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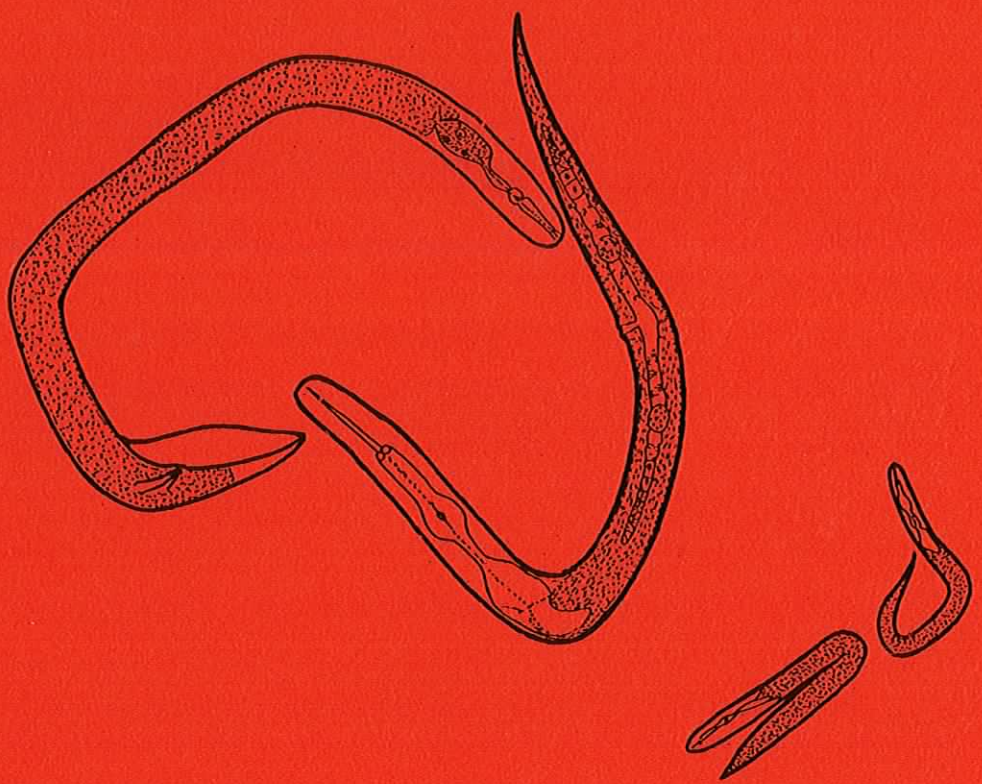
AUSTRALASIAN NEMATOLOGY NEWSLETTER



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From the Editor

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

July Issue
The deadline for the January issue is December 1st. I will notify you a month in advance so please have your material ready once again.

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Association News

FROM THE PRESIDENT

The item of greatest interest to members since the last newsletter may be the number of new members: more than 10 in the last year. The range of interests and geographic locations, from Fiji to PNG to the USA, and all over Australia show the range of interest in nematodes in the region. This augurs well for the society. I trust that Takaniko Ruabete, our member from Fiji, who is located just outside Suva, is safe and well amid the developments there.

On the international scene, preparations are continuing for the Fourth International Congress of Nematology. As Julie Stanton has indicated in an earlier newsletter, AAN is periodically contacted for input for things like sessions for the programme, input of funds, and other things. Having dealt with the issues of funds at the General meeting, if members have any burning issues which they feel should be included in the programme, they can submit them directly to the organizers Thierry Vrain (vraint@em.agr.ca) or Ken Barker (kenneth_barker@ncsu.edu), or through the societies officers.

For those not contemplating attending the international congress, the biennial APPS conference is always a good meeting, and one of only a few opportunities for many of Australia's far-flung nematologists to meet in person. The conference is only a little over a year away now, and the first circular is out, so planning can start now. Any ideas or burning issues that you think should be discussed will be included in the programme if they are sent to me or any other committee members soon. This can be either in the general sessions or the specific nematology workshop. Otherwise, there has been little activity of interest to members since the last newsletter. I can only assume that people are busy collecting data and ideas for next years meeting.

Although much has been written elsewhere, it remains for me to formally express the condolences from the AAN on the death of Alan Bird. All nematologists will be greatly saddened by this sudden event. Alan was an inspirational nematologist for many years, and was still very active, even in retirement. Alan's many achievements are listed elsewhere in this issue, and his contributions to the forerunner of the AAN should also be gratefully acknowledged. Alan's wise and kindly counsel will be greatly missed. On behalf of the Association, I extend the sincerest sympathy to Jean, David and the rest of his family.

Mike Hodda

OBITUARY

Dr Alan Francis Bird

Alan Bird was an international authority in parasitology. Although he specialized in plant parasitic nematodes, his interests were much wider. In his early years at the University of Edinburgh he gave practical classes in helminthology and entomology and lectures on helminthology and nematode physiology. Alan always retained a great respect for the Zoology Dept in Edinburgh and so it is fitting that his PhD (1956) and DSc (1973) were conferred by that University.

Alan joined CSIRO (Commonwealth Scientific and Industrial Research Organization) in 1957 and after a year at Merbein in Victoria, spent the rest of his career in Adelaide. During the '60s and '70s he delivered some lectures to second and third year Zoology students, giving courses in Parasitology at the University of Adelaide. Alan rose to the top research position of Chief Research Scientist in CSIRO.

