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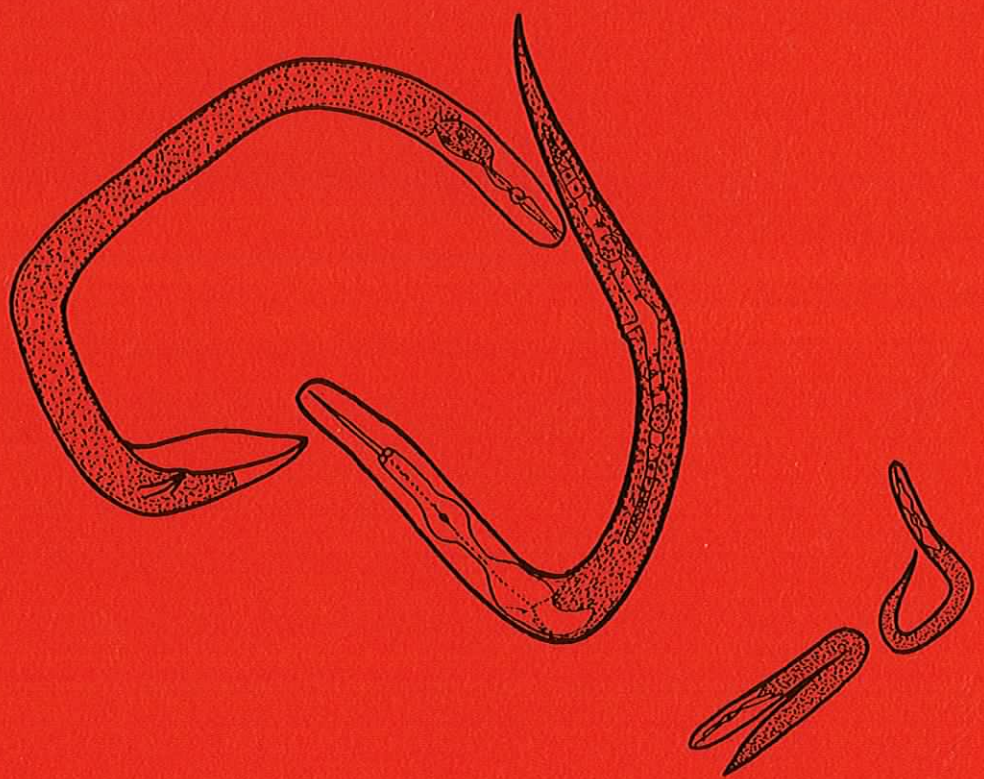
AUSTRALASIAN NEMATOLOGY NEWSLETTER



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From the Editor

As the new editor of the newsletter, I wish to thank all those who made contributions for this edition.

January Issue

The deadline for the January issue is December 10. You will be notified a month in advance so please have material well prepared before hand. Would you please ensure that I have your correct email addresses so that notification can be made simpler?

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Association News

FROM THE PRESIDENT

Another AGM and AAN workshop have come and gone. However, the success of these and the interest that continues to be shown in nematology throughout Australasia assures us that the discipline is alive and well. Even though there are relatively few of us, I feel that our enthusiasm will keep us together and moving forward. Perhaps, being a smallish group enables us to keep in touch with what is going on throughout the region and probably encourages collaboration.

We must apologise for the lack of a 'proper' newsletter in January this year. It took us a bit of time to find a willing editor. This illustrates the importance of current office holders finding suitable replacements before the AGM. Jenny Fanton has been dobbed in and didn't think she could refuse! Fortunately she is doing a great job so we should have some great issues in future.

Annual General Meeting
The AGM was held at Radisson Observation City Hotel at 5:30 pm on Sunday 28 September 1997. A new committee was elected, much to the relief of those who have served two terms! The committee for 1997-1999 is:

President:	Julie Stanton
Secretary:	Ian Riley
Treasurer:	Rob Potter
Newsletter Editor:	Jenny Fanton
Hon Committee Member:	Rob Brown

At the AGM, in the excitement of the moment, I forgot to thank members of the outgoing committee for their excellent work during the previous 2-4 years. Also, thanks to Ian Riley and Rob Potter, the convenors of the AAN workshop in Perth, and to Graham Stirling, Rob Potter and Thierry Vrain who ran the two half-day sessions. It was an excellent format and I personally gained a lot from the day. Thanks to RIRDC and Rob Brown for organising financial support.

Several issues were discussed at the AGM.

1999 Workshop
At present, we are planning the next workshop to be held in conjunction with the APPS conference in Canberra from 27 September - 1 October 1999. At this stage, Robin Bedding, Mike Hodda and John Curran (in his absence!) have agreed in principle to organise the day. It will likely involve a session on some aspect of insect-parasitic nematodes and a session on taxonomy, traditional and/or molecular. It is difficult to know where to start (or stop!) with taxonomy and there is always a need to know more. However, you can help. If there is a particular aspect of taxonomy where you see a need for further training, please let us know.

AAN web site
I undertook to investigate the possibilities of hosting a web site for AAN. QDPI has agreed to host the site and the pages are now written and waiting to go public. The site includes information about AAN, newsletters and links to other useful sites. We will also ask other sites to list ours so that it might be visited more often.

Quarantine
An important issue was raised. The concern is that only seven nematode species are listed by AQIS as being of quarantine importance even though many more species are still absent from Australia. This was illustrated by Ian Riley (AAN newsletter July 1997) who listed 28 other species of Heterodera which have not been recorded in Australia. Some of these pose very serious threats to Australian agriculture. Similar situations exist for other genera.

AQIS can not know of the importance of other species unless we tell them. It was decided that AAN prepare a list of those species which have not been recorded in Australia but which may pose a threat. Future endeavours to maintain freedom from these nematode species will then depend on pest risk analyses by AQIS. A small group including John Thompson, Ian Riley and Rob Brown agreed to coordinate this search. The work will involve many people in their specialty areas so please help when called upon.

Protocols for nematology laboratories
Graham Stirling, Julie Nicol and Frances Reay have nearly completed the book on laboratory protocols entitled "Guidelines for the operation of advisory services for nematode pests". They anticipate that this will be published this year by RIRDC and will be available for $10 per copy.

Julie Stanton, QDPI Indooroopilly.

INTERNATIONAL FEDERATION OF NEMATOLOGY SOCIETIES (IFNS)

Key activities related to the development of this still young Federation during the last nine months have focused on IFNS-Committee structure, outgoing requests to member Societies to consider hosting the Fourth International Congress of Nematology in 2002. Also, as a part of the listing of our Federation in the "YEARBOOK OF INTERNATIONAL ORGANIZATIONS", an initial listing of countries affiliated with one or more member Nematology Societies of IFNS was developed as well as a succinct history, affiliated Societies, aims, structure, finances, major activities, international events, and publications was developed. Providing this information emphasized the opportunities and challenges facing IFNS. Nevertheless, we now have nematologists in more than 110 countries who are represented by the twelve IFNS-affiliated Societies of Nematology. After a review and edit by the IFNS Councillors and Society Presidents, some of this material, along with a list of Society Presidents will be included in a "Home Page" for the Federation.

