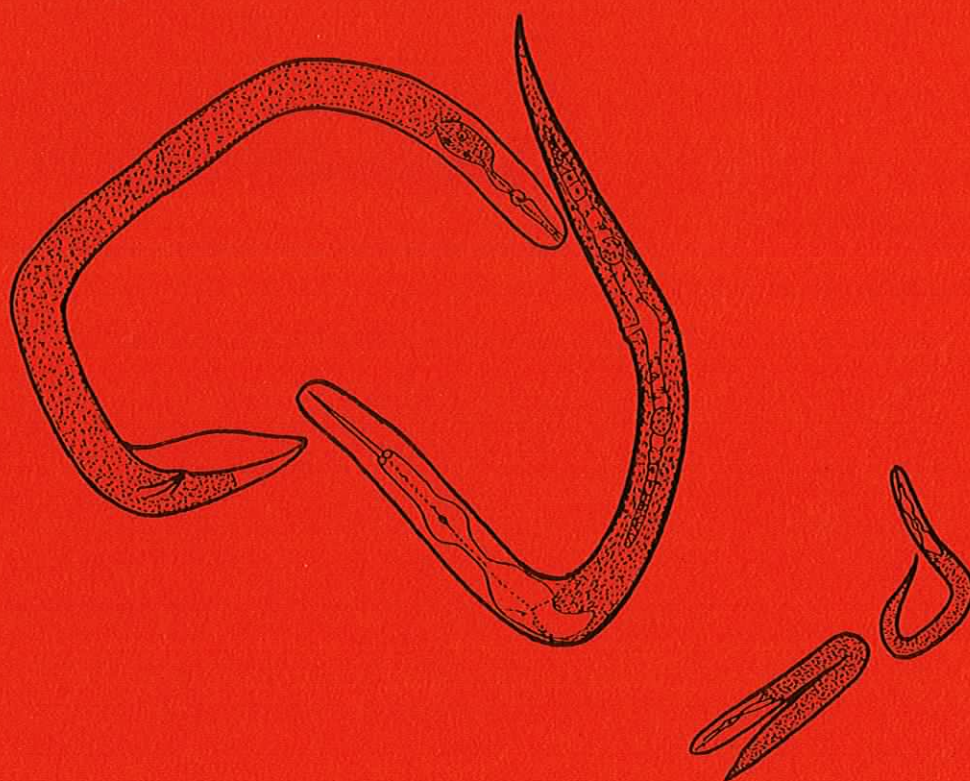


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AUSTRALASIAN NEMATOTOLOGY NEWSLETTER

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From the Editor

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

July Issue

The deadline for the July issue is June 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

Nematologists who have ever wondered about nematodes in aquatic systems should have a look at the new web site hosting my “Key to Aquatic Nematodes of the Murray-Darling River System and Coastal Freshwaters of Southeastern Australia”. It is accessible free through the AAN ,CSIRO Entomology, ABRS and ASL web pages, thanks to the support of ABRS. This is a fully interactive key with the user able to select from over 400 characters of varying difficulty: everything from simple characters like total body length to the position of the hemizonid. I hope this will be a boost for an area of nematology which has been overlooked for a long time; with the exception of Alan Bird and Warwick Nicholas. The last one to sample freshwaters for nematodes was Cobb when he was in Australia over 100 years ago.

One interesting finding of the survey used for sourcing nematode specimens for the key was that some economic plant parasites do occasionally find their ways into our rivers and streams. Presumably they do not survive for very long, but it does emphasise that irrigation water is a potential source of infection and spread for plant-parasitic nematodes. There is a workshop in Albury for professionals monitoring river health, so if interested contact me for further information.

Planning for the biennial meeting of the Association, to be held as usual in conjunction with the Australasian Plant Pathology Society Conference, is now well advanced. This year's conference, to be held in Cairns in September promises to be as interesting as ever, with Gregor Yeates the invited speaker. Gregor will, I'm sure, provide many interesting ideas and perspectives on nematology. The interactions between “hard-core” nematologists, more general plant pathologists, and specialists in other plant pathology disciplines is always useful. In addition to the scientific attractions, the physical surroundings for this year's conference will, I am sure, make the long journey for many of us “southerners” worthwhile.