Following the visit of Dr Wim Seinhorst to Australia in the early '50s, CSIRO accepted the recommendation that research into nematology should be initiated. Alan Bird was one of those early workers who helped to put Australia and Adelaide on the "nematology map". He was, in fact, the first of the post-war Australian nematologists to go abroad for training.

Alan's primary interests were in nematode physiology and morphology. His many publications on nematode ultrastructure reveal several facets of his approach to science – meticulous care, ability to cooperate with other scientists, a wide international involvement and a concern to apply his basic research to practical problems such as biological control of plant nematodes.

He published a book, various papers and book chapters with his wife, Jean Bird who gave him a great deal of support and encouragement. This collaborative work is particularly evident in a chapter on Functional Organization in the book "The Physiology and Biochemistry of Free-living and Plant-parasitic Nematodes" published in 1998.

In December 1997, Alan was made an Honorary Member of the Helminthological Society of Washington. He was only the third Australian parasitologist to receive this award. He had previously been made a Fellow of the Society of Nematologists (1983), and of the Australian Society for Parasitology (1993). He was awarded the Sir Joseph Verco Medal of the Royal Society of South Australia in 1991 and made an Honorary Fellow of that Society in 1999.

In 1981 Alan initiated, and edited for some years, the Australian Nematology Newsletter, thereby promoting communication between nematologists, a sentiment which he always espoused.

At the time of his death, he was an Honorary Research Fellow in CSIRO and was actively engaged in the study of the nematodes of the lakes and rivers in the southern States of Australia. In a recent paper (1999) delivered to the Royal Society of South Australia he made a plea for greater recognition of the importance of nematodes and

other small invertebrates in ecology. A sentiment that other nematologists will surely endorse.

Alan Bird was an active man, a Rugby Union enthusiast – he played scrum-half in his early years and he also played a good game of squash! As well as his loyalty to Edinburgh, he had a deep devotion to Ireland where he went to school. However, his loyalty to Australia never wavered when it came to International sport!

Alan is survived by his wife Jean, daughter Mary, son David and three grandchildren.

By E/Prof. H. R. Wallace

IMPROVING OUR NEMATODE DIAGNOSTIC SERVICES

Most AAN members received a copy of 'Advisory Services for Nematode Pests: Operational Guidelines', after it was published last year by RIRDC. Since the booklet was published, I have been asked by some diagnostic services whether it was possible to check their proficiency with regard to identification and enumeration of nematodes. One way of doing this would be to compare the results obtained by various laboratories on a single set of samples. Similar comparative exercises have been carried out for chemical laboratories in Australia (see Rayment *et al.* 1998, Aust. J. Exp. Agric. 38, 777-784)

If there is sufficient interest, I am prepared to coordinate such an exercise for nematology laboratories. It would be carried out as follows:

1. I would collect soil samples from 10 different agricultural or horticultural situations. Each sample would be mixed thoroughly and then divided so that a sub-sample could be sent to each participating laboratory.

2. Once the samples are ready (sometime in August or September this year), I would dispatch them via overnight air express to each laboratory.

3. Laboratories would process them using their standard techniques and send me the results.

4. I would collate the results and forward them to participating laboratories, which would be identified only by a code number.

The main value of this exercise is that participants would be able to compare their results with those of others. If the results proved useful and there was a continuing demand for such a service, AAN could possibly expand it into a more formal quality assurance program in future.

If you wish to participate in the initial exercise, please contact me by email (biolcrop@powerup.com.au), or telephone (0412 083 489) before 28 July. Because of the costs involved, there will be a charge of $220 per participating laboratory. Laboratories will be invoiced at the time samples are sent to them.

Graham Stirling, Biological Crop Protection Pty. Ltd., Brisbane

WELCOME TO NEW AAN MEMBER

Since our last newsletter we welcome 1 new member

Md. Motiul Quader (University of Adelaide, Adelaide, SA)

NEW WEB SITE ADDRESS

Here is a new web site address that may be of interest to some. The IFNS has a new list server but not many subscribers as yet and virtually no traffic.

http://www.ifns.org/

APPS 2001 NEMATOLOGY WORKSHOP UPDATE

The 13th biennial Australasian Plant Pathology Association conference is being held in Cairns between September 25 and 28, 2001. As part of the conference the AAN will be holding a workshop on Monday, September 24 at the Centre for Wet Tropics Agriculture which is a little more than an hour south of Cairns. It is proposed to have a nematode ecology workshop being led by Dr. Gregor Yeates from Landcare Research, Palmerston North, New Zealand. At this stage it is proposed to have an introduction into some of the nematode ecology terms and indicators, followed by a practical session looking at the nematode ecology in some tropical cropping practices.

As an optional extra it is proposed to have a survey of marine nematodes on the Great Barrier Reef on Sunday, September 23. The additional day will go ahead depending on interest and costs. The marine nematode survey will add a bit more to the cost of the workshop but will be an ideal opportunity to visit the Great Barrier Reef and the Mission Beach area for people who do not often get to tropical Queensland.

Further updates on the nematology workshop will be in later newsletters out lining costs and times.

