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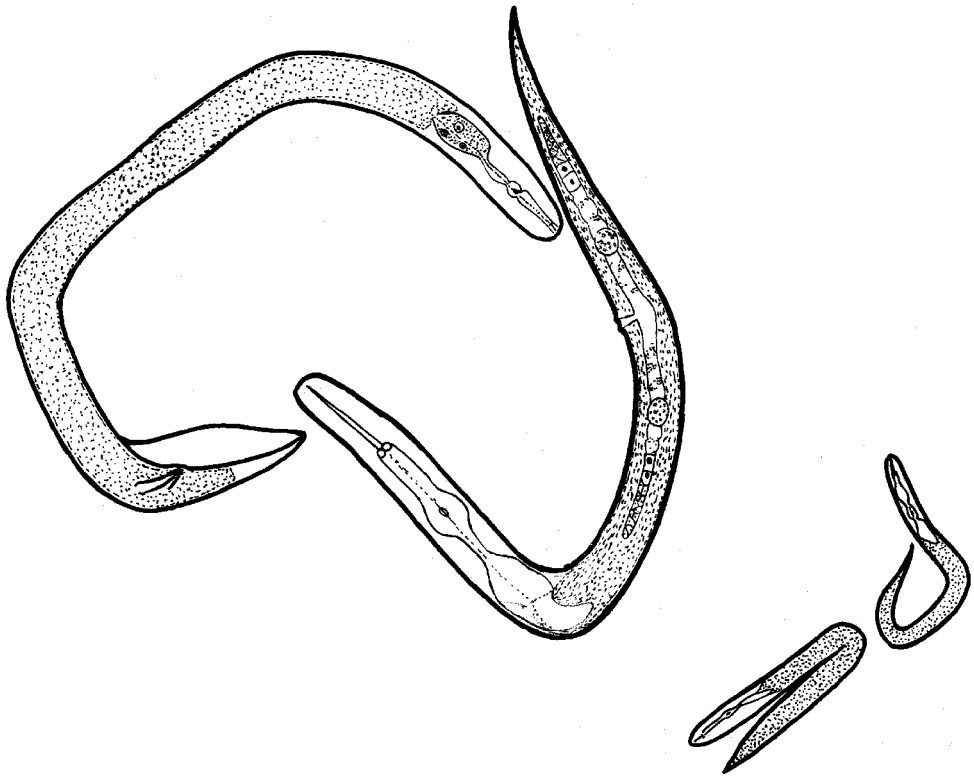
AUSTRALASIAN NEMATOLOGY NEWSLETTER



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From the Editor

The year 2004 was a challenging one for nematology in Australia, and 2005 promises to be equally so. Hopefully, we have all enjoyed a break over the Christmas/New Year period, and are ready to tackle the issues/problems of the next year. Many thanks to the contributors to this newsletter.

Given the recent introduction of the new Privacy Laws, we are not sure of our position with respect to inclusion of membership lists. We may need to ask members for their permission to have their names published in the newsletter. Thus, there is no list in this issue. We are seeking clarification of this issue, and would be pleased to hear from anyone who has already faced a similar problem.

July Issue

The deadline for the July issue will be June 30th. I will be overseas in June, and will remind you at the end of May. Please have your material ready once again.

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From the President

You may recall my last note about the bid put forward by AAN for the 5th International Congress of Nematology. Well…our bid won, and we have been selected to host the Fifth International Congress of Nematology! To paraphrase one of the honorary committee "what the !@#$ have we let ourselves in for?"

I see this as an opportunity to provide nematology in Australia with a much higher profile. If the conference is organised and promoted well, we will have the world experts on every branch of non-helminth nematology (with the possible exception of *C. elegans*), in Australia all at the same time. This result will hopefully see unprecedented publicity, and maybe even identification of some nematode issues we didn't know we had! But seriously, this is an opportunity to use the conference to use the expertise of nematologists from all over the world, and there should be some excellent opportunities for sponsorship of visits to laboratories. If handled well, it should be a big plus. If everyone goes off and does their own thing irrespective of what others are doing, the total sponsorship may be much less. So, please, let's all co-operate on this aspect.

The first thing to do in organising a congress such as this is to rope in as many people to help as possible. Some people have already indicated that they are willing to help, but more hands make light work. There is a list of potential tasks at the bottom of this note, so if anyone feels willing and able to assist in any of these, please let me know. Having got the AAN into this, I will be shouldering a lot of the work, and that includes co-ordinating helpers. I do not claim omniscience on conference organisation, so if there is anything not on the list that should be, again let me know.

Much of the drudgery can be handled by Professional Conference Organisers (PCO's), but the experience of the Organising committee members of other scientific conferences that I have spoken to indicates that someone needs to keep looking over the shoulders and checking that things are being done, not just adequately, but how we want them done. The items labelled "organiser" in the table should only need someone like this to oversee what the organisers are doing.

I look forward to a whirlwind of interest, followed by an avalanche of offers and a flood of names. (If I do too much of the organisation, you will have to put up with a lot more humour like that, so be warned.)

Mike

Tasks/roles to be filled ([1] = oversight of PCO)

deputy convenor
conference organiser selection
liaison with conference organiser
conference finances
AAN finances
legals and AAN
sponsorship & exhibitors
marketing to delegates
logo & theming
scientific program committee
satchells and information
Handbook
government liaison
liaison with other nematology societies
conference tour scientific
conference tour buses etc[1]
associates program[1]
Venue[1]
Accommodation[1]
Catering[1]
conference dinner[1]
audio visual[1]
conference photograph[1]
poster session arrangements[1]
registration arrangements[1]
social events[1]
Web

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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 2005 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 $1300 (+GST) for 1 week, including all course materials. A minimum of 8 participants is required for the course to proceed.

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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We have run this course biennially for the last few years. However, because of changes in the level of support provided for nematology and other commitments by the co-ordinators, this may be the last course run for a few years. So if you are interested, don't wait for the next course, please consider attending this one and contact us as soon as possible.
The dates proposed are from 28th November to 2nd December 2005. This is the week before the Australian Entomological Society and Invertebrate Biodiversity Conference from which is 4th to 9th December.