After rather prolonged inputs by our Councillors via e-mail, the following IFNS committees were organized:

CONGRESS SITE SELECTION/ PROGRAM	COMMUNICATIONS & INFORMATION EXCHANGE
Thierry Vrain, Chair	Safia Siddiqi, Chair
Rodrigo Rodríguez-Kábana	Derek Brown
John Marshall	Luiz Carlos Ferraz
Sanaa Haroon	Forest Robinson
Shashi Sharma	Yasuharu Mamiya
Maurice Moens	David Chitwood
Safia Siddiqi	Maria Vinciguerra

FINANCE	OPERATIONS MANUAL
Martie Botha-Greeff, Chair	Ken Barker, Chair
Nobuyoshi Ishibashi	Mohammad Maqbool
David Chitwood	Sanaa Haroon
Derek Brown	John Marshall
Rodrigo Rodríguez-Kábana	Maurice Moens
Thierry Vrain	Luiz Carlos Ferraz

The Congress Site-Selection/Program Committee, chaired by Dr. Thierry C. Vrain, has been very active since these committees were established. For example, this group developed general guidelines that should be considered by member Societies as they consider the option of developing a proposal to host the Fourth International Congress of Nematology in 2002. These proposals should be submitted to IFNS by August 1, 1998. The Councillors are then scheduled to come to a decision of this important matter by October 31, 1998.

All IFNS Committees will increase their activities during the coming months. These endeavours encompass mechanisms of exchange and sharing educational materials and

general information, initial approaches for fund raising, and the development of an Operations Manual. Thanks to Dr. Thierry Vrain and Dr. Larry Duncan and the cooperation of the Society of Nematologists and North Carolina State University, the available back issues of the Journal of Nematology and a two-volume set of books "An Advanced Treatise on *Meloidogyne* (1985) are offered to anyone worldwide for the cost of surface shipment. Hopefully, similar books and journals can be made available for minimal costs.

We still face the challenge of managing the International Federation of Nematology Societies to provide the maximum benefits to Agriculture, Nematology, and Nematologists. Your sharing ideas on how to meet one or more of these opportunities will be much appreciated. [Please forward inputs to your respective Councillor(s), or Ken Barker, Box 7616, Plant Pathology, N. C. State University, Raleigh, NC 27695-7616 USA].

PLANS FOR A SHORT COURSE IN NEMATOLOGY

DECEMBER 1999

Mike Hodda (CSIRO Entomology, ANIC) and Kerrie Davies (University of Adelaide, Crop Protection) plan to run a 6-day workshop on 'Nematodes in Cropping Systems - Identification and Techniques' from 5-11 December, 1999 at the Waite Campus, The University of Adelaide, Glen Osmond. The course is being designed to suit researchers and professionals working in agriculture, quarantine, green keeping and soil biology, who need to understand the principles and practice of handling soil, plant and insect nematodes. It will provide hands-on experience in sampling, extraction, specimen preparation, culturing, diagnosis, and identification. Cost will depend on number of participants and will be notified closer to the date.

For further information, contact
Mike Hodda (email: Mike.Hodda@ento.csiro.au)
or
Kerrie Davies (email: kdavies@waite.adelaide.edu.au).

GUIDELINES FOR THE OPERATION OF ADVISORY SERVICES FOR NEMATODE PESTS

When I suggested that AAN should produce a book on this topic, I had no idea it would be such a time-consuming task. The publication I had in mind was only 30 or 40 pages, but it eventually grew to occupy more than 120 pages. Nevertheless, it is now finished and will be sent to the printer before the end of June.

The project was funded by RIRDC and on behalf of AAN, I would like to acknowledge the Corporation's contribution. RIRDC will be printing about 250 copies of the book and I understand that all AAN members will receive a copy. I hope you find it useful. The effort in producing it will have been worthwhile if the quality control issues covered in the book are adopted by those who are providing diagnostic services.

Julie Nicol and Frances Reay both made invaluable contributions to the book, and I wish to thank them for their input and support. Julie Stanton, Ian Riley, Kerrie Davies, Wym Wouts, Mike Hodda, Greg Walker, Rob Brown, Maria Scurrah and Vivien Vanstone were some of the many AAN members who also helped and I gratefully acknowledge their contribution.

Like any publication, I am sure there are omissions and areas that can be improved. If you notice any errors while reading the book, or you have suggestions for improvement, please contact me. Your comments will be useful if we decide to update the book in a few years time.

Graham Stirling, Biological Crop Protection, Brisbane

Regional News

NEWS FROM CANBERRA
News from the ANIC Nematode collection

The reference collection of plant-parasitic nematodes in Australia, housed at the Australian National Insect Collection (ANIC) within the CSIRO Division of Entomology in Canberra, has continued to grow with the support of the GRDC. The list of genera and species has expanded, and additional populations from different localities and crops added for species already represented. We have material from all over Australia, and have specimens of many potential pest species from overseas as well. We are maintaining links with overseas institutions such as Riverside, Davis, Wageningen, Rothamsted and the International Institute of Parasitology to keep up with the latest developments in systematics of pest species. As part of our commitment to maintaining the links between the collection and researchers all over Australia, we present this update of the status of the collection.

The collection currently consists of:

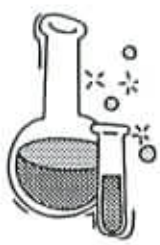
- over 14 500 specimens of plant-parasitic nematodes on permanent slide mounts, covering different life stages, geographic and host variation of many important species;
- more than 750 samples of bulk extracted specimens fixed and stored in glycerol;
- cryopreserved, living specimens of selected species, mainly *Pratylenchus*;
- a computer database of all plant-parasitic nematodes in the collection, plus all other species described from Australia;
- a library of over 2300 articles of relevant literature (books and reprints), including species descriptions, also completely computer catalogued.

The specialist curator is Emily Stewart, while I have responsibility for identifications, management and overall development.

The collection forms the basis for my own systematic work and is actively used. I am still working on morphological variability in *Pratylenchus*, particularly in key characters differing between species and geographically different populations of the same species. I am trying to find reliable ways of distinguishing species, as well as producing a catalogue of the species that occur in Australia. The necessity of doing this was brought home recently when a sample from Western Australia contained some *Pratylenchus*, which did not fit into any currently known species from Australia. The collection is invaluable

when doing this kind of work to check the new specimens against the species currently known, but its usefulness depends on people sending material.

The more comprehensive the collection becomes, the more value it will be used as a resource and repository for nematologists across Australia. I am always seeking further material and specimens. Specimens in culture are best, so that all specimens can be processed in identical fashion and inter-culture variation can be assessed. We will even endeavour to cryopreserve cultures of important species, so that the culture will always be available for genetic analysis, pathogenicity testing and comparison. We can also handle material in other forms (for example bulk soil samples with high counts of particular species of interest, live or fixed sorted specimens, or microscope slides). Original host and location records are also important to the value of the collection. Species identity is a bonus, but if unknown I can identify it for you.



It is important for nematology in this country that the species, specimens and information that people collect now are properly stored so that future nematologists will have access to this resource. In fact it is quite likely that material deposited will be of use to us a few years down the track, so there can be an element of self-help in depositing material. The emphasis on electronic databasing is aimed at ensuring the information remains available and accessible, so with the specialist curator and purpose-built collection halls, the material in the collection will receive the best attention. So take a few minutes to deposit specimens and supporting information will save yourself and the next person a lot of time. And don't let those old slides or tubes sit in the corner until they are broken, dry out or disappear: send them to us, so that they will be cared for and curated until they are needed. Or take a minute to make one extra slide of the material you are currently studying and send it to us. Please feel free to contact myself or Emily to arrange shipment of material - we can be contacted on (02) 6246 4371 (telephone) or (02) 6246 4000 (fax), e-mail mikeh@ento.csiro.au or emilys@ento.csiro.au.