Tony Pattison, QDPI, Centre for Wet Tropics Agriculture, South Johnstone Qld 4859

Regional News

NEWS FROM QUEENSLAND

We have been successful at establishing and maintaining carrot cultures of *Radopholus similis* for many years. We have added to our success by now having 5 different populations of *Radopholus similis* in culture.

But now, we at the DPI at Indooroopilly have managed to establish a pure culture of *Pratylenchus goodeyi*. This carrot culture has taken 6 months to establish from a single female extracted from field roots of banana. Lois Eden of Biological Crop Protection has been successful in establishing a pure culture of *Pratylenchus penetrans*. This lucerne callus culture was established from nematodes extracted from pyrethrum roots from Tasmania.

Jenny Cobon, DPI, Indooroopilly

NEWS FROM NORTH QUEENSLAND

ONTA 2000 Report

The Organisation of Nematologist of Tropical America (ONTA) held their 32nd annual meeting at Auburn University between April 16 and 20. Participants were from throughout Central and South America as well as the USA and Spain. The major discussion topic was the importance of microbial ecology and suppressive soils in managing nematode problems in crops. Examples were given by Professors Dan Kluepfel and Joe Kloepper on the successful isolation, identification and commercialisation of nematode antagonists. A new field emerging in soil ecology is metagenomics, where instead of trying to identify all the organisms present in soils to determine if they may be suppressive, the soil is treated as a single living organism with its own unique DNA. The use of metagenomics is hoped to be able to identify soils with nematode suppressive characteristics.

The phase out of methyl bromide in the USA has caused a lot of concern about the management of plant-parasitic nematodes. The options for the USA are to develop more integrated nematode management techniques or to replace methyl bromide with other broad spectrum fumigants. The reliance on methyl bromide in US agriculture highlights the need to develop a range of strategies for managing plant-parasitic nematodes.

On a business side, *Nematropica*, the journal produced by ONTA currently has a charge of $75 (US) for articles 6 pages or less. Larry Duncan, the chief editor, has informed members that papers in *Nematropica* can be published electronically with no page charges. That is, a paper could be published in the internet issue without page charges, but the title would appear in the bound issue of the journal. However, if the article were to appear in both online and in the bound journal, page charges would be required. This may make *Nematropica* more accessible to institutions that cannot afford the page charges. More information can be gained from the web site.

http://www.ifas.ufl.edu/~onta/NEMATROPICA.HTM

The 33rd ONTA meeting is in Cuba in June in 2001 and would be well worth attending for anyone interested in nematode problems in tropical crops.

Tony Pattison
QDPI
Centre for Wet Tropics Agriculture
South Johnstone Qld 4859

NEWS FROM SOUTH AUSTRALIA

SARDI Horticultural Nematology

We have now passed on all data from field experiments in oranges relating to Rugby 100G® to Crop Care Aust. who are preparing a submission for product registration. This project was mainly concerned with investigating organic methods of nematode control including biofumigation and mulching; an evaluation of cadusafos was included as a secondary aim but expanded in response to industry pressure to become a major focus. We obtained some impressive yield stimulation at some orchards; *Tylenchulus semipenetrans*, *Paratrichodorus* and *Xiphinema* sp. were the main nematodes involved at field sites. However, yield response and hence the economics of using nematicides varied from site to site. Like most nematologists, we remain hopeful of developing reliable non-chemical controls, but recognise that chemicals will be with us for some time yet. The project presented many challenges to us, and I will long remember crawling on the ground beneath orange trees, manually skirting trees when equipment had broken down, and getting torn by thorns and covered in snail slime. But it is always good to get out of the office/laboratory, and away from administration!

Greg Walker, SARDI Plant Research Centre

University of Adelaide

The group at the University of Adelaide has had some successes and growth. Val Kempster is to be congratulated for being awarded her PhD for work on bacterially induced resistance in white clover to *Heterodera trifolii*. Md. Motuil Quader fresh from completing a M. Sc. at USQ, Toowoomba, has started research towards a PhD with the CRC for Viticulture on quantification and management of nematodes in grapevines. Mark Potter is taking up a postdoctoral fellowship from GRDC to further his PhD studies on *Pratylenchus* in canola. GRDC has also provided funding for a new PhD student to work on species interaction in *Pratylenchus*, so if you are keen, do get in touch.

Professor Bill Bowers, University of Arizona, spent six months with the group looking a few aspects of chemical defences of plants to nematodes. Despite being an entomologist, he went away enthused about nematodes and keen to chase grants in US. Primali de Silva arrived on study leave from Sri Lanka and is pursuing aspects of the work Bill started. She is also taking on the challenge, determining the role of the bacteriophage in the relationship between *Rathayibacter toxicus*, its vector *Anguina* and host plant.

Astrid Schmitz returned to Germany to write up her diploma thesis on *Anguina australis*. Despite *A. australis* being quite recalcitrant at times, Astrid enjoyed her time in Adelaide. Interestingly she found that the nematode is a potential vector for *Rathayibacter toxicus* and experiments are in progress to check if the galls in *Ehrharta longiflora* can be colonised and will become toxic.