Regional News

NEWS FROM SOUTH AUSTRALIA

News from The University of Adelaide

Ian Riley's position as Lecturer in Nematology at The University of Adelaide finished at the end of 2004. This essentially brought to an end the long history of plant nematological research and teaching by staff at the Waite Campus of the University. Ian's departure is a significant loss to the pest management group in Plant and Pest Science, and to the study of nematodes in Australia. Despite the difficulties of the past few years, he has maintained a strong commitment to teaching and research. Ian's ties with the University will not be severed completely as he will work one day per fortnight at the University, and will continue to supervise the current nematology postgraduate students**.** Ian will take up a position as a pasture pathologist with SARDI in January.

Congratulations to Imelda Soriano, who graduated in December. The abstract from her Ph D thesis is included under 'Research' in this newsletter.

Kerrie Davies visited Janine Paynter (nee Lloyd) in Auckland in November. In addition to happy hours spent playing with Janine and Quentin's beautiful daughter Jennifer, she did some collecting with Nick Martin, and was successful in isolating the nematode associated with the new species of *Fergusonina* fly found from galls on *Metrosideros excelsa* (pohutukawa) in New Zealand. It is now about 15 months since Nick and Trevor Crosby first collected the fly; very exciting for those in the Ferg world as it was a record from both a new host genus and from a new nation.

Kerrie Davies.

NEWS FROM QUEENSLAND

News from DPI&F

(*Editor's note:* The following was submitted for the July newsletter, and accidentally omitted. My apologies to everyone, and particularly Jenny Cobon, for this 'blue'.)

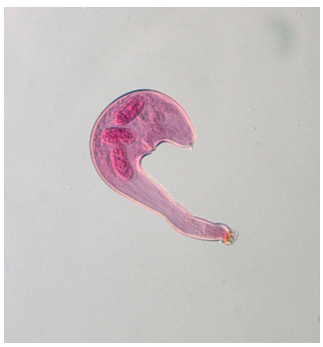
Both Tony Pattison and I attended the 3rd Australasian Soilborne Diseases Symposium in the beautiful Barossa Valley in February, 2004 where Tony gave two presentations on soil health. We had first attended the Nematology Resistance Workshop at the Waite Campus organised by Ian Riley and Sharon Taylor where it was great to catch up on all the news of the nematological

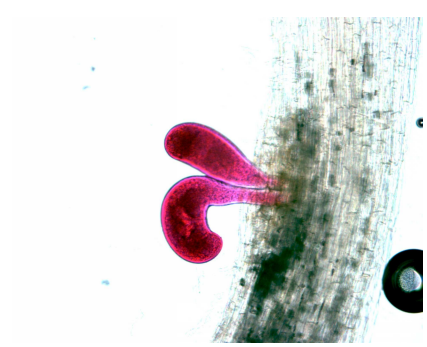
worlds of Australia and New Zealand. The associations' AGM was held following the workshop where I handed over the reins as editor of this newsletter to Kerrie Davies. I have thoroughly enjoyed my time as editor and would like to thank members of the association for that opportunity.

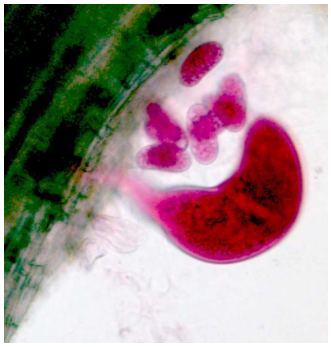
Tony continues to unlock the secrets of soil health in north Queensland in the banana industry while I try the same in the south of the state in bananas, macadamia and pineapples. Recently two students from The University of Queensland have undertaken nematological projects at the Indooroopilly laboratory as part of the course work for their studies.

Ms. Janelle Trot, almost at the completion of a Science/Education dual degree, tested the resistance of three strawberry varieties to four common species of root-knot nematodes. Janelle found that strawberry varieties were equally susceptible to all species of root-knot nematodes and not only to *Meloidogyne hapla* as previously reported. We have submitted an abstract on our findings for inclusion at the upcoming Strawberry Symposium at Coolum on our Sunshine Coast in September. We look forward to presenting a poster on the results of the study at this symposium.

Mr. Jose Rafael Mangue, an entomologist from Mosambique, here on post-graduate studies, looked at the resistance of cotton varieties to *Rotylenchulus reniformis* (reniform nematode). Jose was able to prepare slides of these nematodes and with the help of Dr. Roger Shivas, (Mycologist, DPI&F, Queensland) photograph these incredibly shaped reniform nematodes in the roots of cotton for inclusion in his report.







We wish Janelle and Jose well with their further studies as we get back to secrets of healthy soils.

Jenny Cobon.

NEWS FROM WESTERN AUSTRALIA

News from WA State Agricultural Biotechnology Center (SABC), Murdoch University.

Professor Mike Jones and the Plant Nematology Research Group at the SABC have made excellent progress in the past six months. Over $600K of research funds have been obtained from the Australian Research Council, for one ARC linkage project (commenced July 2004) and two new projects (2005-2007). One research paper has been published in '*Molecular Plant Pathology*' and two others submitted.

Dr. Zhaohui Wang is currently employed on the linkage project "A new approach to control of plant parasitic nematodes". This project was originated from a conversation between Mike and Professor James Dale, from Queensland University of Technology (QUT), during an International Plant Molecular Biology conference in Spain in 2003. These two Principle Investigators (PIs) are combining their knowledge of plant-nematode interactions and plant virology to develop a new strategy to engineer plant resistance against root-knot nematodes. This project is also supported by an industrial partner, Aztech Investment Group, a Perth based company which is developing its business into the biotech area. In September, Mike and Zhaohui visited James and his lab at QUT, together with Mr. Mike Lee (Marketing Manager of Aztech), and the first group meeting of this linkage project was held. The lab work for this project will start in the New Year.

Also in September, Zhaohui was invited to be part of a delegation of about 30 Chinese scholars, who are working at universities and institutes in Australia, organised by the Chinese Embassy, and supported by the Department of Education of China, for a trip to Inner Mongolia and Xinjiang, two provinces in the North-Western region of China. The aim of this trip was to develop collaboration between scientific researchers in these two countries, and to increase the strength of the research work in China, especially in Agriculture. Zhaohui met two nematologists in Xinjiang, where there are some sugar beet cyst nematode problems. They had extensive discussions on potential collaboration, including diagnostics. Funding applications to the Chinese Government have been resulted from this trip.