The Fourth International Congress of Nematology in Tenerife in 2002 is also approaching. Despite being just a little further to get to than Cairns and a little further off in time, it is one of the few opportunities for nematologists from all over the world to interact in one place. Such congresses only occur once every 6 years so start planning now. I have received no comments from members for symposia they would like to participate in, so if you want to plan to attend the congress to discuss particular topics, let me know soon, so that they can be considered before the programme is finalised.

On matters financial, members of AAN can look forward to paying their annual subscription along with their APPS subscriptions. Thanks go to Ian Riley for this innovation to make parting with your money easier.

The short course on “Nematodes in Cropping Systems: Identification & Techniques” will be presented again in Canberra at the end of the year. We anticipate participants from the Pacific Islands again, as well as people from around Australia: the interactions between participants and different perspectives added an extra dimension to the course last time and we hope to achieve the same this time. There is a separate announcement elsewhere in this issue.

In Canberra, we have added more specimens to the collection, which now stands at nearly 20 000 specimens. Notable inclusions have been plant-parasites including *Pratylenchus*, Spiral nematodes and Pin nematodes from wheat, some mermithids from grasshoppers, some aphelenchs from trees and insects, and some dagger nematodes from woody crops. We have also recently received some type specimens of nematomorphs (insect parasites with some macroscopic resemblance to mermithids). We are always keen to accept new specimens of any (non-helminth) nematodes, so once you are finished with your slides or other material, we would love to receive it. We have recently completed an audit of the collection, and all material remains in excellent condition, thanks no doubt to the purpose-built collection hall and the care we have taken in curating material over the years. In addition to recording the nematodes occurring in the various crops and regions, quarantine issues involving nematodes are also likely to be important in the next few years. Having our collections covering as many of the myriad situations in which nematodes are found will be important to meet these needs.

All of the above sounds like a lot going on in nematology in Australia. However, the editor of the newsletter was not exactly rushed with contributions for this issue. I would like to add my voice to the plea to jot down what you are doing and forward it to the newsletter, so that we can all keep abreast of what is going on. Once every 2 years is not very often to meet in person with fellow nematologists, (just as once every 6 years is not very frequent to meet with all ones international colleagues), and we are spread from Cairns to Tasmania, Sydney to Perth, and beyond. The newsletter is important in filling the gaps between meetings and centres of activity.

Mike Hodda

NEW PAYMENT OPTION FOR ANN/APPS MEMBERS

Members of both AAN and the Australasian Plant Pathology Society (APPS) now have a new method of payment for their annual subscriptions. APPS invoices will now include a line - Special Interest Group - Australasian Association of Nematologists - giving you the option to pay ANN fees as part of your payment to APPS. There will be an administrative charge of \$1.00 for this service, nevertheless the convenience and ability

to pay by credit card will make this an attractive option for ANN members.

Furthermore, the additional exposure for ANN should encourage others to join and so strengthen our association. Conversely, ANN members should consider the benefits of joining APPS.

SHORT COURSE ADVERTISEMENT

Nematodes in Cropping Systems: Identification and Techniques

An intensive training course on "Nematode Identification and Techniques" will be held under the joint auspices of ANIC and The Waite Institute, University of Adelaide. The course will be held in December 2001 at The Australian National University, Canberra under co-ordinators Dr Mike Hodda and Dr Kerrie Davies. The course will cover identification of plant, soil and insect nematodes, together with techniques for sampling, extraction, experimentation and analysis. The course is aimed at professionals in plant and insect pathology, pest management, soils and other disciplines dealing with nematodes. Sufficient background will be presented to enable those with limited experience to benefit fully from more advanced aspects. Details of course content will be varied to suit the interests of the participants: please contact the co-ordinators to discuss any specific needs. Anticipated cost is \$1100 (GST inclusive) for 1 week, including all course materials.

Web site: <http://www.ento.csiro.au/research/natres/nematode.htm>

To register your interest or discuss specific needs please contact Dr Mike Hodda at CSIRO Entomology:

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Regional News

NEWS FROM NEW SOUTH WALES

I have attached the summary from my thesis (which appears later in this newsletter). It was submitted on Oct 4th but no news yet! Alamgir Khan has also recently submitted his thesis, also on *Paecilomyces lilacinus*. He's in Bangladesh at present and due back in early January. Other good news on the PhD front is that Rod McLeod's thesis has been accepted. I'm currently not working with nematodes, or even plant pathology! I'm looking at expression of c-fos in rat spinal cord tissue and also doing some biochemical work on saliva of asthmatics, some of who will be getting chiropractic treatment. It's a bit different but nevertheless pays the bills.