Other news from Canberra revolves around arrivals and departures. Frances FitzGibbon, well known to people from Plant Protection at the Waite Institute, was offered a job working on insect pests of sugar cane in a different program within Entomology, and left as curator of the nematode collection in November. Emily Stewart, the new curator is new to nematodes but has lots of experience with vertebrates, so Mike Hodda is currently infecting her with the nematological bug (if that is not mixing metaphors too much). Nora Galway has finished her PhD with John Curran and left to join AQIS.

John Curran's work with Nora and Felice Driver on genetic relationships of plant parasitic nematodes continues, with development of molecular diagnostics for nematodes as a priority. The group is working collaboratively with SARDI and VIDA testing out molecular methods.

In October last year, I finished a 2-year study funded by RIRDC into effects of management practices on soil nematodes in a wheat cropping system, including their use as indicators of soil health. In the study three tillage/mulching systems were compared within sites at Harden and Cowra. The final tally of species from this study was 104, including 2 *Pratylenchus* spp. (*neglectus* most abundant), but also *Tylenchorhynchus*

(Stunt nematode) in considerable numbers, and *Paratrichodorus minor* (Stubby-root nematode). The abundances of *P. neglectus* differed between direct-drill, conventional tillage and stubble incorporation systems at Cowra, but not at Harden. There were large differences in abundance of many of the free-living nematodes among the management systems at both Harden and Cowra, suggesting that there are differences in the decomposition pathways under the different systems, and that nematodes can be sensitive indicators of soil processes which are otherwise very difficult to measure.

Currently, I am completing an electronic key to freshwater aquatic nematodes, sponsored by ABRS. This key will be completed at the end of 1998. Anyone who has an interest in this area who would be willing to test preliminary versions of this key should contact me. All feedback will be very welcome.

Mike Hodda, CSIRO Division of Entomology, Canberra

NEWS FROM QUEENSLAND
Museums are facing a dilemma

Health and workplace safety requirements mean we can no longer store specimens in formalin, but must transfer them to alcohol: for many things this has been the normal practice for years. Empirical evidence, however, shows that small free-living and plant-parasitic nematodes do not remain robust in alcohol, but become increasingly flaccid and consequently lose value as preserved specimens.

The question arises as to what is happening with our specimens. Are they deteriorating? Most of the animal-parasitic nematodes are quite large, but some are little bigger than the free-living and plant-parasitic ones. They are all in alcohol. Are these gradually decaying? And what might be happening to them?

As part of her Ph.D. in chemistry at QUT, Jan Gentner has been collaborating with the Queensland Museum seeking to understand what happens when nematodes are placed in alcohol, with or without prior formalin fixation. Her spectroscopic and electron microscope study of nematode cuticle and the preservation fluids in which the worms rest has revealed some troublesome details. Jan's work has shown structural weakening or change in the body wall accompanied by leaching of chemicals (lipids and proteins) into the preserving fluid.

Damage appears to be slight in the short term and for robust specimens it may not prove to be a serious threat. Her work, however, does provide some experimental evidence as to why storing small nematodes in alcohol is contra-indicated. Of course, in the longer term perhaps all the collections are gently fading away. Her study raises serious questions as to how we in Museums go about our long-term preservation duties. More research - of course, but who pays?

Fortunately, at the Queensland Museum we have been able to arrange for a special cabinet, vented to the exhaust fan system, to be built for the plant parasitic nematodes. We are thus able to comply with the new regulations AND keep the worms in formalin.

Lester Cannon, Queensland Museum, Brisbane

NEWS FROM NORTH QUEENSLAND

Nematology has recently undergone an expansion in north Queensland. Work is continuing with the management of burrowing nematode in bananas with the completion of the fourth year of the current research funded by the Queensland Fruit and Vegetable Growers Association of Queensland and HRDC. Linda Phillips has recently joined Tony Pattison at the Centre for Wet Tropics Agriculture at South Johnstone to help with nematicide and enhanced biodegradation trials, fallow management and investigations of organic products to aid the control of burrowing nematode.

Also joining the Queensland DPI staff at Mareeba is Primitivo Aceret. Primitivo is funded by the Tobacco Research and Development Corporation to investigate alternatives to EDB for controlling root-knot nematode in tobacco. This work also includes the further development of the population dynamics and work on damage thresholds of root-knot nematode in tobacco. Primitivo has recently submitted his PhD thesis at the University of Central Queensland looking at techniques of fermentation of several nematode species.

Tony Pattison, QDPI, South Johnstone

NEWS FROM VICTORIA

The Victorian component of the southern region nematology project is making good progress at screening field crop varieties for their resistance/susceptibility to *Pratylenchus thornei*. Despite the exceptionally dry season in 1997 the field trials still provided useful information. We are continuing with this work in 1998. A summary of the varietal reactions to both *P. thornei* and *P. neglectus* has been published in GRDC research updates.

Last September we travelled to Perth for the Nematology workshop and Australasian Plant Pathology conference. Both were very rewarding; scientifically and socially. The organisers are to be congratulated on all their hard work.

Since the last newsletter Grant has been appointed as the Cereal Pathologist/Agronomist at VIDA. In this role he is continuing as the Victorian leader of the root lesion nematode project, but also takes on responsibility for the Victorian component of the Rhizoctonia project, plus some other minor projects.

Grant Hollaway and Russell Eastwood, Victorian Institute for Dryland Agriculture, Horsham

NEWS FROM INDIA

News from Sugarcane Breeding Institute, Coimbatore, India
Third International Symposium of Afro-Asian Society of Nematologists

Theme: Challenges & Opportunities in 21st Century

The International Symposium was organised by the Sugarcane Breeding Institute, Indian Council of Agricultural Research, Coimbatore, Tamil Nadu, during April 16-19, 1998, under the Chairmanship of Dr Usha K Mehta, Head Division of Crop Protection. Seventy Scientists from USA, UK, Egypt, Iran, India and Sri Lanka participated. More than 60 research papers were presented in 10 scientific sessions. The recommendations and suggestions made during the sessions were put together by a panel and they are given here:

RECOMMENDATIONS

Biodiversity

1. Mapping and documentation of diversity in nematode fauna in different ecosystems of Asia and Africa was identified as a priority area. As India has ample information on nematode fauna associated with agro-ecosystems, the panel recommended that this information must be documented on priority.

2. The panel considered that variety of beneficial nematodes (e.g. insect-parasitic nematodes) and natural parasites of plant-parasitic nematodes that are present in the African and Asian ecosystems are precious natural wealth of the region. The panel endorsed that scientists must exert extreme caution in international sharing of these organisms and they must strictly follow the guidelines developed by their governments. Steps may be initiated to develop national repositories of the beneficial nematodes and natural parasites of harmful nematodes.

Research Methodology

The panel was concerned that the use of standardised research methodologies and protocols by nematologists in the region is still not as common as it should be. This prevents the scientists from making valid conclusions and from comparing results across locations. The panel recommended that guidelines and protocols for conducting and interpreting laboratory, glasshouse, and field studies particularly in the areas of nematode ecology, resistance screening, nematode IPM, and nematode identification schemes should be developed and disseminated.

