AUSTRALASIAN NEMATOLOGY NEWSLETTER

published by : Australasian Association of Nematologists

VOLUME 1 NO. 2 July 1990

FROM THE EDITOR

Well, here we go with the second issue of the Australasian Nematology Newsletter. Thank you to all who contributed for this issue. In May, I was a little worried about contributions but there was a last minute flurry and we have a well-packed issue. Thank you very much to all those who have sent articles and other items. You have certainly made my job much easier.

I find it rather exciting that nematologists from all over Australasia are sharing their thoughts, ideas and results. Even though we are all isolated geographically, this type of communication can help us keep in touch with what is going on in nematology in Australasia. Speaking of which, our association's paper "A review of plant nematology in Australia and New Zealand" is in its first draft and is on schedule for submission for publication at the end of this year.

According to our secretary, we now have 43 members; not bad for what is thought of as a "special interest group". New members names, addresses and interests will be included with each newsletter. You will also find a membership form in the newsletter for any other potential members.

In attempting to maintain some sort of uniformity in the layout of the newsletter, I have been retyping most of the submissions which are not too long. I hope that I have not introduced too many tyopgarphiacl errors in the process! I really don't mind the typing but, if you wish to send your contributions on floppy disks, that would be most welcome. Please use a word processor that produces an ASCII file. Then I can set margins, etc. without wrecking your work.

To those who think that, in the newsletter logo, I have forgotten Tasmania, you are wrong!! If you overlay it with a suitably-sized map of Australia, you will note that Tasmania is included with the mainland. If there are any serious objections to this, I shall try to ignore them!

Contributions for the next newsletter are due on 15 December 1990. If you have ideas now, start writing now!

I have moved to the other side of the country so, in future, please send your contributions to:

Julie M. Stanton
Editor, Australasian Nematology Newsletter
Plant Pathology Branch QDPI,
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INDOOROOPILLY QLD 4068

AUSTRALASIAN ASSOCIATION OF NEMATOLOGISTS

FINANCIAL STATEMENT AS AT 30/5/90

INCOME	$
1990 Subscriptions (43 members)	430.00
1991 Subscriptions	10.00
Bank interest	17.80
EXPENDITURE	Nil
BALANCE AS AT 30/5/90	$457.80

Members will note that because of Rob Brown's generous offer to have ICI produce and mail the newsletter, we have not had any expenses in our first year of operation. I hope to continue to keep costs to a minimum so that we can gradually put AAN on a sound financial footing. However, I would be interested in comments from members on whether that is the most appropriate fiscal policy for the Association.

(G.R. Stirling)
SECRETARY

AUSTRALASIAN ASSOCIATION

OF NEMATOLOGISTS

New Members since 1 December 1989

	INTERESTS
Mr Grant B. Baldwin Market Development Manager AGCHEM Pty. Ltd. GPO Box 122 ADELAIDE SA 5001 Telephone: (08) 258 2233 Facsimile: (08) 281 3697	
Ms Jacqueline M. Balston Bayer Australia Ltd. PO Box 1985 BUNDABERG Q 4670 Telephone: (071) 576 088 Facsimile:	Chemical and biological control of nematodes in horticultural crops.
Ms Leanne Barnes ICI Australia Merrindale Research Centre Newsom Street ASCOT VALE VIC 3032 Telephone: (03) 377 6305 Facsimile: (03) 370 2395	Chemical control, taxonomy, biological control. Host ranges and nematode-disease complexes.
Dr Robin A. Bedding CSIRO Division of Entomology GPO Box 1700 CANBERRA ACT 2601 Telephone: (062) 46 5183 Facsimile: (062) 47 0217	Entomopathogenic nematodes

Dr Rob. H. Brown
ICI Australia
Merrindale Research Centre
Newsom Street
ASCOT VALE VIC 3032

Telephone: (03) 377 6311
Facsimile: (03) 370 2395

General nematology
Synthesis and development of new nematicides. Research administration.

Dr Lester R.G. Cannon
Queensland Museum
PO Box 300
SOUTH BRISBANE Q 4101

Telephone: (07) 840 7724
Facsimile: (07) 846 1918

Curator taxonomic collections

Mr Timothy G. Clewett
Queensland Dept. of Primary Industries
QWRI
13 Holberton Street
TOOWOOMBA Q 4350

Telephone: (076) 34 6644
Facsimile: (076) 33 1943

Pratylenchus thornei in wheat.
Biological control.
Integrated pest management.

Mr Shane R. Dullahide
Department of Primary Industies
Plant Pathology Branch
Granite Belt Horticultural Research Station
PO Box 10
APPLETHORPE Q 4378

Telephone: (076) 811 255
Facsimile: (076) 811 769

Chemical and biological control of parasitic nematodes of deciduous fruit and vegetables.