Dr. Modika Perera has just completed her ARC funded linkage project, jointly with Dr. Vivien Vanstone at Department of Agriculture WA. The focus of this two-year pilot project was to develop 'proof-of-concept' for a novel approach to nematode identification that of protein profiling using MALDI-TOF mass spectrometry. She has developed two methods to generate samples for MALDI-TOF ms analysis. Typical protein profiles and diagnostic peaks have been identified for following nematode species: *Anguina funesta*, *Anguina tritici*, *Ditylenchus dipsaci, Meloidogyne javanica* and *Pratylencus neglectus.* She has also analysed root samples collected from several vegetable crops, trees, and weed plants in the Perth metropolitan area and WA wheatbelt. These have been analysed using esterase isoenzyme phenotyping, protein profiling and DNA profiling to identify RKN species. For example *M. javanica* and two other unidentified variants were found in Paulownia tree plantations in WA.

Kerry Ramsay, an Honours student with Mike and Zhaohui, has submitted her thesis in November and graduated with a First Class degree. Her Honours project was to develop and use Laser Capture Microdissection (LCM) to obtain pure giant cell cytoplasmic contents from root-knot nematode infected roots, and analyse specific gene expression in giant cells using the RNA isolated from the LCM samples. With the successful development of the LCM technique, she has also attempted to construct a cDNA library from the cytoplasm of young giant cells at 4 days post infection. Although the construction of giant cell specific cDNA library still needs to be optimised, the application of the LCM technique on plant-nematode interactions has been published (see Research Abstract). It is an excellent approach to obtain un-contaminated feeding cell contents from early stages in their development.

The Plant Nematology Research Group has one Visiting Scholar, Mr. Jiangyong Zeng, from Tibet. Jiangyong's visiting is supported by the Chinese government, and he will work with us till May, 2005. He has been helping Angela Hollams, a PhD student who is developing *in situ* RT-PCR to localise specific gene transcripts in giant cells and surrounding root cells.

Mike Jones, Zhaohui Wang, Modika Perera, Angela Hollams, Kerry Ramsay, Jiangyong Zeng.

NEWS FROM NEW ZEALAND

News from Dunedin and the Most Southerly Nematologist in the World (maybe?)

Hi! I (David Wharton) am currently on study leave at Miami University in Ohio (no, not Florida – confusing isn't it). I'm working with Prof. Rick Lee Jr. here and his cryolab where they work on freezing tolerant insects, frogs and turtles. I'm, of course, working with nematodes and looking at some species that I can't access in New Zealand. Cultures have been kindly supplied by Paul DeLey, at the Nematology Dept, UCLA, Riverside and by the *Caenorhabditis* Genetics Centre. I visited the Nematology Dept on my way here and gave a seminar (I've given 4 seminars while I've been here: at Riverside, Miami University, Ohio State University and the University of Notre Dame, on 'An Ice-active Protein from an Antarctic Nematode'). The idea of the project is to compare the freezing survival of a variety of nematodes with that of our Antarctic nematode (*Panagrolaimus davidi*), to test them for recrystallisation inhibition (which prevents the growth of ice crystals and seems to be important for the survival of *P. davidi*) and to use an antibody probe against our *P. davidi* ice-active protein to see if they possess similar proteins. So far the work is highlighting the uniqueness of our Antarctic species.

Meanwhile back in New Zealand, Tim Smith (MSc student) has been doing similar work with other nematodes – with similar results. Most don't have recrystallisation inhibition, although he's found one species (*Steinernema carpocapsae*) that does. Sarah Spalding (MSc student) is looking at using nematodes as indicators of biodiversity in soil and comparing organic and conventional kiwifruit farms. Melianie Raymond has just completed a BSc Hons project where she confirmed that *P. davidi* survives intracellular freezing and showed that the nutrient status of the nematode was critical for this ability. Melianie is starting a PhD next year working on Antarctic nematodes but she may also get the opportunity to do some work in the Arctic. Yvonne Bazin is doing an MSc project next year on the freezing tolerance of frogs in New Zealand. I'm co-supervising a Marine Science PhD student next year, part of whose project will look at marine nematodes and a Food Science student working on freezing oyster gametes.

So looks like another busy year but for now I'm looking forward to meeting up with my family next week for a big holiday (Disney World, Florida, New York and San Francisco)!

David Wharton.

Research

MOLECULAR TECHNIQUES CAN BE VALUABLE TOOLS IN STUDYING NEMATODE ECOLOGY – A 2004 PERSPECTIVE

Gregor Yeates

Landcare Research, Private Bag 11052, Palmerston North, New Zealand

Many of the papers at the XXVII International Symposium of the European Society of Nematologists (held in Rome, June 2004) and the 43rd Annual Meeting of the Society of Nematologists (held in Colorado, August 2004) applied molecular techniques to their problems. Previous meetings have been flavoured with 'current' novel techniques, be they transmission electron microscopy, electrophoresis, scanning electron microscopy or computer-based analytical tools, and, of course, the study of plant and soil nematodes was not possible until the application of light microscopy. These are all tools that may be used to address questions of nematode morphology and biology. While each tool may permit new, and possibly highly specific, questions to be addressed or allow observations at a finer scale the fundamental questions of the structure, function, biology and ecological relationships (including pathogenicity to plants, invertebrates and vertebrates) of nematodes remain. Without such understanding, and the framework it provides, detailed knowledge of differences in, for example, metabolic processes or 18S rDNA sequences can only be fragmentary.

The scientific community has seen nematodes as front cover features in high-profile scientific journals in recent years, and awareness of the phylum has increased. *Caenorhabditis elegans* was the first multicellular animal for which the whole genome was sequenced (CSC, 1998) and, while this represented a great advance in knowledge of developmental cues and processes, the natural history (i.e. field biology) of *C. elegans* is unknown. The award of the 2002 Nobel Prize for Medicine to Sydney Brenner, Robert Horvitz and John Sulston truly reflects the intrinsic value of that advance. The 'molecular evolutionary framework' for nematodes described by Mark Blaxter et al. (1998) used results from the initial decade of molecular studies to complement 150 years morphological observation. Some long-held tenets were shattered; many long-standing questions of nematode relationships remained unresolved. Recent soil-related enquiries include relationships within the "Rhabditidae" (Kiontke et al., 2004), "Cephalobina" (Nadler et al., 2004) and "Dorylaimida" (Mullin et al., 2004a).