Human Resources Development

The panel was concerned that many countries in Africa and Asia still do not have sufficient expertise in nematology to identify, assess, manage, and use the nematode fauna. India has the largest trained human resource in nematology in the world. The panel deliberated that India can greatly assist in expanding the nematology base in countries in Asia and Africa by organising short-term and long-term training courses in

nematode diagnosis and management. The International Agriculture Research Centres should be contacted for their input.

Soil Fertility Management

The panel recommended that suppression of nematode-induced damage to plants by soil fertility management must be highlighted. It was evident from the papers presented during the symposium that biological (e.g. mycorrhiza on plantation crops) and chemical (e.g. phosphorus on pigeonpea, gypsum on groundnut) fertilisers tend to reduce the nematode-caused damage. In some cases, increase in crop yield by addition of these fertilisers may be a sum of availability of nutrition to plants and alleviation of nematode-caused damage. The panel also recommended that use of VAM for reducing the losses caused by nematodes must be assessed from a cropping systems perspective.

Research on Entomopathogenic Nematodes

Research on insect-parasitic nematodes needs a greater visibility in Asia and Africa. The work done so far has not made much impact. The panel recommended that a "Working Group" of scientists studying these nematodes in Africa and Asia should be developed to identify partners, research priorities, and to formulate a synergistic workplan. Nematologists at the Tamil Nadu Agricultural University and Sugarcane Breeding Institute, Coimbatore were identified to take the lead.

Nematode Management

The panel was concerned that user-friendly IPM packages for nematode management have either not been developed or not transferred to the growers. Development of IPM packages for important nematode pests (e.g. root-knot, cyst, reniform, burrowing nematodes) of agricultural and horticultural crops must be given high priority.

N.Somasekhar, Ph.D, Organising Secretary,
Third International Symposium of Afro-Asian Society of Nematologists
Nematology Section, Sugarcane Breeding Institute, COIMBATORE-641 007

NEWS FROM NEWS SOUTH WALES

I am Loothfar Rahman and would like to introduce myself to nematologists in Australia. I worked on rice nematodes for 15 years in Bangladesh Rice Research Institute. Now I am working as a Plant Pathologist for the Department of NSW Agriculture since December 1996. Currently I am working on a NSW Ag R & D initiative project entitled "Determination of economic impact of nematodes on grapevine, shoot growth, root health and yield".

Other interest areas: Root-knot and lesion nematodes in grapevines, diagnosis and non-chemical methods of nematode control.

Loothfar Rahman, National Wine and Grape Industry Centre, Wagga Wagga.

Research

NEMATODES ARE IMPORTANT ON SUGARCANE

Graham Stirling, Biological Crop Protection, Brisbane

Although nematodes are known to cause heavy losses to sugarcane in the sandy soils around Bundaberg, they have never been considered an important pest in the other cane-growing districts of Queensland. However, when a research program was established a few years ago to investigate the soil-related factors that were limiting sugarcane yields in Queensland, I took the opportunity to establish a project on nematodes. The work now forms part of the Yield Decline Joint Venture (YDJV), a collaborative research program funded by BSES, CSIRO, DPI and SRDC. Brenden Blair, Peter Whittle, Julie Pattemore and myself carry out the nematological component and our main focus has been to determine whether plant-parasitic nematodes are involved in yield decline. We plan to start publishing our results in the near future, but this article will give AAN members an indication of what we have found.

Summary of progress

- Surveys of more than 500 fields have shown that lesion nematode (*Pratylenchus zeae*) is present in every sugarcane field in Queensland. Populations of more than 1,000 nematodes/g root are common. Root-knot nematode (*Meloidogyne* spp.) and stubby root nematode (*Paratrichodorus* spp.) are present in about 50% and 70% of fields respectively. All three nematodes are known to be pathogenic to sugarcane.

- Lesion nematode occurs in all soil types, with average nematode population densities in sandy soils being similar to those in clay loam and clay soils.

- Surveys of newly planted areas of the Burdekin and data from an experimental site at Tully have shown that lesion nematode increases to high population densities within a few years of planting new land to sugarcane.

- Studies in partially sterilised soil in microplots have shown that lesion nematode destroys secondary and tertiary roots, resulting in a substantial reduction in fine root length.

- When crop losses due to nematodes were assessed at field sites where nematodes would not normally be considered to cause problems, yield responses to nematicides were obtained in 9 out of 10 sites. Yield increases were generally between 10 and 20%, but were sometimes as high as 50%. Responses were obtained in soils ranging in texture from sandy loams to clay loams and clays.

- Data on nematode populations in rotation trials established by the YDJV have shown that 30 months of either bare fallow or a crop that is a poor host of lesion nematode (e.g. soybean) provide a similar level of nematode control to that obtained with registered rates of a nematicide. Although most of the data on the performance of cane following these rotation crops are not yet available, there is visual evidence of responses in treatments in which population densities of lesion nematode have been reduced. At the Bundaberg site, which was the first to be returned to cane, there was a significant correlation between the density of lesion nematodes and yield.

Evidence for the involvement of nematodes in yield decline

Although the nematology project will not be completed for another 18 months, the following evidence suggests that yield decline is caused primarily by nematodes.

- In 1900 it was recognised that the yields obtained in virgin soil were not being achieved 5-8 years later. Thus yield decline has probably existed for more than 100 years and must be caused by a factor which changes relatively quickly after new land is planted to sugarcane. Nematodes are one such factor, as high populations may occur after two years of sugarcane.

- Growth responses to partial sterilisation of soil (using heat at 96°C for 2 hours) were obtained in 1935, demonstrating that yield decline is largely a biological phenomenon. The responses to fumigation which have been obtained more recently, provide similar evidence. Nematodes are one of the major groups of organisms that are controlled by both heat and fumigation.

- A response is obtained in virtually every sugarcane soil that is fumigated. All these soils harbour lesion nematode, and most contain at least three other plant-parasitic species.

- Observations of sugarcane roots in the field have shown that fine roots are often in poor condition, and in many cases they have been destroyed. Such symptoms are typical of those caused by lesion nematode.

- Pre-plant population densities for *P. zeae* on sugarcane usually range from 0.5 to 3 nematodes/g soil. Lesion nematode populations of this magnitude are known to cause significant damage on other crops (e.g. cereals in Queensland in southern Australia).

- When nematicides are applied four times during the season, it is possible to reduce nematode populations by more than 90% and maintain low populations for most of the season. When this is done, consistent yield responses are obtained in a variety of soil types. Long fallow and some crop rotation treatments also provide good control of lesion nematode, and early evidence suggests that yield responses of a similar magnitude to those obtained from nematicides will be obtained from these treatments.

Despite the results that have been obtained recently, it could be argued that previous studies have failed to demonstrate that nematodes are of widespread importance in Queensland. However, careful evaluation of both published and unpublished data reveals that much of this work was not suitable for quantifying crop losses caused by nematodes:

- Nematicides were mainly tested at application rates likely to be economic on sugarcane. At these application rates, limited and temporary nematode control is obtained. In cases where higher application rates were used, yield increases were greater.

- Aldicarb and fenamiphos, the main nematicides used in the sugar industry, tend to be nemastatic rather than nematicidal. At normal application rates, they inhibit egg hatch and prevent nematodes from moving and feeding, but do not kill nematodes directly. Nematode activity resumes once concentrations of the chemical decline below certain critical levels, and this usually occurs after only 4-6 weeks. Since lesion nematode is active for at least nine months of the year and may complete as many as eight generations during this time, the single nematicide application normally used in sugarcane provides protection for only a limited part of the growing season.