Dr Ian D. Kaehne
D.A. Dept. of Agriculture
Northfield Laboratories
GPO Box 1671
ADELAIDE SA 5001

Telephone: (08) 266 8333
Facsimile: (08) 261 4688

Resistance and tolerance breeding
Mechanisms of resistance/tolerance
Legumes and cereals.

Dr John W. Marshall
DSIR
Plant Protection Division
DSIR Private Bag
Christchurch
NEW ZEALAND

Telephone: (03) 252 511
Facsimile: (03) 252 074

Biology and management of
nematodes in temperate crops.
Molecular biology of nematodes.

Mr Christopher F. Mercer
Department of Scientific and
Industrial Research (NZ)
Plant Protection
Private Bag
Palmerston North
NEW ZEALAND

Telephone: (063) 68 019
Facsimile: (063) 62 635

Resistance in white clover
to *M. hapla* and *H. trifolii*.
Effect of grass endophytes on
nematodes.

Dr Gregor W. Yeates
Division of Land and Soil Sciences
DSIR
Private Bag
Lower Hutt
NEW ZEALAND

Telephone: (04) 673 119
Facsimile: (04) 673 114

Ecology, Taxonomy

CURRENT RESEARCH

PAST RESEARCH AT QWRI ON ROOT-LESION NEMATODE (*P. thornei*) OF WHEAT.

My interest in this nematode arose from investigations into the so-called Long Fallow Disorder (LFD). LFD has many aspects. Classical LFD is poor growth of crops planted immediately after a long period (> 10 months) of weed-free fallow. Many crop species are affected to varying degrees. Recent research (Thompson, 1987) has shown classical LFD to result from a decline in viable propagules of vesicular-arbuscular mycorrhizal (VAM) fungi during the fallow. The next crop to be sown is poorly colonized with these symbiotic fungi which improve crop uptake of P and Zn from the soil. The problem is most evident when highly mycorrhizal-dependent crop species, like linseed, are sown in vertisols (dark clay soils) of the northern grain belt. The crop is stunted with symptoms of P or Zn deficiencies and yield is poor.

Another aspect of the LFD complex is that many farmers on the Darling Downs claimed that LFD affected the second wheat crop after long fallow more severely than the first wheat crop. This aspect of LFD was specific to wheat which could yield as little as 1 t/ha when barley treated identically could yield 4 t/ha in the same rotational position. Our initial investigations into this problem showed high populations of root-lesion nematodes in the soil before the problem second wheat crop was sown. This stimulated research directions on RLN which is mentioned briefly here and covered more fully in Thompson (1990).

P. thornei was found to be widely distributed across the Darling Downs but not in the newer wheat areas of Queensland. *P. neglectus* appears to be sporadically distributed in both the Darling Downs and the newer areas. R. McLeod and D. Doyle have shown *P. thornei* to be widely distributed through the vertisols of northern N.S.W. from Dubbo to the Queensland border. Globally it appears the *P. thornei* is encountered more often in clay soils and *P. neglectus* in lighter-textured soils. Interestingly, the age of cultivation to wheat of various areas is Darling Downs, the oldest, followed by northern N.S.W., with Queensland's south-west being the youngest. There appear to be no climatic or edaphic reasons why *P. thornei* could not eventually become a problem to wheat in all vertisols os Queensland and N.S.W.

Our research has shown that high populations of *P. thornei* can be very damaging to wheat yield, e.g. up to 85 % yield loss in cultivar Gatcher.

Chemical control of *P. thornei* was sought by testing 12 nematicides with various rates and modes of application at two sites over 2 years. The most effective was aldicarb (TEMIK^{R}) but the rates needed were uneconomic. Part of the problem for effective chemical control at modest rates is the deep distribution of the nematode populations in the profile of these clay soils. They can occur to 90 cm depth often with peak populations at around 30 cm depth. Obtaining effective leaching of nematicide to these depths in the clay soil will depend in dryland agriculture on the variable rainfall after application.

P. thornei survives well in the clay soils, e.g. 5 % survival in the field

after 4.5 years of clean fallow. However, crop rotation is still the most effective way to manage the problem. Fields with high populations are rotated to sorghum and barley so that no more than one wheat crop is grown every 3 - 5 years.

There are various levels of tolerance among commercial wheat cultivars. Farmers with problem fields learn to grow the more tolerant ones of which Hartog is the best currently available. I will describe our current research on breeding for superior tolerance and resistance to *P. thornei* in a following article.

References

Thompson, J.P. (1987). Decline of vesicular-arbuscular mycorrhizae in long fallow disorder and its expression in phosphorus deficiency of sunflower. *Australian Journal of Agricultural Research 38*, 847-867.