In Rome, August Coomans (2004) emphasised that limited knowledge of functional morphology is a shortcoming of classical taxonomy and noted that, while tools such 'DNA bar-coding' may provide rapid assessment of biodiversity, morphological knowledge is still needed. A comparison of 18S sequences with morphological analysis of nematodes from an arable field at SCRI showed a poor match between data types (Griffiths et al., 2004). Rhabditida represented 30% total numbers but only 2% of clones; Mononchida were 4% of numbers but 33% of clones. However, clone numbers and nematode biovolume showed better fit, being 4% and 35% respectively. That is, at the assemblage level molecular analysis may be as quantitative as the detection of eggs of *Globodera* spp. in soil. Coincidentally, underpinning studies on biovolume in the phylum are emerging (e.g., Yeates & Boag, 2003). Various other groups are 'bar-coding' nematode assemblages with a view to quantitative population studies (e.g. Mullin et al., 2004b)

The distribution of nematode species and the makeup of nematode assemblages have long been known to be influenced by soil structure, and the texture of aquatic sediments. Across "13 pasture sites examined the greater importance of the soil rather than month, year or management practices in determining the composition of the nematode fauna is clear" (Yeates, 1984) and a recent study of experimental perturbation found that "soil factors determined nematode community composition" (Griffiths et al., 2003). The inherent nematode diversity in a particular substrate must affect how that assemblage responds to natural, agricultural or experimental perturbation. Some comparisons of nematode assemblages in nearby cultivated and 'natural' sites are of dubious value because the underlying reason why land managers (i.e., farmers) chose, 50 or 100 years ago, may have been based on differences in the suitability of soils for cultivation (i.e. the baseline nematode assemblages probably differed). It is tempting to suggest that nematode assemblages are most diverse in sandy loam soils, but the dominant effect of plant inputs coupled with more subtle management effects rule out any generality (Yeates & Boag, 2004). In deep-sea marine substrates organic inputs may fall from 5000 m above and high species richness is mediated by non-equilibrium patch dynamic interactions (Lambshead, 2004). Extreme nematode diversity in tropical forests may reflect the interaction of a species-rich vegetation with a multitude of arboreal, litter and soil microhabitats for nematodes. Care is needed in interpreting the distribution of plant-feeding nematodes with moderate host-specificity. Such nematodes will only be common in soils in which the host plant is grown and plant-growth requirements may mean the host is preferentially grown in certain soil types, and it is in such soils the nematode will be likely to be found. Substrate texture, organic inputs and disturbance are all important.

While I have previously suggested that 'disappointing' differences in nematode assemblages among treatments found by Freckman & Ettema (1993) may have been due to samples being collected early in spring before populations became active, the possibility of a low basal diversity may have also contributed. Some management effects on nematodes are totally explicable. For example, following tillage Lenz & Eisenbeis (2000) found 'the density of plant parasitic nematodes decreased and the density and dominance of bacterivorous nematodes increased'. i.e., the live root resource decreased and the bacterial resource, utilising decaying roots, increased. A metadata analysis by Fiscus & Neher (2002) indicated some nematode genera to be more affected by physical stresses and other genera relatively more affected by chemical stresses. That many such differences occurred within many nematode groups (e.g. Mononchida) indicates they do not represent a simple nematode effect and may reflect ecological conditions – perhaps mediated by the various soils in which the original studies were undertaken.

While soil texture is a guide to soil pore size the real factor controlling resource use by nematodes is soil structure, and Jones et al. (1969) clearly drew attention to this, with Elliott et al. (1980) discussing habitable pore space. Soil structure and pore size distribution can be modified by management. In agricultural soils decline in soil structure is associated with a need for greater inputs and lower yields (Sojka et al., 2003). In a classic paper, Ingham et al. (1985) demonstrated that microbial grazing by bacterial-feeding nematodes increased the turnover of plant nutrients (specifically nitrogen). Elliott et al. (1980) showed that where narrow soil pore necks protected bacterial populations from grazing by nematodes protozoa could graze on the bacteria and they, having moved into larger pores, were in turn fed on by nematodes – again biological diversity was important. These positive effects of nematodes on nutrient cycling were reinforced when Yeates et al. (1999) showed that increased 'leakage' of photosynthate from plant roots feed on by a range of stylet-bearing nematodes could results in increased soil microbial biomass. Soil microbial biomass is the 'eye of the needle through which all plant nutrients must pass' (Jenkinson, 1977).

Nematodes in soils typically move in water films, and Yeates (2004) described them as pellicole nematodes. Compared with truly aquatic nematodes, pellicole nematodes benefit from the surface tension of the meniscus in locomotion and from gaseous diffusion between the water films and soil air. Studies in undisturbed soils showed that populations of *Rhabditis*, *Pristionchus* and *Cephalobus* all increased as long as the soil was above wilting point; increasing bacterial concentrations as water content declined was seen as a positive effect (Yeates et al., 2002). Under these conditions relative population growth rates differed from those implied by *c–p* values used in the nematode Maturity Index, but the larger populations of *Cephalobus* agreed with observations in low input field plots, and with experiments under greater moisture tensions. Population studies of bacterial-feeding nematodes on agar plates appear as easily transferred to real soils as do the attractant results of Green (1967) for *Heterodera* and *Globodera* spp. That is not to say that relative reproductive rates of bacterial-feeding nematodes found in a single soil cannot validly be applied across treatments in the same soil (Ferris et al., 2001). Similarly, long-term vegetation plots on uniform mine waste exposed to the same colonising propagules are likely to develop nematode assemblages that can validly be compared (Hohberg, 2003).

Plant cover and soil structure are not homogeneous. Those studying nematode populations in crop fields have long struggled with heterogeneity. On one hand, precision agriculture can allow for intra-field variability. On the other hand, knowledge of short-range variability can be used, for example, to select better performing plants or to investigate factors controlling nematode populations and their interactions (Ettema & Yeates, 2003). One recently documented aspect is that plant species identity, as it affects the quality of resource input to the soil, is more important that resource quantity in terms of different nematode trophic levels in soil food webs (De Deyn et al., 2004). As was said some time ago, 'plants affect nematodes' (Yeates, 1987) as well as plant-feeding nematodes causing economic crop loss.

In the absence of plant cover unintentional alterations in soil moisture have been found by Nkem et al. (2004) to have potentially important implications for nematode assemblages in Antarctic dry valleys – basic observational biology.