- The efficacy of nematicides is markedly affected by soil moisture. When soil is dry, organophosphate and carbamate nematicides remain on the soil surface, while under high rainfall conditions, they readily leach through the soil profile. Thus there is likely to have been considerable variability in efficacy in the nematicide trials previously done in Queensland.

- Non-volatile nematicides move more readily and are more effective in sandy soils than they are in heavy soils. Since responses are therefore easier to obtain in sandy soils, previous nematicide work has concentrated in these soils. The possibility that nematodes are important in heavy soils has rarely been considered.

- Because numerous factors affect the efficacy of nematicides, it is imperative in crop loss assessment work to collect data which demonstrates that nematodes have been controlled and shows the length of time that control has been achieved. Such data have rarely been provided in experimental work with nematicides on sugarcane.

- It is apparent from comments in the literature that nematode damage on sugarcane is largely diagnosed on the basis of root galling. However, such symptoms are produced only by root-knot nematode. Thus the nematode problem currently recognised by the sugar industry is really a root-knot nematode problem. Since root-knot nematode occurs mainly in sandy soils, this has led to the perception that nematodes only cause damage in sandy soils. Symptoms caused by lesion nematode are much more difficult to diagnose. Lesion nematode damage probably occurs throughout the industry, but the root symptoms are likely to be diagnosed as being caused by fungi.

- Short-term pot experiments have commonly been used to determine the relative importance of various root pathogens. However, such experiments under-estimate

the importance of nematodes, because a 6-8 week period is sufficient for little more than one nematode generation. Also, effects on aboveground biomass due to poor roots are often masked by the ready availability of water and nutrients.

A working hypothesis to explain yield decline

On the basis of the information presented in the preceding discussion, the following interpretation of yield decline is proposed.

Yield decline occurs in almost all sugarcane soils in Queensland and its most obvious manifestation is the 5-30% increase in yield, which is obtained following fumigation with a broad-spectrum biocide. This fumigation response is due mainly to the control of plant-parasitic nematodes and soil-borne fungal pathogens. However, because as much as 100 kg/ha of nitrogen is released from microbial biomass when soil is fumigated, and there are also changes in the forms of nitrogen present in soil, some of the initial growth response is due to changes in nutrition. Control of nematodes accounts for most of the fumigation response in sandy soils, and perhaps half the response in heavy soils. The primary causal factor is lesion nematode (*Pratylenchus zeae*). This species may act as a pathogen in its own right, or it may provide entry points into the root for fungi that are normally not considered pathogens. In the latter case, the nematode is most likely to interact with a suite of minor fungal pathogens, including dematiaceous fungi, that are commonly found in association with poor roots of sugarcane.

Root damage caused by lesion nematode results in reduced sugarcane yields, but the level of yield reduction is influenced by the soil environment and the standard of crop management. Yield losses will be greatest in soils with a low water holding capacity, in years of low or erratic rainfall, or in situations where irrigation management is poor. In fertile soils, or in high yielding crops where the standard of crop management is excellent, root function may be markedly diminished but crop losses will be relatively small because the crop's water and nutrient requirements are being satisfied.

Future research

On the basis of results to date, I would argue that the above concept of yield decline is a reasonable working hypothesis. It suggests that the Queensland sugar industry has a chronic and previously unrecognised lesion nematode problem that could be costing at least $100 million per year. The fumigation response is a tangible and widely documented phenomenon and if it is largely due to nematode control, then a research program on nematodes is clearly warranted. That research program should concentrate on the following areas:

1. **Crop loss assessment.** Some crop loss experiments have been established in Mackay, but all other experiments have been done in south Queensland. These field trials need to be extended to central and north Queensland. They should also include both fumigation and nematicide treatments so that more accurate data are obtained on the proportion of the fumigation response that is due to nematode control.

2. Etiology of root diseases involving lesion nematode. Further studies on etiology are needed, particularly with regard to possible interactions between lesion nematodes and fungi.

3. Nematode control. Efforts to improve control measures for nematodes should concentrate on four main areas:

- improved prediction systems (i.e. diagnosis of nematode problems, monitoring procedures, economic thresholds)
- further evaluation of crop rotation (particularly with regard to the benefits of short-term break crops)
- increasing the biological suppressiveness of soils to nematodes (e.g. biological control, increasing soil organic matter, improving soil health)
- breeding and selection of varieties with resistance and/or tolerance to nematodes

WHITE CLOVER SELECTION PROGRAMME FOR TOLERANCE TO PLANT NEMATODES IS SHOWING SUCCESS

Chris Mercer and Richard Watson, AgResearch Grasslands at Palmerston North and Ruakura.

Complementary white clover selection programmes for resistance or tolerance to clover root-knot (*Meloidogyne trifoliophila*) and clover cyst (*Heterodera trifolii*) nematodes have been conducted over a number of years by AgResearch Grasslands at Palmerston North and Ruakura. Selections have expressed superior seedling vigour to existing cultivars when grown in the presence of nematodes. Elite seedlines from both programmes now being evaluated in pastures grazed by sheep or dairy cows at several locations through New Zealand have shown superior vigour to standard cultivars. This work has the potential to dramatically improve the productive efficiency of our pastoral farming. The redundancy of the programme's plant breeder means a shift of emphasis away from nematological aspects of resistance in order to maintain the field trials.

STUDIES ON WHITE AND CAUCASIAN CLOVER

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White clover content in NZ pasture can be unreliable when high summer temperatures, low soil moisture, and clover nematodes combine. A rhizomatous spreading habit, and thus protected growing points in Caucasian clover (*Trifolium ambiguum*) give potential for better summer survival, and thereby at the same time providing a means of managing nematode impacts. Grazed plots of white and Caucasian clover with ryegrass were established in September 1994. Sub-plots containing nematicide and fungicide treatments have been maintained under mowing from February 1995. White clover out performed Caucasian clover in the establishment year, but in the second growing season Caucasian clover was superior, with up to 60% higher growth rates during summer/autumn 1996. Caucasian clover based pasture was superior in the third growing season from October. Caucasian clover was less responsive than white clover to both fungicide and nematicide treatments which have largely accounted for the differences in pasture production. Soil from Caucasian clover plots carry much lower levels of the clover cyst nematode (*Heterodera trifolii*) but slightly higher levels of root knot nematode (mainly *Meloidogyne hapla*). Both nematodes were present at non-detectable levels when the pastures were established after 15 years of maize cropping. Other plant-parasitic nematode populations, *Helicotylenchus labiatus, Pratylenchus* spp., and *Paratrichodorus minor* have changed less dramatically since pasture establishment. During the extremely dry summer of 1998 Caucasian clover stopped growing but white clover disappeared from the pasture. Treatment responses have increased in Caucasian clover and very high levels of root knotting were evident.

Watson, R.N.; Neville, F.J.; Bell, N.L. and Harris, S.L. 1996. Caucasian clover as a pasture legume for dryland dairying in the coastal Bay of Plenty. *Proceedings of the New Zealand Grassland Association 58*: 183-188.

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Review

OPTIONS FOR TRANSGENIC RESISTANCE TO NEMATODES

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Presented at AAN workshop, Perth, September 1997

Finding resistance genes

The successes of breeding for resistance against nematodes have been slow in the last fifty years. The difficulties lie with the complexity of the parasitic relationships that nematodes have with their host plants. And we have not found a large pool of genes for resistance to work from.