Thompson, J.P. (1990). Root-lesion nematodes. Chapter 12 in *Soilborne diseases of wheat in the Australian environment*, 21 pp., L.W. Burgess and B. Summerell (eds), Academic Press: Sydney (*in press*).

(J.P. Thompson Queensland Wheat Research Institute, Box 2282, Toowoomba)

NEMATODES AND KAVA - AN INTERESTING EXPERIENCE IN TONGA

Kava (*Piper methysticum*) is an important traditional and cash crop in Fiji, Tonga, Vanuatu and Western Samoa. An intoxicating, but non-alcoholic beverage is prepared by mixing ground roots and stem bases with water, and this beverage plays a prominent role in social and ceremonial occasions. It is believed to assist in breaking down social barriers, in settling personal conflicts and in enhancing social ties, particularly amongst men. Kava induces relaxation and sleepiness and the lactones which are its active ingredients are known to act as sedatives, anticonvulsants, analgesics, anaesthetics and diuretics.

Piper methysticum is a robust, somewhat succulent perennial shrub that can grow to heights of 2-3 metres. A number of stems arise from a crown at ground level. The plant is usually harvested at 3-5 years of age. Kava is an important cash crop in most countries where it is grown and provides growers with higher economic returns than a number of alternatvie crops. However, production is constrained throughout the South Pacific by wilt-dieback disease of unknown etiology. Plant pathologists in Fiji estimate that dieback causes annual crop losses of about 60 percent.

In January this year I was fortunate to visit Tonga with Dr John Brown, a plant pathologist at the University of New England who is supervising an ACIAR-funded project aimed at determining the cause of the disease. We visited Mr Richard Davis, a former University of Queensland post-graduate student, who is the plant pathologist involved in the project and is based in Tonga.

Kava proved to be interesting nematologically, as it hosted a wide range

and dagger nematodes. We observed serious galling and root-rotting associated with root-knot neamtodes, and lesions on roots probably caused by burrowing nematodes. There have been claims that the incidence of dieback is reduced by nematicide treatment and it is possible that nematodes are involved in the disease, either as the primary cause or in a complex with other soil-borne organisms. I trained Richard Davis in the identification of the nematodes present on kava and established pure cultures of some of the species. Hopefully Richard's work over the next few years will establish whether nematodes play a role in kava dieback.

(G.R. Stirling, QDPI, Indooroopilly)

DEVELOPMENT OF DIAGNOSTIC PROBES TO IDENTIFY SPECIES AND RACES OF ROOT-KNOT NEMATODE

A root-knot nematode (*Meloidogyne* spp.) collection is being established by Plant Pathology Branch, Queensland Department of Primary Industries. The collection will contain 60 populations selected from an Australian survey in which 200 infested sites have been, or are being, sampled. The survey samples are being collected from discrete production areas selected on the basis of geographic location, type of crop production, management system, soil type and climate. Conventional morphological characters of male, female and second-stage juvenile nematodes are being used to select the type populations to be maintained in the collection. Because most root-knot nematode reproduction is by parthenogenesis, nematode cultures are being established from the egg mass associated with a female (single egg mass culture). Preliminary morphological studies have shown morphological differences between and within species of root-knot nematode identified from the samples, i.e. *M. javanica*, *M. hapla*, *M. arenaria* and a range of *M. incognita* types.

Other studies have observed similar variation among species of *Meloidogyne* for proteins, repeated nuclear sequences and mitochrondrial DNA. However, none of these studies included Australian isolates and only the protein study included geographically remote populations within species. Analysis of high copy-number DNA sequences offers the greatest sensitivity and reliability, but there have been no adequate estimates made of variation within species or races. Without this information, it cannot be concluded that observed differences are diagnostic.

A project which has been recommended to the Minister contains five components:

i) Assessment of variation among species of *Meloidogyne* sampled from multiple hosts and localities for restriction fragment patterns (RFLP's) of repeated nuclear sequences and mitochrondrial DNA.

ii) Identification of genetic races from the variation revealed in (i).

iii) Testing of the host ranges of the genetic races revealed in (ii).

iv) Comparison of the genetic races with reference material of the type species of *Meloidogyne* held by CSIRO, Canberra, and North Carolina State University, USA.

v) Development of a rapid assay to diagnose and identify field populations of *Meloidogyne*.

(P. Chris O'Brien, QDPI, Indooroopilly *per* J.M. Stanton)

BREEDING *Trifolium repens* FOR RESISTANCE TO *Meloidogyne hapla* AND *Heterodera trifolii*.

Root knot nematode (*M. hapla*), clover cyst nematode (*H. trifolii*) and clover stem nematode (*Ditylenchus dipsaci*) weaken or kill white clover seedlings and debilitate mature plants. Both clover yield and nitrogen fixation are reduced leading to a general decline in pasture quality and vigour. The reduction in nitrogen fixation is particularly serious as nitrogen is the major nutrient limitation of improved pastures in New Zealand.