Rita Holland, Macquarie University.

NEWS FROM SOUTH AUSTRALIA

University of Adelaide, Applied and Molecular Ecology

After returning to Germany, Astrid Schmitz completed her Diploma thesis for University of Bonn on *Anguina australis* and received pleasing reports from her examiners. Likewise we empathise with Sharyn Taylor's sense of relief having submitted her PhD thesis, and the anxiety of waiting for examiner's reports.

Imelda Soriano from the Philippines has won an International Postgraduate Research Award and will be joining the group early in the year. She is yet to choose a project, but comes with a considerable amount of experience in rice nematology.

Kerrie Davies has recently returned from five weeks touring WA collecting *Fergusobia* from galls of myrtaceous hosts and, to help pay the bills, assessing helicod snail populations for GRDC on the way. Good work if you can get it.

Mark Potter has returned to the University to join the group on a GRDC postdoc to further his studies on *Pratylenchus* resistance in canola. One of our star undergraduate plant pathology students, Tara Sallows, has been beguiled by Mark into doing her honors on isothiocyanates, brassicas and *Pratylenchus* – another welcome addition to the group.

The nematode discussion group continues to be well supported by nematologists from all the Waite Campus institutions. We started second semester with the threesome – Davies, Riley and Kempster – giving accounts of their nematological wanderings in the Northern Hemisphere. Mark Potter and Jackie Nobbs gave presentation on *Pratylenchus* and *Meloidogyne* respectively and finally we were pleased to rope in

Shashi Sharma (new nematologist with AgWA, formerly of India) during his visit to SA to give us an account of nematology research in the semi-arid tropics. The year ended with a great feed at Eagle on the Hill.

Ian Riley, The University of Adelaide.

MORE NEWS FROM SOUTH AUSTRALIA

1) Citrus: follow-up monitoring of yields from field experiments where cadusafos was previously applied to bearing orange trees has been conducted. Some good yield increases have been observed in some, but not all, orchards.

2) Grapevines: greenhouse experiments are being conducted to determine if *Pratylenchus* species obtained from vineyards affect the growth of grapevine rootlings. Earlier testing showed no adverse effects from inoculation with cereal populations of *P. neglectus* and *P. thornei*.

3) Carrots: as part of a national project, a survey is being conducted of South Australian carrot plantings. Most growers are either fumigating soil with metham sodium or are using Nematicur(R). Some crop losses due to *Meloidogyne javanica* have been detected even where metham has been used, but the worst instance of crop damage was observed in a field operated by an organic grower. Four field experiments have been conducted to assess the influence of sampling intensity on reliability/precision, and field/greenhouse experiments are being set up to evaluate effects of nematodes on yields/quality.

Greg Walker, SARDI Plant Research Centre.

AND MORE NEWS FROM SOUTH AUSTRALIA

COFFEE FILTERS AND COWS

**University of Adelaide, Dept of Plant Science, *Prat* Lab
Vivien Vanstone and Michelle Russ**

Most of Vivien's time at the moment is taken up in lobbying for funding, so that the *P. neglectus* field trial program in SA can continue beyond June 2001.

Michelle is processing soil samples and counting nematodes at an amazing rate from the 24 trials we are assessing this year for initial and final *P. neglectus* populations. We are still using the "mister and coffee filter" method for extracting nematodes from soil samples, and keep promising ourselves that we will write up the method for publication (one day). We are now importing coffee filters from the UK, as none available in Australia are suitable. Last month, 28,000 filters embarked from Southampton and arrived safe and dry in Adelaide.

Trial assessments for 2000 include:

- effect of time of sowing on nematode numbers and yield loss;
- effect of P rate (and fluid vs. granular P) on *P. neglectus* population and yield loss;
- effect of P x Zn x N treatments;
- tolerance/yield loss of oat and barley varieties;
- resistance and tolerance/yield loss of new wheat varieties and advanced lines;
- a 2-year pasture/rotation trial examining the effect of *P. neglectus* on the pasture phase following resistant and susceptible cereals, and *vice versa*;
- assessment of Waite Wheat Breeding lines for resistance to *P. neglectus*;
- Jackie Nobbs is looking at samples from about 80 SA sites to determine if there are species other than *P. neglectus* and *P. thornei* present (I am hoping not, but wouldn't be surprised if there are).