Today, we are beginning to understand the intricacies of nematode-plant interactions at the molecular level. Most plants are non-host to most nematodes; they have built-in resistance through the expression of many resistance genes. Because we can transfer any gene from any plant, we now have an unlimited pool of genes for resistance to work from.

We are now breeding for resistance against nematodes at a speed that could not have been imagined twenty years ago. In Nematology, we are witnessing a revolution in the last ten years that is powered by the genetic engineering engine.

To develop resistance against nematodes, we target proteins from the plant or from the nematodes. Sometimes we do not know which protein is affected, but we know which biochemical process is affected. Sometimes we do not even know what processes are affected by the transgenes we use, and we only know the tissues or organs that are affected. For example, I express a lectin in raspberry that is toxic to the root lesion nematode. I do not know precisely the mechanism of toxicity or the target molecule of this lectin. All I know is that this lectin probably interferes with membranes in the lumen of the gut of the nematode.

Sometimes we target the plant with a system of nematode-induced hypersensitivity. We abort the formation of giant cells or syncytia using nematode-sensitive triggers to express antisense messages or toxins. Sometimes we target the nematodes, we feed them lectins, or proteinase inhibitors, or plantibodies, or toxins, or cholesterol oxidase. And we also have to engineer when and where the foreign protein should accumulate so that it is most effective against nematodes, and yet safe for the environment and for humans.

The genes with unknown products: we call them resistance genes. We do not know how they function or what kind of protein they express. However, from the progress of the last few years with the isolation of several genes for resistance against fungal and bacterial pathogens that are quite homologous in function, we suggest that nematode resistance genes are likely involved in the triggering signal, the transduction cascade leading to cell death.

The first gene for resistance against a nematode was isolated a few months ago. And, yes, the sequence of the protein suggests that its role is to detect a chemical signal from the nematode and trigger a hypersensitive reaction. This resistance gene against the sugar beet cyst nematode was isolated from wild beets by Christian Jung of the University of Kiel in Germany with Florian Grundler and two other groups in the Netherlands and in Denmark.

Wild beets are not closely related to cultivated species and so their chromosomes do not pair. So Jung and his colleagues could not use recombination mapping which is the usual approach to locate a gene. Instead they searched a collection of hybrid beets and found that many plants carried the resistance gene on small chromosomal translocation segments from wild beets. They were lucky to find a satellite sequence that always hybridised with the wild beets and the resistant hybrids of cultivated sugar beets. Using this as a probe they identified resistant plants, and they identified those with the smallest chromosome segment from the wild species. They isolated the nematode resistance gene from a cDNA library through cross hybridisation with a yeast artificial chromosome library of the resistant plant. They also tested this gene and found that it is effective against potato cyst nematode.

Cloning resistance genes

As I just mentioned, a basic strategy for cloning plant resistance genes with unknown product is map-based cloning, what we call "chromosome walking". First you identify markers surrounding the gene you want. These can be RFLP markers or RAPDs or AFLP markers that identify a fragment of DNA that always cosegregates with the resistance trait in genetic crosses. The next step is to make a cDNA library and identify pieces of DNA close to the gene, and the more markers you have the closer you are to the gene.

Another strategy to isolate a gene is transposon tagging. Transposable elements move around the genome of plants causing mutations wherever they insert themselves within a gene. Transposons are essentially treated as tags to locate a particular gene.

Valerie Williamson at the University of California in Davis, and Pim Zabel in Wageningen both used a map based cloning strategy to isolate the *Mi* gene this year. Their results are not yet published. This gene confers resistance against several root-knot nematode species.

One can use the sequence information from several resistance genes already isolated. These genes show a leucine-rich repeat motif and a nucleotide binding site with conserved motifs. One can design primers to amplify the conserved sequences in other

plants and pull out homologous sequences. Using this strategy in barley, a group at CSIRO found five independent resistance gene-like sequences with 70 to 99% DNA homology with *Cre* 3, the cereal cyst nematode resistance gene from wheat.

There are several other projects at various stages of development that use this strategy. Most of them are still at the marker identification stage. These are genes for resistance against potato cyst nematode, against soybean cyst nematode and against cereal cyst nematode. We can expect that most of these genes will be cloned, and expressed in several crops in 2 to 5 years.

Function of resistance genes

We are all assuming that these resistance genes will function in a heterologous system. We do not know yet whether a gene for resistance against root-knot nematode in tomato will function effectively in other plants.

The current paradigm for cyst nematodes as well as for root-knot nematodes is that the giant cells or syncytia cells are formed when the juvenile nematodes inject salivary secretions from their oesophageal glands into specific cells near the root tip.

We know that the salivary secretions contain proteins and we believe that one or several of these proteins act as transcription factors with a role in the subsequent gene deregulation of the host cell. The results of the presence of the nematode are striking. Within a few hours the cells undergo enormous changes.

The chromosomes duplicate more than 50 times, and there are numerous nuclei in each cell. The cell walls form digitated ingrowths to accelerate the passage of water and nutrients from the xylem elements. The volume of these cells increases so much that they crush the adjacent tissue, they often damage the xylem and interfere with its flow. These cells function as transfer cells with increased metabolism. The cytoplasm is very rich in amino acids, in fatty acids and small carbohydrate molecules that the nematode feed upon. These giant cells become a permanent feeding site. And the nematode has complete control of its diet. Many genes must be involved to turn a plant cell into a nematode feeding factory. If our assumptions are right, if one of the proteins and polypeptides that are injected into the plant cell at the origin of this transformation, then the induction process is vulnerable, because we can now identify and interfere with the proper function of any one of these nematode proteins.

First, we need to identify the proteins from the salivary secretions. If you use video-enhanced microscopy, you can see these secretions flow from the oesophageal dorsal gland to the stylet and inside plant cells. Several groups in Europe and in the USA are using monoclonal antibodies to localise the oesophageal gland proteins of root-knot and cyst nematodes with immunofluorescent staining. These antibodies can represent a preformed defence in the plant. First you make

nematodes salivate profusely in a solution of 5-methoxy DMT oxalate. You collect the spit and inject it into the spleen of a mouse and you make monoclonal antibodies. You screen the antibodies to find those that bind to a protein from the nematode secretion or to a protein in the oesophageal glands. You can use the monoclonal antibodies to screen expression libraries to isolate nematode genes. You can also clone directly the coding region of the variable domain of an antibody and express it into a plant as a single chain antibody that will, hopefully alter the function of the nematode protein when it is injected in the plant cell through the stylet of the nematode. These people are doing just that, they use monoclonal antibodies to isolate and purify the antigens. They also screen nematode expression libraries to isolate the genes expressing the salivary proteins.

Another strategy is to raise monoclonal antibodies against digestive enzymes. I expect that single chain antibodies will interfere with the active sites of proteinases, impede nematode digestion and assimilation, and seriously affect their development. Many genes from the plant are expressed out of context and out of timing in the giant cell when they become an extension of the nematode.

This is an important strategy, and there is a large effort by several groups in Australia, New Zealand, Europe and the USA, to identify genes specifically expressed in giant cells or syncytia. The goal is to manipulate these genes or manipulate their promoters or their transcription factors, and abort the formation or modify the function of these plant cells.