A selection and crossing programme, in collaboration with plant breeders of DSIR Grasslands, aims to increase resistance to root knot and clover cyst nematodes. Resistance to clover stem nematode has been achieved to some extent in 'Grasslands Kopu' white clover. The present programme screens seedlings against single species inoculum in pots in a glasshouse. Plants with low numbers of root knot nematode galls or clover cyst nematode cysts are crossed. A few plants with high numbers are crossed as a check (susceptibles).

The F_1 progeny means of galls per gram root DWT of pair crossed and open crossed plants which were more resistant to root knot nematode were at 76% and 81% respectively, of the susceptible plants. Response to clover cyst resistance selection was greater; the mean females/cysts per gram root DWT of pair crossed resistant plants was at 37% of susceptible plants.

We are encouraged by this progress after one cycle of crossing. The F_2 progeny are currently being screened. Plans are being made to test the best material under field conditions.

Two other projects also aim to increase the resistance of white clover to nematodes. Resistance has been identified in other species of the genus *Trifolium* and two of these, *T. hybridum* and *T. nigrescens*, are being hybridised using novel genetic techniques. Hybrids, and the progeny of

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(C.F. Mercer, DSIR Plant Protection, Palmerston North)

REGIONAL NEWS

NEWS FROM QUEENSLAND

Julie Stanton was recently appointed to the vacant nematology position with QDPI. Julie expects to move from Perth early in the next financial year and the nematology group at Indooroopilly looks forward to her arrival.

Dr. Ravi Sharma, Research Nematologist from EMBRAPA in Brazil, is currently spending twelve months at Indooroopilly. Ravi is being funded by a fellowship from the Brazilian Government and is hoping to develop better *in vivo* methods of culturing *Pasteuria penetrans*.

My biological control programme has received a boost with the signing of an R&D agreement between QDPI and Incitec Ltd. We are working on a number of bacteria and fungi with biocontrol potential and it is hoped that collaboration with Incitec will hasten progress towards commercialisation. Incitec is an Australian company interested in developing a range of microbial crop protection products and they will use their expertise in fermentation and formulation to develop products from our organisms that are suitable for application in the field.

I have been fortunate to receive a grant through the Australian Academy of Science - Royal Society Exchange Programme, and will be oversease for three months from mid June. Most of my time will be spent in Brian Kerry's laboratory at Rothamsted. However, I also plan to visit Israel to study the biological control work being done there, and to attend the Second International Nematology Congress in The Netherlands.

(G.R. Stirling, QDPI, Indooroopilly)

NEWS FROM VICTORIA

My career in nematology

I arrived in Mildura in 1983, green and enthusiastic and ready to make my mark on the nematology community. Around this time DBCP had been banned for use in Victoria and other states.

My task was to test replacements for DBCP for use against nematodes attacking grapevines and citrus. I embarked on a series of field trials to test the efficacy of a range of systemic nematicides. But just as the results looked promising I was whisked away to work in an area of research which was perceived to be of higher priority than nematology.

You see, it was around this time that nematodes began to vanish from the horticultural districts of North West Victoria. First they were no longer

a problem in citrus and then grapevines were relinquished from this ravaging pest. Or so the story goes.

Since then, I have continued to maintain a base level diagnostic service in nematology in Mildura. And I still have my interest in the nematology of temperate horticultural crops if not an active participation.

(Megan Edwards, Sunraysia Horticultural Centre, Mildura)

I had minimal formal training in nematology, having studied firstly at Longerenong Agricultural College and then at the University of Melbourne, Faculty of Agriculture and Forestry, neither of which offered units in nematology. Since February 1988 I have been employed at the Victorian Crops Research Institute where there has been a long-term interest in cereal cyst nematode, both with regard to its chemical and agronomic control and the development of resistant cereal cultivars. As an integral part of my duties as nematologist at the institute, I am working toward a PhD from the University of Melbourne. My research is focussed on developing alternative sources of resistance to CCN in wheat. Through cooperation with CSIRO Division of Plant Industry, Canberra, we have identified sources of resistance in a wild wheat *Triticum tauschii*. From this I have developed my PhD study which aims to 1) transfer CCN resistance to bread wheat, 2) determine the number and inheritance of resistance genes, 3) study the background effect on expression of these genes, 4) determine the relationship of the genes to genes from *T. ventricosum* (VPM), 5) compare the mechanism of resistance in a range of resistant sources, 6) determine the pathotype structure of the CCN population using known resistance genes, 7) assess the variability of CCN in Australia at the DNA level using Restriction Fragment Length Polymorphisms (RFLP's).