Tricia Franks (postdoctoral fellow) and Adam Clay (PhD student), self acclaimed 'would be nematologists' in the department of Horticulture, Viticulture & Oenology have begun work in a larger project funded by GWRDC and CRC for Viticulture to develop root pest resistances in grapevine cultivars and rootstocks. The project lead by Robyn van Heeswijck targets lesion and root knot nematodes as well as phylloxera. The approach is a molecular one. Beginning with attempts to gain some understanding of grapevine's responses to its root pests by characterising changes in gene expression in the plant roots when pests invade, the ultimate aim is to produce transgenic plants resistant to these pathogens. Tricia says her next update is 2006, we're expecting it much sooner.

After snow on Mount Lofty in the Adelaide hills, Kerrie Davies, Val Kempster and Ian Riley all decided to escaped the South Australian winter to the attend the SON meeting in Quebec. Val presented a poster on her PhD work and Ian a paper with Sean Kelly (Agwest) on cereal nematodes in WA. Ian also visited nematology labs in Japan and the UK. Kerrie visited Florida and Mexico, to check on their worms and catch up with Robin Giblin-Davis and Julie Nicol.

The nematode discussion group has been well supported by nematologists from all the Waite Campus institutions, but was clearly the poorer for the loss of Alan Bird. There were two discussions on *Pratylenchus*, Rachael Hutton on *Pratylenchus* in medics and Ian on WA *Pratylenchus*, an overview of Andreas Hensel's millipede work by Kerrie and presentation by Primali on *Pasteuria* and *Meloidogyne*.

Ian Riley, The University of Adelaide

More news (and a bit of history) from South Australia:

***Pratylenchus* cultures celebrate first decade**

The beginning of the year 2000 marked the 10th anniversary of the *Pratylenchus neglectus* cultures initiated at the University of Adelaide. These cultures were originally established with the assistance of Dr John Fisher (Department of Crop Protection, University of Adelaide).

Nematodes were extracted from roots of susceptible wheat (cv. 'Machete') grown in infested soil from the property of Mr Jeff Eichler (Palmer, South Australia). This property was also the site of the field observations and isolations of *P. neglectus* in 1989 that began the surge of root lesion nematode research in South Australia, and later in Victoria and Western Australia.

The culture method is adapted from Moody *et al.* (1973), as described in Zuckerman *et al.* (1990). The nematodes are cultured aseptically on carrot pieces in 250ml tubs (Nicol and Vanstone, 1993) at 20-25°C, followed by storage at 10-12°C to prolong culture life. New cultures are established by transferring a small segment of infected carrot tissue from 'healthy' cultures. The nematodes have not been surface-sterilised with antibiotic solution since the original cultures were established.

In the early 1990's, cultures were also established using chickpea or medic root callus on White's medium (White, 1963). This method proved far too time consuming in both initial set-up and subsequent culture maintenance. Cultures were more prone to contamination, and nematodes required regular surface-sterilisation and transfer to new plant tissue.

Carrot culture is the preferred method: it is inexpensive; many tubs can be set-up relatively quickly; and sequential inoculation of new tubs ensures continuity of nematode supply. Tubs of aseptic carrots can be stored at 5°C for at least six months, then inoculated in smaller batches as required. One of our secrets of success is the use of FRESH carrots. We are fortunate to be able to obtain high quality, large carrots within a day of picking from Nicol and Son Pty Ltd, Market Gardeners, Virginia, South Australia.

Cultures of this *P. neglectus* isolate are also produced by Sharyn Taylor and her team at the South Australian Research and Development Institute, Waite Campus. Taylor and Szot (1999) have reported no apparent loss of pathogenicity for this *P. neglectus* population, and no difference between nematodes extracted from carrot culture and cultured nematodes freshly extracted from wheat roots.

Progeny of the cultures have been subjected to the indignities of life in the roots of hundreds of cereal, pasture, grain legume and oilseed genotypes; the struggle for survival in weed species; manipulation and experimentation by numerous PhD and Honours students; DNA extraction; and the horrors of death by brassica isothiocyanates.

They have performed admirably through variations in soil type, temperature, nutrient regime, soil water content and host type. Many have given their lives in the pursuit of crop resistance.

In the year of the 121st cultured generation, we thank all the carrot peelers and flamers who have maintained aseptic conditions and continuous carrot supply.

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

Photography by Brenton Brooks, University of Adelaide

PEELERS and FLAMERS – Vivien Vanstone (University of Adelaide). Marzena Kaczmarek (SARDI), Sharyn Taylor (SARDI), Nicole Brooks (SARDI), Michelle Russ (University of Adelaide). Absent - Danuta Szot (SARDI).

Research

YIELD LOSS IN WHEAT CAUSED BY *PRATYLENCHUS THORNEI* IN THE WIMMERA REGION OF VICTORIA

Grant Hollaway, Russell Burns, Graham Exell, Denis Ward
Victorian Institute for Dryland Agriculture, Horsham, Victoria 3401
and
Kathy Ophel-Keller
Field Crops Pathology Unit, South Australian Research and Development Institute, Adelaide, South Australia 5001

Currently in south-eastern Australia there is a paucity of information on the yield loss caused by the root lesion nematode, *Pratylenchus thornei*, in wheat crops. If growers are to make effective management decisions on their disease control strategies they need accurate information on the extent of yield loss caused by a given population density of the pathogen.

To address this lack of information a field trial was conducted during the 1998 and 1999 seasons to generate data on the relationship between population density of *P. thornei* at sowing and subsequent grain yield of an intolerant wheat crop.