Some entomophilic nematodes seem to deny the concept that nematodes require free water for their activity; how *Noctuidonema* survives, indeed generates eggs, when attached to the outside of noctuid Lepidoptera warrants curiosity-driven investigation. The recognition and successful deployment of *Deladenus* in biological control of *Sirex* was a pioneering Australasian advance. Our ignorance of nematode / microbe / arthropod / tree relationships remains great, but there is, in Australasia, awareness of potential problems (e.g., Sathyapala, 2004; Zhao, 2004). Studies on such organism complexes are continuing elsewhere (e.g., Carta et al., 2004), the number of nematodes per arthropod vector is being investigated (Jikumaru & Togashi, 2003; Ćurčić et al., 2004) and there is active microscopic and molecular work to differentiate *Bursaphelenchus* spp. (e.g., Wang et al., 2004; Ye et al., 2004).

Building on the work of Ingham et al. (1985) there have been many demonstrations of the benefits to nutrient turnover of bacterial grazing by nematodes, and of various interactions among nematode feeding or functional groups (Yeates et al., 1993). It is becoming increasingly clear that the functional groups are rather arbitrary; in particular several Tylenchidae can be reared on fungal hyphae (Okada & Kadota, 2003), and 'omnivores' and 'predators' respond similarly to environmental perturbations suggesting similar ecological relations (Yeates et al., 2003; De Deyn et al., 2004). Re-evaluation of nitrogen mineralisation by microbial-feeding nematodes suggests that whereas bacterial-feeding nematodes significantly enhance mineralisation at 'typical' soil C/N ratios (~10–15) for hyphal-feeders that occurs only at C/N ratios >15 (Yeates & Boag, 2004). Bacteria and low soil C/N ratios are associated with 'fast' turnover while fungi and higher C/N ratios relate to slower, apparently more sustainable ecosystems (Ruess, 2003), including organically managed pastures (Yeates et al., 1997). As

agricultural systems move towards lower input, more sustainable management and as nutrient fluxes in natural ecosystems are addressed more attention will need to be paid to the role of hyphal-feeding nematodes in ecosystems. Use of a molecular tool to better understand the role of nematodes in soil food webs is the sequencing of gut contents of microbial-feeding nematodes by Winkler & Adams (2004). In microarthropods, nitrogen isotope ratios have proved useful in differentiating niches among mites (Schneider et al., 2004) and our nematological colleagues at Scottish Crop Research Institute seem to have access to similar facilities (Neilson et al., 2002).

Studies of all nematodes in soils must embrace soil structure, soil moisture, nutrient fluxes, and microbial populations, as well as the plants and soil fauna with which many are familiar. Recent studies significantly advancing our understanding have been in ecosystems as diverse as native forest in New Zealand (Wardle et al., 2001), grazed Dutch pastures (Mulder et al., 2003) and vegetable production in California (Ferris et al., 1996). While molecular techniques are useful tools, scientists must manipulate and interrogate nematode populations using a variety of methods so that they answer meaningful questions.

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APPLICATION OF LASER CAPTURE MICRODISSECTION TO STUDY GENE EXPRESSION IN EARLY STAGES OF GIANT CELLS INDUCED BY ROOT-KNOT NEMATODE

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Despite causing millions of dollars in crop losses annually, very little is known about the complex interactions that occur between root-knot nematodes (*Meloidogyne spp*.) and host plants, which result in the induction of feeding cells called giant cells in host roots. Study of molecular events during the formation and development of giant cells has been limited because of the difficulty of obtaining pure cytoplasm specifically from these highly specialised cells. Until now, extraction of cytoplasmic contents from individual giant cells has only been achieved by micromanipulation at later stages of giant cell development. In this study, laser capture microdissection (LCM) has been used to isolate cytoplasmic contents from paraffin-embedded sections of 4 day old giant cells induced in tomato roots. Total RNA was isolated, and used in RT-PCR to investigate expression of specific genes in giant cells. Two D-type cyclin genes, Le*CycD3*;2 and Le*CycD3*;3, were expressed at higher levels in the giant cells compared to other cell cycle related cyclin genes, suggesting that the induction of the G1 phase of the cell cycle may be triggered in response to stimulation by the infecting nematode.

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NOTES FROM TURKEY

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Since 2001, the Turkish Ministry of Agriculture and Rural Affairs (MARA) and the International Maize and Wheat Improvement Centre (CIMMYT) Turkey office have been conducting a joint project on the importance and control of cereal nematodes including Cyst and Lesion in the winter wheat production areas of Turkey. This research is under the coordination of Australian Nematologist Dr Julie Nicol (CIMMYT Pathologist) and Turkish Pathologist Dr Necmettin Bolat (MARA). As in Australia, there is widespread distribution of *P. thornei* and *P. neglectus*. Unfortunately, the situation with Cyst Nematode is much more complex than in Australia as a number of species have been identified (predominantely *H. filipjevi* and also *H. latipons*) and we have very little information about their population dynamics, possible pathotypes and options for their control.

Key objectives of this National/International collaboration in Turkey on winter wheat are;

1. distribution studies
2. yield loss and population dynamic studies
3. idenification of sources of resistance and their susequent incorporation
4. investigation of other control methods (particularly rotation and crop management practices)
5. training.

The outcomes of this work will have regional implications throughout West, Central Asia and North Africa as part of the CIMMYT/ICARDA/Turkey mandate for winter wheat and also global benefits through the incoporation of identified resistant sources into both (International) spring and winter wheat backgrounds.

As Cyst and Lesion nematodes are also important to Australia we welcome any collaboration and scientific exchange in the area.

Below are three abstracts prepared by Turkish colleagues working in specific areas. The first abstract is currently being prepared for publication in an international journal and the following two abstracts were presented at the National Plant Pathology Congress in Turkey in September 2004.

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The relationship of temperature to the hatching of cereal cyst nematode, *Heterodera filipjevi* Madzhidov *in vitro* and under field conditions

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Heterodera filipjevi is a yield constraint on cereal production in Russia, Sweden, Spain, Bulgaria, Iran, India and Central Asia and Turkey (Nicol *et. al.*, 2004). Hatching of *H. filipjevi* over two growing seasons was monitored in two cereal fields in the Central Anatolian Plateau in Turkey, which is a continental winter wheat growing environment. Relationship of hatching with temperature was investigated. An *in vitro* experiment was conducted on hatching of *H. filipjevi* at constant temperatures.