We collaborate with Minnipa Agricultural Centre, Eyre Peninsula Farming Systems Project, SARDI Field Crop Evaluation, SARDI Oat Breeding, Waite Wheat Breeding and Waite Barley Breeding to make all these trials possible.

Our co-conspirators in Prat research are Grant Hollaway in Victoria and Sharyn Taylor at SARDI.

It has been a busy publishing year for Prats in south-eastern Australia.

We were all relieved, impressed and proud when Sharyn finished her thesis.

A couple of papers currently on the boil will (hopefully) appear next year in Australasian Plant Pathology: broad-leaf and grass weeds as hosts of *P. neglectus* and *P. thornei* (Vanstone and Russ).

“Monitoring *Pratylenchus thornei* densities in soil and roots under resistant (*Triticum turgidum durum*) and susceptible (*Triticum aestivum*) wheat cultivars” (by Talavera and Vanstone) will be published in *Phytoparasitica* 29:1, 2001.

SP Taylor, GJ Hollaway and CH Hunt 2000 – Effect of field crops on density of *Pratylenchus* in south-eastern Australia; Part one: *P. neglectus*. Supplement to *Journal of Nematology* 32(4S) in press.

GJ Hollaway, SP Taylor, RF Eastwood and CH Hunt 2000 – Effect of field crops on density of *Pratylenchus* in south-eastern Australia; Part two: *P. thornei*. Supplement to *Journal of Nematology* 32(4S) in press.

MJ Potter, VA Vanstone, KA Davies and AJ Rathjen 2000 – Breeding to increase the concentration of 2-phenylethyl glucosinolate in the roots of *Brassica napus*. *Journal of Chemical Ecology* 26(8):1811-1820.

Plus all the usual field days, grains expos, GRDC updates, newsletters, press releases, visits by farmer groups, and Brazilian agricultural workers. I had fun with the SA Agricultural Bureaux Better Soils Project and presented a talk titled “Not all nematodes are bad”.

Shashi Sharma, our new colleague in WA, made a quick trip to Adelaide in November, which gave us a good chance to meet and talk about the current SA Prat work, and future chances for collaboration with field trials. Abdol Taheri (former PhD student) made an even quicker visit to the Prat labs in early December. He is now Vice Chancellor of Student Affairs at the University of Sistan and Baluchestan (Iran), and can only do nematode research on rare occasions.

If all the trials go as planned (apart from the one eaten by cows just prior to harvest) we should have some interesting results to report next year.

Vivien Vanstone, The University of Adelaide.

AND EVEN MORE NEWS FROM SOUTH AUSTRALIA

Society of Nematology

The annual meeting of the Society of Nematology was held in Quebec City, Canada 24-28th June 2000. Among others, Valerie Kempster attended this meeting. The meeting was held on the campus of the Université Laval and most delegates were therefore accommodated in student housing, it being long vacation. There were over 400 delegates and a programme covering: Soil Health; Entomopathogenic Nematodes; Biochemistry; Molecular Biology; Resistance; Host-parasite interactions; Ecology and Systematics; Management and Detection and Diagnosis and plant disease loss. Thus a wide sweep through the many aspects that nematologists can delve into in our discipline. There was also a Symposium on GMOs (sponsored by the Monsanto company). This latter was probably (for Val) the liveliest session of the whole conference! Prejudices and biases were aired on both sides. Some scheduled papers were not given due to the absence of their authors, in some cases not notified. However the chairpersons managed to keep the concurrent sessions on track. The dinner was good though very crowded (smallish space) but we got to see the museum free, before the 'cocktail hour'. It was disappointing that a decision was made by the president of the SON not to award a student prize this year. While we have some very well regarded nematologists in SON who have made excellent contributions to the discipline, I felt a 'nod' of encouragement to the upcoming nematologists would not have gone amiss, and would have contributed to the future of the Society.