Methods

Field Trial:
A field trial was established in 1998 at a site in the Wimmera region of Victoria infested with *P. thornei*. Wheat varieties with resistance (Durum Wheat cv. Yallaroi), moderate resistance (Bread Wheat VI 184) and susceptibility (Bread Wheat cv. Ouyen) to *P. thornei* were grown to manipulate the population densities of the nematode in the soil. The trial was sown in a complete randomised block design with five replications. In 1999 the 15 plots were all sown to an intolerant wheat (cv. Ouyen) and grain yield measured.

Nematode quantification:
Each field plot was sampled at sowing in 1999 to determine the initial population density of *P. thornei*. The soil was sampled using a 75 mm earth auger to a depth of 300mm at four locations in the plot. The four soil samples were bulked, sealed in a plastic bag and stored in the dark at 4°C until nematode extraction.

Nematodes were extracted from two 200 g sub-samples of soil from each plot using modified Whitehead trays at 22°C for 72 hrs with nematodes collected on a 20 µm sieve. Nematode suspensions were assessed by counting two sub-samples from each extraction and the mean of the samples was expressed as number of *P. thornei*/g dry soil.

The same soil collected at sowing in 1999 was also tested by the Root Disease Testing Service (RDTS), which is a test based on quantitative DNA technology (available from SARDI) to determine the relationship between population density of *P. thornei* at sowing and subsequent grain yield. These soil samples were, however, stored for several months before being submitted to the RDTS for quantification.

Results

The effect of each of the three cereal varieties grown in 1998 on the population density of *P. thornei* in the soil at the start of the following season is shown in Table 1. This table also shows the effect of these population densities on the grain yield, on farm income and grain protein in a following wheat crop. The relationship between the initial population densities of *P. thornei* and the subsequent grain yield of wheat (cv. Ouyen) as determined by manual counts and the RDTS is shown in Figures 1 and 2 respectively.

Table 1. Effect of cereal varieties with different susceptibility to *Pratylenchus thornei* on the population density in the soil at sowing (determined by manual counts) and the subsequent grain yield and grain protein of an intolerant cereal crop at Swanwater in 1998 and 1999.

Treatment	Crop 1998	Population Ln *P.thornei*/g+1 June 1999	Crop 1999	Root Scores (0-6)[b]	Grain Yield (T/ha) Dec 1999	Income $/ha (assume $150/t)	Grain Protein (%)
Resistant	Yallaroi	1.6 (4.1)[a]	Ouyen	0.2	2.79	$419	11.85
Moderate	VI 184	2.4 (10.1)	Ouyen	0.4	2.66	$400 (-$19/ha)	11.20
Susceptible	Ouyen	3.5 (30.5)	Ouyen	1.2	2.38	$357 (-$62/ha)	11.20
LSD (0.05)		0.73		0.55	0.177		0.370

[a]retransformed mean (*P. thornei*/g soil)
[b]Root Score where 0 = no root disease , 1 = 1-10% of roots truncated with brown pointed tips, 2 = 11–25%, 3 = 26-50%, 4 = 51-75%, 5 = 76-99% and 6 = 100%.

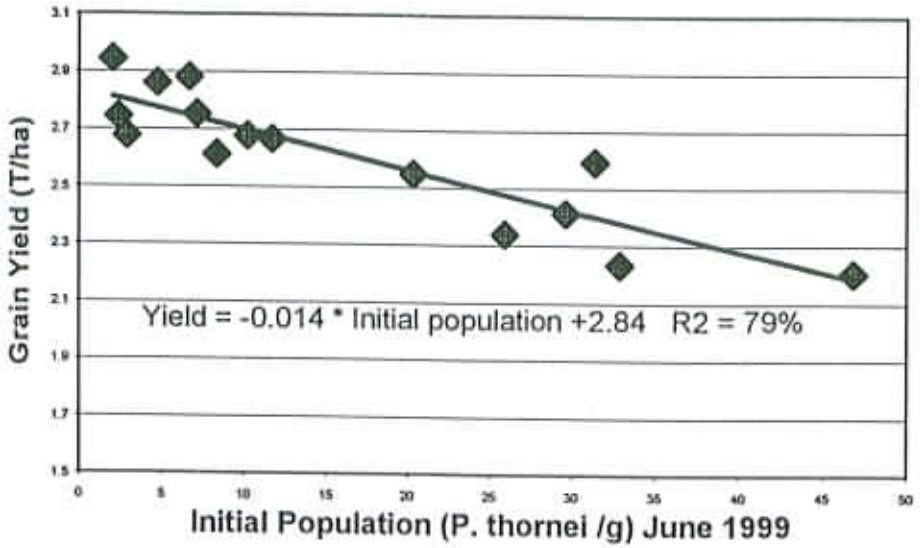

Figure 1. Effect of the initial population of *Pratylenchus thornei* (determined by manual counts) in the soil at sowing on the grain yield of an intolerant wheat (cv. Ouyen) at Swanwater in 1999.