Most of this work is at an early stage, however, many crosses have been made and although results are several months away, one line carrying resistance from *T. tauschii* has laready been introduced into the Victorian wheat breeding program. The RFLP work has shown that variation does exist in the Australian CCN population but this does not necessarily bear any relationship to pathogenicity. If there is variation it may be possible to use RFLP's as markers to identify pathotypes.

I am also working with Lindy Spindler and Phil Larkin (CSIRO division of Plant Industry, Canberra) on a project to transfer a gene for CCN resistance from the Rye complement of Triticale to wheat using tissue culture to induce recombination. My role is in assessing the resistance of the material.

Thus my work focusses on developing alternative sources of resistance to CCN in wheat and understanding their genetic basis and expression.

(Russell Eastwood, Victorian Crops Research Institute, Horsham)

NEWS FROM NEW ZEALAND

Precision woven bolting or nylon cloth

Following the note in ANN 1:1, to assist Australasian nematologists I would like to mention that precision woven cloth is available from Swiss Silk Bolting Mfg. Co. Ltd., Zurich, via their New Zealand agent Ure Pacific Traders Ltd., P.O. Box 20-210, Glen Eden, Auckland: phone (09) 882 182, fax (09) 82 278, Telex (09) 60353.

They have cloth in mesh sizes from 5,000 microns down to 1 micron. Representative costs are mesh pore 22 µ costs $105; mesh 15 µ costs $126, mesh 5 µ costs $144 per metre, one metre width. They will take orders of minimum of half a metre.

I can arrange your order if you wish.

(Gordon S. Grandison, DSIR Entomology, Auckland)

Despite institutional restructuring, Gregor Yeates has been with DSIR at Lower Hutt for almost 20 years; he is now on the staff of DSIR Land Resources which was formed on 1 April. His early work on the distribution and biology of *Heterodera trifolii* and *Meloidogyne hapla* and their effects on white clover was in part responsible for the clover breeding programme at Palmerston North in which Chris Mercer is involved. Gregor's taxonomic work has recently produced surveys of both Plectidae and Mononchoidea under New Zealand pastures, and further groups in preserrved material are to be worked through. Also, he has just submitted a manuscript describing 15 new mononchs from 1988 collections in New Caledonia; again further groups are to be covered (including longidorids with Michel Luc). On the ecological side, his current interests include, firstly, looking at effects of ecosystem disturbance (e.g. soil sterilization, pesticide applications and spills, forest clearance) on the nematode fauna and, secondly, assessing the role of nematodes and other invertebrates in the release of plant nutrients (e.g. in a sustainable forestry (*Pinus radiata*) study and in a pasture sabbatical fallowing study). Gregor is now an assistant editor of *Nematologica* and under the new procedures manuscripts may be sent directly to him; he is also a channel for papers to *Pedobiologia*.

(Gregor Yeates, DSIR Land Resources, Lower Hutt)

The molecular biology of nematodes programme at Lincoln is progressing well with the main emphasis being on the production and characterisation of the probes we have produced. We now have probes which are specific to *Globodera rostochiensis* Ro_1 and Ro_4. This probe when used in dot blot and compared with the results of the Pa_3 rpobe produced earlier, has resulted in some interesting problems.

Pa_3 Probe on:					Ro_1 Probe on:				
Ro_1	Ro_4	Pa_1	Pa_2	Pa_3	Ro_1	Ro_4	Pa_1	Pa_2	Pa_3
-	+	+	+	+	+	+	-	-	-

We are now looking at the sequence of both probes either as an R.F.L.P. map of the probe or a complete sequence of the entire bit.

Our hope is that we can identify the sequence that is common to both probes and be able to construct on Ro_4 specific probe from the information.

The practical application of these probes is also progressing and we are looking at ways of producing up to ten replicate Dot blots from individual cysts and we have successfully used an idea from the *Drosophila* biologists and can crush large numbers of individual cysts. The crushed sample is then digested to release D.N.A. and the sample is transformed to the membrane. This allows us to use a range of probes on a single cyst.

Other nematology

We are also setting up R.F.L.P. studies on the New Zealand collection of entomophagous nematodes. The nematodes are being supplied by Malcolm Garnham, M.A.F. Tech, Wim Wouts, D.S.I.R. Plant Protection, and Michael Surrey, D.S.I.R. Industrial Processing. We have extracted the D.N.A. and have run preliminary restriction enzyme digests.

John Curran C.S.I.R.O. kindly supplied his 7kb Ribosomal DNA probe and the idea is to produce a base line data set to help in the characterisation of species and types from different locations and also reported specific virulence against insect pests.

The molecular nematology programme has benefitted recently by the arrival of Dr. Flerida Carino, a molecular biologist. She is working on the molecular biology of insects and genetic basis of insecticide resistance, and brings a great depth of experience in handling insect D.N.A. all of which has direct application to nematode D.N.A.. Flerida's immediate contribution was to improve the quality of D.N.A. extraction without the use of an ultracentrifuge. The resultant product is high molecular weight with minimal degradation.