Herewith a brief account of highlights from the meeting from one delegate's point of view. Biocontrol sessions included a paper on the successful use of entomopathogenic nematodes to control root-knot nematodes in tomato and soybean plants (Fallon et al.) in the lab and greenhouse. There was an update on work on the *Fergusobia/Fergusonina* gall-forming complex against *Melaleuca quinquenervia* in Florida. There were encouraging papers on the use of green manures and organic amendments for the control of plant-parasitic nematodes and the use of soil bacteria and fungi as suppressors or antagonists to plant parasitic nematodes. There were papers on the testing of soybean cultivars resistant to RKN and SCN for their efficacy against infestations of reniform nematodes (pilot study). There were two papers on mollusc-nematode interactions, one

reporting the discovery of the slug-parasitic nematode *Phasmarhabditis hermaphrodita* in Chile, and tests for its use in biocontrol of slugs. The GMO Symposium had a panel of experts who gave presentations and then a discussion ensued. The panel consisted of Richard Stuckey (Exec. VP Council for Agricultural Science and Technology); Steve Taylor (University of Nebraska, Food Industry Complex); Susan Pitman (International Food Information Council) and Martin Marshall (Dept. of Agricultural Economics, Purdue University). The main points covered were: Stuckey: 'Need' for GMOs to feed the starving 3rd world countries (despite the more than adequate food supply - distribution is the main problem); claim that rain forests are being cleared predominantly by cropping farmers (despite the thousands of hectares cleared by Macdonalds for cattle grazing). Taylor: who spoke of the concept of substantial equivalence (between engineered food crops and conventional crops) which leads to the testing of food safety ONLY with no consideration of environmental or ecological effects. Susan Pitman: who spoke of consumer surveys on reactions to media information on GMOs. I found the survey questions largely biased, incorporating the answers within the question. Ms Pitman is paid for and responsible to the government and industry. Marshall: spoke of the example of Bt corn, and the refuge (20%) strategy. He questioned whether a 20% refuge is acceptable to 3rd world land-hungry countries, and also stated that only when corn borer infestation exceeds 40% does Bt corn become an economic proposition. All in all, an interesting airing of views with sins of both omission and commission!

Joint Meeting of the New Zealand Society for Parasitology and Australian Society for Parasitology

The Joint Meeting of the New Zealand Society for Parasitology and Australian Society for Parasitology was held in Wellington, New Zealand from September 24th to 28th 2000. Valerie Kempster attended this and presented (again, see above) her poster on induced resistance to *Heterodera trifolii* in white clover. There were very few plant-parasitic nematologists (so to say) at the meeting. It is a meeting traditionally dominated by animal parasitologists. However, it is a good forum in which to re-visit the discipline of parasitology. The topics of papers ranged from genetic to ecological and epidemiological and covered such studies as sensory responses of both plant and animal parasitic nematodes to semiochemicals. There were many references in other papers to the valuable contribution of the deciphering of the complete genome of *C. elegans*, which is helping in studies of resistance mechanisms of nematodes to nematicides, including Ivermectin. Warwick Grant gave a refreshing paper on the genomic front, in which he suggested that we do not need to just go on 'stamp collecting' ie. deciphering more and more genomes without first looking at the proteins produced by those genes and also at the ecology and physiology of the organisms concerned. In other words a warning, if you like against reductionism (ad absurdum!). On the whole organism positive side, there were reports of successful breeding programs for animals and plants resistant to various nematode parasites. There were an interesting couple of papers on the potential of the nematode *Strongyloides trichosuri* for the biocontrol of Australian possums which are a major tuberculous -carrying pest in New Zealand. Val met up with Gregor Yeates and Chris Mercer both of whom she 'knew by e-mail' and had some very good discussions with them on their own and her work. After the conference we travelled up the North Island taking in the local wines and sights. Altogether a worthwhile trip.

Research

OCCURRENCE OF ROOT LESION NEMATODES (*PRATYLENCHUS SPP.*) IN WHEAT AND BARLEY CROPS IN THE HIGH RAINFALL REGION OF VICTORIA

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Summary

A survey of wheat and barley crops in south-west Victoria was conducted to determine the occurrence and species of root lesion nematodes infecting plant roots. *Pratylenchus neglectus* was found to be the most prevalent nematode, with the species *P. thornei*, *P. crenatus* and *P. penetrans* also identified. The importance of these nematodes in the high rainfall cropping region is not known.

Introduction

In the higher rainfall regions of south-west Victoria there is a rapid expansion in the area of cereal production occurring with the decline in wool prices. Since this is a relatively new production area it is important to know the diseases that may limit production.

The root lesion nematodes *Pratylenchus thornei* and *P. neglectus* have been shown to reduce the yield of wheat crops in the more traditional cropping areas in south-eastern Australia (Hollaway *et al.* 2000, Vanstone *et al.* 1998). However, the distribution of these nematodes in the higher rainfall cropping zones or the presence of other root lesion nematode species is not known.