Soil samples were collected at fourteen times from 5 designed plots of susceptible winter wheat variety Bezostaja1 in Cifteler, Eskisehir and Haymana, Ankara during the 2002/ 2003 and 2003/ 2004 growing seasons. Cysts and juveniles were extracted and hatching percentages were evaluated.

Mean peak hatching was observed in November (11.4 %) and March (11.1 %) and at soil temperature of 8.8 and 3.7 ^{o}C in Çifteler and Haymana, respectively, in the first growing season. In the second growing season, peak hatchings were observed in November (10.3 %), December (10.6 %) and March (38.4 %) in Haymana. Hatching percentages could not be monitored in the second year in Cifteler because of the low nematode numbers in the field. Total hatching percentages were 18.9 % in Çifteler and 20.1 % in Haymana in the first growing season and 67.6 % in Haymana in the second growing season.

In vitro experiments were carried out with a population of *H. filipjevi* from Karapınar – Konya, in the Central Anatolian Plateau. The optimum hatching percentages were at 10 ^{o}C (40.5%) and 18 ^{o}C (42.8 %) after 15 days. Hatching was significantly lower at 7 ^{o}C (15.2 %) and there was no hatch at 0^{o}C. The *in vitro* and field hatching percentages did not correlate with each other in terms of temperature suggesting that factors other than temperature also contribute to hatching of this species in the field.

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Nicol, J.M., Rivoal, R., Taylor S. & Zaharieva, M. (2004). Global Importance of cyst (*Heterodera spp.*) and lesion nematodes (*Pratylenchus spp.*) on cereals: yield loss, population dynamics, use of host resistance and integration of molecular tools. Nematology Monographs and Perspectives 2, 1-19.

In vitro Inhibition Activity of Actinomycete Isolates Against Second Stage Juvenile Mobility of Cereal Cyst Nematode, *Heterodera filipjevi* (Madzhidov) and Root Lesion Nematode, *Pratylenchus thornei* (Sher and Allen)

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Soil and rhizosphere colonizing microorganisms that promote plant growth and protect roots from pathogens have been promised for nematode antagonistic activity through their metabolic secretions and parasitic activity (Nitao et. al., 1999; Neipp and Becker; 1999; Kerry, 1987). One hundred and twenty six isolates of actinomycetes were screened for *in vitro* nematode inhibitor activity against Cereal Cyst Nematode, *Heterodera filipjevi* and Root Lesion Nematode, *Pratylenchus thornei.*

Culture fluids of the isolates were applied on an average 25 second stage juveniles of *H. filipjevi* and *P. thornei*, respectively, in 24 well microtitre plates, with 4 replications. Motionless juveniles were counted 1 and 3 days later. At the 3rd day, vitality of juveniles was tested with New Blue R stain (0.5 %) (Shepherd, 1962). An insecticidal avermectine ($3x10^{-4}$ g/ ml) compound (Hague and Gowen, 1987) and growth medium were used as positive and negative controls.

Inhibition activities of 81.4, 89.8, 83.8 and 76.5 % were found with isolate 32.09, 33.07, 33.10 and 37.02, respectively, at the 3rd day of the experiment against *H. filipjevi*. The isolates 31.02, 31.05, 32.08 and 33.12 inhibited the juvenile mobility of *H. filipjevi* at the range of 49.5, 36, 79.5 and 42.4 % at the first day of the experiment. Isolate 32.08 and 115.01 had 65.1 and 61.8 % inhibition activity, respectively, against *P. thornei* after 3 days. Isolate 2.07 had the highest inhibition rate (39.7 %) against *P. thornei* after 1 day. Immobile juveniles did not stain with New Blue R after 3 days, neither in actinomycete culture fluid treatments nor in positive control wells for either nematode. All active actinomycete isolates were identified as *Streptomyces* sp. at the genus level. Overall, higher inhibition rates were observed against *H. filipjevi* than *P. thornei*. Susceptibility of *H. filipjevi* second stage juveniles to physicochemical changes in the medium could be a reason for this. The rate of inhibition of second stage juvenile motility of both nematode species increased with time of exposure to the culture fluids. However, the rate of inhibition of *H. filipjevi* juvenile mobility in isolate 32.08 treatments was higher at the first than the 3rd day. Isolate 32.08 had a higher inhibition rate against *H. filipjevi* at first day (79.5 %) and against *P. thornei* second stage juveniles at third day (65.1 %).

Meyer et. al. (2004) pointed to the usefulness of effective compounds as biological control agents against multiple nematode targets. Using this concept, isolate 32.08 could be investigated as a potentially useful biological control agent against multiple nematode targets, including *H. filipjevi* and *P. thornei.*

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Susceptibility of Wheat to Cereal Nematodes through Greenhouse and Field Experimentation

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Cereal cyst nematodes (*Heterodera* spp.) and root lesion nematodes (*Pratylenchus* spp.) are documented to cause significant yield losses around the world. In Turkey, surveys have been conducted and indicate the widespread distribution of both cyst and lesion nematodes on cereals in the Central Anatolian Plateau. Yield loss and susceptibility (hosting ability) of cereal cultivars (10 wheat, 1 triticale and 1 barley) has been assessed under natural nematode populations in wheat fields in Çifteler-Eskişehir and under greenhouse conditions with *Pratylenchus thornei, P. neglectus* and *Heterodera filipjevi*. For *H. filipjevi*, the reaction of cereal cultivars from greenhouse and field studies was highly correlated (R= 0.57). Karma triticale had some degree of resistance, all bread wheat cultivars were considered susceptible, and Kalaycı barley appeared the most susceptible. Data for lesion nematodes was only considered from the greenhouse and clearly demonstrated that there is no relationship between lesion scores and the final numbers extracted for either *P. thornei* or *P. neglectus*. Both species show different varietal reactions to the number and multiplication rate of the nematodes ($P<0.05$) indicating that the pathogenicity of the two species is different. All cereal cultivars can be considered susceptible to both *P. thornei* and *P. neglectus*. Karma triticale had partial resistance to *P. neglectus*, but was highly susceptible to *P. thornei*. A clearly defined screening program is needed to assess resistance to each of these species of nematodes.

Resistance of Selected Strawberry Cultivars to root-knot nematode species (*Meloidogyne* spp.)