The first group to have isolated a gene expressed in abundance in giant cells, can use its promoter to express an antisense sequence and knock out that gene. If they knock out an important function and affect the giant cells, then development and reproduction of the nematodes will be arrested. The promoter can also be used to express a toxin that will abort the formation of the giant cells.

There is obviously a lot of interest here. David Bird at North Carolina State University recently cloned a large number of giant cell specific genes. He isolated 150 giant cells at a similar development stage, from tomato plants infected with root-knot nematode. He made a cDNA library of 2 million recombinants and subjected it to a rigorous subtraction against uninfected mature root cDNA. He had 220 giant cell specific genes that remained after the subtraction step. One advantage of his technique is that the cloning step took place before the subtraction, so only giant cell transcripts were exposed to a replicon. Bird also expected to recover the rare transcripts because recombinants remaining after the subtraction were already cloned.

So what kind of genes are expressed?

Many are expressed in a wide range of tissues other than roots, and at a wide range of relative abundance. For example, there is a gene expressed in giant cells that is expressed strongly in mature leaves. It is clear that giant cells of root-knot nematodes and syncytia of cyst nematodes express many functions not normally expressed in mature roots. Clearly the nematode is driving transcriptional control in the giant cells.

Several groups in Europe and in the USA are using a promoter tagging technique: They isolate those genes expressed exclusively in feeding cells, by first isolating their promoter

with a reporter gene in a promoterless construct. This is a modification of the old promoter - reporter gene fusion technique that we use to examine gene function. Cyst and root-knot nematodes develop in transgenic plants, transformed with a promoterless GUS construct. GUS expression in syncytia or giant cells means that the construct has integrated behind the promoter of a gene that is active in these cells. It is then straightforward to isolate the promoters and the identified genes by inverse PCR.

Charlie Opperman at NC State has been using the promoter of a gene coding for a water channel protein in tobacco. The gene is called RB7, and it was isolated at NC State by Mark Conklin. The two have collaborated for several years to develop the promoter of this gene. They made a deletion series at the 5' end of the Tobacco RB7 promoter. This gene has approximately 1.8 kb of controlling sequences. They used the truncated promoters to drive GUS expression in tobacco.

When the transgenic tobacco plants were infected with root-knot nematodes, those plants transformed with a short promoter sequence of 300 bp showed GUS expression only in the nematode feeding cells. This truncated promoter functions as a nematode specific control element. Their work made the cover of *Science*, a rather exceptional honour in nematological research. They used the nematode specific promoter to direct the expression of barnase but the promoter was not 100% specific to giant cells, expression was leaky and the barnase too toxic. They transformed tobacco to express full-length cDNA antisense constructs of the RB7 gene. Some of the transgenic tobacco plants are resistant. They consistently impede root-knot nematode reproduction in greenhouse and field trials.

Other promoters have been looked at. The 35S promoter, the nopaline syntheses promoter, and several other strong constitutive promoters are highly active in roots without nematodes, but they are silenced or seriously down regulated in nematode feeding cells in tobacco and in *Arabidopsis*. Several people have disagreed with these results. Opperman has not observed silencing of the 35S promoter in giant cells of root-knot nematodes in tobacco.

The green fluorescent protein reporter gene has been used to answer this question. Atkinson in England showed that the 35 S promoter is down regulated in root-knot nematode giant cells. But a group in Wageningen showed that it is not down regulated at all. Perhaps, not everybody is working with the full sequence of this promoter.

Hydroxymethylglutaryl CoA reductase is a defence-related gene that is induced by fungal and bacterial pathogens. In tomato plants infected by root-knot nematodes, the promoter of this gene drives a strong expression of GUS in giant cells. The gene is turned on in the giant cells, but the normal defence function is not effective to prevent nematode development.

The promoter of a haemoglobin gene from a nitrogen-fixing, non-legume plant shows a more complex pattern of control. When root-knot nematodes develops into roots of transgenic tobacco expressing GUS under the control of this promoter, there is little

GUS expression in the giant cells during the first 2 weeks after giant cell initiation in contrast to the high level of expression in other root tissues. However, GUS expression is high in the giant cells at later stages of infection, 3 to 6 weeks after giant cell initiation, while expression in other tissues declines drastically.

We are using a different wound inducible promoter from asparagus, a monocot by the way. This root system is from a transgenic tobacco seedling transformed to express GUS when the tissue is wounded. No wounding, no GUS protein. When the transgenic seedlings are inoculated with root-lesion nematodes, there is a lot of wounding, especially in root hairs. We use this promoter to drive the expression of a proteinase inhibitor and a lectin.

After the rush to isolate plant genes that are upregulated in giant cells or syncytia Opperman and Bird in the US are now transforming soybean cyst nematodes, and with Jones in Scotland they are transforming the potato cyst nematodes. The goal is still to find nematode genes involved in the control of the feeding sites. They transform sperm cells by microinjection into male gonads. They mate virgin females with these injected males. They infect plants with the progeny of the females. The reporter gene green fluorescent protein is under the control of a muscle specific promoter. The transgenic eggs are easy to detect by fluorescence. Transformed nematodes will be used to study the function of a range of genes with important roles in the host-parasite relationship.

Every living cell needs proteinase inhibitors to regulate endogenous proteolytic activity. Plants that accumulate these inhibitors are often protected from pests and parasites. These proteins bind too strongly at the active site of proteinase enzymes. Not only are they not cleaved like other proteins, the enzyme cannot release the inhibitor proteins. This results in a progressive and more or less complete loss of enzymatic activity.

Proteinase inhibitors in the diet of plant parasitic nematodes bind to digestive proteinases in the gut and prevent protein hydrolysis and absorption of amino acids. The nematodes probably excrete undigested proteins along with their own digestive proteinases. A diet containing proteinase inhibitors is deficient in proteins. The excreted digestive proteinases also result in a net loss of amino acids. The starved nematodes do not reproduce or only slowly. The plants expressing proteinase inhibitors are resistant.

The research with proteinase inhibitors goes back to the late 1970's when a breeding line of cowpea was shown to resist several lepidopteran insect pests because of its elevated content of a trypsin inhibitor. The inhibitor was engineered in tobacco and it successfully controlled lepidopteran insect pests placed on the leaves of young transgenic tobacco.

Atkinson in England tested transgenic potato plants expressing this trypsin inhibitor. When he infected these plants with the root-knot or cyst nematodes, the root-knot nematode females produced much fewer eggs. The cyst nematode entered the roots, established themselves and developed. But they showed a noticeable shift in sex ratio. Instead of having equal numbers of male and female nematodes in the transgenic roots, there were about 5 times more males than females. The serine

proteinase inhibitor altered the nutritional status of the plants and influenced the sexual fate of the juvenile nematodes.

We have looked at 3 major species of root-knot nematodes and they all have major cysteine proteinase activity. A cystatin from rice completely inhibits the proteolytic activity of all stages of *Meloidogyne hapla*. But this inhibitor does not bind so tightly to the proteinases of two other species of this nematode. But then we found that rice also produces another cystatin, 100 times more effective than the first one against these two species.

So particular plant inhibitors are more efficient towards particular nematode proteinases than others. If plants express a multitude of cystatin proteinase inhibitors, then we can expect that some of them will be extremely effective against nematodes. However it is very costly to collect a multitude of plants and to extract and test proteinase inhibitors to find those that bind very tightly to nematode proteases. There is a cheaper way to look for variability because we can evolve proteins in a test tube. This protein model shows how a cystatin inhibitor in light blue fits into the proteinase enzyme in dark blue. In effect there are only a few amino acids on each molecule at the reactive site of each molecule that are responsible for the tightness of the protein interaction.