Other nematology programmes underway at Lincoln are on the effect of nematicide application on the yield and quality of Kiwifruit which are infected with root knot nematode. This programme is a joint project between myself, Mike Spink T/O and Mr. Richard Watson, M.A.F. Tech, Ruakara. Richard has done a very thorough study of the bionomics of root knot on the roots of kiwifruit and the yield trials will give a reliable answer to the question, "Do root knot nematodes cause problems on New Zealand kiwifruit plantings?".

(John Marshall, DSIR Plant Protection, Christchurch)

GENERAL ARTICLES

NOTES ON THE FIELD BIOLOGY OF *Achlysiella* N. GEN. (SYN. *Radopholus williamsi* (SIDDIQI, 1964)).

K.J. Chandler

Bureau of Sugar Experiment Stations, P.O. Box 122, Gordonvale QLD 4865, Australia.

SUMMARY

Parasitic nematode species associated with poorly growing sugarcane crops often characterised by a root-rotting syndrome were studied in Far North Queensland from 1976. An unusual and previously unknown feature of the biology of *Radopholus williamsi* (Siddiqi, 1964) was noticed during this study. In this article, observations on the changes in body form of free-living, non-egglaying vermiform female *R. williamsi* into endoparasitic sessile and obese reproductive forms, and the concomitant changes wrought in the host-root tissue are described from field-collected material.

INTRODUCTION

Sugarcane crops throughout Far North Queensland were severely affected by a root-rotting syndrome associated with a previously unknown fungal pathogen during the 1970's. The development of nematode induced root lesions was studied to ascertain any association between the lesions and invasion of roots by the fungal pathogen.

During the course of root dissections, obese, sedentary female nematodes were discovered living within chambers in the cortex layer of sugarcane roots. Numerous eggs, juveniles and recently developed adult nematodes of both sexes were also found in the same chambers. These young nematodes were identical with *Radopholus williamsi* (Siddiqi, 1964), common on sugarcane in North Queensland.

Several series of specimens were submitted to Dr. R.C. (Bob) Colbran at the Department of Primary Industries laboratory, Indooroopilly, Qld. Despite their unusual circumstance, Dr. Colbran consistently identified the obese females as *Rotylenchulus* spp., and the vermiform specimens as *R. williamsi*.

A series of observations and root dissections on field collected sugarcane plants revealed progressive stages of development of both lesions and female nematodes, indicating that vermiform, free-living *R. williamsi* females developed into sedentary, obese, endoparasitic forms resembling *Rotylenchulus*.

The status of *R. williamsi* has recently been questioned (Hunt *et al.*, 1989) with the proposal of the new genus *Achlysiella* to accommodate *R. williamsi*. This proposal followed discussion of the biological data presented below with one of the above authors (J.R. Bridge).

OBSERVATIONS

Lesions began development on young, white coloured actively developing roots, as small, reddened elongate marks almost identical to those caused by *Pratylenchus zeae* (Graham, 1951). Solitary female nematodes dissected from the mid-cortical tissue under these young lesions were microscopically similar to free-living vermiform *R. williamsi*, except that the body had swollen in the area of the posterior intestine and the vulval lips had become protuberant.

Lesions on slightly older roots appeared as purplish-black elongated marks on the epidermis with darkened cortical tissue below the wound. Females dissected from these lesions were solitary, posteriorly swollen and elongate, with a finely pointed tail and very protuberant vulval opening. The anterior body portion was usually lightly embedded into cortical tissue at the base of a cavity developing within the cortex. The anterior body organs still closely resembled those of the free-living form of *R. williamsi*. Posteriorly the ovaries were much enlarged with visible oocytes, and some eggs had been laid.

In mature sugarcane roots, the epidermal layer hardens and darkens, cortical cells degenerate, the cortex tissue develops air cavities and the stele hardens and thickens. Cavities formed by *Achlysiella* extend inward to the stele, and had an external covering composed of dead epidermal cells and soil particles bonded together by a membrane which also formed the external wall of a large egg chamber. In thinner roots, the chamber covering formed an enlarged swelling on the root surface. Dead, blackened cortical cells surrounded the chamber. Usually there was only a single, elongate, obese mature female nematode within each cavity, whose body outwardly resembled *Rotylenchulus*, particularly with regard to the conical, ventrally flexed tail (Willmott *et al.*, 1973). In one case the anterior portion of such a female appeared to be lightly embedded into the stele tissue. Combinations of eggs, larvae and young adult forms of both sexes were usually present in the chamber.