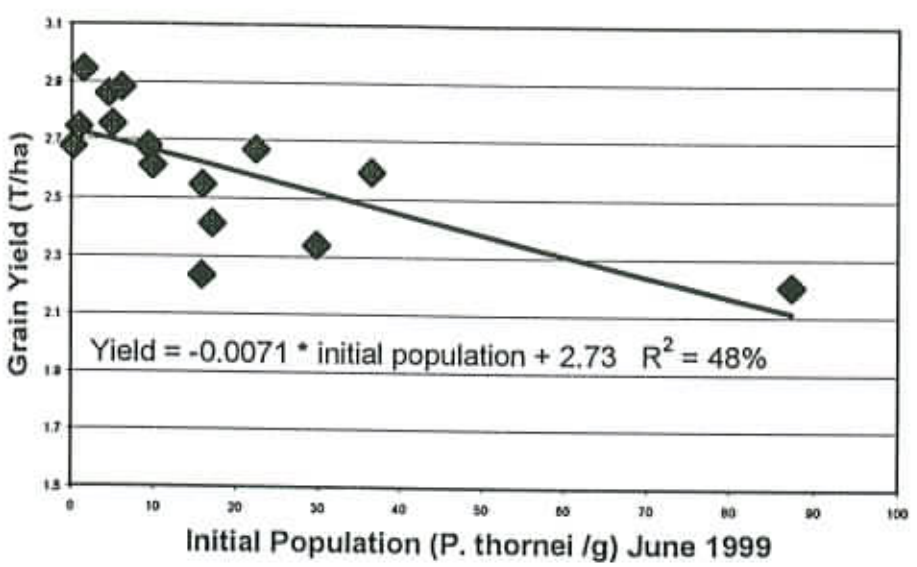

Figure 2. Effect of the population of *Pratylenchus thornei* (determined by RDTS) in the soil at sowing on the grain yield of an intolerant wheat (cv. Ouyen) at Swanwater in 1999.

Discussion

This field trial has demonstrated that growing a crop susceptible to *P. thornei* in 1998 caused a yield loss of 15% (an income reduction of $62/ha) in the 1999 wheat plots relative to where a crop resistant to *P. thornei* had been grown in 1998. The strong negative relationship between initial population density of *P. thornei* and grain yield indicated that most of the yield loss could be attributed to *P. thornei* (Figure 1).

The relationship between initial population of *P. thornei* (determined by manual counts) and grain yield shows that for every increase of one nematode per gram of soil at sowing, grain yield was reduced by 14 kg/ha (approximately 0.5%) and income reduced by $2.10/ha.

Even though the soil samples were stored for five months before processing by the RDTS a negative relationship between initial population density of *P. thornei* and grain yield was again found (Figure 2). The relationship showed that for every increase in initial nematode population density of one nematode per gram of soil there was a subsequent decrease in grain yield of 6 kg/ha (approximately 0.25%) and an income reduction of approximately $0.90/ha.

This trial has demonstrated the yield loss caused by *P. thornei* to an intolerant crop in Victoria. The relationships developed in this study can be used by agronomists and farmers evaluating the risk associated with a given root lesion nematode population. This work is currently been extended to a larger range of cereal, pulse and oilseed varieties.

Acknowledgments

This work was supported by the Grains Research and Development Corporation (DAS229). Thanks to Norm and Richard Bales for provision of land for this field trial and to Jason Mott for technical assistance.

Review

SOIL MICROBES AS POTENTIAL CONTROL AGENTS FOR PLANT-PARASITIC NEMATODES IN PASTURE

University of Adelaide 1999

Summary of Valerie Kempster's PhD thesis

Induced systemic resistance (ISR) is widely manifest in the plant kingdom, but there are few reports of its occurrence against nematodes and, prior to this study, it had not been reported in white clover. It was decided to investigate the induction of resistance to the clover cyst nematode, *Heterodera trifolii* Goffart, an economic pest in white clover pastures that are a key to high milk yields in dairy cattle in Australia and New Zealand. This study aimed to explore the potential of soil and rhizosphere bacteria to induce systemic resistance in white clover, *Trifolium repens* L.

Salicylic acid (SA) and benzo (1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH) are known to induce resistance in both monocotyledonous and dicotyledonous plants against a wide variety of pathogens and pests. In a growth cabinet soil bioassay, both chemicals were applied separately as soil drenches to *T. repens* seedlings, which were subsequently inoculated with infective juveniles of *H. trifolii*. Both chemicals induced resistance to *H. trifolii*, manifest as a reduction in fecundity of the nematode and a higher proportion of abnormal cysts and fewer eggs per cyst, compared to controls treated with water. Resistance was induced in two cultivars of *T. repens*, 'Haifa', and 'Grasslands Huia'. The latter is considered to be very susceptible to *H. trifolii*.