In Canada we have built a phage display library of 3 million variants of a proteinase inhibitor. The wild protein is a typical cystatin from a nematode, which happens to bind quite effectively the proteases of the Colorado potato beetle and the flea beetle. Each variant gene encodes a slightly modified and therefore unique protein, each one a unique proteinase inhibitor. You have to design large numbers of degenerative oligonucleotides to replace the sequence of 5 amino acids at the active site. The gene is modified such that any of the possible 20 amino acids appears in each of the 5 positions. The phage library is panned with insect and nematode proteinases or even crude protein preps to pick the clones that bind tightly.

Atkinson cloned the cysteine proteinase genes from potato and soybean cyst nematodes, and they are like the root-knot nematode proteases, they are quite sensitive to the inhibitor from rice. He also increased the effectiveness of the rice proteinase inhibitor molecule by engineering the protein. He deleted one amino acid at a time at or near the active site of the protein. This technique gave him a small number of variant proteinase inhibitors that he cloned and tested. One of them was ten times more active than the original inhibitor from rice. A single amino acid deletion changed the conformation of the inhibitor protein and made it bind more tightly to the proteinases of the potato cyst nematode. The nematodes do not develop normally in transgenic roots expressing the wild rice inhibitor or the variant protein. The female nematodes in transformed and untransformed roots develop at the same rate for a while, but later the females developing in the roots expressing the mutated protein stay smaller and produce very few eggs.

Bt toxin

You are all familiar with *Bacillus thuringiensis* a common soil bacillus that accumulates large protein crystals when it sporulates. The many kinds of proteins crystallise

separately from the spores, and they are eventually released into the environment. When these bacillus proteins are on plant parts ingested by insects, they dissolve in the insect midgut where they are processed by digestive proteases into smaller polypeptides. These toxins make holes in the insect midgut membranes. The osmotic balance is lost. The cells of the midgut membrane swell and loose their function. The insects stop feeding and die.

Many strains of *Bt* are not toxic to insects so they must rely on other hosts to disseminate, so nematodes swallowing bacteria for a living are susceptible. Many *Bt* strains have been isolated in the last few years that produce polypeptides with nematicidal activity. Belair in Canada and Coomans in Belgium have shown that nematodes that feed on bacteria are killed after ingesting *Bt* spores. The toxicity against nematodes is as specific as with insects. Most *Bt* strains have no effects against a particular nematode species.

The lumen of the stylet of plant parasitic nematodes is too small for bacillus spores to enter. And we do not know enough about the structure and physiology of the intestine of nematodes to predict that plant parasitic nematodes will be affected by these endotoxins. Still, these results are very promising since the mechanisms of toxicity appear to be related in nematodes, and in insects with destruction of intestinal membranes. The close relatedness between bacterial feeders and plant parasitic nematodes makes for a real possibility that the expression of certain *Bat* toxins in transgenic crops will affect nematode pests.

We cannot test directly the effects of *Bt* toxins on plant parasitic nematodes since they cannot ingest bacteria or their spores or even the protein crystals. But instead of making transgenic plants expressing these *Bt* toxins or any other toxic molecules, we can test them *in planta*. Florian Grundler in Germany injects giant cells or syncytia with various proteins and other molecules. He can measure the uptake by nematodes, and he can easily observe the physiological effects on the nematodes.

Lectins are proteins that bind to carbohydrates with extreme specificity. These proteins accumulate in large quantities in many seeds and in other storage organs of plants. We do know the role of lectins in the physiology of plants. They may be involved in transporting carbohydrates, they may be involved in cell wall elongation or cell to cell interactions, perhaps in growth regulation. They may be the instruments to recognize receptors in membranes, or they may function as enzymes or even as storage proteins.

Because many lectins are toxic to many herbivores including insects and humans we now think that lectins may also act as defence proteins. A few Lectins are almost without toxicity to mammals, but they are still very toxic to certain insects. We are expressing a mannose binding lectin, which is toxic to aphids and planthoppers. Paul Burrows at Rothamsted has shown that this lectin is quite toxic to the root lesion nematode and to the potato cyst nematode.

Natural enemies of nematodes

They may be a source of toxic proteins to express resistance in transgenic plants. *Nematoctonus* is a fungal parasite that secretes a toxin while it invades the body of its nematode prey. This toxin causes paralysis and rapid death of the nematodes. *Seinura* is a predatory nematode that injects its digestive secretions into its prey. Presumably one of the proteins injected is the toxin that paralyses the nematode prey in seconds. If these toxins are proteins, and if they are not toxic to mammals, they will be useful to engineer resistance against nematodes.

The enzyme cholesterol oxidase represents a new class of insect control proteins. Monsanto scientists discovered it recently as a protein excreted by *Streptomyces* bacteria that is extremely effective against cotton boll weevil and other insects. 1 ppm of cholesterol oxidase in their diet kills 50% of the larvae and severely retards the other 50%. This is roughly equivalent to the toxicity of *Bt* toxins. Cholesterol oxidase disrupts the insect gut by enzymatic oxidation of cholesterol in the cellular membranes. You can be certain that effects against nematodes have also been tested but Monsanto has released no information.

The engineering strategies with proteinase inhibitors, lectins, cholesterol oxidase or *Bt* toxins, are preformed defences, they do not require induction by nematodes. They may be less innocuous to the environment than engineering strategies where nematode induction is required. We can assume that non-target organisms, including predators of nematodes, will be affected by the toxins. It is highly probable that the ever-present toxic molecules will apply a strong selection pressure on nematode populations.

But we may be lucky: our experience of the last 40 years is that no plant parasitic nematodes have ever evolved field resistance against cholinesterase inhibitors. It is not that nematodes do not evolve virulence, they most probably do. We easily produce in petri dishes populations, bacterial feeders that are resistant to high levels of cholinesterase inhibitors. But nematodes are very restricted in their movements in the soil; many species are parthenogenetic, many nematodes do not develop enough to reproduce, and it would seem that the virulence could be easily lost.

We hear a lot about reservations and fear of genetic engineering technology in agriculture. I suggest that as this technology develops, and is proven safe, we can expect that the doubters will be reassured. After all, introducing alien genes from plants that have learned to defend against pests is not so different from introducing biological control agents into areas where the pests have escaped. And in spite of a few mishaps, the concept of bringing in alien organisms for that purpose is well accepted.

All this said, I think we have good reasons to expect that several genetic engineering strategies will achieve their goal to provide specific and durable resistance against nematodes. If I were to peep at the short-term future I would see several of these strategies as a commercial success. In the next 5 years, we can look forward to a dozen resistance genes against the major cyst nematode and root-knot nematode species.

The data generated by the sequencing projects, for *Caenorhabditis elegans*, rice and *Arabidopsis*, and other plants, will no doubt provide new insights about how plants resist biotic stresses. We shall soon know how the parasitic nematodes are attracted to their hosts, and how they establish themselves inside plants without triggering grand central alarm.

The projected doubling of our population in the next 30 years will necessarily be matched with the same increase in food production. It is becoming obvious that genetic engineering technologies are indispensable to the countries developing their population the fastest. Engineering genetic resistance against plant parasitic nematodes is of necessity one of the prominent tools to double food production in the next 30 years.

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