In the oldest living roots where the stele was the only tissue remaining active, cavities were open and quite visible to the naked eye. In most cases, the cavity had extended slightly into the stele tissue. No nematodes were detected in these old lesions.

DISCUSSION

Large nematode populations including *R. williamsi* were often associated with the root-rotting syndrome, and, although large yield increases could sometimes result following control of the nematodes, it was demonstrated that nematodes were not a predisposing factor in the development of the root-rotting symptoms under investigation (Chandler, 1978, 1980, 1984). *R. williamsi* is considered to be the second most common and prolific nematode associated with sugarcane in Far North Queensland.

In 1986, this nematode species was found during the course of a multi-disciplinary study of sugarcane growth at Ramu Sugar Plantation, Ramu Valley (5° S), Papua New Guinea. Following discussions with this author concerning the above observations on the biology of *R. williamsi*, further work was conducted at the C.A.B.I. Institute of Parasitology, U.K. and similar observations were confirmed in the laboratory. Based on the above biological features and some less distinct physiological differences, the new genus *Achlysiella* has been proposed with *A. williamsi* (Siddiqi, 1964) n. comb. (syn. *R. williamsi*) as the type species (Hunt *et al.*, 1989).

Sher (1968) noted that, in *R. similis* (Cobb, 1893) "some females from the roots of plants appear larger and fatter than specimens from soil around these roots". He also noted the undifferentiated state of the ovaries in described females of at least two species, *R. magniglans* and *R. trilineatus* and speculated that further development may take place before such females commence to produce eggs.

Colbran (1971) described a further eleven species of *Radopholus* from Eastern Australia.

Hunt *et al.* (1989) speculate that, on the basis of oesophageal characters and the "immature" ovaries of the vermiform females, there may be five more species of *Radopholus* for inclusion under *Achlysiella*.

Although no records are given of the numbers of oocytes present in vermiform females, at least seven of the *Radopholus* species described by Sher (1968), and the eleven species described by Colbran (1971) are illustrated as having between 5 - 36 oocytes in each ovary, and none are illustrated with any "mature" oocytes or eggs. The five specified by Hunt *et al* (1989) have been illustrated with between 5 - 20 oocytes per ovary. Therefore this ovarian character seems to be an inconsistent basis for speculation as to what other species of *Radopholus* may belong within *Achlysiella* at this juncture.

Rather, it would seem more pertinent to ask the questions "Is the occurrence of biological differences in the life cycle of species sufficient justification for the creation of separate genera between groups of otherwise similar species?", and "How many other species presently within *Radopholus* have an as-yet-unidentified, sessile, endoparasitic, egg-laying, female morph in their life cycle?".

ACKNOWLEDGEMENTS

Thanks to Dr. R.C. Colbran for helpful discussion on the possible wider significance of this observation. Also Dr. John Bridge and co-authors at C.A.B.I. for bringing some concise nematological taxonomy to bear on this problem.

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A case study in the use of ethylene dibromide by horticulturalists

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Ethylene dibromide (EDB) has been used as a cheap and effective, fumigant nematicide for many years. However, health concerns over the chemical and its gradual phasing out in the United States have clouded its future in this country. Indeed, warnings of its imminent demise have long been circulated. The withdrawal of 1, 3 - dichloropropene left few alternatives to the continued use of EDB (apart from the extremely expensive broad-spectrum fumigants). The author and P Burne (formerly SADA District Advisor, Murray Bridge) recently lobbied Dow Elanco to re-consider their decision not to proceed with re-registration of Telone II(R) soil fumigant. Unfortunately, potential sales volumes were not seen as sufficient to justify provision of the resources needed to meet the company's high standard of product stewardship. For the present then, it is to be hoped that EDB remains available to growers as a useful management tool.

A routine property visit recently provided a case study in the practical use of EDB in horticulture.

Site Details and Methods

The property, situated at Moorook, S.A. had been used for the previous four years for production of lucerne and prior to that, carrots, potatoes and onions had been grown. Soil type was a sandy loam overlying a lime layer at a depth of about 40 cm and had been prepared for fumigation by deep ripping. The grower used his own injection rig with 11 ripper-tynes spaced 25 cm apart to inject Nemarid(R) (2000 g EDB/L) at a depth of 30 - 38 cm at 67.5 L/ha. The fumigant is sufficiently cheap ($473/ha) to be used routinely by the grower, who grows carrots on properties throughout the Riverland.

A 3.2 ha section was fumigated 14 days before planting. A single bay (0.5 ha) was left unfumigated after the grower had run out of fumigant, allowing a comparison to be made of carrot growth in fumigated and unfumigated soils. Carrot seed cv. Longobard (Sunseed Co.) was planted in raised beds on 1 November, 1989. Soil was sampled 21 days after planting, by taking 20 cores (2 cm diameter) at depths 0-20 and 20-40 cm from a fumigated bay and adjacent unfumigated bay . A "zig-zag" sampling pattern was used, cores were bulked and nematodes were extracted from 200 g sub-samples by sieving and centrifugation in a sucrose solution (Jenkins, 1964). Volunteer lucerne growing along sprinkler lines was collected, adult female root-knot nematodes dissected from roots and perineal patterns examined.