Soil samples and white clover plants were collected from pastures known to be infested with *H. trifolii* in Victoria. These and soil samples from South Australia were examined for the presence of *Bacillus* and *Pseudomonas* spp., both known to be potential inducers of ISR in other plants. *Bacillus* strains were isolated on a medium selective for *B. thuringiensis* and *B. cereus*. Fluorescent *Pseudomonas* strains were isolated on King's B medium, further selected as to colony type on tetrazolium chloride agar, and then selected for pectinolysis on crystal violet pectate agar. Two pectinolytic *Pseudomonas* strains, P29 and P80, applied as nutrient broth (NB) cultures, induced resistance in white clover seedlings equivalent to that resulting from application of SA and BTH. Both live and dead cells of strain P29, resuspended in sterile distilled water and applied as a soil drench, had the same effect as the NB culture of the strain. This suggests that there is some plant 'recognition' of the bacterial cell walls which triggers the plant response. Cell-free culture filtrate of strain P29, applied to white clover seedlings, did not induce resistance to *H. trifolii*, suggesting that the bacterial metabolites did not act as inducing

agents. The metabolites had little antagonistic effect against infective juveniles of *H. trifolii in vitro.*

The plant response to BTH and P29 as inducing agents was investigated by quantitative biochemical assays for lignin and callose. At 4 weeks after inoculation with infective juvenile nematodes, there was no difference in the concentrations of lignin or callose in the leaves of plants pre-treated with BTH, P29 or water. Similarly, there was no difference in the concentrations of lignin or callose in the roots of plants pre-treated with BTH, P29 or water, 4 weeks after inoculation with the nematodes.

Bacillus strain B1 was also found to induce resistance against *H. trifolii* in the growth cabinet soil-based bioassays, equivalent to that induced by P29 or BTH.

A greenhouse experiment was carried out to investigate whether resistance could be induced to the blue-green aphid, *Acyrthosiphon kondoi*, in white clover and a medic, *Medicago truncatula*. 'Grasslands Huia', and the medic cultivars 'Sephi 6297' (resistant to the blue-green aphid) and 'Jemalong' (susceptible) were treated with strain P29, BTH or water and 4-5 aphids were released onto the plants. Some resistance to the blue-green aphid was observed. Resistance was manifest as greater plant growth in treated than control plants. Tests of strain P29 as a potential growth-promoting bacterium had shown no increase in growth of white clover plants after 52 days compared to SDW-treated controls. No increase in callose or lignin was found in the leaves and stems of the treated white clover plants.

It is concluded that resistance to the clover cyst nematode can be induced in white clover with a soil drench of BTH, of *Pseudomonas* strains P29 and P80, or of *Bacillus* strain B1 isolated from soil. This is a first report of induced resistance in white clover, and to the clover cyst nematode. These bacterial strains are candidates for further evaluation as biocontrol agents against nematodes in white clover.

THE BIOLOGY OF THE PLANT PARASITIC NEMATODES *PARATYLENCHUS NANUS* AND *PARATRICHODORUS MINOR* IN SOIL UNDER PASTURE

Massey University, 1999.

Abstract from Nigel Bell's PhD thesis

Ectoparasitic nematodes can be the most numerous plant parasitic nematodes in some New Zealand pastures. The effects of *Paratylenchus* and *Paratrichodorus* have been studied in some crops overseas but their effects in pasture, either overseas or in New Zealand, are largely unknown. Implicit in any investigation of plant damage by a pathogen is the need to specifically identify the organism and have an understanding of its spatial and temporal population dynamics. This thesis investigates these factors for

both *Paratylenchus* sp. and *Paratrichodorus* sp. in two soils, under pasture grazed by sheep and cattle, near Hamilton in the Waikato region of New Zealand.

Paratylenchus nanus and *Paratrichodorus minor* were identified from soils under grazed pasture. All hosts of *P. nanus* in host range testing were grasses, while *Paratrichodorus minor* hosts included both grasses and clovers.

Several variations to the Whitehead and Hemming tray extraction method were compared. The optimum variant using a two day extraction period was found to yield *ca* 75% of the total nematode fauna.

Sampling of *P. nanus* populations from 0–10 cm and 10–20 cm soil depth, in grazed pasture, showed that the abundance of *P. nanus* peaked in summer. A Population Age Index, based on developmental stages, showed *P. nanus* population age increased from a minimum in spring to maximum in winter. Positive correlations occurred with soil temperature and negative correlations with soil moisture and rainfall. Accumulated temperature and rainfall (Activity Index) was correlated with *P. nanus* abundance. Evidence is presented for density-dependence in the *P. nanus* population at 0–10 cm depth. Multiple regression models were fitted and their implications in understanding population dynamics discussed.

Seedlings of five grasses were inoculated with one of three rates of *P. nanus*. There was a deleterious effect of the high rate of *P. nanus* inoculum on shoot dry matter only for *Lolium perenne* infected with a selected *Neotyphodium* sp. endophytic fungus. Sampling of soil beneath grazed pasture determined the relationship of *P. nanus* populations with mature *L. perenne* plants. For all samplings, *Lolium perenne* infected with the selected *Neotyphodium* sp supported a consistently greater abundance of *P. nanus* than other plants. Dry matter production and root mass data suggest that greater root production by *Lolium perenne* infected with the selected *Neotyphodium* strain was partly responsible for the greater abundance of *P. nanus*. Implications for field sowing of *Lolium perenne* infected with the selected *Neotyphodium* in the presence of *P. nanus* populations are discussed.

Sampling in soil from a second grazed pasture which contained populations of both *P. minor* and *Paratylenchus nanus* showed the *Paratrichodorus minor* population had no seasonal periodicity while *Paratylenchus nanus* had distinct spring and summer peaks. *Paratrichodorus minor* abundance was correlated with rainfall and Activity Index. There was no evidence for competition occurring between these two nematodes at the population levels studied.