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Introduction

Queensland is the largest strawberry-producing state in Australia, generating earnings of $65 million in 2002. Strawberry production in Queensland is typically over the winter and spring, while other states of Australia produce during the summer and autumn period. This has resulted in a year round supply of strawberries to the Australian market, due to strawberry production in the warmer Queensland winter climate.

Plant-parasitic nematodes have the potential to severely reduce strawberry production worldwide (Brown et al., 1993). Strawberries have traditionally been grown in temperate regions, where *M. hapla* predominates. When strawberry runners are planted into infested soil, the presence of galls 6-8 weeks later are believed to indicate the presence of *M. hapla* (Stirling *et al*.1998). *M. hapla* is considered the only one of four common root-knot nematode species present in Queensland that would parasitise strawberry roots. *M. incognita, M. javanica, and M. arenaria* were originally reported to be a non-host on strawberry (Sasser 1954 and 1966, Kirby *et al*. 1975).

To limit the impact that nematodes have on strawberry production in Australia, the Strawberry Growers Runner Scheme (SGRS) was established. As part of the SGRS, soil samples are sent to DPI&F laboratories in Indooroopilly to determine if *Pratylenchus vulnus,* lesion nematode, or *Meloidogyne hapla,* root-knot nematode (RKN), are present. Because of the difficulty in distinguishing between RKN species using visual methods, it is necessary to determine how virulent the different species are on current strawberry varieties.

Materials and Methods

Tissue cultured strawberry plants of 3 varieties: Jewel, Joy and Sweet Charlie (supplied from Redlands Research Station) were planted into nematode sand mixture in 140mm pots. Four weeks after deflasking the plants respectively were inoculated with 10,000 eggs of *Meloidogyne hapla, M. javanica* and *M. incognita* and with 500 eggs of *M. arenaria.* Nematode eggs used for inoculum had been harvested from Tiny Tim tomato plants on which root-knot nematodes of each species had been maintained in the glasshouse. The plants were grouped into inoculated species groups and kept on separate benches in the glasshouse to avoid cross contamination of the root-knot species. Strawberry plants were fertilised using Osmocote, applied as per the label instructions.

Strawberry plants were harvested six and twelve weeks after inoculating with nematodes. The tops were removed, bagged, oven dried and weighed. The roots were washed free of soil and the fresh weight recorded. The roots were then soaked in a 1% solution of NaOCl for 3 minutes to remove the eggs (Stanton & O'Donnell, 1994). The eggs were collected by washing the solution over a 38µ sieve and counted and the reproduction of each species determined.

The levels of resistance or susceptibility of the strawberry variety was determined by calculating a reproductive factor (RF).

RF = final egg count (Pf) / initial number of eggs inoculated (Pi).

Due to mortality of nematodes, 1/10 of the initial inoculum was used for Pi and the resistance categories assigned as follows: An analysis of variance was performed on transformed (ln(x+1)) reproductive factor and the back-transformed means presented. Resistance ratings were assigned to reproductive factor categories (Table1).

Table 1. Resistance ratings assigned to reproduction factor categories.

Reproductive Factor	Resistance rating
>100	**Highly Susceptible (HS)**
10-100	**Moderately Susceptible (MS)**
1-<10	**Slightly Susceptible (SS)**
0.01<1	**Resistant (R)**
<0.01	**Highly Resistant (HR)**

Results and Discussion

All four common Queensland *Meloidogyne* spp. were found to reproduce on strawberries. There was a significant interaction ($P<0.05$) between strawberry cultivars and the root-knot nematode species at 6 and 12 weeks after harvest (Table 2). The strawberry cultivar Joy was more susceptible than Jewel and Sweet Charlie to all nematode species, except *M. incognita* at the 6 and 12 week harvest (Table 2). The higher nematode reproduction on Joy suggested that this variety was less resistant than other cultivars indicating a greater potential for damage to strawberry crops, and a greater carry over of nematodes to the following crop. However, there was no significant difference in the dry matter production of the strawberry cultivars inoculated with the different root knot nematode species. This suggested that after 12 weeks the strawberry cultivars were tolerant to nematode damage.

The reproduction of *M. hapla* was not significantly greater than the reproduction of other *Meloidogyne* spp. on strawberry roots. It cannot be assumed that if strawberries are infected with galls that it is *M. hapla* causing the infection. Therefore, it is necessary to test for all root-knot nematode species present in the soil as part of the strawberry grower runner scheme process, to ensure that nematodes do not impact on production. However, *M. incognita* was found to have the lowest reproduction rate on strawberries and may be considered less pathogenic.

With the development of new improved varieties for the strawberry industry each year, further evaluation of cultivars for resistance to *Meloidogyne* spp. needs to be undertaken as part of an integrated nematode management programme. Breeding improved varieties may be moving further away from any resistance genes that strawberry varieties contained in past and resistance testing could help breeding programmes identify these nematode resistance genes before they are lost. The testing for all species of *Meloidogyne* in the SGRS may be warranted given the damage potential of these nematodes on strawberries.

Conclusion

The four common species of root-knot nematode *M. hapla, M. incognita, M. javanica, and M. arenaria,* are all able to infect strawberry cultivars. The formation of galls on strawberry roots does not indicate that *M. hapla* is present, as it could be any root-knot nematodes species. The Strawberry Growers Runner Sheme needs to be expanded to prevent the spread of the four common root-knot nematodes species found in Queensland. There is also

a need to evaluate the resistance of current strawberry varieties for resistance as the variety Joy was found to be more susceptible than Jewel and Sweet Charlie to root-knot nematodes.

Table 2. Comparisons of the reproductive factor and the resistance ratings of plants harvested 6 and 12 weeks after inoculation with 4 species of root-knot nematodes.