These bays were re-sampled 33 days after planting when cores (10/plot) were taken at depth 0-20 cm from 1m plots at 10 m intervals along every third bed. Fifteen plots were sampled from each bay. Nematodes were extracted from 100 g sub-samples of soil as before. A spadeful

of seedlings was lifted from the centre of each plot and roots and shoots were weighed for a sub-sample of 6 plants. Root systems were examined under a dissecting microscope for galls. Data were analysed by t-test ($P< 0.05$).

Mean daily maximum and minimum temperatures at the nearest recording station (30 km distant) in the period between planting and final sampling were 26.7° and 13.8°C respectively.

Results

Meloidogyne spp. larvae appeared to be less numerous in fumigated soil than unfumigated soil 21 days after planting (35 days after fumigation). At 33 days after planting, numbers of larvae had declined to low levels in unfumigated soil and there were no significant differences in populations of Meloidogyne spp. between fumigated and unfumigated soils (Table 1).

Perineal patterns from four female root-knot nematodes dissected from lucerne roots were identified as M. hapla Chitwood. Paratrichodorus lobatus (Colbran) Siddiqi, Pratylenchus sp. and Criconemella sp. were also found in soil and populations of the latter nematode were significantly lower in fumigated soil (Table 1).

Table 1. Nematode counts for soil sampled from EDB-fumigated and unfumigated carrot beds 21 and 33 days after planting, Moorook S.A.

Treatment, depth (cm)	Nematodes per 100 g soil			
	Meloidogyne	Paratrichodorus	Criconemella	Pratylenchus
21 d after planting				
Unfumigated				
0-20	130	30	125	65
20-40	533	26	65	136
Fumigated				
0-20	10	10	55	30
20-40	18	54	0	84
33 d after planting[a]				
Unfumigated				
0-20	20.0 (9.2)	31.3 (11.4)	147.3 (32.7)	84.7 (8.2)
Fumigated				
0-20	7.2 (4.6)	29.1 (8.2)	32.7 (15.8)	68.8 (12.1)
P[b]	NS	NS	0.01	NS

[a] Standard errors in brackets.
[b] Probability level for within-column t-test comparing means for fumigated and unfumigated soils 33 days after planting. NS = not significant.

Root and shoot weights were significantly higher in carrots grown in fumigated soil (Table 2). Small galls were infrequently seen 33 days after planting on roots of plants grown in both fumigated and unfumigated soils.

Table 2. Root and shoot fresh weights and incidence of root galls in 33-day-old carrot seedlings grown in EDB-fumigated and unfumigated soils, Moorook SA.

Treatment	Plant fresh weight (mg)		Root galls[A]
	Root	shoot	(%)
unfumigated	56.9 (2.2)[B]	458.6 (25.7)	5.6 (2.7)
fumigated	66.2 (3.9)	544.8 (27.3)	1.1 (1.1)
P[C]	0.05	0.05	NS

[A] % of plants with root galls
[a] standard errors in brackets
[c] probability level of within-column t-test comparing fumigated and unfumigated treatments. NS = not significant.

Discussion

M.hapla causes serious yield loss in carrots, particularly in organic soils (Vrain, 1982), and symptoms include galling, growth retardation, tap-root malformation, root proliferation and plant mortality (Slinger and Bird, 1978). Lucerne is also a susceptible host (Griffin, 1969). Sampling conducted 21 days after planting suggested that fumigation had been effective in reducing soil populations of this nematode, however, this could not be confirmed in the absence of pre-treatment counts. By 33 days after planting, soil populations in unfumigated soil had fallen to the levels of fumigated soil. This finding may have reflected invasion of roots by larvae, however, no significant difference was seen in incidence of galling between plants from fumigated and unfumigated soils at this time.

The benefit of fumigation was demonstrated by the higher shoot and root weights of carrots grown in fumigated soil. Slinger and Bird (1978) showed in green house trials that reduced root weights caused by M.hapla could be observed from 24 days after planting. The most likely explanation for the increased growth rate of carrots in fumigated soil was that it resulted from reduced populations of plant parasitic nematodes, since EDB at the rate used is not considered to be fungicidal (Overman et al., 1970).

The grower concerned injects fumigant at a greater depth than i normally recommended, a practice that could lead to poor efficacy i the surface soil (Van Berkum and Hoestra, 1979), however, nematod counts 21 days after fumigation did not indicate this.

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