This thesis is written as a series of papers, which follow the format of the international journal *Nematology*. Therefore, each chapter contains Summary, Keywords, Introduction, Materials and Methods, Results, Discussion and References. The General Introduction and General Discussion chapters are additional to this format. This thesis has been embargoed until the 30 June 2001 for consideration of matters regarding patent applications.

IMPLEMENTING STRATEGIC CONTROL OF NEMATODES ON BANANA

SUMMARY OF FINAL REPORT ON HRDC PROJECT FR96016

Julie Stanton and Tony Pattison

Burrowing (*Radopholus similis*) and lesion (*Pratylenchus goodeyi*) nematodes are major constraints to banana production throughout the world. In Australia, routine use of nematicides has been the most common control method. However, these chemicals are expensive and toxic. Two previous HRDC projects have addressed this problem in Australia and made good progress in developing better ways of managing these nematodes.

Two field trials evaluated the relative efficacy of the currently registered nematicides in north Queensland. Single applications of nematicide did not reduce the damage caused by *R. similis*. Generally, it required one year of continual application before yield was improved by nematicide. Beneficial effects were due more to reduced toppling rather than to increased bunch weight but all nematicides tested improved yield and increased returns.

All nematicides suffered enhanced biodegradation with continual use. We propose a strategy to reduce the development of enhanced biodegradation by rotating nematicides. We also recommend using the more soluble nematicides, oxamyl and fenamiphos, during the drier months and the less soluble nematicides, terbufos and cadusafos, during the wetter months in north Queensland.

Five unregistered, 'organic' products were evaluated for their effectiveness against *R. similis* compared with Nemacur. None of the unregistered products affected nematode motility except in high concentration. None affected nematode reproduction on bananas in pots or in the field for two years. However, after two years, three products, had some effect against root damage caused by nematodes but none increased yield.

We monitored six banana blocks for reduction in nematode populations during various fallows. Volunteer bananas allowed significant nematode reproduction and represent the greatest carry-over of nematodes. Fallows of more than one year are necessary to allow *R. similis* populations to decline to undetectable levels.

Soil from eight farms in north Queensland and six in south-east Queensland and northern New South Wales were bioassayed for enhanced biodegradation of fenamiphos. Five of the farms in north Queensland had accelerated biodegradation but only one in the subtropics. We found significant correlation between our assay and the one used by Bayer in Germany. The extent of enhanced biodegradation of fenamiphos depends on the frequency of its use and was therefore more prevalent in north Queensland than in the subtropics where other chemicals are more common.

Taylor's power law was used as a model for subtropical and tropical data to develop stop lines for sampling. We found that 20 samples were sufficient to estimate disease

index of a crop in both regions. We were able to assess disease index more precisely than nematode populations in roots.

All growers in the subtropics were sent questionnaires and 28.5% of growers responded. There was a trend in perceived nematode problems increasing from north (25% in Bundaberg) to south (69% in Nambucca) and a corresponding trend in nematicide use (12 and 59%, respectively). Overall, fewer than 40% use a fallow period. Only 42% of respondents said that they had used a nematicide in the previous 12 months. Of those, 69% used it routinely although a significant number used some measure of the state of the crop to decide whether to apply nematicide. In comparison with north Queensland growers, fewer growers used other recommended practices to manage nematodes. The most common reasons for not managing nematodes other than by applying nematicides were that they didn't have enough land to fallow, clean planting material is too expensive and they didn't have the time or skills to use nematode severity to decide whether to apply nematicide.

In addition, 34% of growers who returned questionnaires also sent root samples for the nematode survey. *R. similis* was found on 53% of crops sampled from Rockhampton to Nambucca. *P. goodeyi* was found on 8% of crops from Queensland border to Nambucca. It has also been found in large populations near Tallebudgera and Caboolture. *Meloidogyne* spp., *Helicotylenchus multicinctus* and *H. dihystera* were found in high numbers throughout the subtropics. We also found that *Pratylenchus goodeyi* is fairly common from Caboolture to Nambucca. This nematode causes exactly the same symptoms as *R. similis*.

Also, there were fluctuations in numbers of nematodes between seasons. *R. similis* multiplies faster in higher temperatures so is more prevalent in the warmer months. *P. goodeyi* prefers lower temperatures so multiplies more in the cooler months.

In contrast to the tropics, there was no discernible change in disease index or populations of *R. similis* or *P. goodeyi* from year to year. Economic threshold was determined for four properties to be a root disease index of 25-40. In general, there seemed to be a trend from north to south with a higher economic threshold in warmer areas. So, growers near Bundaberg can use a disease index of about 40 to decide on nematicide application while growers in northern New South Wales should use a lower disease index of about 25 as their economic threshold.

Soil from several crops in the subtropics inhibited *R. similis* reproduction on bananas in pots. Non-pathogenic, endophytic *Fusarium oxysporum* from roots of these crops have been isolated and, so far, one has been found to inhibit nematode reproduction on bananas in pots.

The effect of *Meloidogyne* was tested in the field on plants grown from tissue-cultured plants and those planted as suckers. No effect of root-knot nematode was found at up to 44,000 nematodes/100 g root. We can assume that this nematode will not be a major constraint to banana production even though it may be found in high numbers.

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Environmental physiology of cold tolerance and anhydrobiosis
Nematode ultrastructure

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Identification and management of *Meloidogyne*
Pasture nematodes

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Pratylenchus thornei
Breeding wheat for nematode resistance