RKN species	Strawberry variety	RF (6)		Resistance Rating (6)	RF (12)		Resistance Rating (12)
M. arenaria	Jewel	0.0	a	R	0.3	a	R
M. arenaria	Joy	1.0	b	SS	1.4	b	SS
M. arenaria	Sweet Charlie	0.0	a	R	0.4	a	R
M. arenaria	Tomato	1432.5	f	HS	363.0	f	HS
M. hapla	Jewel	0.0	a	HR	0.0	a	R
M. hapla	Joy	6.6	c	MS	1.2	bc	SS
M. hapla	Sweet Charlie	0.0	a	R	0.0	a	HR
M. hapla	Tomato	393.0	e	HS	43.7	d	MS
M. incognita	Jewel	0.0	a	HR	0.0	a	R
M. incognita	Joy	0.1	a	R	0.3	a	R
M. incognita	Sweet Charlie	0.0	a	HR	0.0	a	R
M. incognita	Tomato	151.0	e	HS	82.0	e	MS
M. javanica	Jewel	0.0	a	HR	0.0	a	HR
M. javanica	Joy	4.6	c	SS	3.4	c	SS
M. javanica	Sweet Charlie	0.1	a	R	0.0	a	HR
M. javanica	Tomato	453.0	e	HS	29.0	d	MS

Means with the same subscript are not significantly different at P=0.05

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NOVEL INDUCIBLE PHYTOCHEMICAL DEFENCES AGAINST PLANT PARASITIC NEMATODES

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The insect moulting hormone, 20-hydroxyecdysone (20E), is inducible in spinach and has been demonstrated to provide defence against insect herbivory. It is not known if such phytoecdysteroids are inducible by and defensive against plant parasitic nematodes. However, given that insects and nematodes belong to the same clade, the ecdysozoa, this is possible. Therefore, plants were tested for the presence of inducible phytoecdysteroids and effects on the nematodes tested.

Induction of possible defence compounds in common cultivars of spinach, two *Briza* spp., *B. maxima* and *B. minor*, oats and lucerne cultivars with varying degree of resistance to stem nematode was undertaken by treatment with methyl jasmonate and by challenging plants with nematodes. The influence of nematode inoculum density on the induction of phytoecdysteroid was also assessed in spinach. In addition, the relationship between the levels of inducible compounds and resistance response of lucerne cultivars to the stem nematode were evaluated.

Treatment with methyl jasmonate induced methanol extractable compounds in all plants tested. *Pratylenchus neglectus* induced the same compounds at levels equivalent to methyl jasmonate induction in all plants except lucerne, which was not tested. An inoculum rate of 500 to 10,000 *P. neglectus* induced similar levels of phytoecdysteroids in spinach. *Heterodera schachtii* induced phytoecdysteroids in both roots and shoots of spinach. *H. avenae* induced methanol extractable compounds in the roots of *B. minor* and shoot and roots of oats. *Meloidogyne javanica* was only found to increase levels of phytoecdysteroids in the shoots of spinach. Among the plants inoculated with the stem nematode, *Ditylenchus dipsaci*, induced compounds were detected only in some lucerne cultivars resistant to the nematode.

The methanol extracts from the induced plants were further tested for biological activity using *Drosophila melanogaster* B_{II} cell microplate-based bioassay to screen and detect biologically active ecdysteroids. The extracts were subjected to mass spectrometry to confirm the presence of ecdysteroids. The biological and chemical characterisation of the inducible compounds in the plants tested provided evidence that spinach, *Briza* spp. and lucerne contained the ecdysteroids 20E and polypodine B, which were biologically active based on the B_{II} cell bioassay except for lucerne. Lucerne shoots appeared to contain compounds or conjugate groups that inhibit ecdysteroids. In addition to the ecdysteroids above, *B. maxima* also contained ecdysone. On the other hand, inducible flavonoids were observed in the shoots and roots of oats.

Two plant parasitic nematodes, *P. neglectus* and *Anguina tritici*, were examined for the presence of similar ecdysteroids induced in the plants tested. This information will corroborate the involvement of these compounds in plant defence against nematodes. Based on HPLC and mass spectrometry data, both nematodes did not contain the ecdysteroids induced in the plants. However, compounds with masses similar to 20,26-dihydroxyecdysone, 20,26-dihydroxyecdysone 22-acetate, makisterone A, and possibly an unreported ecdysteroid were observed in *P. neglectus*. No ecdysteroid was observed in *A. tritici*, which consisted only of second stage juveniles in the anhydrobiotic survival state as opposed to the presence of all stages and actively developing population of *P. neglectus*.

In order to establish that ecdysteroids are potential defence compounds against parasitic nematodes, the effects of direct application of 20E on nematodes was assessed by treating cereal cyst nematode, *H. avenae*, juveniles with concentrations of 20E from 8.2×10^{-8} to 5.2×10^{-5} M before applying to wheat. *H. avenae*, *H. schachtii*, *M. javanica* and *P. neglectus* were treated with 5.2×10^{-5} 20E and incubated in moist sand. To test the protective effects of 20E in plants,

H. schachtii and *H. avenae* were applied to spinach and quaking grass, respectively, and the latter two nematodes in both plants, in which elevated concentrations of 20E had been induced by methyl jasmonate. Abnormal moulting, immobility, reduced invasion, impaired development and death occurred in nematodes exposed to 20E either directly at concentration above $4.2x10^{-7}$ M or in plants. Phytoecdysteroid induction apparently protected spinach and *B. maxima* from plant-parasitic nematodes and may confer a mechanism for nematode resistance.

Green manure is an alternative option to deliver the defence compound, as high constitutive production in a crop plant might impose unacceptable metabolic cost. Induced spinach when applied as green manure suppressed invasion of *H. avenae* in wheat but the direct involvement of 20E was not established because of the highly toxic effects of the treatment on the nematode.

Three inducible compounds, isolated in methanolic root and shoot extracts of oats, were identified as flavone-*C*-glycosides by mass spectrometry. The effect of the flavone-*C*-glycosides on the invasion by and development of cereal cyst nematode, *H. avenae,* was assessed using methanolic extracts of shoots and roots from methyl jasmonate treated plants. Both extracts impaired nematode invasion and development. When the extracts were fractionated by high voltage paper electrophoresis, only one flavone-*C*-glycoside, *O*-methyl-apigenin-*C*-deoxyhexoside-*O*-hexoside, inhibited nematode invasion. The protective effect of the induction of flavone-*C*-glycosides in oats by methyl jasmonate was evaluated against *H. avenae* and *P. neglectus*. Treatment with methyl jasmonate reduced invasion of both nematodes and increased plant mass, compensating for damage caused by the nematodes, and is attributed to the active flavone-*C*-glycoside. The active compound, *O*-methyl-apigenin-*C*-deoxyhexoside-*O*-hexoside, has not been implicated previously in plant defence against any pest or pathogen, and appears to provide protection against the major cereal nematodes *Heterodera* and *Pratylenchus*.