This survey of wheat crops in south-west Victoria was undertaken to establish the distribution and species of root lesion nematodes present.

Methods

In 1999, 43 wheat crops and 3 barley crops were selected at random to be inspected for the severity and cause of disease during the growing season. The cropping history of

each paddock was obtained from the grower. During September 1999, a total of 18 plants from five locations on a 150 m transect were dug from each paddock. The soil was washed from the roots and the plants delivered to the University of Adelaide for nematode extraction.

Roots were chopped into 1 cm lengths, and placed in a misting chamber. Nematodes were extracted from each set of 18 plants over 96 hours. Roots were sprayed with a fine mist of water at 25°C for a duration of 10 seconds at 10 minute intervals. Nematodes in 1 ml of the mister extract were counted, and average number of *Pratylenchus* per plant calculated.

Identification of the *Pratylenchus* species extracted from the wheat roots was conducted on 13 of the samples. Measurements and diagnostic characters were used to identify the species of root lesion nematode present. Characters used to identify the different species included a) tail shape, b) whether the tail was smooth or crenate/annulated, c) the number of head annules, and d) type and number of lateral lines. Measurements included a) length of stylet, b) body length, c) position of vulva as a percentage of body length, and d) ratio of tail length to tail width (c').

Results

The number of nematodes extracted from the plant roots was related to the cropping history (Table 1). The higher numbers of root lesion nematodes were extracted from paddocks with a history of crops (such as canola and wheat) susceptible to the root lesion nematode *P. neglectus* (Taylor *et al.* 2000). The lowest numbers were extracted from roots sampled from paddocks with a history of resistant crops (such as oats, barley or lupins) and pastures.

Table 1. Relationship between paddock history and number of nematodes extracted from the root system of plants collected from paddocks in south-west Victoria in 1999

Crop 1998	Crop 1997	Number of paddocks	Average nematodes per plant extracted
Resistant	Pasture	5	0
Resistant	Resistant	2	2
Wheat or Canola	Pasture	9	17
Pasture	Pasture	9	31
Turnip	Pasture	3	39
Wheat or Canola	Resistant	7	78
Wheat or Canola	Wheat or Canola	11	184

The results from identification of nematodes from the 13 paddocks are shown in Table 2. The predominant nematode identified was *P. neglectus*. *P. thornei*, *P. crenatus* and *P. penetrans* were also identified in some of the samples.

RESEARCH

Table 2. Identification of nematodes extracted from 13 of the paddocks from the survey of cereal paddocks in south-west Victoria in 1999

Paddock Number	Species	Nematodes per plant	Cropping History				
			1999	1998	1997	1996	1995
53	<i>P. thornei</i>	12	Wheat	Canola	Wheat	Wheat	Fodder
21	<i>P. penetrans, P. neglectus</i>	15	Wheat	Wheat	Canola	Wheat	Wheat
55	<i>P. thornei</i>	49	Barley	Wheat	Canola	Fallow	
46	<i>P. penetrans</i>	73	Wheat	Canola	Barley	Wheat	Canola
30	<i>P. neglectus</i>	101	Wheat	Turnip	Pasture	Pasture	Pasture
36	<i>P. penetrans</i>	121	Wheat	Wheat	Pasture	Pasture	Pasture
42	<i>P. neglectus, P. crenatus</i>	121	Wheat	Pasture	Pasture	Pasture	Pasture
56	<i>P. neglectus</i>	138	Wheat	Pasture	Barley	Pasture	Barley
47	<i>P. crenatus</i>	255	Wheat	Wheat	Canola	Wheat	Pasture
23	<i>P. neglectus, P. crenatus</i>	310	Wheat	Canola	Oat	Pasture	Pasture
24	<i>P. neglectus</i>	488	Wheat	Canola	Wheat	Clover	Pasture
20	<i>P. neglectus</i>	699	Wheat	Triticale			
18	<i>P. neglectus</i>	1157	Wheat	Canola	Wheat	Oat	Canola

Discussion

In most paddocks, only low numbers of root lesion nematodes were extracted from the plant roots, suggesting that these nematodes are not causing a major problem to cereal production in the higher rainfall region of Victoria. However, growing a number of susceptible crops in sequence was shown to result in higher nematode numbers, and therefore has the potential to cause yield loss to intolerant crops.

