)/ councilor(s) to IFNS.
- Establishment of the IFNS will most likely depend on approval of at least four Societies, including at least two of the three larger Societies (ESN, ONTA, SON). This minimum number of participating Societies could be changed through inter-Society inputs and agreement at Guadeloupe.
- After the Federation is established, interested but non-participating Societies still may canvas their Executive "Board" and/or membership on the issue of becoming a member of IFNS and join the federation at a later date.

ISSUE #2 - Appointment of councilors to IFNS:

- For participating Societies, these councilors should be appointed by the end of the third day of the Guadeloupe Congress.
- Initially, ESN, ONTA, SON and other Societies with >200 members would appoint two representatives each and two alternates each. Other smaller Societies will appoint one councilor and one alternate.
- Adjustments in numbers of Councilors per Society may be made by the newly formed Council.

ISSUE #3 - Meeting of the first IFNS council:

- The first council will be held during the Guadeloupe Congress (see Agenda).
- Operating Guidelines and needs for a formal Constitution should be addressed (See back of sheet for suggested guidelines).
- Council will elect officers (Chair, Vice Chair, Secretary, Treasurer).

Related issues to ponder:

- Public/scientific images of nematology in our respective countries. (What do our political, scientific, and educational leaders envision when they think of nematology?).
- Increasing global communication among scientists.
- Role of communication in enhancing research and education.
- Competitiveness in world agriculture and its relation to interactions among researchers and educators.
- Evolving research environments in respective countries.
- Communication with our clientele --- local- to world-community.
- Impact of electronic means of communication on nematology around the world.
- Value of a 1- to 2-page International Nematology section in respective Nematology Society Newsletters.
- Usefulness of a World Nematology Directory.
- Need for a standardized mechanism to develop Future "World Congresses of Nematology".
- A statement by Lavoisier "*Those who dwell in the clearer light of the next generation will build better than we have done and will scarcely realize how slowly and painfully many of us groped about for what seems to them so plain.*"

OVER --

Revision: July 18, 1996

TENTATIVE OPERATIONAL GUIDELINES FOR PROPOSED INTERNATIONAL FEDERATION OF NEMATOLOGY SOCIETIES' (IFNS):

Tentative guidelines for the proposed "IFNS" are outlined under the primary headings of 1) Name, 2) Goals/Functions; 3) Structure; and 4) Finances. This rather limited list of guidelines came from communications and suggestions offered by committee members, Society representatives, and other individuals. Note that the scope of activities and functions is rather limited. The goal is to keep operational procedures quite simple and costs to a minimum.

I. **Proposed Name of Organization** - "International Federation of Nematology Societies"

II. **Goals and Objectives**:

The overall goal of the proposed IFNS is to strengthen and advance the science of Nematology worldwide through increased communication and collaboration. Specific objectives/goals are:

A. Promote the overall awareness and image of the Science of Nematology.
B. Foster communication/collaboration among nematologists [through a Newsletter and mechanism(s) to utilize developing electronic media].
C. Establish/maintain International Directory of Nematologists.
D. Facilitate/promote "WORLD (or INTERNATIONAL) CONGRESSES OF NEMATOLOGY."
E. Facilitate information transfer to nematology clientele..

III. **Structure**:

A. *Council* - To be comprised of 6 or more councilors; individual society representatives (or councilors) appointed as follows:
   - Initially, the organizing Council will have one or two appointed representatives per Society. Larger societies, such as ESN, SON and ONTA, will have two representatives, and smaller, but internationally recognized societies will have one representative. Each Society also should name alternate representatives who would participate in council meetings when the primary councilors are unable to attend announced meetings. This Council could function as the first IFNS Council for the first 6-year organization period, starting in 1996.
   - If deemed advisable by a given Society's membership, any member Society may withdraw from the Federation.

B. *Officers:* The Council representatives elect officers to include Chair, Vice-chair, Secretary and Treasurer from amongst themselves (one vote per representative). The term of each officer would be for 6 years to correspond to the World Nematology Congresses..

C. *Committees:*
   1) International Nematology Committee (chaired by "Council Chair") -- identify sites, develop programs, and work with local arrangements committees.
   2) Local Arrangements Committee (for World Congresses).
   3) Newsletter and Information-Exchange Committee, chaired by Secretary [one person to be responsible for Directory]. An alternative to a stand-alone Newsletter could involve an "IFNS-page" for inclusion in Newsletters of participating Societies.
   4) Finance, chaired by Treasurer.

D. Timing of meeting of Council--primarily at "WORLD CONGRESSES OF NEMATOLOGY" but may meet, if practical, at other international meetings of nematologists. The first Council meeting would be in Guadeloupe, possibly in a joint meeting with the "retiring" members of the IFNS Study Group.

IV. **Finances:** -- IFNS to use a very restricted budget -- initially to be limited to "Newsletter" and "WORLD (or INTERNATIONAL) CONGRESSES OF NEMATOLOGY." Member societies could contribute on a per capita (membership) basis [possibly $0.50 U.S. per member per year], *but this will need to be weighted upon a reasonable percentage of each Society's operating budget* (maximum will not exceed 1.0% of a given Society's annual budget). Other sources of funding also should be identified. Any "surplus" funds would be used to foster Congress attendance by students and scientists from developing countries and not by council members.

The decision as to whether an International Umbrella Organization of Nematology Societies is formed should be made at the 1996 Nematology Congress in Guadeloupe. These goals, structure, and guidelines also will be subject to modification by the newly established Council --- establishment of IFNS in Guadeloupe. The possible development of a formal constitution should be considered by the first IFNS Council.

Acknowledgment ... The Study Group expresses appreciation to all past International Nematology Congress Programs as well as Nematology Society Leaders for their contributions to the Study on the Feasibility of an International Federation of Nematology Societies.....Ken Barker, Chair