The identification of species of root lesion nematode other than *P. neglectus* and *P. thornei* in the high rainfall area is of significance. The effect of these nematodes on cereal crop yield in the high rainfall region of southern Victoria is not known. *P. penetrans* occurs in higher rainfall regions and has many hosts (Corbett, 1973), although it is more common in horticultural crops than in broad-acre crops. There is a possibility that several pathotypes of *P. penetrans* exist (Loof, 1991). *P. crenatus* causes damage to cereals in light sandy soils in Europe (Loof, 1991) and also attacks vegetable crops. It has been associated with the poor establishment of pastures on old meadow sites (Loof, 1991).

Further studies are required to determine the economic importance and host range of these other species of root lesion nematode in the higher rainfall regions of Victoria. Their identification using both morphological and molecular criteria may also need to be investigated, as complications may arise using the current diagnostic methods.

Acknowledgments

This work was supported by the Grains Research and Development Corporation through projects DAV428, DAS229 and DAS281.

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Review

PAECILOMYCES LILACINUS AS A BIOCONTROL AGENT

Macquarie University, Sydney, 2000

Summary of Rita Holland's PhD thesis

The studies presented in this thesis are based on the fungal species *Paecilomyces lilacinus*. This fungus is under development as a microbial biocontrol agent against plant parasitic nematodes, in particular root-knot nematodes, species *Meloidogyne*. Biocontrol of these pest nematodes offers an attractive alternative to the chemical nematicides currently in use. Effective biocontrol can only be achieved with a full understanding of the microbial pest control agent and the studies presented here go a long way towards achieving that aim.

P. lilacinus infection of eggs of *Meloidogyne javanica* was examined, mainly at the ultrastructural level. This work was preceded by the development of a new method for preparing nematode eggs of known age. It is clear that fungal hyphae of *P. lilacinus* penetrate eggshells of *M. javanica* with enzymes and pressure following the formation of a simple appressorium. The entire contents of the egg are then used as a food resource by the fungus, completely destroying the embryo/larva in the process. Eggs containing embryos or larvae can become infected by the fungus.

The preparation of *P. lilacinus* used in the field consists mainly of spores. Therefore the condition of the spores is important. The fungus was cultured under two sets of defined conditions to produce aerial spores, cultured on agar plates, and submerged spores, grown in a liquid medium. These two types of spores were then examined and compared in various ways. Submerged spores were found to be larger than aerial spores though with more variability in their size. Although not previously reported, rodlet layers were found on aerial spores of *P. lilacinus* and were found to be absent on the submerged spores. The ultrastructure of both spore types is described. No difference in nematode infectivity was found between spore types when used in a nematophagy assay. Viability of spores after drying suggested that aerial spores were more robust when compared to submerged spores.

There are many isolates of *P. lilacinus*. To test the ability of each isolate to infect nematode eggs, an assay using egg masses of *M. javanica* was developed. The assay was used to test over 140 isolates of *P. lilacinus*. Considerable variations in nematophagous abilities were found, with isolates varying in the percentage of infected nematode eggs and in the rate of infection of those eggs.

For any microbial biocontrol agent, its level of specificity to the target pest is important. For *P. lilacinus* to be used successfully against plant parasitic nematodes it should not parasitise other organisms in the soil environment to which it is applied. This

information is also required before a biocontrol agent can be registered. Many plant species were tested and fungal hyphae were never found in healthy roots, even when very large amounts of fungal inoculum were used. Additionally, the behaviour of hyphae of *P. lilacinus* on root surfaces and on egg surfaces was very different at the ultrastructural level. Invertebrate specificity was extensively studied and when *P. lilacinus* was used at 100x the dose invertebrates would be expected to encounter in the field, a few species suffered low mortality. However at a normal field dose *P. lilacinus* did not cause any mortality to the invertebrates tested.

It is important to know what will happen to a biocontrol agent after it has been applied. The persistence of *P. lilacinus* after application to soil has been estimated and results indicate that levels fall after application until after a few months it is very difficult to isolate the fungus from the soil. This suggests that *P. lilacinus* will only cause a short-term disturbance to the soil biota and will not have any long-term effects.

The genetic relationships of many isolates of *P. lilacinus* were explored using the technique of long-primer random amplified polymorphic DNA analysis. A large amount of variation was detected among the isolates tested, with no correlation between site of isolation and genetic relatedness. Results generally supported earlier work carried out using allozyme electrophoresis. Using five primer pairs it was possible to differentiate the isolates tested from each other. This method would therefore be suitable for the identification of proprietary strains.